Early Anti-inflammatory Treatment in Patients with Acute ACL Tear and Painful Effusions

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DECLARATION OF SPONSOR

This study protocol was subject to critical review. The information it contains is consistent with:

- The current risk-benefit evaluation of the investigational product
- The moral, ethical, and scientific principles governing clinical research as set out in the World Medical Association Declaration of Helsinki (Edinburg, October 2000) and the principles of GCP and described in the International Conference of Harmonisation Tripartite Guidelines Top E6: "Guidelines for Good Clinical Practice," as well as in the applicable local guidelines.

The investigator will be supplied with details of any significant or new finding, including adverse events, related to the treatment with the investigational product.

Signature: _____ Date: _____

Printed Name: Christian Lattermann, MD

DECLARATION OF INVESTIGATOR

I confirm that I have read the above protocol. I understand it, and I will work according to the principles of GCP as described in 21 CFR parts 50, 54, 56 and according to applicable local requirements and the World Medical Association Declaration of Helsinki (Edinburg, October 2000).

I agree to disclose any proprietary interest I may hold in the investigational product or the Sponsor foundation.

Investigator Signature: _____ Date: _____

Printed Name (Typed or blocked letters): _____

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BACKGROUND AND RATIONALE

Background and Study Rationale

Patients who suffer anterior cruciate ligament (ACL) tears commonly undergo surgical reconstruction. While surgical techniques have consistently been improved to be more anatomic, the long term consequences such as posttraumatic osteoarthritis and subsequent pain have not been significantly reduced. In fact, over 50% of all young patients with ACL tears will develop radiographic OA osteoarthritis (OA) within 10 years after injury.

The long-term consequences of post-traumatic OA 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. 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 the risk of post-traumatic OA 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 articular cartilage in the knee joint. We propose to disrupt the inflammation-driven cascade with Triamcinolone (Kenalog®). We hypothesize that Tiamcinolone 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. We will test our hypothesis in a multicenter, randomized, placebo controlled, double blinded, clinical trial.

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 and amenability of pain control in acute ACL injury. Our proposed study will also generate robust preliminary data that will be used to inform a larger randomized clinical trial with our collaborators at the Multicenter Orthopaedic Outcomes Network (MOON; PI K. Spindler, Vanderbilt University, TN) cohort of ACL injury.

Study Drug - Triamcinolone, is manufactured by Bristol-Meyer-Squibb, New York NY (Kenalog), is available as 1ml (40mg) vials and is FDA approved for acute gouty arthritis, acute and subacute bursitis, acute nonspecific tenosynovitis, epicondylitis, rheumatoid arthritis, synovitis, or osteoarthritis.

Pediatric dosing -The efficacy and safety of corticosteroids in the pediatric population are based on the well established course of effect of corticosteroids which is similar in pediatric and adult populations. Published studies provide evidence of efficacy and safety in pediatric patients for the treatment of nephrotic syndrome (>2 years of age), and aggressive lymphomas and leukemias (>1 month of age). Other indications for pediatric use of corticosteroids, e.g., severe asthma and wheezing, rheumatoid arthritis, acute inflammatory arthritis are based on adequate and well-controlled trials conducted in adults, on the premises that the course of the disease.

The adverse effects of corticosteroids in pediatric patients are similar to those in adults. Like adults, pediatric patients should be carefully observed for local symptoms after injection. Pediatric patients who are treated with corticosteroids by any route, including systemically administered corticosteroids, may experience a decrease in their growth velocity. This negative impact of corticosteroids on growth has been observed at low systemic doses and in the absence of laboratory evidence of HPA axis suppression (i.e., cosyntropin stimulation and basal cortisol

plasma levels). Since in this study only two single time local administrations at much lower systemic dosage will be carried out this side effect is not likely and not expected.

For the purpose of comparison, the following is the equivalent milligram dosage of the various glucocorticoids:

Cortisone, 25	Triamcinolone, 4
Hydrocortisone, 20	Paramethasone, 2
Prednisolone, 5	Betamethasone, 0.75
Prednisone, 5	Dexamethasone, 0.75
Methylprednisolone, 4	

These dose relationships apply only to oral or intravenous administration of these compounds. When these substances or their derivatives are injected intramuscularly or into joint spaces, their relative properties may be greatly altered locally, however, systemic doses are significantly lower.

A total of 1ml (40 mg) will be injected intra-articularly after synovial fluid aspiration 'to dryness' either once (at study entry or 2 weeks after injury) or twice (at both study entry and 2 weeks after injury).

Rational for including pediatric population

The specific reason for enrolling the age group of children aged 14-17 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]
- 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.

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 14-17 from this important trial.

RESEARCH OBJECTIVES OF THE CURRENT STUDY:

The research objectives of this study are:

1. Test the efficacy of Triamcinolone to alleviate knee pain (KOOS, IKDC) and improve patient-reported outcomes (PRO) following ACL rupture in a randomized, placebo controlled, double blinded, clinical trial. Triamcinolone will be administered intra-articularly within 1 week of ACL injury or at 2 weeks after ACL injury, or at both time points and compared with saline control injections. Patients will be evaluated at 5 post-injury time points (1-2 days, 12-14 days, 2 weeks post-op, 4 weeks post-op and 6 months

post-op) following ACL reconstruction.

2. Determine if intra-articular Triamcinolone therapy improves levels of a panel of inflammatory (cytokine), meniscus and cartilage metabolism and oxidative stress biomarkers as measured in synovial fluid from patients at relatively short-term time points after ACL injury (1-7 days, 10-17 days after injury and at time of surgery). Biomarkers to be tested include IL-1α, IL-1β, Collagen type I (NtxI) and collagen type II (CTXII) breakdown products, cartilage oligomeric protein (COMP) and glycomsaminoglycan (GAG) and Xanthine Oxidase. Additionally, the change in IL-1α and IL-1β levels in the Triamcinolone treated and placebo individuals will be evaluated specifically for association with pain and function outcomes.

RESEARCH OBJECTIVES OF EXTENDED FOLLOW-UP SUB-STUDY:

1. Test the efficacy of a treatment protocol using triamcinolone acetate and knee arthrocentesis to improve patient-reported outcomes (PRO) following ACL rupture in a randomized, placebo controlled, double blinded, clinical trial at 24 months. All knees have undergone arthrocentesis and have received either the study drug or placebo within 1 week of ACL injury. The aim is to evaluate patients at the 24 time point following ACL reconstruction using established validated instruments for ACL injuries and PTOA (KOOS, IKDC, MARX).

2. Determine if specific synovial fluid biomarker levels and their response to early intervention with triamcinolone acetonide (Kenalog) correlates with improved clinical outcomes as measured by the KOOS. Early clinical trial results demonstrate that the collagen break down product CTX-II, the chondroprotective cross-linking molecule TSG-6 and cartilage oligomeric protein COMP show synovial fluid patterns similar to established models of early Osteoarthritis in humans and mice in patients treated with placebo. These patterns reverse in patients who received Kenalog at one or two time points prior to surgery. The goal of this aim is to identify if these biomarker levels may be predictive of better or worse outcome as measured by the KOOS at 24 months.

3. Determine if joint health as evaluated by MRI at 24 months following ACL injury is influenced by the initial treatment with Kenalog. Two years following ACL injury MRI analysis using standardized T2-mapping will be performed will be performed on 10 control subjects and 10 subjects who have received Kenalog injections.

INVESTIGATIONAL PLAN

Description of Overall Study Design and Plan (See Figure 1 for study timeline)

This study is a multicenter, randomized, placebo controlled, double blinded, clinical trial. This trial will enroll 68 subjects between the ages of 14 and 33. This trial will be conducted at two clinical centers, the University of Kentucky and Vanderbilt University, as well as established criteria and support of the Multicenter Orthopaedic Outcome Network (MOON). A sub-study involving extended follow-up of patients, 24 months post-surgery will occur at the University of Kentucky clinical center only. This sub-study will involve 20 subjects of the initial University of Kentucky cohort returning for one additional follow-up time point.

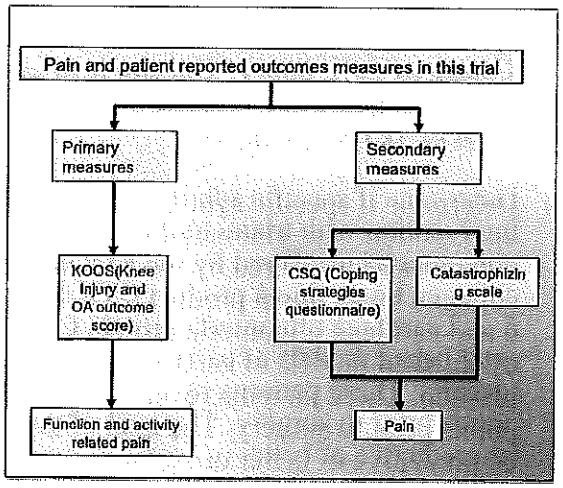
Description of observations and measurements to be made in this study:

Assessment of Pain and Function by KOOS – This trial will use the standardized patient 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 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 subjects' 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 (44). This trial will supplement the KOOS with function score assessments from the International Knee Documentation Committee (IKDC) form for evaluation of knee ligament injuries [45]. In addition to the KOOS and IKDC, those completing the extended follow-up sub-study will also complete the MARX activity scale as an assessment of current physical activity level at the 24 month follow-up.

Specific Pain assessments - Pain coping strategies are important in understanding improvements in pain and psychological disability [46-48]. This trial will use a simple Likert pain scale, the Coping Strategies Questionnaire (CSQ) and 'pain catastrophizing' as pain assessment tools (Figure 2). 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." Not only do individuals who catastrophize, experience more experimental pain [49-52] 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. It has been associated with heightened anxiety, depression, increased pain behaviors, increased use of health care services and analgesic medication and thus, "catastrophizing has risen to the status of one of the most important psychological predictors of the pain experience."[49] In a study of patients undergoing anterior-cruciate ligament surgery, Pavlin, et al. [50] found that high pain catastrophizers were much more likely to report high levels of post-operative pain, had longer durations of moderate to severe pain, and were less likely to report adequate pain control at home.

Figure 2:



Knee Aspiration: All patients will undergo a standard of care knee joint aspiration upon presentation to the clinic, and a study related aspiration two weeks after injury as well as on the day of surgery. Once the study subject undergoes surgery, no further scheduled aspirations are planned. However, if the subject has a substantial post-operative effusion the physician may choose to aspirate that effusion, per their standard of care. In that case the aspirated fluid will be kept for study purposes.
Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will received treatment according to this protocol but, aspiration at time of surgery will be omitted.

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 asceptically with local anaesthesia 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. Serum and urine samples will also be collected but will not be tested in this study. Samples will be stored and archived in the UK-Orthopaedics Cryostorage facility for analysis. Samples can be destroyed if a subject requests this.

IL-1α, IL-1ß, 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 [53], the standard curve will be extended in the low range for the Aristospanα 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 [54]. 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).[61] CTX-II will be measured in synovial fluid by ELISA (IDS, Herlev, Denmark).[55]

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 [55]. We will measure NTX-I in synovial fluid by ELISA.[55] 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 [56]. Although other joint tissues express COMP [57], it is most abundant in articular cartilage. Serum COMP levels are representative of cartilage catabolism [58,59] 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.

Laboratory Analysis of Study Samples:

The supernatants will be stored, pending analysis, in the UK-Orthopaedics biomarker sample repository located in Dr. Lattermann's laboratory.

All biomarker analyses in synovial fluid will be performed by co-Investigator Dr. Kraus (Duke University).

A portion of previously collected samples will be de-identified and shipped on dry ice to Children's National Medical Center at George Washington University where they will undergo the SOMAscan™ assay. The latest version of the SOMAscan assay measures 1,129 protein analytes in only 150 µl of serum. The assay offers exceptional dynamic range, quantifying

proteins that span over 8 logs in abundance (from femtomolar to micromolar), with low limits of detection (38 fMol media LOD) and excellent reproducibility (5.1% median %CV) (http://www.somalogic.com/Technology/SOMAscan-basic-info.aspx). Once the analyses are complete any remaining samples at National Medical Center will be destroyed. At no time will anyone at Children's National Medical Center have access to subject identifiers or any other subject data

Randomization: Patients will be randomized into one of four groups, and there will be 17 subjects per group.

- Group 1: will receive injection of Triamcinolone (40 mg) at time point 1(1-2 days post injury) and saline placebo at timepoint 2)12-14 post injury).
- Group 2: will receive a saline placebo injection at time point 1 (1-2 days post injury) and 40 mg Triamcinolone at time point 2 (12-14 days post injury.)
- Group 3: will receive two consecutive injections of 40 mg Triamcinolone, the first at time point 1 and the second at time point 2.
- Group 4 (control group): will receive two consecutive injections of saline placebo. The first at time point 1 and the second at time point 2.

Duration of Patient Enrollment: All subjects enrolled in the study will be followed for six months. An additional sub-sample of 20 subjects from the main study will be recruited and enrolled in the extended follow-up sub-study at approximately 24 months following surgery.

Duration of Study: Study duration will be 36 months from the commencement date. This duration may be extended to reach desired enrollment.

Data Safety and Monitoring Board (DSMB) - A DSMB consisting of 5 individuals (will monitor the study semi-annually and review reportable adverse events to the FDA as wells as Vanderbilt and University of Kentucky Institutional Review Boards (IRBs). One of these members will maintain the allocation strategy, available upon request from the DSMB.

PATIENT POPULATION: Subjects in this trial will typically be referred by an Emergency Room or walk-in clinic to the Orthopaedics/Sports Medicine Clinic within 48 hours of injury. The study is open to male and female subjects who are between the ages of 14 and 33, who have ACL tears.

Recruitment Method: Principal investigator, co-investigator and his respective study personnel will recruit potential patients who have been referred to them for ACL injuries. All subject recruitment materials must be reviewed and approved by the site IRB/IEC prior to use. Potential subjects will be given a written description of the study in the Informed Consent Form or Assent, and will have the opportunity to ask questions about the study prior to participation. Subjects must sign an IRB approved Informed Consent Form or assent prior to undergoing any study procedures for screening.

Inclusion Criteria: Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

- 14-33 years of age
- Currently participating in a sporting activity
- Injury occurred while playing in a sporting activity
- Normal contralateral knee status
- Documentation of closed growth plates as noted on the screening x-rays

Exclusion Criteria: Subjects presenting with any of the following will not be included in the study:

- underlying inflammatory disease (i.e. Rheumatoid Arthritis, Psoriatic Arthritis etc)
- currently have any infections, including infection of the skin, or have signs and symptoms of an infection, including fever.
- have a disease that weakens your immune system such as diabetes, cancer, HIV or AIDs
- other major medical condition requiring treatment with immunosuppressant or modulating drugs.
- A history of chronic use of non-steroidal anti-inflammatory drugs
- Received corticosteroid injections into the injured knee within three months of enrollment
- previous exposure or allergic reaction to Kenalog®
- prior knee surgery (Ipsilateral or contralateral)
- have received any investigational drug with 4 weeks of study Visit 1
- additionally, subjects experiencing an injury or re-injury to the involved or contralateral knee following primary ACL reconstruction will be excluded from the extended follow-up sub-study.

Early Termination of Subjects from Study:
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 study withdrawal may include the following:

- Adverse event
- Non-compliance with protocol/protocol deviation
- Sponsor's decision
- Lost to follow up
- Withdrawal of consent

Subjects who are withdrawn or terminate early from the study will be preplaced under certain circumstances. These circumstances are withdrawal prior to the completion of all three blood and synovial fluid draws (the study is powered to show a difference in these markers and will be underpowered if these subjects cannot be replaced)

The date and reason must be recorded for all subjects who withdraw from the study. If the reason for withdrawal is a treatment-emergent AE, the specific AE will be identified on the CRF and every attempt will be made to follow the event until resolution.

In addition, every attempt should be made to complete the Visit 6/Early Termination assessments.

If a subject discontinues the study for any reason prior to Visit #5/12-14 weeks post injury visit, then the collected data will be included in the safety analysis, however will not be included in the overall efficacy analysis and a replacement subject will be enrolled in the study.

STUDY PROCEDURES/EXAMINATIONS (See Appendix A)

Demographics, medical history and medication history: Demographic data to be collected on screening visit will include height, weight, date of birth, age, gender and race. Detailed past medical history and medication history will be collected on screening visit. The medical history or concurrent medication history will be noted on each visit.

Knee Aspiration: All subjects undergo knee aspiration to dryness at initial encounter (1-4 days after ACL injury), (+/- 4 days), 12-14 days after injury, at the time of surgery. Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will received treatment according to this protocol but, aspiration at time of surgery will be omitted. Planned aspiration postoperatively will not be performed unless clinically necessary for significant postoperative effusions. If an aspiration is done, a sample will be retained for analysis.

Visit 1 Screening (1-4 days post injury) (+/- 4 days): Upon arriving at the study clinic potential subjects will have the following assessments: range of motion, knee instability (Lachman's test) and standardized Flexion weight bearing x-rays. At this time the subjects will also be asked to fill in a standard questionnaire covering the KOOS, IKDC, SF-36 and, the catastrophizing scale, CSQ and a Likert pain scale.

All subjects must have a clinical exam that is consistent with an ACL tear. Following the review of above assessment results, review of inclusion/exclusion and after signing the informed consent/assent the following assessments will take place:

Collection of urine and 10 cc of blood for laboratory testing, partial Medical history, concomitant medications, demographic, BMI, smoking status, outcomes, and pain questionnaires will also be collected.

Subjects will then be randomized into and one of four treatment groups and undergo a knee aspiration. Following randomizing, the subject will receive their first dose study medication.

Visit 2 (12-14 days post injury):
- Lab tests(urine and 10 cc of blood)
- Pain Questionnaires administered
- Knee aspiration
- Study medication injection
- Patient reported outcomes (PROs) administered

Visit 3 (day of surgery)
- Lab tests(urine and 10cc of blood)
- Pain Questionnaires administered prior to surgery
- Knee aspiration (in the operating room under anesthesia)
- Patient reported outcomes (PROs) administered prior to surgery

Visit 4 (1-2 weeks post surgery)
- Pain Questionnaires administered
- Knee aspiration (if clinically indicated)
- Patient reported outcomes (PROs) administered

Visit 5 (4-6 weeks post surgery)
- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered

Visit 6 (6 months follow-up/Early Termination Visit)
- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered

Optional Extended Follow-Up Sub-Study (To Include 20 University Of Kentucky Subjects)

Visit 7 (24 (+/- 3) month follow-up

- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered
- Research Specific MRI

ADVERSE EVENTS

Any significant change in a subject's health from screening will be recorded and reported as adverse events while the subject is taking part in the study. Adverse Events (AEs) will include but are not limited to severe post-administration pain requiring treatment, infection of the injection site or the knee joint, ER visit following the administration of the study drug at visit 1 or 2. Due to the short half life of the drug we do not expect any SAE's related to the drug administration later than after the date of ACL surgery. During the observation time (time of surgery to 6 months post-op) other, non-drug related, SAEs may occur which will be recorded and reported.

Any reported SAE occurring during the study must be reported by the sponsor to the Food and Drug Administration and the appropriate Institutional Review Board. Any SAE due to any cause, whether or not related to the study drug, must be reported within 24 hours of when the Investigator becomes aware of the event. Investigators who need to report SAEs to the sponsor may do so by utilizing FDA Form 3500A the sponsor provided SAE reporting form and faxing report to (859) 323-2412.

Plan for unexpected adverse event (AE) reporting. Serious AEs will be reported to Human Subjects/IRB within 48 hr. Unanticipated events will be reported to the IRB.

Monitoring of adverse events. Adverse events will be monitored via exams, vital signs, review of subject's medical chart, and documented. Each visit will be documented with a progress note in the research chart.

Adverse Event Follow-up

All AEs, including clinically significant changes in physical examinations findings, or isolated clinically significant laboratory findings must be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up. If resolved or stabilized, a resolution date should be documented on the CRF.

Adverse events ongoing at the final visit or early termination visit will be followed until that adverse event is resolved.

Potential Risk:

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

Benefit Vs Risk: Triamcinolone is a fist line treatment of established OA. In this trial, we expect that an early intervention with Triamcinolone may block the deleterious cascade of joint degradation events after joint injury. We expect a significant effect of Triamcinolone on post-injury pain and concomitantly an increased improvement in early function.

Risks associated with Triamcinolone: The following are the risks associated with Kenalog®: Subjects may experience an allergic reaction (difficulty breathing; closing of the throat; swelling of your lips, tongue, or face; or hives). In rare cases, people receiving Kenalog® have developed serious infections. Subjects should notify the study doctor immediately if they develop a fever, flu-like symptoms, or any other sign of infection.
Other less serious side effects may be more likely to occur.

- nausea or diarrhea;
- a headache;
- sinus irritation or infection; or
- redness, bruising, pain, or swelling at the injection site.

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

- Dose: 40 mg of Triamcinolone is a standard concentration given in patients with acute joint inflammation. Cortisone is cleared systemically within several days after administration while locally it may persist longer. A second booster injection 10 days after initial administration is not likely to have a harmful effect on cartilage or any other intraarticular tissue.

- 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.

Statistics, Quality Control and Minimization of bias:

Quality Control - All data that we collect will be carefully analyzed with respect to variability, linear range of standard, and need for repeat analyses. Controls provided with commercially available ELISA kits are used with every run. For assays for which no control is available or provided, aliquots of serum from normal human subjects have been aliquoted and frozen at -80°C for this purpose. Each assay day, a fresh aliquot of this control serum is thawed and used on every plate to calculate intra- and inter-assay variance of the assay. In addition to the standard curve run in duplicate for each assay, this control will be run with each assay and the results used to determine the precision of the assay and to establish an acceptable control range for the assay. The mean of the control sample for all assays plus or minus 2SD's is defined as the acceptable control range. Any samples on a plate in which the control falls outside of this range will be excluded and repeated. Samples will be run in duplicate and reanalyzed if the CV >15%. For values that are below the level of detection, defined by the lowest standard, ½ LLOQ (lowest level of quantification) will be reported for statistical purposes.

Sample Size and Power Analysis - We used the VAS and WOMAC data from Chevalier et al. as the template for power calculations [4] and the change in KOOS pain and ADL from our study. Chevalier reported a 53% response rate by VAS. Response was defined as a 50% improvement in VAS pain score from baseline. We observed comparable differences in our study (20-30% between outcomes in the IL-1Ra and placebo groups); therefore this dataset is reasonable to estimate study power. Based upon the change in VAS pain score in the Chevalier study, and conservatively estimating a placebo response rate of 30%, the effect size was 0.47 for a dichotomous outcome. At this effect size power for a sample size of 17/group is reasonable (65%) for the weakest possible outcome (i.e., one that only generates a "yes" or a "no" for each patient). Moreover, power is excellent for the continuous KOOS outcomes. With 17 patients per

group we achieve 90% power for effect sizes of approximately 0.77 and 80% power for effect sizes of approximately 0.65. These latter effect sizes are in standard deviation multiples. So, for example, if the standard deviation of the VAS is approximately 20, and we expect an improvement of approximately 20 units in the intervention group, we can tolerate an improvement of approximately 7 units in the placebo group and still have 90% power (i.e. the effect size is (20-7)/20 = 0.65). Moreover, using the effect size standard benchmarks of 0.50 as moderate and 0.80 as large, we will be powered to detect moderate-to-large effects of the intervention, which is consistent with intuition for a study of this size. Power calculations based on our pilot KOOS data confirm this estimate. The change in KOOS (days 0-4) comparing treatment and placebo groups yielded an effect size of 0.62 for pain and 1.08 for ADL. At a group size of 17 we will have 80% power to detect a difference in KOOS pain and >99% power to detect a difference in KOOS ADL.

The power calculations include several caveats. The power calculation was performed based upon the use of a IL-1 receptor antagonist. There is no clinical data available in a young patient population with knee ligament injuries regarding their pain improvement after cortisone injection. The IL1RA data is the most comparable. Cortisone is a wider spectrum anti-inflammatory than IL-1RA and thus may have a larger effect on pain control that may be longer lasting. In that case our study may be over-powered. First, for purposes of power calculation, we have not assumed a full repeated measures analysis, but have instead analyzed change from baseline at a single time point. 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 and IKDC 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 three 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. For those subjects enrolled in the extended follow-up sub-study the 24 month time point will be examined as an additional time point. Additionally, correlation analyses will be used to examine the relationship between biomarkers collected at Visits 1-3 and KOOS and IKDC values and MRI findings at 24 month follow-up.

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.

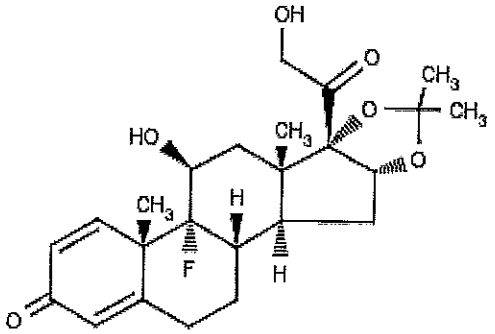
STUDY MEDICATION:

Formulation, Packaging and Labeling:

Kenalog® is a sterile suspension containing 40 mg/mL of micronized triamcinolone acetonide in the following inactive ingredients:

Polysorbate 80	0.20% w/v
Sorbitol Solution USP	50.00% w/v
Water for Injection qs ad	100.00% V
Hydrochloric Acid and Sodium Hydroxide, if required, to adjust pH to	4.0-8.0
Preservative: Benzyl Alcohol	0.90% w/v

The chemical name for triamcinolone acetonide is 9α-Fluoro-11β,16α, 17,21- tetrahydroxyp-pregna-1,4-diene-3, 20-dione cyclic 16, 17-acetal with acetone. Its structural formula is:



CLINICAL PHARMACOLOGY

Glucocorticoids, naturally occurring and synthetic, are adrenocortical steroids that are readily absorbed from the gastrointestinal tract.

Naturally occurring glucocorticoids (hydrocortisone and cortisone), which also have salt-retaining properties, are used as replacement therapy in adrenocortical deficiency states.

Their synthetic analogs are primarily used for their anti-inflammatory effects in disorders of many organ systems. When injected intraarticularly, triamcinolone acetonide can be expected to be absorbed slowly from the injection site.

Shipping, Storage, Handling: Unblinded study drug will be shipped from the manufacturer directly to the site. The syringes with Kenalog will be prepared at the site after study participants have been identified and consented and randomization has been performed. An unblinded nurse, nursing assistant or athletic trainer that has no primary involvement with this study will prepare a 10cc syringe with 40mg of Kenalog and 9cc of saline solution in standard aseptic technique. The syringe will be fitted with an opaque sleeve that does not allow for the visual distinction between study drug and placebo.

The study drug will be kept at the research site at The University of Kentucky (or Vanderbilt University respectively) and stored at controlled room temperature, 20°-25°C (68°-77°F), avoiding freezing and protected from light.

Under aseptic clean conditions, a total of 40 mg (1 ml) of Kenalog® will be drawn into a sterile 10 ml B&Dsyringe, A standard dilution into 9ml of injectable saline solution will be performed.

For the placebo we will utilize 10 ml of 0.9%NACL sterile saline solution drawn up in the same type of syringe as for the Kenalog® administration in order to maintain double blinding of the study investigator and patients.

Once a subject is identified and consented, the study physician will utilize the syringe corresponding to the assigned patient ID to administer the study medication.

STUDY MANAGEMENT:

Regulatory Guidelines: This study will be performed in accordance with US 21 Code of Federal Regulations Parts:

50, Protection of Human Subjects;
54, Financial Disclosure by Clinical Investigators and
56, Institutional Review Boards

Good Clinical Practice (GCP): Consolidated Guideline (International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, May 1996).

Declaration of Helsinki, concerning medical research in humans ("Recommendations Guiding Physicians in Biomedical research Involving Human Patients," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989 and revised version of Sumerset West, Republic of South Africa, Oct, 1986).

IRB Approval

This study must have initial and at least annual approval from an Institutional Review Board (IRB) responsible for approving clinical studies. Furthermore, screening and enrollment of subjects into the trial will not commence until Investigator receives the IRB approval letter. In addition, a copy of the IRB approval letter must be filed on-site in the investigator's study binder. When appropriate, amendments to the protocol must be submitted for IRB review and approval before being implemented.

Informed Consent/Assent (Title 21, Section 50 and 312.62 of the CRF)

- Study enrollment will not begin until the Investigator has received an approved and validated informed consent and Assent from the IRB.
- All subjects enrolling in the study:
  1. Will be informed of the investigational nature of the study
  2. Must be given a copy of the Informed Consent, and if required, the Assent Form
  3. Must be given the opportunity to ask any questions regarding the study treatment
  4. Will have voluntarily and willingly signed the Informed Consent Form/Assent Form

prior to initiation of any study related test/procedure. Children age 14 and older must have an IRB approved assent in place in order to be considered enrolled into the study.

A separate consent will be utilized to enroll subjects in the extended follow-up sub-study. All subjects enrolling in the sub-study:

1. Will be informed of the investigational nature of the study
2. Must be given a copy of the Informed Consent addendum, and if required, the Assent Form addendum
3. Must be given the opportunity to ask any questions regarding study procedures
4. Will have voluntarily and willingly signed the Informed Consent Form/Assent Form prior to initiation of any study related test/procedure. Children age 12 and

older must have an IRB approved assent in place in order to be considered enrolled into the study.

Pre-study Documentation Requirements

The investigator is responsible for assembling, and sending to the Sponsor, the following documents before study ignition can occur:

- Signed and dated protocol signature page
- Copy of approved ICF and Assent
- Copy of the IRB approval of the protocol
- The IRB composition and/or written IRB compliance statement
- Signed Clinical Trial Agreement
- Curricula Vitae of all investigators and sub-investigators (signed and dated)
- Signed and dated FDA Form 1572
- Lab certifications (if required)
- Signed and dated Financial Disclosure for everyone listed on the site's FDA Form 1572
- Additional documentation, as required by the sponsor.

Protocol Adherence

The Investigator agrees to conduct the study in accordance with this protocol. Prior to beginning the study, the Investigator(s) must sign both the Investigator Agreement and the protocol signature page.

An Investigator must not make any changes to the study without first receiving written approval from the Sponsor and IRB, except when necessary to eliminate apparent immediate hazard to a subject. As soon as possible, the implemented deviation or change, reasons for it, and if appropriate, the proposed protocol amendment(s) should be submitted to the IRB for review and approval.

Protocol Amendments

Protocol amendments must be made only with the prior approval of the Sponsor (Dr. Lattermann). Agreement from the principal investigator must be obtained for all protocol amendments and amendments to the ICF. The IRB must be informed of all amendments and give approval for any amendments likely to affect the safety of the subjects or the conduct of the study. The principal investigator must send a copy of the approval letter from the IRB to the Sponsor and/or designee.

Both the Sponsor and the principal investigator reserve the right to terminate the study according to the study contract. The principal investigator should notify the IRB/IEC in writing of the study's completion or early termination and send a copy of the notification to the Sponsor and/or designee.

Study Monitoring and Auditing

The Sponsor and/or designee representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the study (eg, CRFs and other pertinent data) provided that subject confidentiality is respected, in accordance with the International Conference on Harmonization, (ICH) Good Clinical Practices (GCP) guidelines (ICH GCP E6 Section 5.18). The Investigator will permit representatives of the Sponsor's monitoring team or FDA to inspect facilities and records relevant to this study.

The Sponsor and/or designee Monitor and/or clinical research associate (CRA) is responsible for inspecting the CRF/eCRFs at regular intervals throughout the study to verify that the clinical trial is conducted in an organized manner according to the protocol provided.

Monitoring visits will assess:

- adherence to the protocol
- progress in the conduct of the study
- completeness, accuracy, and consistency of the data
- AE reporting to the Sponsor and the IRB/IEC
- adequacy of the facilities and availability of equipment required to conduct the study (ie,
- local laboratory, ECG equipment)
- adherence to local regulations on the conduct of clinical research

The Monitor should have access to each subject's permanent medical records and other study related records needed to verify the entries on the CRF/eCRFs.

The principal investigator will permit the Sponsor's personnel (or designees) to audit all CRF/eCRFs and supporting documentation, eg, hospital, office, clinic, pharmacy, and laboratory records for each subject. The principal investigator agrees to cooperate with the Monitor to ensure that any problems detected in the course of these monitoring visits are resolved.
In accordance with the ICH, GCP and the Sponsor and/or its designee audit plans, this study may be selected for audit. Inspection of site facilities (eg, pharmacy, drug storage areas, laboratories, etc.) and review of study-related records will occur in order to evaluate the study conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

Data Safety Monitoring Board

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 University of Kentucky Center Center for Clinical and Translations 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.

Study Stopping Criteria

- local intolerance of the administered Kenalog® 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.

Data Recording and Record Retention

The PI must maintain all Essential Documents (as listed in the ICH Guideline for GCP) until notified by the Sponsor and in accordance with all local laws regarding retention of records.

The Investigator should maintain a list of appropriate qualified persons to whom he/she has delegated trial duties. All persons authorized to make entries and/or corrections to the case report forms should be included on the list of qualified persons. Source documents are original documents, data, and or records from which the participant's case report form data are obtained.

These include, but are not limited to, hospital records, clinical and office charts, laboratory and pharmacy records, microfiches, radiographs and correspondence.

The Investigator and study staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the University of Kentucky and/or applicable regulatory authorities. Elements should include:

- Participant files containing complete case report forms, ICFs, assents, HIPAAs, and supporting copies of source documentation;
- Study files containing the protocol with all amendments, Kineret® package insert, copies of pre-study documentation, and all correspondence to and from the IRB and;
- Proof of receipt, Investigational Product Accountability Record, Return of Investigational Product for Destruction, Final Investigational Product Reconciliation Statement, and all drug-related correspondence.

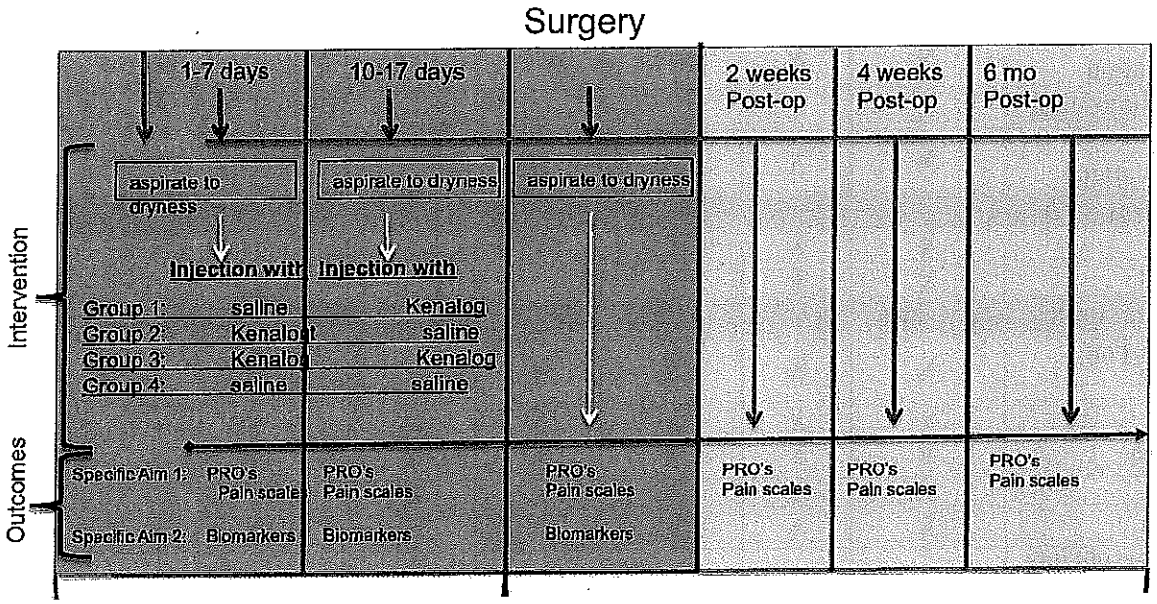
No study documentation should be destroyed without prior written agreement between Doctor Lattermann and the investigator. Should the investigator wish to assign the study record to another party or move them to another location, he/she may do so only with the prior written approval of Dr. Lattermann.

SAE reporting to the Sponsor:
Adverse reactions will be reported promptly to the Principal Investigator 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.

SUBJECT CONFIDENTIALITY

The Investigator and the Principal Investigator affirm a subject's right to protection against invasion of privacy. In compliance with United States federal regulations, the Principal Investigator requires the Investigator(s) to permit, when necessary, representatives from the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws. Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent/assent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

Figure 1: Study Design and Plan



Appendix A: Study Flow Chart

	Study Drug Treatment			Follow-up			Extended Follow-up
	Screening Baseline 1-2 days post injury (+/- 4 days)	12-14 days post injury	Surgery[5]			6 Months Follow-up/Early Termination Visit	Sub-Study
Study Time points	Time point 1	Time point 2		1-2 Weeks post surgery	4-6 weeks post surgery		24 Months (+/- 3) post surgery
Procedures	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Informed Consent	X						X
Partial Medical History	X						X
Medication History	X	X	X	X	X	X	X
Smoking Status	X						
BMI Measurement	X						

Demographics	X						
X-Ray[1]	X						
MRI[2, 6]	X						X
Range of Motion	X						X
Knee Aspiration	X	X	X[3&5]	X[3&5]			
Randomization	X						
Study Medication	X	X					
Laboratory Tests							
Urine	X	X	X				
Blood	X	X	X				
Questionnaires And Scales							
Likert Pain Scale[4]	X	X	X	X	X	X	X
CSQ	X	X	X	X	X	X	X
KOOS	X	X	X	X	X	X	X
IKDC	X	X	X	X	X	X	X
SF 36	X	X	X	X	X	X	X
Catastrophizing Scale	X	X	X	X	X	X	X
MARX Activity Scale							X

1 = All subjects must have standardized flexion weight bearing x-rays. *Documentation of closed growth plates at screening will be noted on the routine SOC x-rays.*
2 = All subjects enrolled will have an MRI performed as a routine diagnostic tool 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.
3 = Knee aspiration will only be performed if the subject has post-op fluid on the knee. Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, aspiration at time of surgery will be omitted.
4 = All subjects enrolled into the study must have been diagnosed with an ACL tear.
5 = Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will receive treatment according to this protocol.
6 = Subjects enrolled into the extended follow-up sub-study will undergo a research specific MRI, that is not considered standard of care.

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Early Anti-inflammatory Treatment in Patients with Acute ACL Tear and Painful Effusions

Date: 4 May 2015

Version 7

Sponsor/Principle Investigator: Christian Lattermann, MD
University of Kentucky
Dept. of Orthopaedic Surgery and Sports Medicine
Kentucky Clinic K 401
740 South Limestone Street
Lexington KY 40536

Early anti-inflammatory Treatment in Patients with Acute ACL Tear and Painful Effusions

DECLARATION OF SPONSOR

This study protocol was subject to critical review. The information it contains is consistent with:

- The current risk-benefit evaluation of the investigational product
- The moral, ethical, and scientific principles governing clinical research as set out in the World Medical Association Declaration of Helsinki (Edinburg, October 2000) and the principles of GCP and described in the International Conference of Harmonisation Tripartite Guidelines Top E6: "Guidelines for Good Clinical Practice," as well as in the applicable local guidelines.

The investigator will be supplied with details of any significant or new finding, including adverse events, related to the treatment with the investigational product.

Signature: _____ Date: _____

Printed Name: Christian Lattermann, MD

DECLARATION OF INVESTIGATOR

I confirm that I have read the above protocol. I understand it, and I will work according to the principles of GCP as described in 21 CFR parts 50, 54, 56 and according to applicable local requirements and the World Medical Association Declaration of Helsinki (Edinburg, October 2000).

I agree to disclose any proprietary interest I may hold in the investigational product or the Sponsor foundation.

Investigator Signature: _____ Date: _____

Printed Name (Typed or blocked letters): _____

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BACKGROUND AND RATIONALE

Background and Study Rationale

Patients who suffer anterior cruciate ligament (ACL) tears commonly undergo surgical reconstruction. While surgical techniques have consistently been improved to be more anatomic, the long term consequences such as posttraumatic osteoarthritis and subsequent pain have not been significantly reduced. In fact, over 50% of all young patients with ACL tears will develop radiographic OA osteoarthritis (OA) within 10 years after injury.

The long-term consequences of post-traumatic OA 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. 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 the risk of post-traumatic OA 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 articular cartilage in the knee joint. We propose to disrupt the inflammation-driven cascade with Triamcinolone (Kenalog®). We hypothesize that Tiamcinolone 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. We will test our hypothesis in a multicenter, randomized, placebo controlled, double blinded, clinical trial.

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 and amenability of pain control in acute ACL injury. Our proposed study will also generate robust preliminary data that will be used to inform a larger randomized clinical trial with our collaborators at the Multicenter Orthopaedic Outcomes Network (MOON; PI K. Spindler, Vanderbilt University, TN) cohort of ACL injury.

Study Drug - Triamcinolone, is manufactured by Bristol-Meyer-Squibb, New York NY (Kenalog), is available as 1ml (40mg) vials and is FDA approved for acute gouty arthritis, acute and subacute bursitis, acute nonspecific tenosynovitis, epicondylitis, rheumatoid arthritis, synovitis, or osteoarthritis.

Pediatric dosing -The efficacy and safety of corticosteroids in the pediatric population are based on the well established course of effect of corticosteroids which is similar in pediatric and adult populations. Published studies provide evidence of efficacy and safety in pediatric patients for the treatment of nephrotic syndrome (>2 years of age), and aggressive lymphomas and leukemias (>1 month of age). Other indications for pediatric use of corticosteroids, e.g., severe asthma and wheezing, rheumatoid arthritis, acute inflammatory arthritis are based on adequate and well-controlled trials conducted in adults, on the premises that the course of the disease.

The adverse effects of corticosteroids in pediatric patients are similar to those in adults. Like adults, pediatric patients should be carefully observed for local symptoms after injection. Pediatric patients who are treated with corticosteroids by any route, including systemically administered corticosteroids, may experience a decrease in their growth velocity. This negative impact of corticosteroids on growth has been observed at low systemic doses and in the absence of laboratory evidence of HPA axis suppression (i.e., cosyntropin stimulation and basal cortisol

plasma levels). Since in this study only two single time local administrations at much lower systemic dosage will be carried out this side effect is not likely and not expected.

For the purpose of comparison, the following is the equivalent milligram dosage of the various glucocorticoids:

Cortisone, 25	Triamcinolone, 4
Hydrocortisone, 20	Paramethasone, 2
Prednisolone, 5	Betamethasone, 0.75
Prednisone, 5	Dexamethasone, 0.75
Methylprednisolone, 4	

These dose relationships apply only to oral or intravenous administration of these compounds. When these substances or their derivatives are injected intramuscularly or into joint spaces, their relative properties may be greatly altered locally, however, systemic doses are significantly lower.

A total of 1ml (40 mg) will be injected intra-articularly after synovial fluid aspiration 'to dryness' either once (at study entry or 2 weeks after injury) or twice (at both study entry and 2 weeks after injury).

Rational for including pediatric population

The specific reason for enrolling the age group of children aged 14-17 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]
- 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.

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 14-17 from this important trial.

RESEARCH OBJECTIVES OF THE CURRENT STUDY:

The research objectives of this study are:

1. Test the efficacy of Triamcinolone to alleviate knee pain (KOOS, IKDC) and improve patient-reported outcomes (PRO) following ACL rupture in a randomized, placebo controlled, double blinded, clinical trial. Triamcinolone will be administered intra-articularly within 1 week of ACL injury or at 2 weeks after ACL injury, or at both time points and compared with saline control injections. Patients will be evaluated at 5 post-injury time points (1-2 days, 12-14 days, 2 weeks post-op, 4 weeks post-op and 6 months

post-op) following ACL reconstruction.

2. Determine if intra-articular Triamcinolone therapy improves levels of a panel of inflammatory (cytokine), meniscus and cartilage metabolism and oxidative stress biomarkers as measured in synovial fluid from patients at relatively short-term time points after ACL injury (1-7 days, 10-17 days after injury and at time of surgery). Biomarkers to be tested include IL-1α, IL-1β, Collagen type I (NtxI) and collagen type II (CTXII) breakdown products, cartilage oligomeric protein (COMP) and glycomsaminoglycan (GAG) and Xanthine Oxidase. Additionally, the change in IL-1α and IL-1β levels in the Triamcinolone treated and placebo individuals will be evaluated specifically for association with pain and function outcomes.

RESEARCH OBJECTIVES OF EXTENDED FOLLOW-UP SUB-STUDY:

1. Test the efficacy of a treatment protocol using triamcinolone acetate and knee arthrocentesis to improve patient-reported outcomes (PRO) following ACL rupture in a randomized, placebo controlled, double blinded, clinical trial at 24 months. All knees have undergone arthrocentesis and have received either the study drug or placebo within 1 week of ACL injury. The aim is to evaluate patients at the 24 time point following ACL reconstruction using established validated instruments for ACL injuries and PTOA (KOOS, IKDC, MARX).

2. Determine if specific synovial fluid biomarker levels and their response to early intervention with triamcinolone acetonide (Kenalog) correlates with improved clinical outcomes as measured by the KOOS. Early clinical trial results demonstrate that the collagen break down product CTX-II, the chondroprotective cross-linking molecule TSG-6 and cartilage oligomeric protein COMP show synovial fluid patterns similar to established models of early Osteoarthritis in humans and mice in patients treated with placebo. These patterns reverse in patients who received Kenalog at one or two time points prior to surgery. The goal of this aim is to identify if these biomarker levels may be predictive of better or worse outcome as measured by the KOOS at 24 months.

3. Determine if joint health as evaluated by MRI at 24 months following ACL injury is influenced by the initial treatment with Kenalog. Two years following ACL injury MRI analysis using standardized T2-mapping will be performed will be performed on 10 control subjects and 10 subjects who have received Kenalog injections.

INVESTIGATIONAL PLAN

Description of Overall Study Design and Plan (See Figure 1 for study timeline)

This study is a multicenter, randomized, placebo controlled, double blinded, clinical trial. This trial will enroll 68 subjects between the ages of 14 and 33. This trial will be conducted at two clinical centers, the University of Kentucky and Vanderbilt University, as well as established criteria and support of the Multicenter Orthopaedic Outcome Network (MOON). A sub-study involving extended follow-up of patients, 24 months post-surgery will occur at the University of Kentucky clinical center only. This sub-study will involve 20 subjects of the initial University of Kentucky cohort returning for one additional follow-up time point.

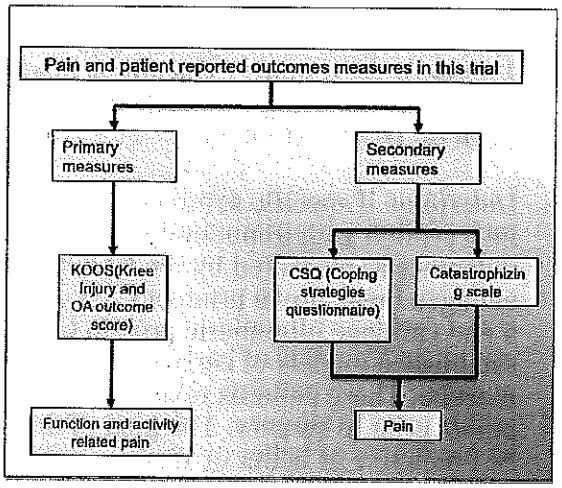
Description of observations and measurements to be made in this study:

Assessment of Pain and Function by KOOS – This trial will use the standardized patient 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 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 subjects' 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 (44). This trial will supplement the KOOS with function score assessments from the International Knee Documentation Committee (IKDC) form for evaluation of knee ligament injuries [45]. In addition to the KOOS and IKDC, those completing the extended follow-up sub-study will also complete the MARX activity scale as an assessment of current physical activity level at the 24 month follow-up.

Specific Pain assessments - Pain coping strategies are important in understanding improvements in pain and psychological disability [46-48]. This trial will use a simple Likert pain scale, the Coping Strategies Questionnaire (CSQ) and 'pain catastrophizing' as pain assessment tools (Figure 2). 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." Not only do individuals who catastrophize, experience more experimental pain [49-52] 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. It has been associated with heightened anxiety, depression, increased pain behaviors, increased use of health care services and analgesic medication and thus, "catastrophizing has risen to the status of one of the most important psychological predictors of the pain experience."[49] In a study of patients undergoing anterior-cruciate ligament surgery, Pavlin, et al. [50] found that high pain catastrophizers were much more likely to report high levels of post-operative pain, had longer durations of moderate to severe pain, and were less likely to report adequate pain control at home.

Figure 2:



Knee Aspiration: All patients will undergo a standard of care knee joint aspiration upon presentation to the clinic, and a study related aspiration two weeks after injury as well as on the day of surgery. Once the study subject undergoes surgery, no further scheduled aspirations are planned. However, if the subject has a substantial post-operative effusion the physician may choose to aspirate that effusion, per their standard of care. In that case the aspirated fluid will be kept for study purposes.
Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will received treatment according to this protocol but, aspiration at time of surgery will be omitted.

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 asceptically with local anaesthesia 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. Serum and urine samples will also be collected but will not be tested in this study. Samples will be stored and archived in the UK-Orthopaedics Cryostorage facility for analysis. Samples can be destroyed if a subject requests this.

IL-1α, IL-1ß, 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 [53], the standard curve will be extended in the low range for the Aristospanα 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 [54]. 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).[61] CTX-II will be measured in synovial fluid by ELISA (IDS, Herlev, Denmark).[55]

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 [55]. We will measure NTX-I in synovial fluid by ELISA.[55] 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 [56]. Although other joint tissues express COMP [57], it is most abundant in articular cartilage. Serum COMP levels are representative of cartilage catabolism [58,59] 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.

Laboratory Analysis of Study Samples:

The supernatants will be stored, pending analysis, in the UK-Orthopaedics biomarker sample repository located in Dr. Lattermann's laboratory.

All biomarker analyses in synovial fluid will be performed by co-Investigator Dr. Kraus (Duke University).

A portion of previously collected samples will be de-identified and shipped on dry ice to Children's National Medical Center at George Washington University where they will undergo the SOMAscan™ assay. The latest version of the SOMAscan assay measures 1,129 protein analytes in only 150 µl of serum. The assay offers exceptional dynamic range, quantifying

proteins that span over 8 logs in abundance (from femtomolar to micromolar), with low limits of detection (38 fMol media LOD) and excellent reproducibility (5.1% median %CV) (http://www.somalogic.com/Technology/SOMAscan-basic-info.aspx). Once the analyses are complete any remaining samples at National Medical Center will be destroyed. At no time will anyone at Children's National Medical Center have access to subject identifiers or any other subject data

Randomization: Patients will be randomized into one of four groups, and there will be 17 subjects per group.

- Group 1: will receive injection of Triamcinolone (40 mg) at time point 1(1-2 days post injury) and saline placebo at timepoint 2)12-14 post injury).
- Group 2: will receive a saline placebo injection at time point 1 (1-2 days post injury) and 40 mg Triamcinolone at time point 2 (12-14 days post injury.)
- Group 3: will receive two consecutive injections of 40 mg Triamcinolone, the first at time point 1 and the second at time point 2.
- Group 4 (control group): will receive two consecutive injections of saline placebo. The first at time point 1 and the second at time point 2.

Duration of Patient Enrollment: All subjects enrolled in the study will be followed for six months. An additional sub-sample of 20 subjects from the main study will be recruited and enrolled in the extended follow-up sub-study at approximately 24 months following surgery.

Duration of Study: Study duration will be 36 months from the commencement date. This duration may be extended to reach desired enrollment.

Data Safety and Monitoring Board (DSMB) - A DSMB consisting of 5 individuals (will monitor the study semi-annually and review reportable adverse events to the FDA as wells as Vanderbilt and University of Kentucky Institutional Review Boards (IRBs). One of these members will maintain the allocation strategy, available upon request from the DSMB.

PATIENT POPULATION: Subjects in this trial will typically be referred by an Emergency Room or walk-in clinic to the Orthopaedics/Sports Medicine Clinic within 48 hours of injury. The study is open to male and female subjects who are between the ages of 14 and 33, who have ACL tears.

Recruitment Method: Principal investigator, co-investigator and his respective study personnel will recruit potential patients who have been referred to them for ACL injuries. All subject recruitment materials must be reviewed and approved by the site IRB/IEC prior to use. Potential subjects will be given a written description of the study in the Informed Consent Form or Assent, and will have the opportunity to ask questions about the study prior to participation. Subjects must sign an IRB approved Informed Consent Form or assent prior to undergoing any study procedures for screening.

Inclusion Criteria: Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

- 14-33 years of age
- Currently participating in a sporting activity
- Injury occurred while playing in a sporting activity
- Normal contralateral knee status
- Documentation of closed growth plates as noted on the screening x-rays

Exclusion Criteria: Subjects presenting with any of the following will not be included in the study:

- underlying inflammatory disease (i.e. Rheumatoid Arthritis, Psoriatic Arthritis etc)
- currently have any infections, including infection of the skin, or have signs and symptoms of an infection, including fever.
- have a disease that weakens your immune system such as diabetes, cancer, HIV or AIDs
- other major medical condition requiring treatment with immunosuppressant or modulating drugs.
- A history of chronic use of non-steroidal anti-inflammatory drugs
- Received corticosteroid injections into the injured knee within three months of enrollment
- previous exposure or allergic reaction to Kenalog®
- prior knee surgery (Ipsilateral or contralateral)
- have received any investigational drug with 4 weeks of study Visit 1
- additionally, subjects experiencing an injury or re-injury to the involved or contralateral knee following primary ACL reconstruction will be excluded from the extended follow-up sub-study.

Early Termination of Subjects from Study:
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 study withdrawal may include the following:

- Adverse event
- Non-compliance with protocol/protocol deviation
- Sponsor's decision
- Lost to follow up
- Withdrawal of consent

Subjects who are withdrawn or terminate early from the study will be preplaced under certain circumstances. These circumstances are withdrawal prior to the completion of all three blood and synovial fluid draws (the study is powered to show a difference in these markers and will be underpowered if these subjects cannot be replaced)

The date and reason must be recorded for all subjects who withdraw from the study. If the reason for withdrawal is a treatment-emergent AE, the specific AE will be identified on the CRF and every attempt will be made to follow the event until resolution.

In addition, every attempt should be made to complete the Visit 6/Early Termination assessments.

If a subject discontinues the study for any reason prior to Visit #5/12-14 weeks post injury visit, then the collected data will be included in the safety analysis, however will not be included in the overall efficacy analysis and a replacement subject will be enrolled in the study.

STUDY PROCEDURES/EXAMINATIONS (See Appendix A)

Demographics, medical history and medication history: Demographic data to be collected on screening visit will include height, weight, date of birth, age, gender and race. Detailed past medical history and medication history will be collected on screening visit. The medical history or concurrent medication history will be noted on each visit.

Knee Aspiration: All subjects undergo knee aspiration to dryness at initial encounter (1-4 days after ACL injury), (+/- 4 days), 12-14 days after injury, at the time of surgery. Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will received treatment according to this protocol but, aspiration at time of surgery will be omitted. Planned aspiration postoperatively will not be performed unless clinically necessary for significant postoperative effusions. If an aspiration is done, a sample will be retained for analysis.

Visit 1 Screening (1-4 days post injury) (+/- 4 days): Upon arriving at the study clinic potential subjects will have the following assessments: range of motion, knee instability (Lachman's test) and standardized Flexion weight bearing x-rays. At this time the subjects will also be asked to fill in a standard questionnaire covering the KOOS, IKDC, SF-36 and, the catastrophizing scale, CSQ and a Likert pain scale.

All subjects must have a clinical exam that is consistent with an ACL tear. Following the review of above assessment results, review of inclusion/exclusion and after signing the informed consent/assent the following assessments will take place:

Collection of urine and 10 cc of blood for laboratory testing, partial Medical history, concomitant medications, demographic, BMI, smoking status, outcomes, and pain questionnaires will also be collected.

Subjects will then be randomized into and one of four treatment groups and undergo a knee aspiration. Following randomizing, the subject will receive their first dose study medication.

Visit 2 (12-14 days post injury):
- Lab tests(urine and 10 cc of blood)
- Pain Questionnaires administered
- Knee aspiration
- Study medication injection
- Patient reported outcomes (PROs) administered

Visit 3 (day of surgery)
- Lab tests(urine and 10cc of blood)
- Pain Questionnaires administered prior to surgery
- Knee aspiration (in the operating room under anesthesia)
- Patient reported outcomes (PROs) administered prior to surgery

Visit 4 (1-2 weeks post surgery)
- Pain Questionnaires administered
- Knee aspiration (if clinically indicated)
- Patient reported outcomes (PROs) administered

Visit 5 (4-6 weeks post surgery)
- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered

Visit 6 (6 months follow-up/Early Termination Visit)
- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered

Optional Extended Follow-Up Sub-Study (To Include 20 University Of Kentucky Subjects)

Visit 7 (24 (+/- 3) month follow-up

- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered
- Research Specific MRI

ADVERSE EVENTS

Any significant change in a subject's health from screening will be recorded and reported as adverse events while the subject is taking part in the study. Adverse Events (AEs) will include but are not limited to severe post-administration pain requiring treatment, infection of the injection site or the knee joint, ER visit following the administration of the study drug at visit 1 or 2. Due to the short half life of the drug we do not expect any SAE's related to the drug administration later than after the date of ACL surgery. During the observation time (time of surgery to 6 months post-op) other, non-drug related, SAEs may occur which will be recorded and reported.

Any reported SAE occurring during the study must be reported by the sponsor to the Food and Drug Administration and the appropriate Institutional Review Board. Any SAE due to any cause, whether or not related to the study drug, must be reported within 24 hours of when the Investigator becomes aware of the event. Investigators who need to report SAEs to the sponsor may do so by utilizing FDA Form 3500A the sponsor provided SAE reporting form and faxing report to (859) 323-2412.

Plan for unexpected adverse event (AE) reporting. Serious AEs will be reported to Human Subjects/IRB within 48 hr. Unanticipated events will be reported to the IRB.

Monitoring of adverse events. Adverse events will be monitored via exams, vital signs, review of subject's medical chart, and documented. Each visit will be documented with a progress note in the research chart.

Adverse Event Follow-up

All AEs, including clinically significant changes in physical examinations findings, or isolated clinically significant laboratory findings must be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up. If resolved or stabilized, a resolution date should be documented on the CRF.

Adverse events ongoing at the final visit or early termination visit will be followed until that adverse event is resolved.

Potential Risk:

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

Benefit Vs Risk: Triamcinolone is a fist line treatment of established OA. In this trial, we expect that an early intervention with Triamcinolone may block the deleterious cascade of joint degradation events after joint injury. We expect a significant effect of Triamcinolone on post-injury pain and concomitantly an increased improvement in early function.

Risks associated with Triamcinolone: The following are the risks associated with Kenalog®: Subjects may experience an allergic reaction (difficulty breathing; closing of the throat; swelling of your lips, tongue, or face; or hives). In rare cases, people receiving Kenalog® have developed serious infections. Subjects should notify the study doctor immediately if they develop a fever, flu-like symptoms, or any other sign of infection.
Other less serious side effects may be more likely to occur.

- nausea or diarrhea;
- a headache;
- sinus irritation or infection; or
- redness, bruising, pain, or swelling at the injection site.

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

- Dose: 40 mg of Triamcinolone is a standard concentration given in patients with acute joint inflammation. Cortisone is cleared systemically within several days after administration while locally it may persist longer. A second booster injection 10 days after initial administration is not likely to have a harmful effect on cartilage or any other intraarticular tissue.

- 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.

Statistics, Quality Control and Minimization of bias:

Quality Control - All data that we collect will be carefully analyzed with respect to variability, linear range of standard, and need for repeat analyses. Controls provided with commercially available ELISA kits are used with every run. For assays for which no control is available or provided, aliquots of serum from normal human subjects have been aliquoted and frozen at -80°C for this purpose. Each assay day, a fresh aliquot of this control serum is thawed and used on every plate to calculate intra- and inter-assay variance of the assay. In addition to the standard curve run in duplicate for each assay, this control will be run with each assay and the results used to determine the precision of the assay and to establish an acceptable control range for the assay. The mean of the control sample for all assays plus or minus 2SD's is defined as the acceptable control range. Any samples on a plate in which the control falls outside of this range will be excluded and repeated. Samples will be run in duplicate and reanalyzed if the CV >15%. For values that are below the level of detection, defined by the lowest standard, ½ LLOQ (lowest level of quantification) will be reported for statistical purposes.

Sample Size and Power Analysis - We used the VAS and WOMAC data from Chevalier et al. as the template for power calculations [4] and the change in KOOS pain and ADL from our study. Chevalier reported a 53% response rate by VAS. Response was defined as a 50% improvement in VAS pain score from baseline. We observed comparable differences in our study (20-30% between outcomes in the IL-1Ra and placebo groups); therefore this dataset is reasonable to estimate study power. Based upon the change in VAS pain score in the Chevalier study, and conservatively estimating a placebo response rate of 30%, the effect size was 0.47 for a dichotomous outcome. At this effect size power for a sample size of 17/group is reasonable (65%) for the weakest possible outcome (i.e., one that only generates a "yes" or a "no" for each patient). Moreover, power is excellent for the continuous KOOS outcomes. With 17 patients per

group we achieve 90% power for effect sizes of approximately 0.77 and 80% power for effect sizes of approximately 0.65. These latter effect sizes are in standard deviation multiples. So, for example, if the standard deviation of the VAS is approximately 20, and we expect an improvement of approximately 20 units in the intervention group, we can tolerate an improvement of approximately 7 units in the placebo group and still have 90% power (i.e. the effect size is (20-7)/20 = 0.65). Moreover, using the effect size standard benchmarks of 0.50 as moderate and 0.80 as large, we will be powered to detect moderate-to-large effects of the intervention, which is consistent with intuition for a study of this size. Power calculations based on our pilot KOOS data confirm this estimate. The change in KOOS (days 0-4) comparing treatment and placebo groups yielded an effect size of 0.62 for pain and 1.08 for ADL. At a group size of 17 we will have 80% power to detect a difference in KOOS pain and >99% power to detect a difference in KOOS ADL.

The power calculations include several caveats. The power calculation was performed based upon the use of a IL-1 receptor antagonist. There is no clinical data available in a young patient population with knee ligament injuries regarding their pain improvement after cortisone injection. The IL1RA data is the most comparable. Cortisone is a wider spectrum anti-inflammatory than IL-1RA and thus may have a larger effect on pain control that may be longer lasting. In that case our study may be over-powered. First, for purposes of power calculation, we have not assumed a full repeated measures analysis, but have instead analyzed change from baseline at a single time point. 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 and IKDC 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 three 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. For those subjects enrolled in the extended follow-up sub-study the 24 month time point will be examined as an additional time point. Additionally, correlation analyses will be used to examine the relationship between biomarkers collected at Visits 1-3 and KOOS and IKDC values and MRI findings at 24 month follow-up.

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.

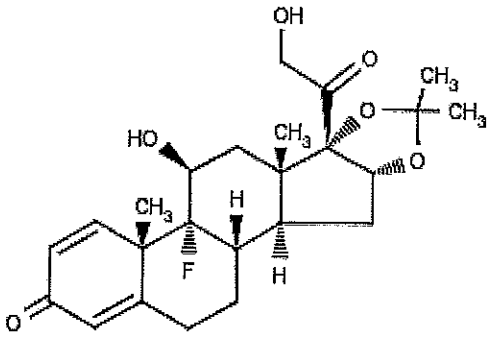
STUDY MEDICATION:

Formulation, Packaging and Labeling:

Kenalog® is a sterile suspension containing 40 mg/mL of micronized triamcinolone acetonide in the following inactive ingredients:

Polysorbate 80	0.20% w/v
Sorbitol Solution USP	50.00% w/v
Water for Injection qs ad	100.00% V
Hydrochloric Acid and Sodium Hydroxide, if required, to adjust pH to	4.0-8.0
Preservative: Benzyl Alcohol	0.90% w/v

The chemical name for triamcinolone acetonide is 9α-Fluoro-11β,16α, 17,21- tetrahydroxyp-pregna-1,4-diene-3, 20-dione cyclic 16, 17-acetal with acetone. Its structural formula is:



CLINICAL PHARMACOLOGY

Glucocorticoids, naturally occurring and synthetic, are adrenocortical steroids that are readily absorbed from the gastrointestinal tract.

Naturally occurring glucocorticoids (hydrocortisone and cortisone), which also have salt-retaining properties, are used as replacement therapy in adrenocortical deficiency states.

Their synthetic analogs are primarily used for their anti-inflammatory effects in disorders of many organ systems. When injected intraarticularly, triamcinolone acetonide can be expected to be absorbed slowly from the injection site.

Shipping, Storage, Handling: Unblinded study drug will be shipped from the manufacturer directly to the site. The syringes with Kenalog will be prepared at the site after study participants have been identified and consented and randomization has been performed. An unblinded nurse, nursing assistant or athletic trainer that has no primary involvement with this study will prepare a 10cc syringe with 40mg of Kenalog and 9cc of saline solution in standard aseptic technique. The syringe will be fitted with an opaque sleeve that does not allow for the visual distinction between study drug and placebo.

The study drug will be kept at the research site at The University of Kentucky (or Vanderbilt University respectively) and stored at controlled room temperature, 20°-25°C (68°-77°F), avoiding freezing and protected from light.

Under aseptic clean conditions, a total of 40 mg (1 ml) of Kenalog® will be drawn into a sterile 10 ml B&Dsyringe, A standard dilution into 9ml of injectable saline solution will be performed.

For the placebo we will utilize 10 ml of 0.9%NACL sterile saline solution drawn up in the same type of syringe as for the Kenalog® administration in order to maintain double blinding of the study investigator and patients.

Once a subject is identified and consented, the study physician will utilize the syringe corresponding to the assigned patient ID to administer the study medication.

STUDY MANAGEMENT:

Regulatory Guidelines: This study will be performed in accordance with US 21 Code of Federal Regulations Parts:

50, Protection of Human Subjects;
54, Financial Disclosure by Clinical Investigators and
56, Institutional Review Boards

Good Clinical Practice (GCP): Consolidated Guideline (International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, May 1996).

Declaration of Helsinki, concerning medical research in humans ("Recommendations Guiding Physicians in Biomedical research Involving Human Patients," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989 and revised version of Sumerset West, Republic of South Africa, Oct, 1986).

IRB Approval

This study must have initial and at least annual approval from an Institutional Review Board (IRB) responsible for approving clinical studies. Furthermore, screening and enrollment of subjects into the trial will not commence until Investigator receives the IRB approval letter. In addition, a copy of the IRB approval letter must be filed on-site in the investigator's study binder. When appropriate, amendments to the protocol must be submitted for IRB review and approval before being implemented.

Informed Consent/Assent (Title 21, Section 50 and 312.62 of the CRF)

- Study enrollment will not begin until the Investigator has received an approved and validated informed consent and Assent from the IRB.
- All subjects enrolling in the study:
  1. Will be informed of the investigational nature of the study
  2. Must be given a copy of the Informed Consent, and if required, the Assent Form
  3. Must be given the opportunity to ask any questions regarding the study treatment
  4. Will have voluntarily and willingly signed the Informed Consent Form/Assent Form

prior to initiation of any study related test/procedure. Children age 14 and older must have an IRB approved assent in place in order to be considered enrolled into the study.

A separate consent will be utilized to enroll subjects in the extended follow-up sub-study. All subjects enrolling in the sub-study:

1. Will be informed of the investigational nature of the study
2. Must be given a copy of the Informed Consent addendum, and if required, the Assent Form addendum
3. Must be given the opportunity to ask any questions regarding study procedures
4. Will have voluntarily and willingly signed the Informed Consent Form/Assent Form prior to initiation of any study related test/procedure. Children age 12 and

older must have an IRB approved assent in place in order to be considered enrolled into the study.

Pre-study Documentation Requirements

The investigator is responsible for assembling, and sending to the Sponsor, the following documents before study ignition can occur:

- Signed and dated protocol signature page
- Copy of approved ICF and Assent
- Copy of the IRB approval of the protocol
- The IRB composition and/or written IRB compliance statement
- Signed Clinical Trial Agreement
- Curricula Vitae of all investigators and sub-investigators (signed and dated)
- Signed and dated FDA Form 1572
- Lab certifications (if required)
- Signed and dated Financial Disclosure for everyone listed on the site's FDA Form 1572
- Additional documentation, as required by the sponsor.

Protocol Adherence

The Investigator agrees to conduct the study in accordance with this protocol. Prior to beginning the study, the Investigator(s) must sign both the Investigator Agreement and the protocol signature page.

An Investigator must not make any changes to the study without first receiving written approval from the Sponsor and IRB, except when necessary to eliminate apparent immediate hazard to a subject. As soon as possible, the implemented deviation or change, reasons for it, and if appropriate, the proposed protocol amendment(s) should be submitted to the IRB for review and approval.

Protocol Amendments

Protocol amendments must be made only with the prior approval of the Sponsor (Dr. Lattermann). Agreement from the principal investigator must be obtained for all protocol amendments and amendments to the ICF. The IRB must be informed of all amendments and give approval for any amendments likely to affect the safety of the subjects or the conduct of the study. The principal investigator must send a copy of the approval letter from the IRB to the Sponsor and/or designee.

Both the Sponsor and the principal investigator reserve the right to terminate the study according to the study contract. The principal investigator should notify the IRB/IEC in writing of the study's completion or early termination and send a copy of the notification to the Sponsor and/or designee.

Study Monitoring and Auditing

The Sponsor and/or designee representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the study (eg, CRFs and other pertinent data) provided that subject confidentiality is respected, in accordance with the International Conference on Harmonization, (ICH) Good Clinical Practices (GCP) guidelines (ICH GCP E6 Section 5.18). The Investigator will permit representatives of the Sponsor's monitoring team or FDA to inspect facilities and records relevant to this study.

The Sponsor and/or designee Monitor and/or clinical research associate (CRA) is responsible for inspecting the CRF/eCRFs at regular intervals throughout the study to verify that the clinical trial is conducted in an organized manner according to the protocol provided.

Monitoring visits will assess:

- adherence to the protocol
- progress in the conduct of the study
- completeness, accuracy, and consistency of the data
- AE reporting to the Sponsor and the IRB/IEC
- adequacy of the facilities and availability of equipment required to conduct the study (ie,
- local laboratory, ECG equipment)
- adherence to local regulations on the conduct of clinical research

The Monitor should have access to each subject's permanent medical records and other study related records needed to verify the entries on the CRF/eCRFs.

The principal investigator will permit the Sponsor's personnel (or designees) to audit all CRF/eCRFs and supporting documentation, eg, hospital, office, clinic, pharmacy, and laboratory records for each subject. The principal investigator agrees to cooperate with the Monitor to ensure that any problems detected in the course of these monitoring visits are resolved.
In accordance with the ICH, GCP and the Sponsor and/or its designee audit plans, this study may be selected for audit. Inspection of site facilities (eg, pharmacy, drug storage areas, laboratories, etc.) and review of study-related records will occur in order to evaluate the study conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

Data Safety Monitoring Board

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 University of Kentucky Center Center for Clinical and Translations 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.

Study Stopping Criteria

- local intolerance of the administered Kenalog® 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.

Data Recording and Record Retention

The PI must maintain all Essential Documents (as listed in the ICH Guideline for GCP) until notified by the Sponsor and in accordance with all local laws regarding retention of records.

The Investigator should maintain a list of appropriate qualified persons to whom he/she has delegated trial duties. All persons authorized to make entries and/or corrections to the case report forms should be included on the list of qualified persons. Source documents are original documents, data, and or records from which the participant's case report form data are obtained.

These include, but are not limited to, hospital records, clinical and office charts, laboratory and pharmacy records, microfiches, radiographs and correspondence.

The Investigator and study staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the University of Kentucky and/or applicable regulatory authorities. Elements should include:

- Participant files containing complete case report forms, ICFs, assents, HIPAAs, and supporting copies of source documentation;
- Study files containing the protocol with all amendments, Kineret® package insert, copies of pre-study documentation, and all correspondence to and from the IRB and;
- Proof of receipt, Investigational Product Accountability Record, Return of Investigational Product for Destruction, Final Investigational Product Reconciliation Statement, and all drug-related correspondence.

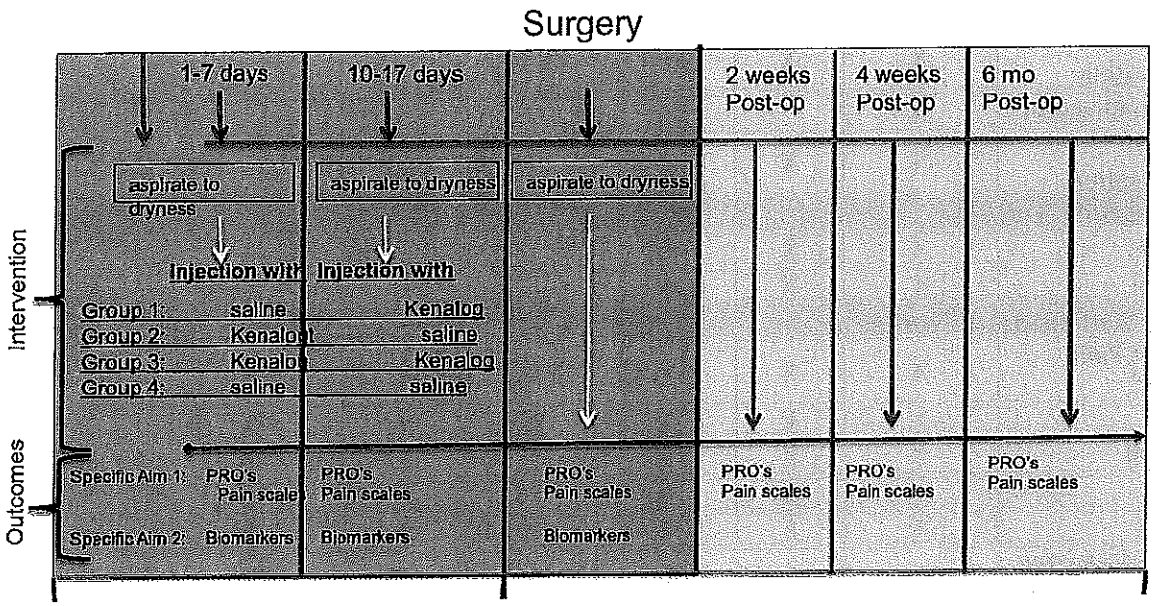
No study documentation should be destroyed without prior written agreement between Doctor Lattermann and the investigator. Should the investigator wish to assign the study record to another party or move them to another location, he/she may do so only with the prior written approval of Dr. Lattermann.

SAE reporting to the Sponsor:
Adverse reactions will be reported promptly to the Principal Investigator 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.

SUBJECT CONFIDENTIALITY

The Investigator and the Principal Investigator affirm a subject's right to protection against invasion of privacy. In compliance with United States federal regulations, the Principal Investigator requires the Investigator(s) to permit, when necessary, representatives from the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws. Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent/assent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

Figure 1: Study Design and Plan



Appendix A: Study Flow Chart

	Study Drug Treatment			Follow-up			Extended Follow-up
	Screening Baseline 1-2 days post injury (+/- 4 days)	12-14 days post injury	Surgery[5]			6 Months Follow-up/Early Termination Visit	Sub-Study
Study Time points	Time point 1	Time point 2		1-2 Weeks post surgery	4-6 weeks post surgery		24 Months (+/- 3) post surgery
Procedures	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Informed Consent	X						X
Partial Medical History	X						X
Medication History	X	X	X	X	X	X	X
Smoking Status	X						
BMI Measurement	X						

Demographics	X						
X-Ray[1]	X						
MRI[2, 6]	X						X
Range of Motion	X						X
Knee Aspiration	X	X	X[3&5]	X[3&5]			
Randomization	X						
Study Medication	X	X					
Laboratory Tests							
Urine	X	X	X				
Blood	X	X	X				
Questionnaires And Scales							
Likert Pain Scale[4]	X	X	X	X	X	X	X
CSQ	X	X	X	X	X	X	X
KOOS	X	X	X	X	X	X	X
IKDC	X	X	X	X	X	X	X
SF 36	X	X	X	X	X	X	X
Catastrophizing Scale	X	X	X	X	X	X	X
MARX Activity Scale							X

1 = All subjects must have standardized flexion weight bearing x-rays. *Documentation of closed growth plates at screening will be noted on the routine SOC x-rays.*
2 = All subjects enrolled will have an MRI performed as a routine diagnostic tool 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.
3 = Knee aspiration will only be performed if the subject has post-op fluid on the knee. Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, aspiration at time of surgery will be omitted.
4 = All subjects enrolled into the study must have been diagnosed with an ACL tear.
5 = Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will receive treatment according to this protocol.
6 = Subjects enrolled into the extended follow-up sub-study will undergo a research specific MRI, that is not considered standard of care.

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Early Anti-inflammatory Treatment in Patients with Acute ACL Tear and Painful Effusions

Date: 4 May 2015

Version 7

Sponsor/Principle Investigator: Christian Lattermann, MD
University of Kentucky
Dept. of Orthopaedic Surgery and Sports Medicine
Kentucky Clinic K 401
740 South Limestone Street
Lexington KY 40536

Early anti-inflammatory Treatment in Patients with Acute ACL Tear and Painful Effusions

DECLARATION OF SPONSOR

This study protocol was subject to critical review. The information it contains is consistent with:

- The current risk-benefit evaluation of the investigational product
- The moral, ethical, and scientific principles governing clinical research as set out in the World Medical Association Declaration of Helsinki (Edinburg, October 2000) and the principles of GCP and described in the International Conference of Harmonisation Tripartite Guidelines Top E6: "Guidelines for Good Clinical Practice," as well as in the applicable local guidelines.

The investigator will be supplied with details of any significant or new finding, including adverse events, related to the treatment with the investigational product.

Signature: _____ Date: _____

Printed Name: Christian Lattermann, MD

DECLARATION OF INVESTIGATOR

I confirm that I have read the above protocol. I understand it, and I will work according to the principles of GCP as described in 21 CFR parts 50, 54, 56 and according to applicable local requirements and the World Medical Association Declaration of Helsinki (Edinburg, October 2000).

I agree to disclose any proprietary interest I may hold in the investigational product or the Sponsor foundation.

Investigator Signature: _____ Date: _____

Printed Name (Typed or blocked letters): _____

CONTACT DETAILS OF KEY PERSONNEL

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BACKGROUND AND RATIONALE

Background and Study Rationale

Patients who suffer anterior cruciate ligament (ACL) tears commonly undergo surgical reconstruction. While surgical techniques have consistently been improved to be more anatomic, the long term consequences such as posttraumatic osteoarthritis and subsequent pain have not been significantly reduced. In fact, over 50% of all young patients with ACL tears will develop radiographic OA osteoarthritis (OA) within 10 years after injury.

The long-term consequences of post-traumatic OA 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. 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 the risk of post-traumatic OA 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 articular cartilage in the knee joint. We propose to disrupt the inflammation-driven cascade with Triamcinolone (Kenalog®). We hypothesize that Tiamcinolone 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. We will test our hypothesis in a multicenter, randomized, placebo controlled, double blinded, clinical trial.

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 and amenability of pain control in acute ACL injury. Our proposed study will also generate robust preliminary data that will be used to inform a larger randomized clinical trial with our collaborators at the Multicenter Orthopaedic Outcomes Network (MOON; PI K. Spindler, Vanderbilt University, TN) cohort of ACL injury.

Study Drug - Triamcinolone, is manufactured by Bristol-Meyer-Squibb, New York NY (Kenalog), is available as 1ml (40mg) vials and is FDA approved for acute gouty arthritis, acute and subacute bursitis, acute nonspecific tenosynovitis, epicondylitis, rheumatoid arthritis, synovitis, or osteoarthritis.

Pediatric dosing -The efficacy and safety of corticosteroids in the pediatric population are based on the well established course of effect of corticosteroids which is similar in pediatric and adult populations. Published studies provide evidence of efficacy and safety in pediatric patients for the treatment of nephrotic syndrome (>2 years of age), and aggressive lymphomas and leukemias (>1 month of age). Other indications for pediatric use of corticosteroids, e.g., severe asthma and wheezing, rheumatoid arthritis, acute inflammatory arthritis are based on adequate and well-controlled trials conducted in adults, on the premises that the course of the disease.

The adverse effects of corticosteroids in pediatric patients are similar to those in adults. Like adults, pediatric patients should be carefully observed for local symptoms after injection. Pediatric patients who are treated with corticosteroids by any route, including systemically administered corticosteroids, may experience a decrease in their growth velocity. This negative impact of corticosteroids on growth has been observed at low systemic doses and in the absence of laboratory evidence of HPA axis suppression (i.e., cosyntropin stimulation and basal cortisol

plasma levels). Since in this study only two single time local administrations at much lower systemic dosage will be carried out this side effect is not likely and not expected.

For the purpose of comparison, the following is the equivalent milligram dosage of the various glucocorticoids:

Cortisone, 25	Triamcinolone, 4
Hydrocortisone, 20	Paramethasone, 2
Prednisolone, 5	Betamethasone, 0.75
Prednisone, 5	Dexamethasone, 0.75
Methylprednisolone, 4	

These dose relationships apply only to oral or intravenous administration of these compounds. When these substances or their derivatives are injected intramuscularly or into joint spaces, their relative properties may be greatly altered locally, however, systemic doses are significantly lower.

A total of 1ml (40 mg) will be injected intra-articularly after synovial fluid aspiration 'to dryness' either once (at study entry or 2 weeks after injury) or twice (at both study entry and 2 weeks after injury).

Rational for including pediatric population

The specific reason for enrolling the age group of children aged 14-17 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]
- 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.

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 14-17 from this important trial.

RESEARCH OBJECTIVES OF THE CURRENT STUDY:

The research objectives of this study are:

1. Test the efficacy of Triamcinolone to alleviate knee pain (KOOS, IKDC) and improve patient-reported outcomes (PRO) following ACL rupture in a randomized, placebo controlled, double blinded, clinical trial. Triamcinolone will be administered intra-articularly within 1 week of ACL injury or at 2 weeks after ACL injury, or at both time points and compared with saline control injections. Patients will be evaluated at 5 post-injury time points (1-2 days, 12-14 days, 2 weeks post-op, 4 weeks post-op, 6 months

post-op, and 24 months post-op) following ACL reconstruction.

2. Determine if intra-articular Triamcinolone therapy improves levels of a panel of inflammatory (cytokine), meniscus and cartilage metabolism and oxidative stress biomarkers as measured in synovial fluid from patients at relatively short-term time points after ACL injury (1-7 days, 10-17 days after injury and at time of surgery). Biomarkers to be tested include IL-1α, IL-1β, Collagen type I (NtxI) and collagen type II (CTXII) breakdown products, cartilage oligomeric protein (COMP) and glycomsaminoglycan (GAG) and Xanthine Oxidase. Additionally, the change in IL-1α and IL-1β levels in the Triamcinolone treated and placebo individuals will be evaluated specifically for association with pain and function outcomes.

RESEARCH OBJECTIVES OF EXTENDED FOLLOW-UP SUB-STUDY:

1. Determine if joint health as evaluated by MRI at 24 months following ACL injury is influenced by the initial treatment with Kenalog. Two years following ACL injury MRI analysis using standardized T2-mapping will be performed will be performed on 10 control subjects and 10 subjects who have received Kenalog injections.

INVESTIGATIONAL PLAN

Description of Overall Study Design and Plan (See Figure 1 for study timeline)

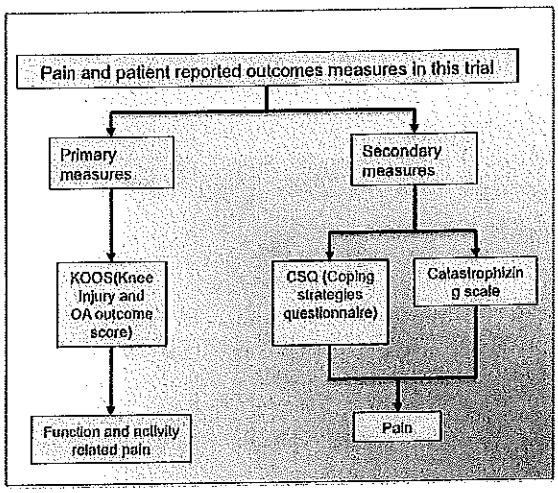
This study is a multicenter, randomized, placebo controlled, double blinded, clinical trial. This trial will enroll 68 subjects between the ages of 14 and 33. This trial will be conducted at two clinical centers, the University of Kentucky and Vanderbilt University, as well as established criteria and support of the Multicenter Orthopaedic Outcome Network (MOON). A sub-study involving extended follow-up of patients, 24 months post-surgery will occur at the University of Kentucky clinical center only. This sub-study will involve 20 subjects of the initial University of Kentucky cohort returning for one additional follow-up MRI.

Description of observations and measurements to be made in this study:

Assessment of Pain and Function by KOOS – This trial will use the standardized patient 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 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 subjects' 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 (44). This trial will supplement the KOOS with function score assessments from the International Knee Documentation Committee (IKDC) form for evaluation of knee ligament injuries [45]. In addition to the KOOS and IKDC, current physical activity level at the 24 month follow-up study will be evaluated using the MARX activity scale.

Specific Pain assessments - Pain coping strategies are important in understanding improvements in pain and psychological disability [46-48]. This trial will use a simple Likert pain scale, the Coping Strategies Questionnaire (CSQ) and 'pain catastrophizing' as pain assessment tools (Figure 2). 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." Not only do individuals who catastrophize, experience more experimental pain [49-52] 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. It has been associated with heightened anxiety, depression, increased pain behaviors, increased use of health care services and analgesic medication and thus, "catastrophizing has risen to the status of one of the most important psychological predictors of the pain experience."[49] In a study of patients undergoing anterior-cruciate ligament surgery, Pavlin, et al. [50] found that high pain catastrophizers were much more likely to report high levels of post-operative pain, had longer durations of moderate to severe pain, and were less likely to report adequate pain control at home.

Figure 2:



Knee Aspiration: All patients will undergo a standard of care knee joint aspiration upon presentation to the clinic, and a study related aspiration two weeks after injury as well as on the day of surgery. Once the study subject undergoes surgery, no further scheduled aspirations are planned. However, if the subject has a substantial post-operative effusion the physician may choose to aspirate that effusion, per their standard of care. In that case the aspirated fluid will be kept for study purposes.

Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will received treatment according to this protocol but, aspiration at time of surgery will be omitted.

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 asceptically with local anaesthesia 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. Serum and urine samples will also be collected but will not be tested in this study. Samples will be stored and archived in the UK-Orthopaedics Cryostorage facility for analysis. Samples can be destroyed if a subject requests this.

IL-1α, IL-1ß, 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 [53], the standard curve will be extended in the low range for the Aristospanα 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 [54]. 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).[61] CTX-II will be measured in synovial fluid by ELISA (IDS, Herlev, Denmark).[55]

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 [55]. We will measure NTX-I in synovial fluid by ELISA.[55] 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 [56]. Although other joint tissues express COMP [57], it is most abundant in articular cartilage. Serum COMP levels are representative of cartilage catabolism [58,59] 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.

Laboratory Analysis of Study Samples:

The supernatants will be stored, pending analysis, in the UK-Orthopaedics biomarker sample repository located in Dr. Lattermann's laboratory.

All biomarker analyses in synovial fluid will be performed by co-Investigator Dr. Kraus (Duke University).

A portion of previously collected samples will be de-identified and shipped on dry ice to Children's National Medical Center at George Washington University where they will undergo the SOMAscan™ assay. The latest version of the SOMAscan assay measures 1,129 protein analytes in only 150 µl of serum. The assay offers exceptional dynamic range, quantifying proteins that span over 8 logs in abundance (from femtomolar to micromolar), with low limits of detection (38 fMol media LOD) and excellent reproducibility (5.1% median %CV) (http://www.somalogic.com/Technology/SOMAscan-basic-info.aspx). Once the analyses are complete any remaining samples at National Medical Center will be destroyed. At no time will anyone at Children's National Medical Center have access to subject identifiers or any other subject data.

Randomization: Patients will be randomized into one of four groups, and there will be 17 subjects per group.

- Group 1: will receive injection of Triamcinolone (40 mg) at time point 1(1-2 days post injury) and saline placebo at timepoint 2)12-14 post injury).
- Group 2: will receive a saline placebo injection at time point 1 (1-2 days post injury) and 40 mg Triamcinolone at time point 2 (12-14 days post injury.)
- Group 3: will receive two consecutive injections of 40 mg Triamcinolone, the first at time point 1 and the second at time point 2.
- Group 4 (control group): will receive two consecutive injections of saline placebo. The first at time point 1 and the second at time point 2.

Duration of Patient Enrollment: All subjects enrolled in the study will be followed for six months. Those subjects enrolled at the University of Kentucky site will be followed for 24 months, and 20 subjects from the main study will be recruited and enrolled in the extended follow-up MRI sub-study.

Duration of Study: Study duration will be 36 months from the commencement date. This duration may be extended to reach desired enrollment.

Data Safety and Monitoring Board (DSMB) - A DSMB consisting of 5 individuals (will monitor the study semi-annually and review reportable adverse events to the FDA as wells as Vanderbilt and University of Kentucky Institutional Review Boards (IRBs). One of these members will maintain the allocation strategy, available upon request from the DSMB.

PATIENT POPULATION: Subjects in this trial will typically be referred by an Emergency Room or walk-in clinic to the Orthopaedics/Sports Medicine Clinic within 48 hours of injury. The study is open to male and female subjects who are between the ages of 14 and 33, who have ACL tears.

Recruitment Method: Principal investigator, co-investigator and his respective study personnel will recruit potential patients who have been referred to them for ACL injuries. All subject recruitment materials must be reviewed and approved by the site IRB/IEC prior to use. Potential subjects will be given a written description of the study in the Informed Consent Form or Assent, and will have the opportunity to ask questions about the study prior to participation. Subjects must sign an IRB approved Informed Consent Form or assent prior to undergoing any study procedures for screening.

Inclusion Criteria: Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

- 14-33 years of age
- Currently participating in a sporting activity
- Injury occurred while playing in a sporting activity
- Normal contralateral knee status
- Documentation of closed growth plates as noted on the screening x-rays

Exclusion Criteria: Subjects presenting with any of the following will not be included in the study:

- underlying inflammatory disease (i.e. Rheumatoid Arthritis, Psoriatic Arthritis etc)
- currently have any infections, including infection of the skin, or have signs and symptoms of an infection, including fever.
- have a disease that weakens your immune system such as diabetes, cancer, HIV or AIDs
- other major medical condition requiring treatment with immunosuppressant or modulating drugs.

- A history of chronic use of non-steroidal anti-inflammatory drugs
- Received corticosteroid injections into the injured knee within three months of enrollment
- previous exposure or allergic reaction to Kenalog®
- prior knee surgery (Ipsilateral or contralateral)
- have received any investigational drug with 4 weeks of study Visit 1
- additionally, subjects experiencing an injury or re-injury to the involved or contralateral knee following primary ACL reconstruction will be excluded from the extended follow-up sub-study.

Early Termination of Subjects from Study:
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 study withdrawal may include the following:

- Adverse event
- Non-compliance with protocol/protocol deviation
- Sponsor's decision
- Lost to follow up
- Withdrawal of consent

Subjects who are withdrawn or terminate early from the study will be replaced under certain circumstances. These circumstances are withdrawal prior to the completion of all three blood and synovial fluid draws (the study is powered to show a difference in these markers and will be underpowered if these subjects cannot be replaced)

The date and reason must be recorded for all subjects who withdraw from the study. If the reason for withdrawal is a treatment-emergent AE, the specific AE will be identified on the CRF and every attempt will be made to follow the event until resolution.

In addition, every attempt should be made to complete the Visit 6/Early Termination assessments.

If a subject discontinues the study for any reason prior to Visit #5/12-14 weeks post injury visit, then the collected data will be included in the safety analysis, however will not be included in the overall efficacy analysis and a replacement subject will be enrolled in the study.

STUDY PROCEDURES/EXAMINATIONS (See Appendix A)

Demographics, medical history and medication history: Demographic data to be collected on screening visit will include height, weight, date of birth, age, gender and race. Detailed past medical history and medication history will be collected on screening visit. The medical history or concurrent medication history will be noted on each visit.

Knee Aspiration: All subjects undergo knee aspiration to dryness at initial encounter (1-4 days after ACL injury), (+/- 4 days), 12-14 days after injury, at the time of surgery. Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will received treatment according to this protocol but, aspiration at time of surgery will be omitted. Planned aspiration postoperatively will not be performed unless clinically necessary for significant postoperative effusions. If an aspiration is done, a sample will be retained for analysis.

Visit 1 Screening (1-4 days post injury) (+/- 4 days): Upon arriving at the study clinic potential subjects will have the following assessments: range of motion, knee instability

(Lachman's test) and standardized Flexion weight bearing x-rays. At this time the subjects will also be asked to fill in a standard questionnaire covering the KOOS, IKDC, SF-36 and, the catastrophizing scale, CSQ and a Likert pain scale.

All subjects must have a clinical exam that is consistent with an ACL tear. Following the review of above assessment results, review of inclusion/exclusion and after signing the informed consent/assent the following assessments will take place:

Collection of urine and 10 cc of blood for laboratory testing, partial Medical history, concomitant medications, demographic, BMI, smoking status, outcomes, and pain questionnaires will also be collected.

Subjects will then be randomized into and one of four treatment groups and undergo a knee aspiration. Following randomizing, the subject will receive their first dose study medication.

Visit 2 (12-14 days post injury):
- Lab tests(urine and 10 cc of blood)
- Pain Questionnaires administered
- Knee aspiration
- Study medication injection
- Patient reported outcomes (PROs) administered

Visit 3 (day of surgery)
- Lab tests(urine and 10cc of blood)
- Pain Questionnaires administered prior to surgery
- Knee aspiration (in the operating room under anesthesia)
- Patient reported outcomes (PROs) administered prior to surgery

Visit 4 (1-2 weeks post surgery)
- Pain Questionnaires administered
- Knee aspiration (if clinically indicated)
- Patient reported outcomes (PROs) administered

Visit 5 (4-6 weeks post surgery)
- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered

Visit 6 (6 months follow-up/Early Termination Visit)
- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered

Visit 7 (24 (+/- 3) month follow-up – completed via mail)
- Patient reported outcomes (PROs) administered
- Pain Questionnaires administered

Visit 8 (24 (+/- 3) OPTIONAL EXTENDED FOLLOW-UP SUB-STUDY TO INCLUDE 20 SUBJECTS)
- Research Specific MRI

ADVERSE EVENTS

Any significant change in a subject's health from screening will be recorded and reported as adverse events while the subject is taking part in the study. Adverse Events (AEs) will include

but are not limited to severe post-administration pain requiring treatment, infection of the injection site or the knee joint, ER visit following the administration of the study drug at visit 1 or 2. Due to the short half life of the drug we do not expect any SAE's related to the drug administration later than after the date of ACL surgery. During the observation time (time of surgery to 6 months post-op) other, non-drug related, SAEs may occur which will be recorded and reported.

Any reported SAE occurring during the study must be reported by the sponsor to the Food and Drug Administration and the appropriate Institutional Review Board. Any SAE due to any cause, whether or not related to the study drug, must be reported within 24 hours of when the Investigator becomes aware of the event. Investigators who need to report SAEs to the sponsor may do so by utilizing FDA Form 3500A the sponsor provided SAE reporting form and faxing report to (859) 323-2412.

Plan for unexpected adverse event (AE) reporting. Serious AEs will be reported to Human Subjects/IRB within 48 hr. Unanticipated events will be reported to the IRB.

Monitoring of adverse events. Adverse events will be monitored via exams, vital signs, review of subject's medical chart, and documented. Each visit will be documented with a progress note in the research chart.

Adverse Event Follow-up

All AEs, including clinically significant changes in physical examinations findings, or isolated clinically significant laboratory findings must be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up. If resolved or stabilized, a resolution date should be documented on the CRF.

Adverse events ongoing at the final visit or early termination visit will be followed until that adverse event is resolved.

Potential Risk:

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

Benefit Vs Risk: Triamcinolone is a fist line treatment of established OA. In this trial, we expect that an early intervention with Triamcinolone may block the deleterious cascade of joint degradation events after joint injury. We expect a significant effect of Triamcinolone on post-injury pain and concomitantly an increased improvement in early function.

Risks associated with Triamcinolone: The following are the risks associated with Kenalog®: Subjects may experience an allergic reaction (difficulty breathing; closing of the throat; swelling of your lips, tongue, or face; or hives). In rare cases, people receiving Kenalog® have developed serious infections. Subjects should notify the study doctor immediately if they develop a fever, flu-like symptoms, or any other sign of infection.

Other less serious side effects may be more likely to occur.

- nausea or diarrhea;
- a headache;
- sinus irritation or infection; or
- redness, bruising, pain, or swelling at the injection site.

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

- Dose: 40 mg of Triamcinolone is a standard concentration given in patients with acute joint inflammation. Cortisone is cleared systemically within several days after administration while locally it may persist longer. A second booster injection 10 days after initial administration is not likely to have a harmful effect on cartilage or any other intraarticular tissue.

- 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.

Statistics, Quality Control and Minimization of bias:

Quality Control - All data that we collect will be carefully analyzed with respect to variability, linear range of standard, and need for repeat analyses. Controls provided with commercially available ELISA kits are used with every run. For assays for which no control is available or provided, aliquots of serum from normal human subjects have been aliquoted and frozen at -80°C for this purpose. Each assay day, a fresh aliquot of this control serum is thawed and used on every plate to calculate intra- and inter-assay variance of the assay. In addition to the standard curve run in duplicate for each assay, this control will be run with each assay and the results used to determine the precision of the assay and to establish an acceptable control range for the assay. The mean of the control sample for all assays plus or minus 2SD's is defined as the acceptable control range. Any samples on a plate in which the control falls outside of this range will be excluded and repeated. Samples will be run in duplicate and reanalyzed if the CV >15%. For values that are below the level of detection, defined by the lowest standard, ½ LLOQ (lowest level of quantification) will be reported for statistical purposes.

Sample Size and Power Analysis - We used the VAS and WOMAC data from Chevalier et al. as the template for power calculations [4] and the change in KOOS pain and ADL from our study. Chevalier reported a 53% response rate by VAS. Response was defined as a 50% improvement in VAS pain score from baseline. We observed comparable differences in our study (20-30% between outcomes in the IL-1Ra and placebo groups); therefore this dataset is reasonable to estimate study power. Based upon the change in VAS pain score in the Chevalier study, and conservatively estimating a placebo response rate of 30%, the effect size was 0.47 for a dichotomous outcome. At this effect size power for a sample size of 17/group is reasonable (65%) for the weakest possible outcome (i.e., one that only generates a "yes" or a "no" for each patient). Moreover, power is excellent for the continuous KOOS outcomes. With 17 patients per group we achieve 90% power for effect sizes of approximately 0.77 and 80% power for effect sizes of approximately 0.65. These latter effect sizes are in standard deviation multiples. So, for example, if the standard deviation of the VAS is approximately 20, and we expect an improvement of approximately 20 units in the intervention group, we can tolerate an improvement of approximately 7 units in the placebo group and still have 90% power (i.e. the effect size is (20-7)/20 = 0.65). Moreover, using the effect size standard benchmarks of 0.50 as moderate and 0.80 as large, we will be powered to detect moderate-to-large effects of the intervention, which is consistent with intuition for a study of this size. Power calculations based on our pilot KOOS data confirm this estimate. The change in KOOS (days 0-4) comparing treatment and placebo groups yielded an effect size of 0.62 for pain and 1.08 for ADL. At a

group size of 17 we will have 80% power to detect a difference in KOOS pain and >99% power to detect a difference in KOOS ADL.

The power calculations include several caveats. The power calculation was performed based upon the use of a IL-1 receptor antagonist. There is no clinical data available in a young patient population with knee ligament injuries regarding their pain improvement after cortisone injection. The IL1RA data is the most comparable. Cortisone is a wider spectrum anti-inflammatory than IL-1RA and thus may have a larger effect on pain control that may be longer lasting. In that case our study may be over-powered. First, for purposes of power calculation, we have not assumed a full repeated measures analysis, but have instead analyzed change from baseline at a single time point. 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 and IKDC 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 three 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. For those subjects enrolled in the extended follow-up sub-study the 24 month time point will be examined as an additional time point. Additionally, correlation analyses will be used to examine the relationship between biomarkers collected at Visits 1-3 and KOOS and IKDC values and MRI findings at 24 month follow-up.

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.

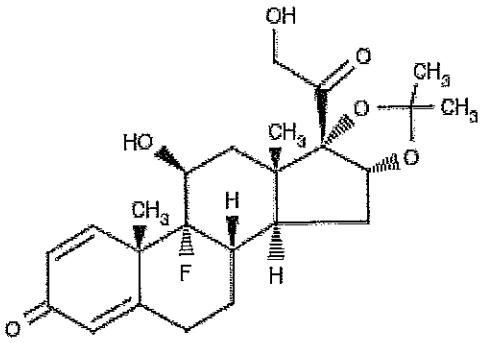
STUDY MEDICATION:

Formulation, Packaging and Labeling:

Kenalog® is a sterile suspension containing 40 mg/mL of micronized triamcinolone acetonide in the following inactive ingredients:

Polysorbate 80	0.20% w/v
Sorbitol Solution USP	50.00% w/v
Water for Injection qs ad	100.00% V
Hydrochloric Acid and Sodium Hydroxide, if required, to adjust pH to	4.0-8.0
Preservative: Benzyl Alcohol	0.90% w/v

The chemical name for triamcinolone acetonide is 9α-Fluoro-11β,16α, 17,21- tetrahydroxyp-pregna-1,4-diene-3, 20-dione cyclic 16, 17-acetal with acetone. Its structural formula is:



CLINICAL PHARMACOLOGY

Glucocorticoids, naturally occurring and synthetic, are adrenocortical steroids that are readily absorbed from the gastrointestinal tract.

Naturally occurring glucocorticoids (hydrocortisone and cortisone), which also have salt-retaining properties, are used as replacement therapy in adrenocortical deficiency states.

Their synthetic analogs are primarily used for their anti-inflammatory effects in disorders of many organ systems. When injected intraarticularly, triamcinolone acetonide can be expected to be absorbed slowly from the injection site.

Shipping, Storage, Handling: Unblinded study drug will be shipped from the manufacturer directly to the site. The syringes with Kenalog will be prepared at the site after study participants have been identified and consented and randomization has been performed. An unblinded nurse, nursing assistant or athletic trainer that has no primary involvement with this study will prepare a 10cc syringe with 40mg of Kenalog and 9cc of saline solution in standard aseptic technique. The syringe will be fitted with an opaque sleeve that does not allow for the visual distinction between study drug and placebo.

The study drug will be kept at the research site at The University of Kentucky (or Vanderbilt University respectively) and stored at controlled room temperature, 20°-25°C (68°-77°F), avoiding freezing and protected from light.

Under aseptic clean conditions, a total of 40 mg (1 ml) of Kenalog® will be drawn into a sterile 10 ml B&Dsyringe, A standard dilution into 9ml of injectable saline solution will be performed.

For the placebo we will utilize 10 ml of 0.9%NACL sterile saline solution drawn up in the same type of syringe as for the Kenalog® administration in order to maintain double blinding of the study investigator and patients.

Once a subject is identified and consented, the study physician will utilize the syringe corresponding to the assigned patient ID to administer the study medication.

STUDY MANAGEMENT:

Regulatory Guidelines: This study will be performed in accordance with US 21 Code of Federal Regulations Parts:

50, Protection of Human Subjects;
54, Financial Disclosure by Clinical Investigators and
56, Institutional Review Boards

Good Clinical Practice (GCP): Consolidated Guideline (International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, May 1996).

Declaration of Helsinki, concerning medical research in humans ("Recommendations Guiding Physicians in Biomedical research Involving Human Patients," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989 and revised version of Sumerset West, Republic of South Africa, Oct, 1986).

IRB Approval

This study must have initial and at least annual approval from an Institutional Review Board (IRB) responsible for approving clinical studies. Furthermore, screening and enrollment of subjects into the trial will not commence until Investigator receives the IRB approval letter. In addition, a copy of the IRB approval letter must be filed on-site in the investigator's study binder. When appropriate, amendments to the protocol must be submitted for IRB review and approval before being implemented.

Informed Consent/Assent (Title 21, Section 50 and 312.62 of the CRF)

- Study enrollment will not begin until the Investigator has received an approved and validated informed consent and Assent from the IRB.
- All subjects enrolling in the study:
  1. Will be informed of the investigational nature of the study
  2. Must be given a copy of the Informed Consent, and if required, the Assent Form
  3. Must be given the opportunity to ask any questions regarding the study treatment
  4. Will have voluntarily and willingly signed the Informed Consent Form/Assent Form

prior to initiation of any study related test/procedure. Children age 14 and older must have an IRB approved assent in place in order to be considered enrolled into the study.

A IRB addendum will be utilized to enroll subjects in the extended follow-up sub-study. All subjects enrolling in the sub-study:

1. Will be informed of the investigational nature of the study
2. Must be given a copy of the Informed Consent addendum, and if required, the Assent Form addendum
3. Must be given the opportunity to ask any questions regarding study procedures
4. Will have voluntarily and willingly signed the Informed Consent Form/Assent Form prior to initiation of any study related test/procedure. Children age 12 and older must have an IRB approved assent in place in order to be considered enrolled into the study.

Pre-study Documentation Requirements

The investigator is responsible for assembling, and sending to the Sponsor, the following documents before study ignition can occur:

- Signed and dated protocol signature page
- Copy of approved ICF and Assent
- Copy of the IRB approval of the protocol
- The IRB composition and/or written IRB compliance statement

- Signed Clinical Trial Agreement
- Curricula Vitae of all investigators and sub-investigators (signed and dated)
- Signed and dated FDA Form 1572
- Lab certifications (if required)
- Signed and dated Financial Disclosure for everyone listed on the site's FDA Form 1572
- Additional documentation, as required by the sponsor.

Protocol Adherence

The Investigator agrees to conduct the study in accordance with this protocol. Prior to beginning the study, the Investigator(s) must sign both the Investigator Agreement and the protocol signature page.

An Investigator must not make any changes to the study without first receiving written approval from the Sponsor and IRB, except when necessary to eliminate apparent immediate hazard to a subject. As soon as possible, the implemented deviation or change, reasons for it, and if appropriate, the proposed protocol amendment(s) should be submitted to the IRB for review and approval.

Protocol Amendments

Protocol amendments must be made only with the prior approval of the Sponsor (Dr. Lattermann). Agreement from the principal investigator must be obtained for all protocol amendments and amendments to the ICF. The IRB must be informed of all amendments and give approval for any amendments likely to affect the safety of the subjects or the conduct of the study. The principal investigator must send a copy of the approval letter from the IRB to the Sponsor and/or designee.

Both the Sponsor and the principal investigator reserve the right to terminate the study according to the study contract. The principal investigator should notify the IRB/IEC in writing of the study's completion or early termination and send a copy of the notification to the Sponsor and/or designee.

Study Monitoring and Auditing

The Sponsor and/or designee representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the study (eg, CRFs and other pertinent data) provided that subject confidentiality is respected, in accordance with the International Conference on Harmonization, (ICH) Good Clinical Practices (GCP) guidelines (ICH GCP E6 Section 5.18). The Investigator will permit representatives of the Sponsor's monitoring team or FDA to inspect facilities and records relevant to this study.

The Sponsor and/or designee Monitor and/or clinical research associate (CRA) is responsible for inspecting the CRF/eCRFs at regular intervals throughout the study to verify that the clinical trial is conducted in an organized manner according to the protocol provided.

Monitoring visits will assess:

- adherence to the protocol
- progress in the conduct of the study
- completeness, accuracy, and consistency of the data
- AE reporting to the Sponsor and the IRB/IEC
- adequacy of the facilities and availability of equipment required to conduct the study (ie,

- local laboratory, ECG equipment)
- adherence to local regulations on the conduct of clinical research

The Monitor should have access to each subject's permanent medical records and other study related records needed to verify the entries on the CRF/eCRFs.

The principal investigator will permit the Sponsor's personnel (or designees) to audit all CRF/eCRFs and supporting documentation, eg, hospital, office, clinic, pharmacy, and laboratory records for each subject. The principal investigator agrees to cooperate with the Monitor to ensure that any problems detected in the course of these monitoring visits are resolved. In accordance with the ICH, GCP and the Sponsor and/or its designee audit plans, this study may be selected for audit. Inspection of site facilities (eg, pharmacy, drug storage areas, laboratories, etc.) and review of study-related records will occur in order to evaluate the study conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

Data Safety Monitoring Board

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 University of Kentucky Center for Clinical and Translations 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.

Study Stopping Criteria

- local intolerance of the administered Kenalog® 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.

Data Recording and Record Retention

The PI must maintain all Essential Documents (as listed in the ICH Guideline for GCP) until notified by the Sponsor and in accordance with all local laws regarding retention of records.

The Investigator should maintain a list of appropriate qualified persons to whom he/she has delegated trial duties. All persons authorized to make entries and/or corrections to the case report forms should be included on the list of qualified persons. Source documents are original documents, data, and or records from which the participant's case report form data are obtained. These include, but are not limited to, hospital records, clinical and office charts, laboratory and pharmacy records, microfiches, radiographs and correspondence.

The Investigator and study staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the University of Kentucky and/or applicable regulatory authorities. Elements should include:

- Participant files containing complete case report forms, ICFs, assents, HIPAAs, and supporting copies of source documentation;
- Study files containing the protocol with all amendments, Kineret® package insert, copies of pre-study documentation, and all correspondence to and from the IRB and;
- Proof of receipt, Investigational Product Accountability Record, Return of Investigational Product for Destruction, Final Investigational Product Reconciliation Statement, and all drug-related correspondence.

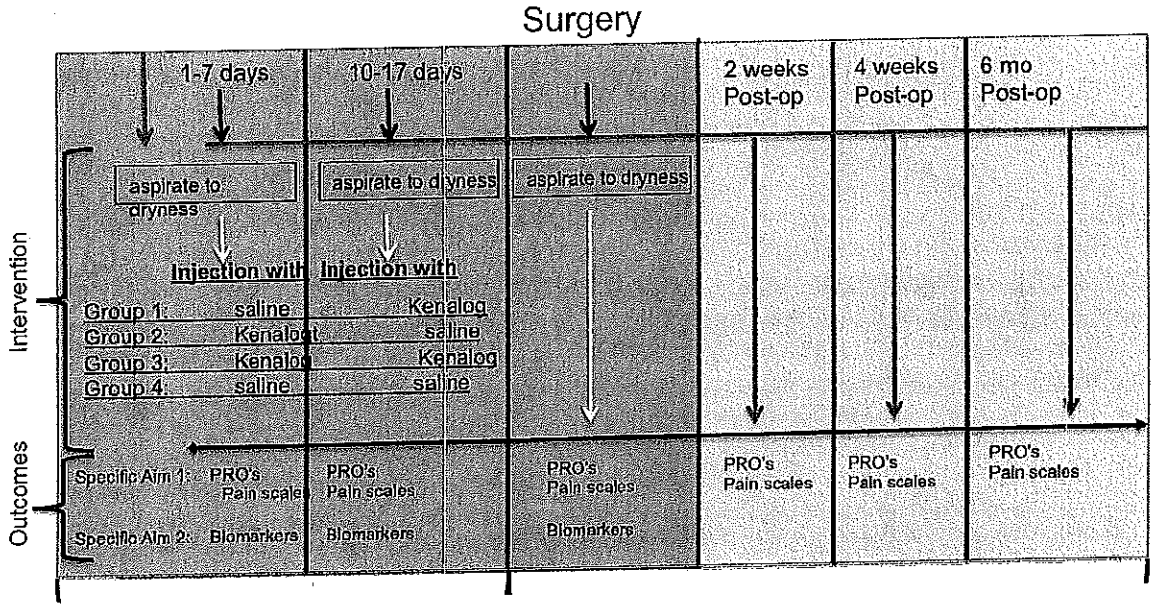
No study documentation should be destroyed without prior written agreement between Doctor Lattermann and the investigator. Should the investigator wish to assign the study record to another party or move them to another location, he/she may do so only with the prior written approval of Dr. Lattermann.

SAE reporting to the Sponsor:
Adverse reactions will be reported promptly to the Principal Investigator 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.

SUBJECT CONFIDENTIALITY

The Investigator and the Principal Investigator affirm a subject's right to protection against invasion of privacy. In compliance with United States federal regulations, the Principal Investigator requires the Investigator(s) to permit, when necessary, representatives from the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws. Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent/assent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

Figure 1: Study Design and Plan



Appendix A: Study Flow Chart

	Study Drug Treatment		Surgery [5]	Follow-up				Extended Follow-up Sub-Study
	Screening Baseline 1-2 days post injury (+/- 4 days)	12-14 days post injury		1-2 Weeks post surgery	4-6 weeks post surgery	6 Months Follow-up/Early Termination Visit	24 Months (+/-3) post surgery	24 Months (+/-3) post surgery
Study Time points	**Time point 1**	**Time point 2**						
Procedures	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7 (out of office)**	**Visit 8 (MRI)**
Informed Consent	X							X
Partial Medical History	X						X	
Medication History	X	X	X	X	X	X	X	
Smoking Status	X							
BMI Measurement	X							
Demographics	X							

X-Ray[1]	X							
MRI[2, 6]	X							X
Range of Motion	X							
Knee Aspiration	X	X	X[3&5]	X[3&5]				
Randomization	X							
Study Medication	X	X						
Laboratory Tests								
Urine	X	X	X					
Blood	X	X	X					
Questionnaires And Scales								
Likert Pain Scale[4]	X	X	X	X	X	X		
CSQ	X	X	X	X	X	X		
KOOS	X	X	X	X	X	X	X	
IKDC	X	X	X	X	X	X	X	
SF 36	X	X	X	X	X	X		
Catastrophizing Scale	X	X	X	X	X	X		
MARX Activity Scale							X	

1 – All subjects must have standardized Flexion weight bearing x-rays. *Documentation of closed growth plates at screening will be noted on the routine SOC x-rays.*

2 - All subjects enrolled will have an MRI performed as a routine diagnostic tool 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.

3 – Knee aspiration will only be performed if the subject has –post-op fluid on the knee. Should a subject be enrolled into the study and choose not to undergo ACL reconstruction aspiration at time of surgery will be omitted.

4 - All subjects enrolled into the study must have been diagnosed with an ACL tear.

5 - Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will received treatment according to this protocol

6- Subjects enrolled in the extended follow-up sub-study will undergo a research specific MRI, that is not considered standard of care.

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