ITLE: A Phase I/II Randomized Double-Blinded Standard of Care (Corticosteroid) vs. Sterile Amniotic Fluid for Osteoarthritis

Principal Investigator	David Petron, MD University Orthopaedic Center University of Utah 590 Wakara Way Salt Lake City, UT 84108 david.petronl@hci.utah.edu
Sub-Investigators	Bob Toth, PA,ATC University Orthopaedic Center University of Utah 590 Wakara Way Salt Lake City, UT 84108 robert.toth@hsc.utah.edu Nick Monson, DO University Orthopaedic Center University of Utah 590 Wakara Way Salt Lake City, UT 84108 nick.monson@hsc.utah.edu John Phillips, PhD Division of Hematology and Hematologic Malignancies 5C330 SOM, 30 N. 1900 E., University of Utah School of Medicine, Salt Lake City UT 84132 john.phillips@hsc.utah.edu Jan Pierce, MBA Cell Therapy & Regenerative Medicine University of Utah Health Sciences 675 Arapeen Drive, Suite 300 Salt Lake City, Utah 84108 jan.pierce@hsc.utah.edu Christopher Gee, MD, MPH University Orthopaedic Center University of Utah 590 Wakara Way

	Salt Lake City, UT 84108 christopher.gee@hsc.utah.edu
Sub-Investigators (continued)	Emily J. Harold, MD University Orthopaedic Center University of Utah 590 Wakara Way Salt Lake City, UT 84108 Emily.Harold@hsc.utah.edu Joy English, MD, RMSK University Orthopaedic Center University of Utah 590 Wakara Way Salt Lake City, UT 84108 Joy.English@hsc.utah.edu Daniel Cushman, MD University Orthopaedic Center University of Utah 590 Wakara Way Salt Lake City, UT 84108 Dan.Cushman@hsc.utah.edu Amy Powell, MD University Orthopaedic Center University of Utah 590 Wakara Way Salt Lake City, UT 84108 Amy.Powell@hsc.utah.edu
Statistician	John VanBuren, PhD Assistant Professor University of Utah – Division of Pediatric Critical Care 295 Chipeta Way Salt Lake City, UT 84108 john.vanburen@hsc.utah.edu

SYNOPSIS

Title	A Phase I/II Randomized Double-Blinded Standard of Care (Corticosteroid) vs. Sterile Amniotic Fluid for Osteoarthritis
Short Title	pAF for the treatment of Osteoarthritis
IRB Number	128491
IND	IND # 26443
Phase	Phase I/II
Design	This is a Phase I/II Randomized Double-Blinded Standard of Care (Corticosteroid) vs. Sterile Amniotic Fluid for Osteoarthritis
Study Duration	3 years
Study Center(s)	University of Utah Orthopedic Center
Objectives	Primary Objectives: • To determine the safety of using processed Amniotic Fluid (pAF) in patients with osteoarthritis • To determine the need for repeat intra-articular injection Secondary Objectives: • To determine the pain level following the injection of AF relative to corticosteroids • To evaluate functional outcomes
Number of Subjects	60 patients

Diagnosis and Main Eligibility Criteria	Inclusion: 1. Patients who are between the ages of 18-75 years; and 2. A confirmed diagnosis of knee osteoarthritis based on clinical and radiographic findings consistent with Kellgren-Lawrence Stage2-3 disease; and 3. Patients who have failed conservative treatment (e.g. steroid, activity modification, therapy, etc.) within 3 months; and 4. Unilateral or bilateral chronic knee joint pain >4 months; and 5. Patients who are able to ambulate (i.e. not wheelchair bound); and 6. Patient reported a typical pain of at least 4 out of 10 during the past week using VAS numeric pain scale (0-10) Exclusion: 1. Subjects who have had a previous injection (i.e. steroid, platelet rich plasma, or other) within the last 3 months; or 2. BMI >40 as defined by NIH Clinical Guidelines Body Mass Index; or 3. Concurrent participation in another investigational trial involving systemic administration of agents (within the previous 30 days) or plans to participate in any other allogeneic stem cell therapy trial during the 12 month follow-up period; or 4. Clinical suspicion of infection at injection site; or 5. Any surgeries within 4 weeks, other than diagnostic surgery; or 6. Insulin or self-reported non-insulin dependent diabetic evident of HgA1c ≥8% among known diabetics; or 7. Unable to consent to an English Language Consent Form; or 8. Frank mechanical issues (i.e. locking of the knee); or 9. Workman's Compensation cases; or

	10. Rheumatoid arthritis; or 11. Patients with a known allergy to local anesthetics or components of the study drug (pAF or steroid injection); or 12. Patients with vascular claudication or neurologic disorders affecting the index lower limb; or 13. Patients with inflammatory arthropathies or connective tissue disorders; or 14. Patients with known alcohol or drug abuse or dependence, recreational use of illicit drug or prescription medications, or have used medical marijuana within 7 days of study enrollment; or 15. Patients with history of active cancer/malignancy within 2 years of screening, apart from adequately treated basal cell or squamous cell carcinoma of the skin not associated with the target knee; or 16. Women who are nursing or pregnant; or 17. Patients of childbearing potential who are unwilling to use adequate contraception for 90 days after study drug injection.
Study Product, Dose, Route, Regimen	Standard of care steroid injection includes corticosteroids (40mg/cc Kenolog, 2ml of Ropivacane [3mL total]). The amniotic fluid injection will also be given as 3mL of drug injected directly into the knee.
Statistical Methodology	The primary endpoint (i.e. need for a repeat injection) will be compared between the two arms using Fisher's Exact test. An alpha 0.05 will be used as the threshold for statistical significance.

STUDY SUMMARY

This study will look at blinded standard of care (SOC) steroid injection vs. amniotic fluid injection (pAF) to treat and reduce osteoarthritis (OA) inflammation and pain. The main objectives of this study are to establish the safety and tolerability of allogeneic intra-articular pAF injections. Secondary objectives include pain levels and functional outcome scoring in patients over a 12 month time frame.

OUTCOMES OF THE STUDY

Primary Endpoint

- The need for a repeat allogeneic intra-articular injection [Time Frame: 6 months].

  Participants in both the SOC and pAF treatment arms may require and/or request rescue medication (i.e. SOC injection) at any time and will be given per PI discretion as part of standard of care. The clinicians will not know which study arm the study participant is in but will treat the participant with the SOC injection. This information will be documented and collected in the Electronic Medical Record (EMR), as well as the study's electronic data capture system. Participants will not be given any additional pAF injections throughout the 12-month study period.

  The participant will continue to be treated with SOC injections as needed. The outcome will be an indicator of whether or not a subject received a rescue medication within 6 months. Sensitivity analyses will also be performed to assess the number of rescue medications given over the course of a year.

Secondary Endpoints

- Pain level following the injection based on Visual Analog Scale scoring. [Time Frame: 6 months]

  Pain will be measured by the Visual Analog Scale for pain (VAS Pain). This will consist of a comparison of mean values for VAS scoring scales pre-injection and at the 6 month follow-up assessment. Clinically significant outcomes will be determined based on the minimal clinically important difference (MCID) occurring for each respective scale, which is defined as the smallest change score needed for the effect to be considered clinically relevant; described below. Specifically, VAS represents a patient-reported measurement tool used to quantify perceived level of pain, graded on a scale from 0 to 10 with 0 representing no pain and 10 representing the worse pain possible, and with precise measurement of the marked pain level's distance from 0 comprising the pain level.

- Functional outcomes scoring using Single Assessment Numerical Evaluation (SANE), PROMIS Physical Function CAT (PF CAT), and Knee Injury and Osteoarthritis Outcome Score (KOOS) scoring scales. [Time Frame: 6 months]

  Functional outcomes will be evaluated throughout the study period. The SANE asks the patient to evaluate their percentage of normal on the affected joint or region of interest. This is a one-question survey with a scale from 0-100%. The PFCAT is a bank of questions related to physical function. It is a computerized adaptive test that asks the patient to self-report capabilities rather than actual performance of physical activities. It includes functioning of upper extremities, lower extremities, and central regions and also assess instrumental activities of daily living, such as running errands. The KOOS is a survey that assesses joint symptoms, pain, pain during daily activities, physical function in daily living, as well as physical function during sports or

recreational activities. It also assesses quality of life. These functional outcome scores will be assessed at time points: 1, 3, 6 and 12 months.

Exploratory Endpoints

- Number of treatment emergent adverse events (AEs) directly related to the injection. [Time Frame: 1 day, 2 days, 1 month, 3 months, 6 months and 12 months]
- Pain level following the injection based on Visual Analog Scale scoring. [Time Frames: 1, 3, 6 and 12 months]
- Functional outcomes scoring using Single Assessment Numerical Evaluation (SANE), PROMIS Physical Function CAT (PF CAT), and Knee Injury and Osteoarthritis Outcome Score (KOOS) scoring scales. [Time Frames: 1, 3, 6 and 12 months]

BACKGROUND AND RATIONALE

Osteoarthritis (OA):
Osteoarthritis (OA) is the most common type of joint disease, affecting more than 30 million individuals in the United States alone [1]. It is the leading cause of chronic disability in older adults, costing the US greater than $185 billion annually [2]. Some people call it degenerative joint disease or "wear and tear" arthritis. It occurs most frequently in the hands, hips, ankles and knees. With OA, the cartilage within a joint begins to break down and the underlying bone begins to change. These changes usually develop slowly and get worse over time. OA can cause pain, stiffness, and swelling. In some cases, it also causes reduced function and disability; some people are no longer able to do daily tasks or work. Current treatments include intra-articular oral pharmacologic, Nonsteroidal anti-inflammatory drugs (NSAIDS), corticosteroid injections, and arthroplasty (partial or full joint replacement).

The progression of osteoarthritis has been divided into three stages including proteolytic breakdown of cartilage, fibrillation and erosion of cartilage surface with release of proteoglycan and collagen fragments into the synovial fluid, and cartilage breakdown inducing a chronic inflammatory response in the synovium, creating further cartilage breakdown.

We are interested in the clinical effects of human amniotic fluid's natural anti-inflammatory, immunomodulatory and growth-promoting properties to reduce and/or eliminate the inflammatory cascade of events leading to the degradation of cartilage; as well as, the possibility of using AF as an alternative to current treatments that can have a long-term detrimental effect on tissues such as corticosteroids.

Amniotic fluid (AF): Preclinical Data
Early after conception and until the mother's water breaks for the delivery of their infant, the fetus is bathed in amniotic fluid. AF functions as a supportive cushion to the fetus and provides a protective environment. AF is a rich source of nutrients, cytokines and growth factors that are required for fetal development and maturation [3]. AF also contains stem cells with the potential to differentiate along multiple cell lineages [4, 5]. The protective and regenerative properties of AF are achieved via the exchange of water and solutes with surrounding tissues. This is accomplished via the utilization of

different pathways during the course of a pregnancy that likely contribute to changes in the composition of the AF with gestational age [3].

A report that concentrates of AF inhibited the development of peritonitis was among some of the first evidence that AF had protective biological properties [6]. This was followed by a publication by Shimberg and co-workers that AF accelerates defense-repair mechanisms within damaged joints [6, 7]. Since these early publications, more sophisticated evaluations have revealed the presence of antimicrobial, immunomodulatory, and growth-promoting activities in AF [3]. Reports about antimicrobial activity in AF differ [8] among investigators. Some studies show that AF is inhibitory, while others show no effect against the same microorganisms. Yet, others show evidence that AF with low antimicrobial activity is associated with a high incidence of an infectious syndrome in pregnant women [9]. Components with antimicrobial, antiviral and antifungal activity that are present in AF include lysozyme, peroxidase, transferrin, β-lysin, immunoglobulins and zinc-peptide complexes [8], just to name a few.

Immunomodulatory properties of AF are evident from studies showing that enteral feeding of AF suppresses the pro-inflammatory responses in preterm pigs with necrotizing enterocolitis [9]. While growth promoting activities of AF are supported by animal studies as well as by in vitro studies showing that AF can enhance neochondrogenesis [10], regenerate peripheral nerves [11] and bone [12], accelerate re-epithelialization in corneas [13], and promote healing of human skin wounds [14]. Some of the factors that are found in AF that may contribute to these activities include inflammatory mediators that include, but are not limited to TNF-a, IL-6, IL8, and IL-10 [15], trophic factors that include EGF, IGF-1, FGF, HGF and TGF-a [16-17], and HA, an important factor in promoting re- epithelialization in human skin wounds [14].

Based on the hypothesis that nutrients, cytokines and growth factors contained in the non-cellular fraction of AF are useful for reparative and regenerative treatments in patients, Pierce et al., conducted a study at the University of Utah to address three issues. The first was to determine the feasibility of consenting and screening volunteer donors for the routine collection of AF from full-term pregnant women scheduled for caesarean- section (C-sections) and then processing the AF for clinical applications. The second aim was to develop a processing method that resulted in a cell-free AF preparation suitable for clinical applications. The third goal was to gain a better understanding about components of AF procured from full-term pregnancies.

With the above 3 goals in mind, human AF was collected by the staff of the Obstetrical and Gynecological department at the University of Utah hospital and was processed by technical staff of the Cell Therapy and Regenerative Medicine (CTRM) facility at the University of Utah. Physician executed abdominal incisions were performed through the abdominal and uterine muscles without cutting into the amnion membrane. Using a sterile soft suction catheter connected to a sterile MediVac Suction Container (Cardinal Health, Waukegan, IL), a blunt end insertion with a catheter was made into the amnion membrane and the AF was aseptically suctioned into a MediVac Container. The container was labelled, wrapped in frozen Insul-ice mats (Fisher Scientific, Hanover Park, IL) and placed in a temperature-monitored shipper that was validated for transport between 2 and 8 °C. The AF was transported to the Cell Therapy and Regenerative Medicine (CTRM) facility at the University of Utah. Upon arrival at the CTRM facility, the product was immediately placed into a refrigerator at 2–8 °C until

processing occurred. At the time of processing, the MediVac container with AF was aseptically placed in a biological safety cabinet and the AF was transferred via aseptic techniques into sterile centrifuge tubes. The total volume and gross appearance of the AF were recorded and samples were removed for sterility testing, cell counts and other relevant testing. The AF was centrifuged at 1400Xg for 20 min at 4 °C. Once centrifugation was complete, the supernatant was expressed into a new transfer pack and the remaining cell pellet was characterized and cultured as described below. The supernatant from the AF was processed using a proprietary filtration technology to sterilize and eliminate cellular debris from the final product. AF collections and final products were evaluated for total volume, fluid chemistries, total protein, and hyaluronic acid (HA) levels. Final products of processed AF (pAF) were also assessed for their cellular content and for their protein profiles using quantitative antibody arrays.

To validate the above described approach for collecting and processing AF, 36 pregnant women consented and passed the donor screening criteria. AF was successfully collected from 17 individuals. Median AF volumes were 70 mL (range 10–815 mL; n = 17). Fluid chemistries were similar, but some differences were noted in HA levels and cytokine profiles. Cytokine arrays revealed that an average of 304 ± 20 (mean ±SD; n=3) of 400 proteins tested were present in AF with a majority of cytokines associated with host defense. Some of the peptides encountered and classified according to their function are found on **Table 1**.

Table 1

Pro-inflammatory	OPN, PAI-I, CD163, RAGE, IL17, IL1R3
Host defense	IL-27, LAG-3, GITR, PD1
Innate Immunity	hCGb, Galectin-3, TLR-2, Osteoactivin
Antimicrobial	TSP-1, lactoferrin, CXCL14, Trappin-2, CCL-28, MIG
Anti-inflammatory	IL1-ra, MBL
Embryonic development	DKK1, DKK3
Angiogenesis	VEGF R1, Transferring, TIMP-2
Wound healing	OPN, PAPP-A, FAP

Processed Amniotic Fluid (pAF): Preliminary Clinical Data
pAF has been clinically used in over 2000 applications for over 100 different conditions. A majority of treatments have been for wounds and burns with over 100 patients receiving pAF for the treatment of large joint osteoarthritis. No adverse events have been directly associated with the injection of pAF or when pAF has been injected into the joint space for the treatment of osteoarthritis.

For the 3 patients treated for osteoarthritis at the University of Utah Orthopedic Center, each patient received a dose of 2.0 ml injected into the affected knee joint, and no toxicities or adverse events were observed. One patient with mild unilateral patellofemoral compartment osteoarthritis experienced mild effusion for 24 hours after pAF injection, but had elimination of pain with activity after 48 hours; and reports no pain in the joint 12 months after injection. This patient continues to exercise with no pain. Another patient had moderate bilateral patellofemoral compartment osteoarthritis and had a steroid injection 3 months prior to pAF injection. This patient experienced mild effusion for 24 hours, and had continued pain and discomfort for approximately 2 months. After 2 months, the patient reported a

significant reduction in pain. This patient reports no swelling and reports not taking NSAIDS anymore, and his knees continue to improve after 9 months post pAF injection. The patient continues to ride bikes and do strengthening exercises. The third patient had moderate bilateral osteoarthritis. This patient experienced effusion for 24 hours after pAF injection. The patient had minimal effect from the pAF injection until 2 months after the pAF injection, and then reported significant reduction in pain for the past 9 months. The patient is able to exercise because of her reduced pain.

ELIGIBILITY CRITERIA

Eligible participants will be identified by study staff.

Inclusion criteria are:

1. Age between 18-75 years; AND
2. Confirmed diagnosis of knee osteoarthritis based on clinical and radiographic findings consistent with Kellgren-Lawrence Stage 2-3 disease; AND
3. Patients who have failed conservative treatment (e.g. steroid, activity modification, therapy, etc.) within 3 months; AND
4. Unilateral or bilateral chronic knee joint pain >4 months; AND
5. Must be able to ambulate (not wheelchair bound); AND
6. Patient reported a typical pain of at least 4 out of 10 during the past week using VAS numeric pain scale (0-10)

Exclusion criteria are:

1. Subjects who have had a previous injection (steroid, Platelet Rich Plasma (PRP), or other) within the last 3 months; OR
2. BMI > 40 as defined by NIH Clinical Guidelines Body Mass Index; OR
3. Concurrent participation in another investigational trial involving systemic administration of agents (within the previous 30 days) or plans to participate in any other allogeneic stem cell therapy trial during the 12 month follow-up period; OR
4. Clinical suspicion of infection at injection site; OR
5. Any surgeries within 4 weeks, other than diagnostic surgery; OR
6. Insulin or self-reported non-insulin dependent diabetic evident of HgA1c ≥8% among known diabetics; OR
7. Unable to consent to an English Language Consent Form; OR
8. Frank mechanical issues (i.e. locking of the knee); OR
9. Workman's Compensation cases; OR
10. Rheumatoid arthritis; OR
11. Patients with a known allergy to local anesthetics or components of the study drug (pAF or steroid injection); OR
12. Patients with vascular claudication or neurologic disorders affecting the index lower limb; OR
13. Patients with inflammatory arthropathies or connective tissue disorders; OR

14. Patients with known alcohol or drug abuse or dependence, recreational use of illicit drug or prescription medications, or have used medical marijuana within 7 days of study enrollment; OR
15. Patients with history of active cancer/malignancy within 2 years of screening, apart from adequately treated basal cell or squamous cell carcinoma of the skin not associated with the target knee; OR
16. Women who are nursing or pregnant; OR
17. Patients of childbearing potential who are unwilling to use adequate contraception for 90 days after study drug injection.

OVERALL STUDY DESIGN

The blinded standard of care-steroid injection (SOC) vs. amniotic fluid injection (pAF) to treat and reduce osteoarthritis (OA) inflammation and pain will consist of 60 patients, wherein half of the patients will be in the SOC treatment arm and half of the patients will be in the AF arm. The PI (Dr. Petron) and patients will be blinded as to the arm in which they will be participating.

Screening and Enrollment

Patients will be screened and consented at the University of Utah Orthopedic Center and all participating sub-investigator's clinics when they come for a standard of care physical exam. During this exam, the patient will be asked about their health and lifestyle, medical history, and current medications. As part of the SOC physical exam, the patient will also have standard of care pre-treatment x-rays, neurological assessment, and a diagnosis of mild to severe OA in the knee joint assessment. The patient will also complete standard of care questionnaires including pain assessment and outcome surveys which are described below:

- Pain will be measured by the Visual Analog Scale for pain (VAS Pain). VAS Pain is a patient-reported measurement tool used to quantify perceived level of pain, graded on a scale from 0 to 10 with 0 representing no pain and 10 representing the worse pain possible, and with precise measurement of the marked pain level's distance from 0 comprising the pain level.

- Functional outcomes will be assessed using Single Assessment Numerical Evaluation (SANE), PROMIS Physical Function CAT (PF CAT), and Knee Injury and Osteoarthritis Outcome Score (KOOS) scoring scales.
    - The SANE asks the patient to evaluate their percentage of normal on the affected joint or region of interest. This is a one-question survey with a scale from 0-100%.
    - The PFCAT is a bank of 160 questions related to physical function. It is a computerized adaptive test that asks the patient to self-report capabilities rather than actual performance of physical activities. It includes functioning of upper extremities, lower extremities, and central regions. It also assesses instrumental activities of daily living, such as running errands.

  - The KOOS is a survey with 42 questions that assesses joint symptoms, pain, pain during daily activities, physical function in daily living, as well as physical function during sports or recreational activities. It also assesses quality of life.

- Review of systems questionnaire (will only be completed during the 3, 6, and 12 months follow up period).

If the patient is found to be eligible for the study, research staff will approach the patient for informed consent. Patients with bi-lateral knee OA can participate but only one knee will receive the study treatment. The treating physician/principal investigator and the patient will discuss which knee should be treated with a study injection. Patients who have bi-lateral knee pain will need to consent that only one knee will be treated with study injection and are willing to forego steroid injection in the non-target knee for the duration of the study. Those who have previously undergone steroid injection will have to wait 3 months after last injection before participating in the study Medications, such as anti-inflammatories, pain relievers, etc. may be used throughout the duration of the study and will be recorded as concomitant medications throughout the study period and at each follow-up visit.

Randomization
Consented patients will be randomized into two groups at a 1:1 ratio using block permutations of 2 and 4. Randomization will be done using either an online randomization service or envelope system. Randomization will occur following the patient's consent into the study, either at the initial visit or the day of the planned injection. Once the randomization number is obtained, unblinded research staff will prepare the appropriate study drug. The injections will be blinded to the research coordinator in charge of data entry, the treating physician and the patient. Clinical staff will prepare the study drug injection and maintain the blind by placing a blank label over the syringe to ensure that the treatment is blinded to the patient and treating physician. Once a patient has been randomized, they become an enrolled subject and will be included in the intention to treat analysis of the trial outcomes, regardless of whether or not they receive study drug.

Study Enrollment Staggering
In order to help ensure patient safety and to help limit unreasonable risk the first six subjects will be enrolled in staggered intervals. One subject may be enrolled per week for the first six subjects. Half of the six subjects will be randomized to pAF and the other half will be randomized to SOC steroid. Preliminary safety data for the first six subjects will be gathered and reviewed by the Data Safety and Monitoring Board (DSMB). If the DMSB expresses no concern over the preliminary data, the study may proceed with enrollment.

Study Drug Administration
Study Drug Administration will only occur at the Orthopedic Center. Patients that are screened and consented at participating sub-investigator clinics will need to go to the Orthopedic Center to receive study drug. Patient eligibility will be reviewed prior to study drug administration The standard of care

steroid injection includes corticosteroids (40mg/cc Kenolog, 2ml of Ropivacane [3mL total]). The amniotic fluid injection will also be given as 3mL of drug injected directly into the knee.

Frozen processed amniotic fluid will be provided in a validated container with dry ice, by the CTRM each day that a consented or potential patient is seen in the Orthopedic Center. If the patient has been randomized to receive the amniotic fluid injection, unblinded research staff will prepare the injection and place a blank label over the syringe to ensure the blind. The amniotic fluid will be room temperature (same as the steroid injection) prior to being given to the treating physician to further ensure the blind. The assignment of study drug will be randomly selected. Neither the patient, nor their providers will know what the patient is receiving.

Prior to the injection, aspiration of the knee may be indicated. The treating physician will perform the aspiration and the volume and characteristics of the aspiration will be recorded. After the aspiration is performed, the syringe is removed from the needle while the needle remains in place. The injectate is then screwed onto the same needle and its contents are injected into the knee per the standard of care. If no aspiration needed, then a 18g to 25g 1.5-inch needle is used to inject the injectate.

If the treating provider suspects an infection of the knee due to the appearance of the aspirate the aspirate will be sent for cultures and the patient will not receive a study drug injection. The subject will still be required to complete the follow up assessments as detailed in the patient study calendar per the intention to treat principle.

For the **Corticosteroid injection**, the skin is prepped with Chloraprep. The skin is anesthetized using a freezing spray. Then a mixture of 1 mL of Kenalog (corticosteroid) and 2 mL of ropivacaine is injected into intra-articular space of the knee.

For the processed **Amniotic Fluid injection**, the pAF injectate is prepared. The skin is prepped with Chloraprep. The skin is anesthetized using a freezing spray. 3 mLs of the pAF injectable solution is injected into the intra articular space of the knee.

Ultrasound guidance will be used for all study injections.

Following injection, patients will receive post injection strength training recommendations as determined by the treating PI and as the patient tolerates.

Follow-up Visits/Emails/Phone Calls/Texts:
The patient will have one (1) standard of care in-person clinic visit and five (5) follow up questionnaires /phone call visits as part of research. The patient may come to the clinic for an evaluation at any time if they are experiencing any pain or side effects as part of standard of care.

The schedule of follow up visits are:

- **24 hours:** Follow up phone call will be made 1 day after study drug injection. The phone call will be made to assess AEs. The study team will instruct the subject to contact the study team at any

point if new or worsening AEs arise. The subject reserves the right to come back into clinic for an in-person visit per standard of care.

- **48 hours:** Follow up phone call will be made 2 days after study drug injection. The phone call will be made to assess AEs. The study team will instruct the subject to contact the study team at any point if new or worsening AEs arise. The subject reserves the right to come back into clinic for an in-person visit per standard of care.
- **1 month**: Follow up in-person clinic visit with associated physical exam and questionnaires. This visit is part standard of care. If the patient is unable to return to clinic, the patient may receive a phone call by the study team to do remote clinic visit and complete questionnaires.
- **3 Months**: Patient questionnaires sent by mail or email/text. If the patient is non-responsive to initial contact additional follow up contact (e.g. additional email/phone calls/text) will be made by the study team. The patient may receive additional follow up contact (e.g. email/phone call/text) by the study team to clarify any responses. The study team will instruct the subject to contact the study team at any point if new or worsening AEs arise. The subject reserves the right to come back into clinic for an in-person visit per standard of care. This is a study related follow up.
- **6 Months**: Patient questionnaires sent by mail or email/text. If the patient is non-responsive to initial contact additional follow up contact (e.g. additional email/phone calls/text) will be made by the study team. The patient may receive additional follow up contact (e.g. email/phone call/text) by the study team to clarify any responses. The study team will instruct the subject to contact the study team at any point if new or worsening AEs arise. The subject reserves the right to come back into clinic for an in-person visit per standard of care. This is a study related follow up.
- **12 Months:** Patient questionnaires sent by mail or email. If the patient is non-responsive to initial contact additional follow up contact (e.g. additional email/phone calls/text) will be made by the study team. The patient may receive additional follow up contact (e.g. email/phone call/text) by the study team to clarify any responses. The study team will instruct the subject to contact the study team at any point if new or worsening AEs arise. The subject reserves the right to come back into clinic for an in-person visit per standard of care. This is a study related follow up.

The patient may also receive additional phone calls by the study team to assess any adverse events that occur in between the scheduled 1-month follow-up visit and subsequent emails,phone calls, and/or text messages.

PATIENT STUDY CALENDAR

	Screening /Baseline	Day 1 (24± 5 hours post injection)	Day 2 (48 ± 5 hours post injection)	1-Month (± 1 week)	3-Months (± 2 weeks)	6-Months (± 1 month)	12-Months (± 1 month)	As needed
Eligibility [A]	X							
Informed Consent	X							
Medical History	X							
Concomitant Medications	X			X	X	X	X	X
Physical Exam	X			X				X
Laboratory tests[B]	x							
Randomization	X							
Study Drug Administration	X							
Follow-up Contact[C]		X	X	X[D]	X	X	X	X
In Person Follow-up Visit				X[D]				X
Pain Assessment [E]	X			X	X	X	X	
Outcome Assessment[F]	X			X	X	X	X	
Review of systems questionnaire[G]					X	X	X	
Review of adverse events[H]		X	X	X	X	X	X	X
Knee Aspiration[I]	X							
Aspiration Characteristics[J]	X							

[B] Laboratory tests include: PT/INR (only applicable to patients taking blood thinners) and urine hCG (only applicable to women of childbearing potential)
[C] Follow up Email/phone call/text may occur to clarify any responses and/ or assess AEs and/ or establish contact with the patient.
[D] The patient may receive a phone call by the study team to do remote clinic visit and complete questionnaires if the patient is unable to return to clinic to do in-person visit.
[E] Visual Analog Scale (VAS).
[F] Single Assessment Numerical Evaluation (SANE), PROMIS Physical Function CAT (PF CAT), and Knee Injury and Osteoarthritis Outcome Scale (KOOS). These surveys may be sent via email/text or done over the phone.
[G] This questionnaire may be sent via email/text or done over the phone.
[H] The study team may call the participant to assess adverse events in between the scheduled follow-up visit and phone calls.
[I] If deemed applicable by the treating provider.
[J] Only applicable if there is a suspicion of infection as determined by the treating provider.

WITHDRAWAL FROM STUDY

A patient may withdraw from the study at any time, without a penalty or loss of benefits. Data collected up until the point of withdrawal will be still be used in efficacy and safety analyses.

Patients should not be withdrawn from the trial for AEs. Patients who discontinue the study will be followed for safety according to the safety monitoring plan and attempts to complete all safety assessments will be made, unless the subject withdraws consent.

STUDY STOPPING/ DISCONTINUATION

Events used to trigger discontinuation or stopping of the study pending a safety investigation include:

- If there is one death, irrespective of attribution
- If there is a SAE, that is possibly or probably related to study drug injection (pAf or steroid injection)
- Signs of severe hypersensitivity in a least 10% of enrolled patients
- If there is one case of hypersensitivity, defined as an acute inflammatory reaction requiring invasive intervention
- If there an adverse event that is classified as Severe per protocol that is possibly or probably related to the IP Injection (AF or steroid)

In the event study enrollment has been suspended due to the above criteria or any other reason the FDA, the DSMB, IRB and the Sponsor must be notified. Enrollment and treatment will be resumed after a review of the incident and of any corrective and preventive actions have been established with consultation between the study team, the Sponsor, and the DMSB. The study may not resume without FDA approval.

DATA COLLECTION

Patients will be evaluated at baseline in the clinic. At 1 month after the injection an in-person clinic follow-up visit will occur. At this visit, concomitant medications, pain assessment, and outcomes will be assessed, as well as a physical exam and review of adverse events. Three, six and twelve months post injection, research staff will send an email/text to patients with a link to the surveys. If patients do not complete surveys within a week, research staff will follow up with a phone call to the patients inquiring about their knee(s) and instruct them to complete the various assessment tools online (VAS, SANE, PFCAT, and KOOS). Research staff may call patients after they complete surveys to clarify any responses. Information about repeat injections will be collected, including date of injection. Additional visits, which include a physical exam, review of concomitant medications and adverse events, will be done as needed.

Medical history, concomitant medications and physical exam will be collected at the baseline visit, prior to randomization. Concomitant medications will also be collected at the following time points: 1, 3, 6 and 12 months. Adverse events will also be collected at all follow-up time points (1 day, 2 days, 1 month, 3 months, 6 months and 12 months after baseline).

At the end of 12 months, the pain and outcomes assessments for both treatment arms will be compared and assessed using the Visual Analog Scale (VAS), Single Assessment Numerical Evaluation (SANE), PROMIS Physical Function CAT (PF CAT), and Knee Injury and Osteoarthritis Outcome Score (KOOS).

DATA ANALYSIS

The primary endpoint (need for a repeat injection) will be compared between the two arms using Fisher's Exact test. An alpha 0.05 will be used as the threshold for statistical significance. Missing data will remain missing rather than be imputed since this is a pilot study assessing preliminary safety and feasibility.

The secondary endpoint (pain) will consist of a comparison of mean values for VAS scoring scales pre-injection and at follow-up visits at 1, 3, 6 and 12-months. Clinically significant outcomes will be determined based on the minimal clinically important difference (MCID) occurring for each respective scale, which is defined as the smallest change score needed for the effect to be considered clinically relevant.

Continuous secondary and exploratory endpoints will be compared between arms using Wilcoxon Rank Sum tests. Categorical endpoints (including safety outcomes) will be compared between arms using Chi-squared tests. In cases of small counts, exact tests may be used.

SAMPLE SIZE CALCULATIONS AND STATISTICAL POWER

The primary endpoint is the need for a repeat injection. Based on historic data, about 50% of individuals who receive a steroid injection return for an additional injection within six months due to pain. Assuming a significance level of 0.05 and a need for a repeat injection in the AF arm of 10%, with 60 total patients (30 per arm), we have about 90% power to detect a difference in need for repeat injections at 6 months between the two arms. Expecting approximately 5 randomizations per week, 60 patients will take approximately 3 months to enroll.

DATA MANAGEMENT

Clinical Site Data Management

The clinical site will maintain study records in locked file cabinets. The site will maintain an Essential Documents Binder, which may be in paper or electronic form. Copies of all informed consent documents will be kept on file and be available for site monitoring inspection (on site or remote).

DATA COORDINATING CENTER

The Data Coordinating Center (DCC) in the Department of Pediatrics at the University of Utah School of Medicine provides data coordination and management services for a variety of national research networks. Anchoring these services is a state-of-the-art, energy efficient data center. The data center facility supports more than 3500 users around the country and provides a secure, reliable, enterprise-wide infrastructure for delivering mission critical DCC systems and services.
The data center was built using high industry standards and energy efficient cooling solutions. The data center is cooled by an LCP (Liquid Cooling Package) inline cooling technology; providing efficiency, redundancy and the modularity required. Cooling is based upon a hot/cold aisle design that allows for

even air distribution with minimal hot spots. The data center electrical power system contains an uninterruptible power system (UPS) with a diesel backup generator. The data center is protected with a FM200 fire suppression system to act as a secondary system to the smoke detectors. The data center provides enhanced security to safeguard the equipment and the data within in it. Entry into the data center is restricted by card access and layered security measures and controls. The data center and external building access points are monitored with video surveillance.

The data center has a virtualized environment. The virtual environment consists of more than 400 virtual servers and nearly 25 physical servers. The data center's virtualization solution provides key advantages; 1) high availability – in the event of hardware failure, virtual servers automatically go back online in a seamless process. 2) flexible infrastructure – disk storage, memory and processor capacity can be increased or reallocated at any time. 3) rapid deployment – servers can be provisioned on-demand.

Production servers running mission critical applications are clustered and configured for failover events. Servers are backed up with encryption through a dedicated backup server. Our storage area networking (SAN) applications, clusters, and switch-to-switch links are on a 10 gigabit network. Incremental backups occur Monday through Friday. A full system backup occurs weekly. Full backups are taken off site on a weekly basis to an off-site commercial storage facility. The data center currently manages over 150 terabytes of data.

Our information systems are available 24 hours a day, 7 days a week to all users, unless a scheduled maintenance interruption or mitigation of an unexpected event is required. If this occurs, we notify all users of the relevant systems, and data entry can be deferred until after the interruption is over. Our critical systems availability has exceeded 99.9% for the past two years.

Security, Support, Encryption and Confidentiality

The data center coordinates the network infrastructure and security with University Information Technology (UIT) at the University of Utah. This provides us with effective firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University. Centralized authentication and communication over public networks is encrypted using transport layer security (TLS) or virtual private network (VPN) technologies, both of which provide at least 128 bit encryption. Direct access to data center machines is only available while physically located inside our offices, or via a VPN client.

All network traffic is monitored for intrusion attempts, security scans are regularly run against our servers, and our IT staff are notified of intrusion alerts. Security is maintained with Windows user/group domain-level security. Users are required to change their passwords every 90 days. All files are protected at user/group levels; database security is handled in a similar manner with group-level access to databases, tables, and views in Microsoft SQL Server. Finally, all laptop computers in use in the DCC/division are whole-disk encrypted.

The data center uses control center tools to continuously monitor systems and failure alerts. Environmental and network systems are also monitored to ensure up time. And highly trained system administrators on staff are available to respond in high risk emergency events.

All personnel involved with data coordinating centers have signed confidentiality agreements concerning data encountered in the course of their daily work. All personnel (including administrative staff) have received Human Subjects Protection and Health Information Portability and Accountability Act (HIPAA) education. We require all users to sign specific agreements concerning security, confidentiality, and use of our information systems, before access is provided.

ELECTRONIC DATA CAPTURE SYSTEM

The Data Coordinating Center (DCC) will develop an electronic data capture system for this trial. Currently the DCC uses multiple applications, such as OpenClinica or REDCap, and will elect to use the most appropriate application at the time of implementation of the study. Data will be entered by the clinical site.

The DCC will use an electronic discrepancy management system to notify sites of inconsistent or erroneous data entry, which will be corrected by the clinical site. The discrepancy management system maintains an audit trail of all discrepancy resolution.

STUDY MONITORING

The investigators recognize the importance of ensuring data of excellent quality. Study monitoring is critical to this process. Monitoring has been a very effective tool for maintaining data quality in previous Data Coordinating Center studies, and we will utilize this process to ensure excellent quality data in the proposed study. The DCC utilizes risk-based methodology to identify and correct problems that may arise at sites. The risk-based approach to monitoring focuses on oversight activities and preventing or mitigating key risks to data quality, as well as to processes critical to human subject protection and integrity of the trial or study.

Study monitors must be provided with appropriate access to study materials and the medical records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the study monitor's review of data in the electronic medical record.

Site Monitoring Plan

A supplemental study-specific risk-based monitoring plan, separate from the protocol will be completed which outlines specific criteria for monitoring. This plan may include the number of planned site visits, criteria for focused visits, additional visits or remote monitoring, a plan for chart review and a follow up plan for non-compliant sites, if applicable. The monitoring plan also describes the type of monitoring that will take place (e.g., sample of all subjects within a site; key data or all data), the schedule of visits, how they are reported and a time frame to resolve any issues found.

Clinical Site Monitoring

Site monitoring visits may be performed by a trained site monitor during the study period to ensure regulatory compliance, patient safety, and to monitor the quality of data collected. Essential document binders, regulatory documents and data collection forms may be reviewed. Interim visits will take place depending on budget, site enrollment, and compliance issues identified. The site monitor will provide each site with a written report, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (prior to

patient enrollment), interim visits, and a close out visit. The site initiation may take place as group training made up of site investigators and research assistants.

Remote Monitoring
The Data Coordinating Center may supplement on-site monitoring with remote monitoring activities. Remote monitoring involves detailed review of the data entered by the Clinical Center and consultations with the Clinical Center investigator and/or research coordinator to review safety and data quality. This may require uploading copies of medical records, patient study specific regulatory documentation, or other source documents for the monitor to review. Alternatively, other methods, such as remotely viewing source documentation, may be utilized. This helps assure protocol compliance and accurate data collection. The Data Coordinating Center may conduct more remote monitoring activities early in the trial to assure protocol compliance and identify any training issues that may exist. Remote monitoring documents will be retained in accordance with federal requirements. Safety of subjects will be monitored and ensured in accordance with the Data and Safety Monitoring Board (DSMB) plan.

Pharmacy Monitoring
The Cell Therapy and Regenerative Medicine (CTRM) must maintain adequate records of all dispensed study drug. The CTRM may be monitored and requested to send copies of these documents to the Data Coordinating Center.

RecordAccess
The medical record and study files (including informed consent documents) must be made available to authorized representatives of the Data Coordinating Center, upon request, for source verification of study documentation. In addition, medical information and data generated by this study must be available for inspection upon request by representatives (when applicable) of the Food and Drug Administration (FDA), other Federal funders or study sponsors, and the Institutional Review Board (IRB).

PROTECTION OF HUMAN SUBJECTS

Institutional Review Board (IRB) Approval
IRB approval must be obtained prior to participating in the study. The Data Coordinating Center will track IRB approval status and will not permit subject enrollment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project.

Informed Consent
For all subjects ≥ 18 years of age, informed consent is required. Subjects who are capable of giving consent and who are alert and competent, will be asked, following an appropriate discussion of risks and benefits, to give consent to the study. For those with diminished mental capacity, a Legal Authorized Representative will be used.

Potential Risks
Loss of confidentiality of the subject is a potential risk of the study; however, safeguards are in place to protect against this.

Regarding loss/breach of privacy and confidentiality, all applicable parties (e.g. clinical sites, DCC) will be responsible for ensuring that appropriate data security procedures are in place.

Potential Benefits
The benefits of active drug therapy in this population are unknown; however, it is postulated that there may be a potential benefit in pain and functional outcomes for patients with osteoarthritis of the knee.

DATA AND SAFETY MONITORING PLAN

Data Safety Monitoring Board (DSMB)

The study will have a Data Safety Monitoring Board (DSMB). The DSMB will have a charter, will approve the protocol prior to implementation, and will review interim analyses as applicable. The purpose of the DSMB is to advise the sponsors and Principal Investigator(s) regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study. The DSMB is responsible for monitoring accrual of study subjects, adherence to the study protocol, assessments of data quality, performance of the individual Clinical Center, review of serious adverse events and other subject safety issues, and review of formal interim statistical analyses of treatment efficacy.

ADVERSE EVENT REPORTING

The site investigator is responsible for evaluating all adverse events at their Clinical Center and ensuring that they are properly reported. All adverse events will be captured during the study period. The nature of each experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment will be documented.

Definition of Adverse Event and Serious Adverse Event

Adverse Event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom.

Serious Adverse Event (SAE): A serious adverse event (SAE) for this population is an adverse event that:

- results in death; or
- is life-threatening (the patient was, in the view of the site investigator, in immediate danger of death from the event as it occurred); or
- requires inpatient hospitalization or prolongs an existing hospitalization; or
- results in persistent or significant disability or incapacity; or
- results in congenital anomaly/birth defect; or
- any other event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in these definitions.

Classification of an Adverse Event (Relatedness, Severity, and Expectedness)

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the site investigator using the criteria below. Relatedness must be assessed by an investigator and may not be assessed by a research coordinator.

- **Not Related:** The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

- **Possibly Related**: The event follows compatible temporal sequence from the time of beginning the assigned study intervention, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

- **Probably Related:** The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and cannot be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Severity: The severity, which is a measure of intensity, of clinical adverse events and laboratory abnormalities will be recorded by the site investigator and categorized. The following guidelines will be used to describe severity.

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

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information described for the study intervention.

- **Expected:** An event is considered expected if it is known to be associated with the underlying condition or is related to the study intervention and is mentioned in the protocol, informed consent, or other study documents. An event may be expected despite the study subject's clinical state immediately prior to the event.

  Expected adverse events for this study are:
  - Pain at injection site
  - Swelling or effusion at injection site
  - Soreness
  - Bleeding at the injection site

- **Unexpected:** An event is considered unexpected if there are no prior data linking this event

with either the condition or intervention under study or an event that occurred unexpectedly in the course of treatment.

Treatment or Action Taken: For each adverse event, the site investigator will record whether an intervention was required:

- Medical or surgical procedure
- Concomitant medication: started, changed, or discontinued
- Other, specify
- No action taken

Outcome of Event: Finally, the site investigator will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the patient returned to baseline status
- Recovered with permanent sequelae
- Symptoms persist

Time Period for Adverse Events

For purposes of this study, events that occur following randomization through the last follow-up visit at 12 months will be reported as adverse events. Serious adverse events, unexpected medically attended events, and new onset chronic illnesses will be recorded from randomization through twelve months after the last study dose. Specifically, events that occur following informed consent to participate in the study, but prior to actual randomization, are not adverse events. These should be recorded as baseline conditions.

Data Collection Procedures for Adverse Events

After patient randomization all adverse events (including serious adverse events) will be recorded according to relatedness, severity, and expectedness, as well as their duration and any treatment prescribed. Any medical condition present at the time of randomization, recorded in the patient's baseline history at study entry, which remains unchanged or improves (unless the clinician feels it is clinically relevant), will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the Data Coordinating Center as this requires specific training.

Unanticipated Problems (UP)

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related or possibly to participation in the study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The site investigator will report unanticipated problems to the Data Coordinating Center within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the Data Coordinating Center within 3

working days of the event. After receipt of the complete report, the Data Coordinating Center will review these unanticipated problems to determine if there is a safety concern. In accordance with local IRB requirements, the site investigator may be required to report such unanticipated problems to the IRB in addition to notifying the Data Coordinating Center. The CTRM, medical monitor or DSMB may be notified of these events. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, and DSMB staff cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator (Dr. Petron) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent by the DSMB.

Amendments

Any amendments or administrative changes to an IRB approved protocol will not be initiated without submission of an amendment for IRB review and approval. Any amendments to the protocol that significantly affect the safety of subjects, the scope of the investigation, or the scientific quality of the study are required to submit the amendment for FDA review.

Monitoring Serious Adverse Events and IND Safety reports

A qualified physician will be designated to fulfill the function of the medical monitor for this study. Site investigators and/or research coordinators will report serious adverse events to the Data Coordinating Center within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event, and the medical monitor will assess all serious adverse events reported from site investigators.

For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The medical monitor will sign off on each SAE report after review. All SAE reports will be retained at the Data Coordinating Center, and all SAE reports will be available for review by DSMB members. The SAE reporting process may be incorporated into the Electronic Data Capture (EDC) System in use for the study.

In the unlikely event that the medical monitor believes an unexpected and study- related SAE warrants emergent cessation of enrollment in the trial, the DSMB chairperson will be immediately consulted. If this individual concurs with the judgment of the medical monitor, or if the DSMB chairperson cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator (Dr. Petron), the CTRM, and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent by the DSMB.

In accordance with local IRB requirements, the site investigator may be required to report such events to the IRB in addition to notifying the Data Coordinating Center.

After notification of the CTRM, and the DSMB chairperson, of serious, unexpected, and study-related adverse events or unanticipated problems (UP), decisions will be made whether to continue the study without change, and whether to convene the entire DSMB for an emergent meeting. If a decision is made to suspend enrollment in the trial, this will be reported to the study investigator (Dr. Petron), who will be instructed to report this to their local IRB.

The DSMB will review all adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings. The Data Coordinating Center will prepare a Summary Report of Adverse Events for the DSMB meetings, classified with the MedDRA coding system.

During these routine meetings of the DSMB, they will review eligibility and recruitment, adverse events, safety analyses, and study data. The DSMB has the ability to use their discretion on suspending enrollment in the trial due to reported adverse events, safety analyses and findings from the study data. The DSMB will be un-blinded to the treatment arm.

Under 21 CFR 312.32(c), the Sponsor is required to notify FDA and all participating investigators in an IND safety report (i.e., 7- or 15-day expedited report) of potentially serious risks from clinical trials or any other source as soon as possible, but no later than 15 calendar days after the Sponsor receives the safety information and determines that the information qualifies for reporting.

Follow-up of Serious, Unexpected and Related Adverse Events
All serious, unexpected and related adverse events, that are unresolved at the time of the patient's termination from the study, will be followed by the Clinical Center investigators until the events are resolved, subject is lost to follow-up, the adverse event is otherwise explained or has stabilized, or 12 months have passed from the time of last study dose.

STUDY TRAINING
A formal training program for investigators and research staff will be held prior to the start of enrollment. The training program will cover regulatory topics and Good Clinical Practice. The training will also provide in depth explanations regarding study procedures, clinical care, adverse event reporting, data entry procedures, quality assurance, site monitoring, and the informed consent process. A manual of operations will be provided to each investigator prior to the start of enrollment. The manual will detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically. The Data Coordinating Center, in collaboration with the study investigator (Dr. Petron), will be the main contact for study questions.

REGULATORY CONSIDERATIONS

Food and Drug Administration
This trial is being conducted under an Investigational New Drug application approved by the Food and Drug Administration (Investigational New Drug application #26443). The clinical investigator at each participating site will complete a Form FDA 1572, "Statement of Investigator."

An annual progress report will be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect (21 CFR 312.33).

Health Insurance Portability and Accountability Act
Data elements collected may include the date of birth and date of admission. Prior to statistical analyses, dates will be used to calculate patient age at the time of the study events. The final data sets (used for study analyses and archived at the end of the study) will be de–identified, and will exclude these specific dates.

Data elements for race, ethnicity, and gender are also being collected. These demo- graphic data are required for Federal reporting purposes to delineate subject accrual by race, ethnicity, and gender.

Inclusion of Women and Minorities
There will be no exclusion of patients based on gender, race, or ethnicity.

ClinicalTrials.gov Requirements
This study will be registered on clinicaltrials.gov.

Retention of Records
For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

References
1. Osteoarthritis Fact Sheet. Center for Disease Control and Prevention. https//www.cdc.gov/arthritis/basics/osteoarthritis.htm

2. Kotlarz H, Gunnarsson CL< Fang H, Rizzo JA,. Insurer and Out of Pocket Costs of Osteoarthritis in the US. Evidence from national survey data. Arthritis Rheum. 2009, Dec 60 (12) 3546-53.

3. Underwood, M.A., W.M. Gilbert, and M.P. Sherman, Amniotic fluid: not just fetal urine anymore. J Perinatol, 2005. 25(5): p. 341-8.

4. Prusa, A.R., et al., Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? Hum Reprod, 2003. 18(7): p. 1489-93.

5. Bottai, D., et al., Third trimester amniotic fluid cells with the capacity to develop neural phenotypes and with heterogeneity among sub-populations. Restor Neurol Neurosci, 2012. 30(1): p. 55-68.

6. Johnson, H.L., Peritoneal Immunization. The American Journal of Surgery, 1936. 34(2): p. 266-271.

7. Shimberg, M., The Use of Amniotic Fluid Concentrate in Orthopedic Conditions. The Journal of Bone and Joint Surgery, 1938(20): p. 167-177.

8. Ismail, M.A., G.I. Salti, and A.H. Moawad, Effect of amniotic fluid on bacterial recovery and growth: clinical implications. Obstet Gynecol Surv, 1989. 44(8): p. 571-7.

9. Ojo, V.A., E.E. Okpere, and E.E. Obaseiki-Ebor, Antimicrobial properties of amniotic fluid from some Nigerian women. Int J Gynaecol Obstet, 1986. 24(2): p. 97-101.

10. Siggers, J., et al., Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotizing enterocolitis in preterm neonates. Am J Physiol Gastrointest Liver Physiol, 2013. 304(10): p. G864-75.

11. Ozgenel, G.Y., G. Filiz, and M. Ozcan, Effects of human amniotic fluid on cartilage regeneration from free perichondrial grafts in rabbits. Br J Plast Surg, 2004. 57(5): p. 423-8.

12. Ozgenel, G.Y. and G. Filiz, Combined application of human amniotic membrane wrapping and hyaluronic acid injection in epineurectomized rat sciatic nerve. J Reconstr Microsurg, 2004. 20(2): p. 153-7.

13. Karacal, N., et al., Effect of human amniotic fluid on bone healing. J Surg Res, 2005. 129(2): p. 283-7.

14. Castro-Combs, J., et al., Corneal wound healing is modulated by topical application of amniotic fluid in an ex vivo organ culture model. Exp Eye Res, 2008. 87(1): p. 56-63.

15. Nyman, E., et al., Hyaluronic acid, an important factor in the wound healing properties of amniotic fluid: in vitro studies of re-epithelialisation in human skin wounds. J Plast Surg Hand Surg, 2013. 47(2): p. 89-92.

16. Weissenbacher, T., et al., Influence of maternal age, gestational age and fetal gender on expression of immune mediators in amniotic fluid. BMC Res Notes, 2012. 5: p. 375.

17. Merimee, T.J., M. Grant, and J.E. Tyson, Insulin-like growth factors in amniotic fluid. J Clin Endocrinol Metab, 1984. 59(4): p. 752-5.

18. Hung M, Bounsanga J, Voss MW, Saltzman CL. Establishing minimum clinically important difference values for the Patient-Reported Outcomes Measurement Information System Physical Function, hip disability and osteoarthritis outcome score for joint reconstruction, and knee injury and osteoarthritis outcome score for joint reconstruction in orthopaedics. World J Orthop. 2018;9(3):41–49. Published 2018 Mar 18. doi:10.5312/wjo.v9.i3.41

19. Winterstein AP, McGuine TA, Carr KE, Hetzel SJ. Comparison of IKDC and SANE Outcome Measures Following Knee Injury in Active Female Patients. Sports Health. 2013;5(6):523–529. doi:10.1177/1941738113499300

20. Roos EM, Toksvig-Larsen S. Knee injury and Osteoarthritis Outcome Score (KOOS) - validation and comparison to the WOMAC in total knee replacement. Health Qual Life Outcomes. 2003;1:17. Published 2003 May 25. doi:10.1186/1477-7525-1-17