Phase 1/2 Prospective Double-blind, Placebo-controlled Randomized Clinical Trial Using Losartan to Treat Grade II and III Hamstring Strains

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IND APPLICATION:

CLINICAL STUDY PROTOCOL FOR

The Use of Losartan to Treat Grade II and III Hamstring Strains

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C. CLINICAL PROTOCOL: Summary Information

Clinical Protocol Title:

The Use of Losartan to Treat Grade II and III Hamstring Strains

Protocol number:

Version number and date:

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Phase of clinical investigation:

Phase I

IND number:

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Investigational drug(s):

Losartan Potassium

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C. Clinical Protocol

1. Introduction:

1.1 Background:

Muscle injuries stem from a variety of events, including direct trauma (i.e., muscle lacerations, contusions, or strains) and indirect causes (i.e., ischemia or neurological dysfunction)[1-15]. They account for a large number of all injuries sustained by participants in professional and recreational sports. In fact, muscle injuries constitute between 10% and 55% of all injuries sustained by athletes, depending on the type of sport[1-16]. The hamstring muscle complex is the most commonly involved[11, 17, 18] and mainly occurs in the sports requiring bursts of speed or rapid acceleration[19]. Although muscles can undergo regeneration after injury, the healing process is slow and often culminates in incomplete functional recovery[1, 2] and fibrosis. The formation of dense scar tissue can impair muscle function and lead to muscle contracture and chronic pain and diminish the individual's ability to achieve full recovery and return to prior levels of performance. Despite the considerable medical need, there has been relatively little progress in the development of therapeutic approaches to enhance healing following muscle injury.

A growing understanding of the cellular and molecular events that commonly occur during fibrosis in various tissues, including skeletal muscle, has provided a strong foundation for the development of effective therapies to prevent fibrosis and improve tissue healing. Because TGF-β1 plays such a crucial role in tissue fibrosis[20-32], particularly in skeletal muscle[33-38], it warrants attention as a key target for anti-fibrotic applications. In an effort to test various methods of antagonizing the effect of TGF-β1, we have investigated the abilities of decorin, suramin, IFN-γ, relaxin and an angiotensin II receptor blocker, to neutralize TGF-β1's effect and improve muscle healing after injury. Our preliminary animal studies have shown that blocking TGF-β1 can decrease fibrosis and improve muscle healing[33, 39-45]. Of the agents to block TGF-β1, Losartan (Merck), which is an angiotensin II receptor blocker, is particularly attractive for clinical application because it is FDA approved and has minimal side effects. Losartan has been in clinical use for over 20 years and has been associated with a reduction in fibrosis in several tissues[46-48], improved myoblast transplantation[49] and protection against sarcopenia[50].

To evaluate the effects of Losartan on muscle healing and prevention of fibrosis we plan to conduct a phase 1 randomized double blind placebo-controlled trial to compare Losartan to placebo for the treatment of grade II and III hamstring injuries in athletes or military personnel, veterans or their families that have sustained a sports-related hamstring injury. We hypothesize that subjects who receive Losartan will have decreased

formation of fibrosis and a faster return to their prior level of activity and muscle function.

1.2 Rationale:

Developed in 1990, losartan potassium is an FDA-approved drug for the treatment of hypertension, left ventricular hypertrophy and diabetic nephropathy. Losartan and EXP3174 have been referenced in 184 studies according to PubMed (United States Library of Medicine). These reports have shown losartan is a specific blocker of the type-1 angiotensin II receptor[51, 52], which regulates the expression of TGF-β1[53]. Our research shows strong expression of TGF-β1 in injured skeletal muscle, which clearly implicates TGF-β1 in the fibrotic cascade that occurs after the onset of muscle disease or trauma[54, 55]. Indeed, the use of anti-fibrotic agents that inactivate TGF-β1 reduce muscle fibrosis, improves muscle healing and leads to enhanced recovery of injured muscle[56-62]. Losartan potassium (Merck™) is an FDA approved drug whose use has been associated with a reduction in fibrosis in several tissues[46-48]. Furthermore, it has been used to improve myoblast transplantation[49] and protect against sarcopenia[50].

Based on our promising experimental data in a murine muscle injury model, the goal of this phase 1 randomized double blind placebo-controlled clinical trial is to test the effects of Losartan on muscle healing after sports, military training or combat injuries. Muscle injury caused by vigorous physical activity occurs in the US Active Duty Army at rates 3 to 4 fold higher than the civilian population[63]. Subject recruitment will include athletes or military personnel, veterans or their families that have sustained an acute sports-related hamstring injury. This is relevant to the injured military population, since advances in combat field medicine translates into high rates of survival and increased numbers of wounded warriors[64], with the majority of injuries occurring in the upper and lower extremities[65].

Recently, we performed a preliminary phase 1 case study involving two adult males with acute/recurrent hamstring muscle injury[66]. In both patients, we initiated treatment with Losartan after the acute period of necrosis/degeneration and inflammation. Both patients demonstrated normal hamstring flexibility and strength recovery by 6-9 weeks after injury. To date (> 2 years after treatment) neither individual has suffered any re-injury to their hamstring muscles, which may represent the most important feature of the study. Furthermore, both individuals tolerated the Losartan with no reported side effects and remained normotensive throughout the 30 day course of treatment. Despite the limitations of making treatment decisions based on two case reports, we are encouraged by the results of these interventions.

Based on our basic science results and preliminary clinical experience, we hypothesize that subjects who receive Losartan for a short duration along with the current standard of care treatment after sustaining an acute grade II or III

hamstring muscle injury will have less formation of fibrosis and a faster return to their prior level of activity and muscle function when compared to a placebo control group who only receives the current standard of care treatment. To test this hypothesis we propose a phase 1 randomized double blind placebo-controlled clinical trial to compare the use of Losartan (50mg/day) to placebo in 20 individuals with an acute grade II or III hamstring injury between the ages of 18-35. The main goal of this study is to determine safety and tolerability and to provide pilot data to determine the magnitude of the effect and variability in the outcome of Losartan in comparison to placebo, which will allow a more accurate prediction of sample size for a future definitive clinical trial. Subjects enrolled in this study that are randomized to the active drug arm of the study will be given 50 mg of Losartan to be taken daily by mouth. This dose was chosen as it is the prescribed starting dose for the FDA-approved uses of losartan. It is also the dose prescribed for a case study of 2 patients with a severe hamstring strain that demonstrated improved muscle structure and function[66].

In conducting this phase 1 study, we will utilize the multidisciplinary collaboration of an experienced group of scientists, surgeons and physical therapists in a unique environment at the University of Pittsburgh and the USAISR (United States Army Institute of Surgical Research) at Brooke Army Medical Center (BAMC) San Antonio, Texas. The main outcome measures will be to (a) quantify the length and volume of fibrosis in the injured hamstring muscle, based on a baseline 3 Tesla MRI and a subsequent follow-up MRI examination 6 months post-injury and (b) prospectively follow the subjects to evaluate the amount of scarring and time to return to prior activity[66]. Safety and tolerability will be assessed by monitoring adverse events, symptoms (dizziness, new fatigue, peripheral edema or urinary symptoms), hypotension, and laboratory measures of liver and renal function, hemoglobin and hematocrit counts and serum potassium and uric acid levels.

A 1999 study in the United Kingdom[67] examined the safety of losartan potassium post-marketing in over 14,000 patients. The results of this study indicate losartan potassium, like other angiotensin II receptor antagonists (ARAs), does not present any significant adverse events. The most commonly cited specific adverse events include dizziness, headache, malaise, nausea, lassitude, cough, diarrhea, dyspnea and impotence. A literature review conducted by Sica *et al*.[68] found other adverse events being decreases in hemoglobin and hematocrit counts, dysgeusia and ageusia, migraines and acute psychosis, hepatic abnormalities, angioedema, increase in serum potassium, decrease in serum uric acid, and changes in renal function. As is the case with all drugs directly affecting the renin-angiotensin system, Losartan should not be taken by anyone who is pregnant or thinks they may be pregnant. The use of these drugs can cause neonatal injury, hypotension, neonatal skull hypoplasia, anuruia, renal failure and death. To our knowledge losartan potassium has not been withdrawn from research or marketing in the United States or any other country.

2. Clinical Study Objectives:

2.1 Primary objective:

Our long term objective is to improve muscle healing by decreasing pathologic fibrosis that occurs after skeletal muscle injury limiting effective muscle regeneration. Our **overall hypothesis** is that Losartan, which is an angiotensin II receptor blocker, will improve muscle healing and function after acute hamstring injuries in athletes and military personnel.

In this **phase 1 prospective double-blind placebo-controlled randomized clinical** our objective will be to **evaluate safety and tolerability** and to **provide pilot data** to determine the magnitude of the effect and variability in the outcome of Losartan in comparison to placebo, which will allow a more accurate prediction of sample sized needed for a future definitive trial. The specific aims of the study are to:

Specific Aim #1: To determine safety and tolerability of Losartan when used for treatment of an acute grade II or III hamstring strain.

Hypothesis 1a: Use of Losartan will have an acceptable adverse event profile that is comparable to placebo. To test this hypothesis we will monitor adverse and serious adverse events throughout the duration of the study.

Hypothesis 1b: The side effect profile of Losartan will be comparable to placebo. To test this hypothesis we will monitor subject complaints, symptoms, hypotension, and laboratory values.

Specific Aim #2: To determine the effect of Losartan on recovery of hamstring muscle function and structure following an acute grade II or III hamstring strain.

Hypothesis 1.1: Subjects who receive Losartan will have a quicker return of normal hamstring muscle function, as evidenced by better flexibility and strength of the hamstring muscle when compared to the control group who receives the current standard of care treatment without Losartan.

Hypothesis 1.2: Subjects who receive Losartan will have less formation of fibrosis compared to the control group who receives the current standard of care treatment without Losartan.

Specific Aim #3: To determine the effect of Losartan on time to return to prior level of function following an acute grade II or III hamstring strain.

Hypothesis 3: Subjects who receive Losartan for a short duration along with the current standard of care treatment after sustaining an acute grade II or III

hamstring muscle injury will have a faster return to their prior level of sports activity when compared to the control group who receives the current standard of care treatment without Losartan.

Specific Aim #4: To determine the effect of losartan on recurrence of injury following an acute grade II or III hamstring strain.

Hypothesis 4: Subjects who receive Losartan will have a lower incidence of recurrent injury after return to their prior level of sports and military activity compared to the control group who receives the current standard of care treatment without Losartan.

3. Study Design:

We will conduct a **phase 1 prospective double-blind placebo-controlled randomized clinical trial** to evaluate the safety and tolerability and to provide preliminary data related to the the magnitude and variability of the effect of Losartan, an angiotensin II receptor blocker, for the treatment of acute grade II and III hamstring strains in athletes and military personnel. Safety will be evaluated in terms of the adverse event profile and tolerability (Specific Aim 1). The effect of Losartan will be evaluated in terms of function and structure of the muscle (Specific Aim 2), time to return to sports (Specific Aim 3), and recurrence of injury (Specific Aim 4). Subjects will be followed over the course of 12 months after injury.

3.1 Study design schematic

After signed informed consent is provided, subjects will undergo screening that will include collection of baseline demographics, medical history, activity level, baseline vital signs (blood pressure and heart rate), blood tests including a chemistry panel with electrolytes and complete blood cell count (CBC), pregnancy test for women, physical examination, measurements of pain and hamstring flexibility and strength and a 3.0 Tesla MRI. After screening to confirm eligibility, subjects will be randomized to receive Losartan or placebo for four weeks. All subjects will undergo standard of care rehabilitation. During the period of active intervention, subjects will be followed weekly. Blood work will be repeated 2, 4, and 6 weeks after randomization. Pregnancy tests for females will also be repeated 2, 4, and 6 weeks after randomization. Throughout the remainder of the year-long study period, subjects will undergo follow-up testing at 6 and 12 weeks, 4, 6, 9 and 12 months. The study procedures over time are summarized in the table (**Table 1**) below.

Table 1 – Summary of Study Procedures

	Pre-treat	Day 0	1w	2w	3w	4w	6w	12w	4m	6m	9m	12m
Informed Consent	X											
Demographic Questionnaire	X											
Activity Level	X		X	X	X	X	X	X	X	X	X	X
Medical History	X											
Vital Signs (standard and orthostatic blood pressure and heart rate)	X		X	X	X	X	X			X		
Physical Examination	X											
Blood Work	X			X		X	X					
Numerical Pain Rating Scale	X		X	X	X	X	X	X		X		
Hamstring flexibility	X		X	X	X	X	X			X		
Isometric hamstring strength testing	X		X	X	X	X	X			X		
Isokinetic hamstring strength testing							X			X		
3.0 Tesla MRI	X									X		
Randomization		X										
Day of 1st Dose		X										
Medication Log Review			X	X	X	X						
Telephone follow-up								X	X		X	X
	Legend: pre-treat = pre-treatment; w = week; m = months											

Figure 1 – Flow Chart for Study Design

3.2 Allocation to treatment

Subjects will be randomized in a 1:1 ratio to either treatment with Losartan or placebo. Both groups will otherwise undergo the same standard of care rehabilitation for treatment of an acute hamstring injury. The randomization list will be generated using a random number generator. An equal number of subjects will be allocated to each group in random block sizes of two and four. Group assignments will be placed in sequentially numbered opaque envelopes, which will be opened by the Project Coordinator at each site once the participant has agreed to participate in the study and baseline testing has been completed to confirm subject eligibility. Thus the treatment allocation will be concealed from the Project Coordinator and others responsible for recruitment. To minimize bias throughout the study, both the subject and individuals responsible outcome measure collection/analysis will remain blinded to the group assignment.

3.3 Breaking the blind

Unblinding of subjects will be limited to reduce the potential for bias. Unblinding of a subject will only be considered in cases in which there is a significant safety or health concern to the subject and only after consultation with the treating physician, the study Medical Director (Dr. Vonda Wright MD) and Principal Investigator (Dr. Johnny Huard). Only those individuals who need to know the subject's group assignment to ensure the health and safety of the subject will be unblinded. To the extent possible, all of the other study staff will remain blinded to the subject's group assignment.

A statement will be placed in the electronic medical record of the subject stating the subject is enrolled in a double-blinded placebo controlled trial using losartan potassium as an investigational drug. In the event of a medical emergency, a health care provider can contact the study Medical Director or other designated study investigator to obtain information about the assigned study drug during the dosing period.

If unblinding becomes necessary, the following information will be recorded:

- Randomization identification number of the unblinded subject;
- Reason for unblinding;
- Study staff member that is responsible for unblinding the subject and
- A list of person(s) who are not blinded.

4. Subject Selection:

Subjects will be drawn from the population of patients with an acute grade II or grade III hamstring injury that present to the UPMC Center for Sports Medicine or Athletic Training Rooms at the University of Pittsburgh or to the Brooke Army Medical Center. A total of 20 subjects will be enrolled in the study. A subject will consider to be enrolled in the study if he/she has provided informed consent for study participation.

4.1 Subject inclusion criteria

Subjects will be eligible for participation in this study if they are between 18 and 35 years of age and if they have had a grade II or III hamstring injury in the previous 7 days of enrolling in the study. Only those individuals who are regular participants (greater than 100 hours per year) in Level I (football, basketball or soccer) or Level II (racquet sports, skiing, manual labour occupations/heavy physical work) activities will be eligible for participation in this study. Individuals from BAMC that suffer a grade II or III hamstring injury in the previous 7 days of enrolling in the study that are involved in military training that at least is equivalent to heavy physical work in the civilian subjects will be eligible to participate in the study. To be eligible to participate in the study, subjects will need to agree to take the study medications as prescribed.

4.2 Subject exclusion criteria

Subjects will be excluded if they: 1) have had a previous hamstring injury on the same side or chronic symptoms; 2) have an injury that requires surgical intervention (e.g. avulsion with associated bony involvement, grade III complete tears); 3) have concurrent lower back symptoms; 4) are pregnant or breast feeding; 5) are a smoker; 6) have contraindications to Losartan therapy (e.g. known hypersensitivity to losartan, hepatic impairment); 7) currently use angiotensin I converting enzyme inhibitor (ACEI) / angiotensin II receptor blocker (ARB); 8) have hypertension (blood pressure greater than or equal to 140 mm Hg systolic pressure or greater than or equal to 90 mm Hg diastolic pressure); 9) have hypotension (blood pressure less than or equal to 90 mm Hg systolic pressure or less than or equal to 60 mm disastolic pressure); 10) have orthostatic hypotension defined as a drop in systolic pressure greater than or equal to 20 mm Hg or a drop in diastolic blood pressure greater than or equal to 10 mm Hg or reports of lightheadedness or dizziness would be considered abnormal upon standing or 11) have diabetes mellitus, cardio-vascular, renal or hepatic co-morbidities.

Additionally, because participation in this study requires undergoing MRI, subjects will be excluded if they: 1) had prior surgery for an aneurysm; 2) have a cardiac pacemaker; 3) have metal fragments in the eyes, brain or

spinal cord from shrapnel, metal work or welding; 4) have surgical implants, such as an ear implant or neurostimulator; 5) have a history of claustrophobia or 6) have a history of not tolerating previous MRI scans without medication.

For the military personnel, individuals recruited to participate in this study should not have a scheduled deployment within 6 months following initial injury to ensure adequate follow up.

5. Study Drug(s):

As described in section 1.2, losartan potassium is an FDA-approved drug for the treatment of hypertension, left ventricular hypertrophy and diabetic nephropathy. It is prescribed for oral intake at doses from 25-100 mg per day with a usual starting dose of 50 mg per day which is what will be used for this study. Losartan potassium was developed by Merck under the proprietary name Cozaar® and is now generically available.

Prior to starting the study medication, a study physician will review the effects of losartan, its side-effects and how the medication should be taken with all subjects. At this time, the physician will answer any questions the subject has regarding the study medication. Subjects will be evaluated weekly while they are taking the medication. Blood work will be repeated 2, 4 and 6 weeks after randomization to identify any clinically relevant abnormalities.

Following randomization, subjects will begin a closed label course of 50mg of Losartan daily for 4 weeks or placebo for the same duration of time. Losartan will be initiated within 7 days of injury after initial tests are performed and any abnormalities are ruled out. No initial dosage adjustment is necessary. The drug will be self-administered by the subject with or without food and will be ingested once a day. With the dose that will be used in this study, no tapering is necessary at the end of the intervention phase or if the subject needs to be withdrawn from the study.

5.1 Study drug compliance/adherence

During the weekly patient encounters, a member of the research team will answer any questions the subject might have about the medication, will assess and promote medication adherence and monitor any side effects that the patient might be experiencing. The subject will be instructed to bring the medication so that pill counting can be performed as a measure to assess adherence. Also, at the time of randomization and at each weekly encounter, the subject will be given a medication log to complete. The medication log will allow the patient to record the day and time of day the study medication and any concomitant medications were taken during that time period.

The medication log will become part of the research records, but will not be placed in the medical record. A statement will be placed in the electronic medical record of the subject stating the subject is enrolled in a double-blinded placebo controlled trial using losartan potassium as aninvestigational drug. In the event of a medical emergency, a health care provider can contact the study Medical Director or designated study investigator to obtain information about the assigned study drug during the dosing period.

5.1.1 Withdrawal of subjects due to non-compliance/ adherence

Because we are interested in evaluating efficacy, we will use a per-protocol approach, as opposed to an intention-to-treat approach for the conduct of this study. As such, subjects that are non-compliant with the medication regimen (defined as taking less than 80% of the medication doses) will be withdrawn from the study and additional subjects will be recruited to replace subjects that have been withdrawn due to non-compliance. Subjects that are withdrawn for non-compliance will be instructed to terminate administration of the study medication without tapering down the dose and to return unused medication to the investigators.

5.2 Study drug supplies

5.2.1 Formulation and packaging

Losartan will be ordered through HC central pharmacy and billed to the study. The UPMC Investigational Drug Service will provide the Losartan or placebo. The placebo will be formulated to have the same appearance as the Losartan. Study medications will be provided to the subjects free of charge.

5.2.2 Preparing and dispensing

IDS will blind the IP and matching placebo. The IDS will dispense per physician order.

5.2.3 Drug administration

The drug is self-administered orally with or without food and will be ingested once a day as per protocol

5.4 Study drug storage and accountability

The IDS will store IP under controlled room temperature which is monitored daily and will maintain accountability records. Upon completion of the study or expiration of the medication, destruction will occur per IDS policy #103.

5.5 Concomitant Medications

At the time of randomization and at each weekly encounter with the study staff, the subject will be given a medication log to complete. The medication log will allow the patient to record the times/days the study medication and any concomitant medications taken during that time period. During the review of the log, the study staff will ask and document questions about concomitant medications and the reasons for taking them. Adverse events related to concomitant medications will be recorded.

5.5.2 Rescue Medication

6. Research Study Procedures:

6.1 Recruitment Procedures

This prospective randomized phase 1 trial is a joint effort of the UPMC Center for Sports Medicine/University of Pittsburgh and the Department of Orthopaedics and Rehabilitation at BAMC.

Subjects recruited through the UPMC Center for Sports Medicine will be informed of and referred to the study by their athletic trainer and/or physician. With permission of the subject, a research assistant will then contact them and initiate the study process. Alternatively, potential subjects will sign a Health Insurance Portability and Accountability Act (HIPAA) authorization for sharing of contact information, which will be forwarded to the study coordinator, who will in turn contact the subject to provide additional information concerning the study.

Subjects recruited through the Department of Orthopaedics and Rehabilitation at Brooke Army Medical Center (BAMC) in San Antonio, Texas that meet the inclusion criteria (grade II/III hamstring strains due to sports or duty related injury) will be referred to the Research Coordinator in the BAMC Department of Orthopaedics and Rehabilitation for possible recruitment. All testing and evaluation of recruited subjects will be conducted at the Center for the Intrepid in the Department of Orthopaedics and Rehabilitation at BAMC,

MRI's will be performed at the BAMC Department of Orthopaedics and Rehabilitation MRI Clinic.

6.2 Informed Consent Process

The Research Coordinator will explain the details of the study, including the purpose of the study, procedures that will be utilized and the associated risks and benefits. Potential subjects will be assured that the decision of whether or not to participate in this study will not have any effect on their care. All questions that the subject may have prior to signing the informed consent form will be answered to the subject's satisfaction by one of the investigators. If the subject is willing to participate in the study, the subject will sign an informed consent form that has been approved by the University of Pittsburgh Institutional Review Board (for subjects recruited at the UPMC Center for Sports Medicine) or by the BAMC-IRB (for subjects recruited at Brooke Army Medical Center) prior to participating in any research-related activities.

6.3 Screening procedures

6.3.1 Baseline Demographics and Activity Level

Demographic information that will be recorded include age, sex, weight, height, body mass index (BMI), education level, sports and work activity level, smoking history and marital status.

Sports Activity - We will use the sports activity rating scale developed by Marx et al.[69] to measure the subject's level of sports activity in the year prior to injury. This scale consists of 4 questions (running, cutting, decelerating and pivoting) that are rated in terms of the frequency with which the subject performed the activity (less than once per month, once per month, once per week, 2 or 3 times per week or 4 or more times per week) in the prior year. Test retest reliability for this scale over a two-week period was high (ICC=0.97) and it had moderately strong correlations with the Cincinnati and Lyhsolm scores[69].

Work Activity - Work activity will be measured with the Occupational Rating Scale of the Cincinnati Knee Rating System[70]. This is a patient-reported measure of work activity in which individuals will report the duration or frequency of sitting, standing and walking, walking on uneven ground, squatting, climbing, lifting and carrying as well as the amount of weight that they carry while at work. Each item is scored on a scale that ranges either from 0 to 5 or 0 to 10 such that higher scores indicate less sedentary activity and more physically demanding work activity. The item scores are summed and multiplied by 2 to create a score that ranges from 0 to 100. Cincinnati Occupational Rating Scale has demonstrated high test-retest reliability in both patients (ICC=.97) and uninjured subjects (ICC=.87)[70].

6.3.2 Baseline Assessment of Injury

At the time of study enrollment, the subject will be interviewed about the mechanism of injury (movement that was made during injury), presence or absence previous symptoms at the injury site, presence or absence of ecchymosis and the ability to walk and run. An examination will include inspection to confirm the presence or absence of ecchymosis. Palpation of the posterior thigh will be performed with the subject in prone and the knee extended[71]. The point of greatest pain will be marked and the distance to the ischial tuberosity will be measured[71] and the presence or absence of a palpable defect will be recorded. Pain during resisted knee flexion will be graded on an 11 point (0 to 10) numerical pain rating scale.

Subjects will be eligible for participation in the study if the physical examination reveals: 1) localized tenderness with or without a palpable defect; 2) pain and weakness with resisted knee flexion with the individual in the prone position and 3) pain and a limitation (in comparison to the contralateral leg) in the popliteal angle with the hip at 90 degrees of flexion. Presence of a grade II/III hamstring strain will be confirmed on the baseline MRI that is performed prior to randomization. MRI evidence of a grade II/III hamstring strain includes edema with architectural disruption indicating a partial tear (grade II) or edema with complete rupture of the muscle or tendon (grade III). Excellent intra- and inter-rater reliability has been reported for this MRI grading system of acute hamstring injuries[72].

6.3.3 Baseline Vital Signs, Medical History and Physical Examination

Blood pressure will measured according to the American Heart Association Guidelines[73]. The subject will be comfortably seated, with his legs uncrossed. The cuff will be placed on the upper arm that will be at the level of the midpoint of the sternum and all measurements will be performed with an aneroid sphygmomanometer. For the initial visit, measurements will be performed in both upper extremities. The midline of the bladder of the cuff will be placed over the brachial artery. The stethoscope will be placed over the brachial artery but in the antecubital fossa. The cuff will then be inflated until the brachial artery is occluded (usually until 30 mm Hg above the point at which the radial pulse disappears). The cuff is then deflated and the first and last sounds should are the systolic and diastolic pressures.

We will also measure orthostatic blood pressure using procedures described by the Centers for Disease Control. After lying in the supine position for 5 minutes, heart rate and blood pressure will be measured using the procedure described in the paragraph above. Following this, the subject will stand and blood pressure and heart rate will be measured again after 1 and 3 minutes. A drop in systolic pressure greater than or equal to 20 mm Hg or a drop in diastolic blood pressure greater than or equal to 10 mm Hg or reports of lightheadedness or dizziness would be considered abnormal. Individuals with orthostatic hypotension will be excluded from further participation in the study.

A medical history, review of systems and physical examination will be performed for all subjects during screening. The medical history will include a review of the chief complaint, history of present injury, past medical history, allergies, medications, and social and family history.

6.3.4 Blood Draw Procedures

For the blood draws, the subject will be comfortably seated and identification of the subject will be confirmed. Blood will be drawn from the median cubital vein. Initially a tourniquet will be placed on the arm, proximal to the location where the blood will be drawn. The skin around this area will be wiped with an alcohol swab (or iodine prep). A needle will be inserted, bevel facing upwards, into the median cubital vein. Once the needle is in the vein, a vacutainer is pushed onto the needle holder and this will be repeated until all blood samples required are completed. Once the needle is removed, clean cotton wool will be pressed onto the wound. The tubes will be labeled immediately. Sufficient blood will be drawn to permit a chemistry panel with electrolytes and CBC. Hemoglobin and hematocrit counts, serum potassium and uric acid, liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase), renal function (blood urea nitrogen [BUN] and serum creatinine), creatine phosphokinase (CPK) to assess muscle injury, and pregnancy in women (human chorionic gonadotropin (hCG)) will be assessed.

6.3 Study drug procedures

Following randomization, subjects will begin a closed label course of 50mg of losartan daily for 4 weeks or placebo for the same duration of time. Losartan will be initiated within 7 days of injury after initial testing has been performed and any abnormalities are ruled out. No initial dosage adjustment is necessary. The drug is self-administered with or without food and will be ingested once a day.

Prior to starting the study medication, a study physician will review the effects of Losartan, its side-effects and how the medication should be taken with all subjects. At this time, the physician will answer any questions the subject has regarding the study medication.

The UPMC Investigational Drug Service will provide the Losartan or placebo. The placebo will be formulated to have the same appearance as the Losartan. Study medications will be provided to the subjects free of charge. Subjects will be evaluated weekly while they are taking the medication. Blood work will be repeated 2, 4, and 6 weeks after randomization.

During the weekly patient encounters, a member of the research team will answer any questions the subject might have about the medication, will assess and promote medication adherence and monitor any side effects that the patient

might be experiencing. Also, the subject will be instructed to bring the medication to each visit so that pill counting can be performed as a measure to assess adherence. At each weekly encounter with the study staff, the subject will be given a medication log to complete. The medication log will allow the patient to record the times/days the study medication and any concomitant medications taken during that time period.

6.4 Follow-up procedures *(Incorporate only if follow-up procedures will be performed)*

6.4.1 Standard of Care Rehabilitation

All subjects will undergo a standardized post-injury rehabilitation program, supervised by a physical therapist or athletic trainer. The rehabilitation protocol will be similar to that described by Sherry[74]. The rehabilitation will be divided into 2 phases. The initial phase will focus on static stretching, isometric hamstring exercises and active range of motion. This will include 10 minutes of low-intensity cycling on a stationary ergometer with no resistance, static supine and long-sit hamstring stretching exercises (30 second hold, 4 repetitions each), submaximal isometric hamstring contractions at 20° and 90° of knee flexion (10 second hold, 10 repetitions at each angle) and prone knee flexion without resistance (30 repetitions). Ice will be applied at the end of the exercise session for 20 minutes. The phase 1 exercise program will be performed daily.

Subjects will progress to phase 2 when they can walk with the same stride length and stance time on the injured and un-injured legs and can do a high knee march in place without pain. In phase 2, the focus will be on dynamic stretching and concentric and eccentric hamstring strengthening. Subjects will ride a stationary ergometer for 15 minutes with a moderate level of resistance, during which the subject shoulder perceive a moderate level of exertion (12-13 on Borg scale of perceived exertion). This will be followed with standing, supine and long-sit dynamic hamstring stretching exercises. Prone leg curls will be performed to strengthen the hamstring muscles using a daily adjustable progressive resistance exercise (DAPRE) method as describe by Knight[75]. In this method, subjects will perform 4 sets of resisted hamstring exercises against variable levels of resistance and repetitions as described in the table below (**Table 2**). The number of repetitions completed in the 3rd set will be utilized to determine the amount of weight used for the 4th set and the number of repetitions performed in the 4th set will determine the working weight for the next exercise session **(Table 3).** The "foot catch exercise"[76] will be performed in the standing position. In this exercise, subjects will simulate the swing phase of walking or running as they quickly contract the quadriceps and catch or stop the lower leg before reaching terminal knee extension using an eccentric hamstring contraction. During phase 2, subjects will be permitted to run and jog without high-speed maneuvers as tolerated by pain. Ice will be applied at the end of the exercise session for 20

minutes. Except for the prone leg curls, which will be performed every other day, the phase 2 exercise program will be performed daily.

Table 2 – Sets, Weight and Number of Repetitions for DAPRE Program

Set	Portion of Working Weight Used	Number of Repetitions
1	50%	10
2	75%	6
3	100%	As many as possible
4	Adjusted based on number of repetitions in 3rd set	As many as possible

Subjects will be permitted to begin high-speed running and agility drills when: 1) they have no palpable tenderness in the posterior thigh, 2) hamstring flexibility is within 3° of the un-injured leg and 3) resisted hamstring strength is 85% of the un-injured leg. Subjects will be permitted to return to full practice or military activity when they can tolerate high-speed running and agility drills for 3 consecutive days with no exacerbation of symptoms and when they can perform a 40-yard sprint with no posterior thigh tightness or twinges. Upon return to sports, subjects will be encouraged to continue with the rehabilitation program 3 days a week for at least 2 months after returning to sports.

To ensure consistency in the rehabilitation program for all subject, we will create educational training materials (video and written instructions) demonstrating the correct performance and progression of the rehabilitation program. This information will be distributed to all physical therapists and athletic trainers responsible for rehabilitation of the subjects participating in this study. Compliance with the rehabilitation program will be assessed by reviewing the physical therapy or athletic training rehabilitation records. Additionally, subjects will independently track exercise compliance by recording the rehabilitation tasks that are performed on a daily basis in an exercise log. This log will be reviewed at each follow-up visit to ensure compliance with the rehabilitation program. To control for co-interventions, applications of heat and ultrasound and use of

Table 3 – Change in Working Weight for Fourth Set and Next Session Based Upon Number of Repetitions Performed

Number of Repetitions Performed During Third Set	Fourth Set	Next Session
0-2	Decrease 5-10 lbs.	No change
3-4	Decrease 0-5 lbs.	Increase 5 lbs.
5-7	No change	Increase 5-10 lbs.
8-12	Increase 5-10 lbs.	Increase 10-15 lbs.
> 13	Increase 10-15 lbs.	Increase 15-20 lbs.

neuromuscular electrical will be avoided.

6.4.2 Outcome Measures

The outcomes measures will be collected at the time of study enrolment and at the 1, 2, 3, 4 and 6 weeks and 3, 4, 6, 9 and 12 month follow-up as described in **Table 1** above, using procedures described in a Manual of Operations and Procedures. We will use a **trained** Clinical Research Assistant at each site, who has a background as a physical therapist or athletic trainer, to collect the measures of hamstring flexibility and strength and to conduct all follow-up telephone calls. Prior to data collection, co-I Irrgang (who has greater than 30 years of experience with collection of clinical outcomes data) will train the Clinical Research Assistants.

A musculoskeletal radiologist will read and interpret the MRI scans to determine the presence and extent of fibrosis and muscle atrophy. A bilateral magnetic resonance imaging (MRI) of both thighs will be conducted. The amount of fibrosis atrophy will be determined from the MRI using MIPAV software (Medical Imaging Processing, Analysis and Visualization, NIH, Bethesda, MD).

All individuals responsible for collecting outcome measures will be blinded to the subject's group assignment throughout the duration of the study.

6.4.2.1 Return to Previous Level of Sports Activity and Recurrence of Injury (Specific Aims 3 and 4)

The primary outcome for return to sports will be the number of days from injury to full, unrestricted return to practice or military training. The secondary outcome for level of sports activity will be the Marx Activity Scale Score. At 4 and 6 weeks, the Marx Sports Activity Scale will be modified to measure sports activity in the last week and at 4, 6 and 12 months the scale will be modified to measure sports activity in the past month.

Recurrence of injury will be monitored over the course of 12 months after study enrolment and randomization. A subject will be considered to have a recurrent injury if there was a specific mechanism of injury that caused a return of posterior thigh pain, tenderness to palpation along the muscle-tendon unit, pain with resisted knee flexion and the inability to participate in sports activities. Recurrent injuries will be monitored weekly during the course of rehabilitation and at each follow-up study visit (1, 2, 3, 4 & 6 weeks, 6 months) and follow-up phone call (3, 4, 9 and 12 months). After return to sports participation, any subject experiencing a recurrent injury will be instructed to contact the Project Coordinator and a return visit will be scheduled to confirm the presence and severity of re-injury.

6.4.2.2 Function and Structure of Hamstring Muscle (Specific Aim 2)

Pain – An 11 point (0 to 10) numeric pain rating scale will be used to rate pain at rest and walking at the time of study enrolment and at 1, 2, 3, 4 and 6 weeks after enrolment. Pain with jogging and sprinting will be rated starting when these activities are initiated through the remainder of the in-person and telephone

follow-up visits. The numerical pain rating scale is anchored so that 0 corresponds with no pain and 10 corresponds with worst imaginable pain. The psychometric properties of the numeric pain rating scale compare favorably to other pain rating scales and are the preferred method of rating pain by young and old individuals in a study of experimentally induced pain[77].

Measurement of Hamstring Flexibility – Hamstring length will be assessed by measuring the popiteal angle as described by ten Berge et al[78]. This will be performed by placing the ipsilateral hip at 90° of flexion and passively extending the knee to the end range of motion. To eliminate the possibility of initial muscle stiffness, the knee will be moved to end range extension 3 times before taking the measurement. A standard goniometer will be used to measure the angle of knee flexion. The proximal arm of the goniometer will be aligned with the lateral midline of the thigh on a line between the lateral epicondyle and the center of the greater trochanter of the femur and the distal arm will be positioned parallel to the lateral midline of the leg on a line between the lateral malleolus and lateral epicondyle of the femur. To account for variability between subjects, hamstring flexibility will be expressed as the difference in popliteal angle between the hamstring-injured and contra-lateral normal leg. Using this methodology, ten Berge et al[78] demonstrated adequate levels of intra- and inter-observer reliability (ICC=.82 and .72 respectively) in healthy subjects between 20 and 29 years of age.

Isometric Hamstring Strength – A Lafayette hand-held dynamometer (Lafayette Instrument Company, Lafayette IN) will be used to measure isometric hamstring strength at the time of study enrolment and at 1, 2, 3, 4, and 6 weeks after enrolment. For these measurements, the subject will be positioned in the prone position. The dynamometer will be placed on the distal leg approximately 2 cm proximal to the level of the lateral malleolus. The distance from the point of application of the hand-held dynamometer to the lateral joint line will be recorded in centimeters. The amount of force generated by the subject will be resisted by the examiner (i.e. a “make test” will be performed). The test will be performed at 30° and 90° of knee flexion, first on the un-injured leg than on the injured leg. To calculate torque, the force will be multiplied times the distance from the point of application of the hand-held dynamometer to the lateral joint line of the knee. Isometric strength (Newton-meters) of the involved hamstrings will be expressed as an absolute number and as a percentage of strength of the un-injured leg. Trudelle-Jackson et al[79] demonstrated high levels of intra- (ICC ranging from .97-.98) and inter-day (ICC ranging from .85-.87) reliability when using a hand-held dynamometer to test hamstring strength in healthy subjects. Additionally, measurements with the hand-held dynamometer were strongly related to concurrent measurements of isometric strength on an isokinetic dynamometer (r from 0.83 - 0.85).

Isokinetic Hamstring Strength – Isokinetic hamstring strength at 60 and 180° per second will be measured on the Biodex isokinetic dynamometer (Biodex Medical Systems, Shirley NY) 4 and 6 weeks after study enrolment as well as at

the 6 month follow-up visit. Prior to performing the test, the subject will ride a stationary ergometer for 5 minutes at a moderate intensity level and will perform static hamstring stretching exercises (30 second hold, 4 repetitions) for each leg. The subject will be seated on the isokinetic dynamometer so that the axis of rotation of the dynamometer is aligned with the lateral epicondyle of the femur. The length of the input arm will be adjusted so that the pad is positioned just above the lateral malleolus. The dynamometer settings will be recorded to ensure consistency during subsequent testing sessions. To familiarize the subject with the testing protocol prior to performing the maximal effort tests, the subject will perform 2 sub-maximal contractions and 1 maximal contraction at 60°/sec and 6 sub-maximal and 3 maximal contractions at 180°/sec. For the test that is recorded, the subject will perform 3 maximal concentric quadriceps and hamstring contractions at 60°/sec and 20 maximal concentric quadriceps and hamstring contractions at 180°/sec. Verbal encouragement will be provided during testing to ensure maximal effort.

The primary outcome variable of interest will be the involved to un-involved ratio of hamstring peak torque at 60°/sec. Secondary isokinetic variables that will be evaluated include the involved to un-involved ratio of total hamstring work at 60°, the involved hamstring to quadriceps ratio in peak torque at 60°/sec, peak torque and total work at 180°/sec and the ratio of work in the last 3 repetitions to work in the first 3 repetitions at 180°/sec. Pincivero et al[80] demonstrated high levels of test re-test reliability (ICCs ranging from) over a 7 day period in normal healthy subjects using the Biodex System 2 Isokinetic Dynamometer and a testing protocol similar to that which we will be using.

6.4.2.3 Evaluation by 3.0 Tesla MRI (Specific Aim 2)

Magnetic Resonance Imaging Procedures - Participants in this study will undergo 3T MRI at the UPMC Magnetic Resonance Research Center or the Magnetic Resonance Facility, BAMC, Wilford Hall, San Antonio before treatment is initiated and 6 months after injury. Rigorous procedures to ensure high quality MRI that are capable of reliably detecting small changes in muscle structure over time will be undertaken. These include standardized procedures to position and minimize motion of the subject, establishment of criteria to assess the quality of the magnetic resonance images and daily, weekly, monthly and annual quality control procedures for maintenance of the magnetic resonance scanner.

Images of both thighs will be acquired from the ischial tuberosity to just below the knee so that the entire length and volume of the hamstrings will be imaged. A torso coil will be used. Total scan time is approximately 30 minutes. Following a conventional 3-plane localizer, axial, sagittal and coronal T1-weighted non-fat saturated, axial and coronal short inversion recovery time (STIR) and axial and sagittal T2 fat saturated (FS) images will be obtained. The details for each of the sequences are specified in the Table 4 below.

Table 4 – Summary of MRI Parameters			
Sequence	Slice/Gap Thickness (mm)	Matrix	Field of View
Axial T1[a]	8/1	512 x 180	40 cm/phase 75
Sagittal T1[a]	5/2	194 x 384	38 cm/phase 84
Coronal T1[a]	5/2	192 x 512	40 cm/phase 75
Axial STIR[b]	8/1	256 x 256	40 cm/phase 75
Axial T2 FS[c]	8/1	203 x 384	40 cm/phase 75
Sagittal T2 FS[c]	5/2	194 x 384	38 cm/phase 84
Coronal STIR[b]	5/2	256 x 256	40 cm/phase 75
[a] T1 weighted non-fat saturated. [b] Short inversion recovery time. [c] T2 weighted fat saturated.			

The primary MRI outcome measures for analysis will be the maximal cranio-caudal length and volume of the injury/scar tissue. To calculate volume of injury/scar tissue, the area of injury/scar tissue will be determined on each slice. This area will be multiplied by the slice thickness and summed across all slices in which the injury/scar tissue is visible. Additionally, total volume of the involved compared to the non-involved hamstring muscles will be determined as a measure of muscle atrophy.

6.5 Schedule of activities (Study Table)

Table 1 – Summary of Study Procedures

	Pre-treat	Day 0	1w	2w	3w	4w	6w	12w	4m	6m	9m	12m
Informed Consent	X											
Demographic Questionnaire	X											
Activity Level	X		X	X	X	X	X	X	X	X	X	X
Medical History	X											
Vital Signs (standard and orthostatic blood pressure and heart rate)	X		X	X	X	X	X			X		
Physical Examination	X											

Blood Work	X			X		X	X					
Numerical Pain Rating Scale	X		X	X	X	X	X	X		X		
Hamstring flexibility	X		X	X	X	X	X			X		
Isometric hamstring strength testing	X		X	X	X	X	X			X		
Isokinetic hamstring strength testing							X			X		
3.0 Tesla MRI	X									X		
Randomization		X										
Day of 1st Dose		X										
Medication Log Review			X	X	X	X						
Telephone follow-up								X	X		X	X
	Legend: pre-treat = pre-treatment; w = week; m = months											

7. Safety and Effectiveness Assessments:

7.2 Safety assessments

Vital signs (including blood presure and heart rate) will be monitored daily prior to each rehabilitation session. Blood work, including a chemistry panel with electrolytes and complete blood cell count (CBC) and pregnancy tests for women will be performed prior to randomization and at 2, 4, and 6 weeks after randomization. The blood work will be used to assess hemoglobin and hematocrit counts, serum potassium and uric acid, liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase), renal function (blood urea nitrogen [BUN] and serum creatinine), creatine phosphokinase (CPK) to assess muscle injury, and pregnancy in women (human chorionic gonadotropin (hCG)).

The profile of adverse and serious adverse events will also be monitored to assess safety throughout the duration of the subject's participation in the study.

7.2 Effectiveness assessments

Our overall hypothesis is that Losartan, which is an angiotensin II receptor blocker, will improve muscle healing and function after acute hamstring injuries in athletes and military personnel. The effectiveness assessments for each specific aims of the study are to:

Specific Aim #2: Recovery of Hamstring Muscle Function and Structure.

Recovery of muscle function will be assessed in terms of hamstring flexibility and strength. Hamstring length will be assessed by measuring the popiteal angle as described by ten Berge et al[78]. To account for variability between subjects, hamstring flexibility will be expressed as the difference in popliteal angle between the hamstring-injured and contra-lateral normal leg. To assess hamstring strength, isometric testing with the knee flexed to 30˚ and 90˚ will be performed before treatment and at 1, 2, 3, 4 and 6 weeks and 6 months after enrolment and isokinetic testing at 60˚ and 180˚/sec will be performed 6 weeks and 6 months after enrolment. The detailed procedures for measuring hamstring flexibility and strength are described in section 6.4.2.2.

3 Tesla MRI will be performed at enrolment and at 6 months follow-up to assess hamstring structure in terms of the length and volume of the injury and scarring. Additionally total volume of the injured and contra-lateral hamstrings will be calculated to determine the degree of atrophy. The detailed procedures for use of 3T MRI to assess hamstring structure are described in section 6.4.2.3.

Specific Aim #3: Time to Return to Prior Level of Function

The primary outcome for return to sports will be the number of days from injury to full, unrestricted return to practice. The secondary outcome for return to sports activity will be the Marx Activity Scale Score at 4 and 6 weeks and 3, 4, 6 and 12 months. At 4 and 6 weeks, the Marx Sports Activity Scale will be modified to measure sports activity in the last week and at 4, 6 and 12 months the scale will be modified to measure sports activity in the past month.

Specific Aim #4: Recurrence of Injury

Recurrence of injury will be monitored over the course of 12 months after study enrolment and randomization. A subject will be considered to have a recurrent injury if there was a specific mechanism of injury that caused a return of posterior thigh pain, tenderness to palpation along the muscle-tendon unit, pain with resisted knee flexion and the inability to participate in sports activities. Recurrent injuries will be monitored weekly during the course of rehabilitation and at each follow-up study visit (1, 2, 3, 4 & 6 weeks, 6 months) and follow-up phone call (3, 4, 9 and 12 months).

8. Adverse Event Reporting:

8.1 Adverse event definitions

Adverse event means any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related.

Adverse reaction means any adverse event caused by a drug.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction".

- *Reasonable possibility.* For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.

Life-threatening, suspected adverse reaction. A suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include a suspected adverse reaction that had it occurred in a more severe form, might have caused death.

Serious, suspected adverse reaction. A suspected adverse reaction is considered "serious" if, in the view of the Investigator (i.e., the study site principal investigator) or Sponsor, it results in any of the following outcomes: death, a life-threatening adverse reaction, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

- Important drug-related medical events that may not result in death, be life-threatening, or require hospitalization may be considered "serious" when, based upon appropriate medical judgment, they may jeopardize the research subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

  Unexpected, suspected adverse reaction. A suspected adverse reaction is considered "unexpected" if it is not listed in the general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

8.2 Recording/Reporting requirements

8.2.1 Eliciting adverse event information

Throughout the duration of the study subjects will be monitored for adverse events. At each study visit (weeks 1, 2, 3, 4, 6 and 6 months) and follow-up phone call (3, 4, 9 and 12 months), subjects will be asked to provide any information about possible adverse events that may have occurred since the last visit. Subjects will be asked to provide information about any reaction to the study medication, any new health-related symptoms they may be experiencing, and any new events or injury that may impact their compliance with the study protocol. If during the follow-up phone call the subject reports a new or ongoing adverse event, a follow-up clinic visit will be scheduled.

8.2.2 Recording requirements

All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study drug(s) will be recorded in the subjects' case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a *serious adverse event*) and; 2) an assessment of the casual relationship between the adverse event and the study drug(s).

Adverse events or abnormal test findings felt to be associated with the study drug(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the Sponsor-Investigator.

8.2.2.1 Abnormal test findings

An abnormal test finding will be classified as an *adverse event* if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy.
    - Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.

- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study.

- The test finding is considered an adverse event by the Sponsor-Investigator of the IND application.

8.2.2.2 Causality and severity assessment

The Sponsor-Investigator of the IND application will promptly review documented adverse events and abnormal test findings to determine: 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s); and 3) if the adverse event meets the criteria for a *serious adverse event*.

If the Sponsor-Investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s)", the adverse event will be classified as *associated with the use of the study drug(s)* for reporting purposes. If the Sponsor-Investigator's final determination of causality is "unknown but not related to the study drug(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

8.3 Reporting of adverse reactions

8.3.1 Reporting of adverse reactions to the FDA

8.3.1.1 Written IND Safety Reports

The Sponsor-Investigator will submit a written IND Safety Report (i.e., completed FDA Form 3500 A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a *serious and unexpected, suspected adverse reaction*. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the

Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Sponsor-Investigator will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., "Follow-up IND Safety Report").

If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor-Investigator will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

8.3.1.2 Telephoned IND Safety Reports – Fatal or life-threatening suspected adverse reactions

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor-Investigator will notify the responsible review division of the FDA by telephone or facsimile transmission of any *unexpected, fatal or life-threatening suspected adverse reaction.*

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

8.3.2 Reporting adverse events to the responsible IRB

In accordance with applicable policies of the University of Pittsburgh Institutional Review Board (IRB), the Sponsor-Investigator will report, to the IRB, any observed or volunteered adverse event that is determined to be: 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the sponsor-investigator's receipt of the respective information. Adverse events which are: 1) *associated with the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3) *unexpected* will be reported to the IRB within 24 hours of the Sponsor-Investigator's receipt of the respective information.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information becomes available. If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the Sponsor-Investigator will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

8.4 Withdrawal of subjects due to adverse events

Vital signs, including blood pressure and heart rate will be monitored daily prior to each rehabilitation session. Individuals with symptomatic hypotension (defined as symptoms consistent with hypotension including dizziness, lightheadedness etc. with systolic or diastolic blood pressure less than 90 and 60 mm Hg respectively) will be discontinued from the study. Withdrawal of a subject due to symptomatic hypotension will be reported as an adverse event.

Blood work, including a chemistry panel with electrolytes and complete blood cell count (CBC) will be performed prior to randomization and at 2, 4, and 6 weeks after randomization. The blood work will be used to assess hemoglobin and hematocrit counts, serum potassium and uric acid levels, liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase), renal function (blood urea nitrogen [BUN] and serum creatinine), creatine phosphokinase (CPK) to assess muscle injury, and pregnancy in women (human chorionic gonadotropin (hCG)). Additional blood work will be ordered if subjects report new fatigue, peripheral edema or urinary symptoms. Follow-up blood work will be performed 4-6 weeks (±3 days) after discontinuing medication to ensure that lab values have returned to normal ranges after discontinuation of the study medication. Abnormal lab values resulting in subject withdrawal from the study will be reported as an adverse event.
Human chorionic gonadotropin (hCG) be determined to identify pregnant women will be assessed prior to randomization and at 2, 4 and 6 weeks after randomization. Women that are pregnant prior to randomization will be discontinued from the study, but will not be reported as an adverse event. Human chorionic gonadotropic will also be determined for females enrolled in the study who expect that they For females enrolled in the study that have become pregnant, the study medication will be stopped immediately and the subject will be withdrawn from the study. Pregnancy necessitating withdrawal from the study will be reported as an adverse event.

Adverse events or abnormal test findings felt to be associated with the study drug(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the Sponsor-Investigator.

The severity of an adverse event will be determined by the study medical director based on the criteria stated in section 8.1. Additionally, the medical director will assign the event a grade of mild, moderate, or severe. If it becomes medically necessary to withdraw the subject from the study, the study medication will be discontinued. The subject will be followed clinically until the adverse event or abnormal test findings felt to be associated with the study drug resolves or stabilizes at a level acceptable to the Sponsor-Investigator. This follow-up will include assessment of vital signs, physical examination and assessment of blood work. Follow-up blood work will be performed 4-6 weeks (±3 days) after discontinuing medication to ensure that lab values have returned to normal ranges after discontinuation of the study medication. The subject will be treated for any medication related problems as clinically appropriate. Information from this appointment will be documented in the subject's case file. The patient will be terminated from the study, and will not complete any further follow-up appointments for the study. Subjects that are terminated from participation in the study due to an adverse event will be replaced by recruitment of another subject.

9. Statistical Methods/Data Analysis:

9.1 Study endpoints

9.1.1 Primary endpoint(s)

Specific Aim #1: To determine safety and tolerability of Losartan when used for treatment of an acute grade II or III hamstring strain.

To assess safety, vital signs will be monitored daily and we will also monitor the frequency of abnormal lab values as well as adverse and serious adverse events during period of active intervention as well as throughout the subjects' participation in the study. To assess the side effect profile of Losartan in this population, we will monitor the frequency of subject complaints, symptoms (dizziness, new fatigue, peripheral edema, urinary symptoms) and laboratory measures of liver and renal function, haemoglobin and haematocrit counts and serum potassium and uric acid levels. The frequency of abnormal lab values, adverse and serious adverse events and side effects will be monitored separately for the Losartan and placebo control groups.

Specific Aim #2: Recovery of Hamstring Muscle Function and Structure

The primary endpoints to assess hamstring muscle function are the injured to contra-lateral leg differences in the popliteal angle at 6 weeks and 6 months and the injured to contra-lateral ratio of peak isokinetic hamstring torque at 6 weeks and 6 months.

The primary endpoint to assess hamstring structure will be the length and volume of fibrosis within the hamstrings at 6 months as evidence on 3 Tesla MRI.

Specific Aim #3: Time to Return to Prior Level of Function

The primary outcome for return to sports will be the number of days from injury to full, unrestricted return to practice.

Specific Aim #4: Recurrence of Injury

The primary outcome for recurrence of injury will be the incidence of recurrent injury. A recurrent injury will be considered to have occurred if there was a specific mechanism of injury that caused a return of posterior thigh pain, tenderness to palpation along the muscle-tendon unit, pain with resisted knee flexion and the inability to participate in sports activities. Recurrent injuries will be monitored weekly during the course of rehabilitation and at each follow-up study visit (1, 2, 3, 4 & 6 weeks, 6 months) and follow-up phone call (3, 4, 9 and 12 months).

9.1.2 Secondary endpoints

Specific Aim #2: Recovery of Hamstring Muscle Function and Structure

Secondary outcomes for recover of hamstring muscle function include isometric strength (Newton-meters) of the involved hamstrings expressed as a percentage of strength of the un-injured at 1, 2, 3, 4 and 6 weeks and 6 months. Secondary isokinetic variables that will be explored include the injured to contra-lateral leg ratio of total hamstring work at 60°/sec, involved hamstring to quadriceps ratio in peak torque at 60°/sec, injured to contra-lateral leg ratio of peak torque and total work at 180°/sec and the ratio of work in the last 3 repetitions to work in the first 3 repetitions at 180°/sec at 6 weeks and 6 months.

The secondary outcome for structure of the hamstring will include atrophy of the hamstrings as determined by the ratio of the volume of the injured and contra-lateral leg 6 months after injury.

Specific Aim #3: Time to Return to Prior Level of Function

The secondary outcome for return to sports activity will be the Marx Activity Scale Score at 4 and 6 weeks and 3, 4, 6 and 12 months. At 4 and 6 weeks, the Marx Sports Activity Scale will be modified to measure sports activity in the last week

and at 4, 6 and 12 months the scale will be modified to measure sports activity in the past month.

9.2 Sample size determination

With an approved sample size of 20 patients (10 in each group), we will not have enough statistical power to detect statistically significant differences in clinical measures between the Losartan and placebo groups. Therefore the objective of this phase 1 trial is to observe trends by group and estimate effect sizes that can be used to inform power calculations in future clinical trials.

9.3 Definition(s): Analysis population(s)

All analyses for treatment group comparisons will use the original treatment assignment as randomized for each participant (intent-to-treat) regardless of the subject's actual compliance with taking the study medications. Secondary analyses will consider per protocol analysis, in which compliance with the study medication regimen will be defined as taking greater than 80% of the doses**.**

9.4 Effectiveness analysis

The distribution of baseline characteristics and important prognostic factors (age, sex, body mass index, pre-injury activity level, etc) will be summarized overall, and by treatment (versus control) group to assess the effectiveness of the randomized allocation. Continuous variables will be summarized with means and standard deviations, and medians and inter-quartile ranges. Categorical data will be summarized using frequencies and percentages. We will then graphically explore the distributions of the primary outcome variables (for normality or skewedness and potential outliers) using q-q plots and histograms before conducting any tests to compare the two groups. When appropriate, transformations will be applied to outcome measures that are not normally distributed.

We anticipate minimal loss to follow-up (<5% of patients) at the 6 month assessment and only 10-15% loss to follow-up at 12 months post-injury. The primary outcome measures (safety and tolerance, recovery of muscle structure and function, return to prior level of function) will be evaluated between 6 weeks and 6 months; therefore the only outcome that could appreciably be affected by lost to follow-up is recurrence of injury within 12 months. We will compare baseline characteristics between participants and drop-outs, obtain reasons for

study drop (to investigate the possible missing data mechanism), and will also conduct a sensitivity analysis (assigning poor scores and good scores to those persons with missing information differentially by treatment assignment) to evaluate the impact on our study results.

For outcome variables that are measured repeatedly, we will use mixed models to adjust for correlation among the observations from the same person as well as a clustering effect for each site. In these models, subject characteristics such as age, sex, and treatment group will be fixed effect predictors, and subject and site identification will be included as random effects. Depending on the type of outcome variable of interest, we will employ linear mixed models or mixed effect logistic regression. Though we do not anticipate statistically significant differences between the Losartan and placebo groups due to the small sample size, these analyses will guide us in developing future clinical trials.

For time to recurrence, which will be the focus of Specific Aim 4, we expect 50%-60% of subjects in control group will have recurrence within one year. Although the subjects will report whether they had recurrence or not at intermittent time points (3, 4, 6, 9 and 12 months after treatment), we will have subjects report the actual date of recurrence. We will perform logistic regression (recurred/not recurred), and if we obtain reliable information about recurrence date and we have enough number of recurrences, we will consider Cox regression model to investigate the effect of treatment on time to recurrence, though we do not anticipate having enough power to achieve statistically significant results with either logistic or Cox modeling.

Any deviations from the previously described statistical plan will be described and justified in a protocol amendment and/or in the final report submitted to the IND application.

9.5 Safety analysis

To assess safety, vital signs will be monitored daily and we will also monitor the frequency abnormal lab values as well as adverse and serious adverse events during period of active intervention as well as throughout the subjects' participation in the study. To assess the side effect profile of Losartan in this population, we will monitor the frequency of subject complaints, symptoms (dizziness, new fatigue, peripheral edema, urinary symptoms) and laboratory measures of liver and renal function, hemoglobin and hematocrit counts and serum potassium and uric acid levels. The frequency of abnormal lab values, adverse and serious adverse events and side effects will be monitored separately for the Losartan and placebo control groups. Differences between groups will be assessed using Fisher exact tests.

Any deviations from the previously described statistical plan will be described and justified in a protocol amendment and/or in the final report submitted to the IND application.

9.6 Interim analysis

No interim analysis is planned.

9.7 Data and Safety Monitoring Committee

9.7.1 Type of Monitoring Body

The data and safety monitoring activities for this phase 1 clinical trial will be under the oversight of a Data and Safety Monitoring Committee (DSMC) consisting of the principal investigators from each site, the study statistician, and other appointed investigators. The goals of this DSMC are to ensure the safety of the study participants, integrity of the data and the validity of the study results. The data and safety monitoring activities will focus on safety of the study participants; performance of the investigative team with respect to participant recruitment, retention and follow-up, adherence to the protocol, and quality of the data; and to evaluate if the study should continue.

9.7.2 Membership of the Data and Safety Monitoring Committee

The DSMC will be comprised of the principal investigators from each site, study statistician, and other appointed investigators. A DSMC member will be obligated to bring an issue of any potential conflict of interest to the attention of the full DSMC for open discussion and resolution.

Dr. Irrgang **w**ill act as the committee chairperson. He will be responsible for overseeing the meetings and working with the project coordinator at the coordinating site to develop the agenda and summarize the meeting.

Dr. Doperak **w**ill serve as the Safety Officer for this study. She will be the contact person for serious adverse event reporting and study safety issues. Dr. Doperak will review of events that might impact the safety of participants and/or the safety of the data for this study.

9.7.3 Responsibilities of the Data and Safety Monitoring Committee

The DSMC will be responsible for assuring that subjects are not exposed to unnecessary or unreasonable risks and that the investigator conducts the clinical trial according to the highest scientific and ethical standards.

Initially, in the planning and development phase of this study, the DSMC will review and make recommendations regarding the research protocol, informed

consent documents, quality control plans, and plans for data and safety monitoring prior to the study initiation.

Once the study is implemented, ongoing responsibilities of the DSMC will be to:

- Evaluate the progress of trial, including periodic assessments of data quality and timeliness; participant recruitment, accrual and retention; participant risk versus benefit ratio; performance of the trial site; and other factors that can affect study outcome;
- Consider the impact of factors external to the study when new information, such as scientific or surgical developments become available and may affect the safety of participants, their willingness to participate in the study, or the conduct of the trial;
- Review study performance, make recommendations and assist in the resolution of problems reported by the investigators;
- Protect the safety and privacy of study participants;
- Report on the safety and scientific progress of the trial;
- Make recommendations concerning continuation, termination, or other modifications of the trial based on the observed adverse effects of the treatment under study;
- Ensure data integrity;
- Ensure confidentiality of the trial data and the results of monitoring;
- Assist by commenting on any problems with study conduct, enrollment, sample size, statistics, and/or data collection; and
- Review and evaluate requests for protocol modifications after the trial begins to evaluate whether the study should continue as approved or undergo a protocol modification.

9.7.4 Data and Safety Monitoring Committee Processes and Procedures

9.7.4.1 Meetings of the Data and Safety Monitoring Committee

The DSMC will meet at designated intervals to review accumulated data on safety. Meetings will be convened as conference calls or in person, although it is recommended that the initial meeting and meetings to discuss interim analyses be face-to-face. An emergency meeting of the DSMC will be called at any time by the investigators should questions of patient safety arise. Notes and minutes of each meeting of the DSMC will be recorded and stored in the study regulatory files.

Each meeting will include a recommendation to continue or to terminate the study made by DSMC majority or unanimous vote. Should the DSMC decide to issue a termination recommendation, the full vote of the DSMC will be required. In the event of a split vote, the majority vote will rule and a minority report should

be appended. In the event of a 50-50 split vote, the DSMC Chairperson will provide the tie breaker.

9.7.4.2 Interim Analyses

No interim analyses are planned. However, any data reviewed by the DSMC will be limited to as small a group as possible. Data files to be used for review will have undergone established editing procedures to the extent possible.

9.7.4.3 Termination of Study

A recommendation to terminate the study may be made by the DSMC at any time.

9.7.4.4 Confidentiality

All materials, discussions, and proceedings of the DSMC will be completely confidential. Members and other participants in DSMC meetings will be expected to maintain confidentiality at all times.

10. Quality Control and Quality Assurance:

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically (i.e., at a minimum of annually) by qualified staff of the Education and Compliance Office – Human Subject Research, Research Conduct and Compliance Office, University of Pittsburgh.

The Sponsor-Investigator and the University of Pittsburgh and UPMC will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

Data integrity and credibility of the study are dependent on strict adherence to the protocol, obtaining complete follow-up information on all participants and establishing and adhering to quality control measures to maintain high standards for data quality. The quality control procedures that have been developed and implemented for this study include following established procedures for the conduct of research and patient care at the University of Pittsburgh and University of Pittsburgh Medical Center (UPMC) in Pittsburgh, PA and at the Brooke Army Medical Center in San Antonio, Tx. Additionally, we will closely monitor data collection and form completion at both research sites.

All study staff will receive initial and ongoing training related to study procedures to maximize adherence to the protocol and to achieve high quality data. Additionally, all study investigators and staff and the University of Pittsburgh will complete training as required, provided by the Collaborative Institutional Training

Institute (CITI) (www.citi.pitt.edu). The following training modules will be completed:

- Research Integrity
- Human Subjects Research
- HIPAA and
- Good Clinical Practice

All study investigators and staff at Brooke Army Medical Center will complete training as required, provided by the Collaborative Institutional Training Institute (CITI) (https://www.citiprogram.org/default.asp). The following training modules will be completed:

- Avoiding Group Harms: U.S. Research Perspectives
- Unanticipated Problems and Reporting Requirements in Biomedical Research
- Students in Research
- History and Ethical Principles
- Basic Institutional Review Board (IRB) Regulations and Review Process
- Informed Consent Social and Behavioral Research for BiomedicalResearchers
- Records-Based Research
- Genetic Research in Human Populations
- Research With Protected Populations – Vulnerable Subjects: An Overview
- Vulnerable Subjects - Research Involving Prisoners
- Vulnerable Subjects - Research Involving Children
- Vulnerable Subjects - Research Involving Pregnant
- Women, Human Fetuses, and Neonates
- International Studies
- FDA-Regulated Research
- Human Subjects Research at the VA
- Research and HIPAA Privacy Protections

This research project is conducted by faculty and staff at the University of Pittsburgh and staff at the UPMC in facilities operated by the UPMC and University of Pittsburgh as well as by faculty and staff at Brooke Army Hospital. Investigators and study staff at the University of Pittsburgh and UPMC must adhere to the policies and procedures of the University of Pittsburgh, which can be found at http://www.bc.pitt.edu/policies/, and the UPMC, which can be found at http://policymanuals.infonet.upmc.com/. Additionally we strictly adhere to all University of Pittsburgh IRB policies and procedures, which can be found at http://www.irb.pitt.edu/pandp/default.aspx. Faculty and staff at Brooke army Hospital must adhere to the policies and procedures of the Brooke Army Medical Center, which can be found at http://fhpr.dhhq.health.mil/dmrn.aspx.

11. Data Handling and Record-Keeping:

11.1 Data recording/Case Report Forms

A Case Report Form (CRF, see Appendix 1) will be completed for each subject enrolled into the clinical study. The Sponsor-Investigator will review, approve and sign/date each completed CRF; the Sponsor-Investigator's signature serving as attestation of the Sponsor-Investigator's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

This study will utilize paper CRFs for study visits. These forms will include patient reported outcomes (demographics, Marx Activity Scale, and Cincinnati Occupational Rating Scale); vital signs, including blood pressure and heart rate; numerical pain rating scale; strength measurements and hamstring flexibility; medication logs; telephone follow-up logs, and any narrative notes written by the study staff. Study data will be recorded directly on these CRFs. The CRFs will be signed and dated by investigators and will serve as source documentation for this study.

The paper-based CRF will be created using word processing software but saved as .pdf files to prevent changes. The finalized forms will be marked as such by the generation of a unique form/screen identification number. Revisions to finalized forms and screens will not be encouraged once the study is underway. Reasons for revisions will be recorded and stored with the finalized versions of the forms and screens and all subsequent revisions. Dates of revisions will be recorded on the data collection forms as they occur. Forms with variable names and codes for the responses for each variable will be used to define all variables included in the study data set.

The following guidelines will be used to complete the study CRF:

- Forms will be completed legibly using black or blue ink;

- No participant identifiers will be recorded on patient questionnaires, with the exception of the Identification and Contact Information Form;
- Header information, including the participant identification number and date will be completed on all forms;
- If historical dates are required, the month/day/year should be provided. If the individual cannot recall the specific date of the month, the date should be set to the 15th of the month in which the event occurred;
- Abbreviations will not be used unless defined on the form;
- Responses should only be written in the spaces provided. Extraneous writing on the form should be avoided;
- To correct an error, a single line will be drawn through the error and the change will be dated and initialed by the individual that made the correction;
- When answering patient-reported forms, the Clinical Research Assistant will not help the subject answer the questions. Rather subjects will be instructed to answer all questions to the best of their ability. If the participant states they do not perform an activity and there is no opt out answer, the subject will be instructed to answer the question as if they were to do the activity;
- All questions should be answered. No questions should be left blank;
- If answers are illegible, every effort will be made to read the form. If possible, the participant will be contacted to answer the question or clarify the response;
- If data are not available to complete the form, the item for which the data is not available should be circled and the reason for the unavailability of data should be documented on the form near the appropriate field using the following conventions:
    - If the evaluation was not done, write “Not Done” or “ND” and provide a reason;
    - If the evaluation was done but the data is not available at the time the forms are being completed, indicate “Not Available” or “NAV” and document the reason, initial and date;
    - If an evaluation does not apply to the situation, indicate “Not Applicable” or “NA” and document the reason and initial and date;
- If an entire page is not complete, draw a diagonal line through the form & indicate “Not Done”, “Not Available”, or “Not Applicable” as appropriate along with the reason.

To ensure patient confidentiality, patient identifiers will not be written on the CRF at any time. Any list linking patient names to study identification numbers will be stored in locked file cabinets and /or on secure servers behind firewalls. All

paper based study documents will be stored in secure locked file cabinets. Any computerized records will be stored on secure servers behind firewalls.

11.2 Record maintenance and retention

The Sponsor-Investigator will maintain records in accordance with Good Clinical Practice guidelines; to include:

- FDA correspondence related to the IND and clinical protocol, including copies of submitted Safety Reports and Annual Reports
- IRB correspondence (including approval notifications) related to the clinical protocol; including copies of adverse event reports and annual or interim reports
- Current and past versions of the IRB-approved clinical protocol and corresponding IRB-approved consent form(s) and, if applicable, subject recruitment advertisements
- Signed FDA Form 1572 Statements of Investigator (i.e., for the Sponsor-Investigator)
- Financial disclosure information (i.e., for the Sponsor-Investigator and for sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the research study data])
- Curriculum vitae (i.e., for the Sponsor-Investigator)
- Certificates of required training; e.g., human subject protections, Good Clinical Practice, etc. (i.e., for the Sponsor-Investigator and for all sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data])
- Listing of printed names/signatures. (i.e., for the Sponsor-Investigator and for all sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data])
- Normal value(s)/range(s) for medical/laboratory/technical procedures or tests included in the clinical protocol
- Laboratory certification information
- Instructions for on-site preparation and handling of the investigational drug(s), study treatment(s), and other study-related materials (i.e., if not addressed in the clinical protocol)
- Responsibility delegation log
- Signed informed consent forms

- Completed Case Report Forms; signed and dated by Sponsor-Investigator
- Source Documents or certified copies of Source Documents
- Monitoring visit reports
- Copies of Sponsor-Investigator correspondence (including notifications of safety information) to sub-investigators
- Subject screening and enrollment logs
- Subject identification code list
- Investigational drug accountability records, including documentation of drug disposal.
- Final clinical study report

- Decoding procedures for blinded trials
- Master randomization list

- Signed FDA Form 1572 Statements of Investigator (i.e. for Investigators responsible for the conduct of the clinical research study at external study sites).
- Financial disclosure information (i.e., for Investigators responsible for the conduct of the clinical research study at external study sites; also for all study site sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data])
- Curriculum vitae (i.e., for Investigators responsible for the conduct of the clinical research study at external study sites)
- Certificates of required training; e.g., human subject protections, Good Clinical Practice, etc. (i.e., for Investigators responsible for the conduct of the clinical research study at external study sites; also for all study site sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data])
- Listing of printed names/signatures (i.e., for Investigators responsible for the conduct of the clinical research study at external study sites; also for all study site sub-investigators who will be involved in the administration of the study drugs and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data])
- Copies of initial and continuing IRB approval notifications (i.e., for each of the external study sites)
- Normal value(s)/range(s) for medical/laboratory/technical procedures or tests included in the clinical protocol (i.e., for each of the external study sites)
- Laboratory certification information (i.e., for each of the external study sites)
- Monitoring visit reports (i.e., for all external study sites)
- Copies of Sponsor-Investigator correspondence (including notifications of safety information) to Investigators responsible for the conduct of the clinical research study at external study sites

The Sponsor-Investigator will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

12. Ethics:

12.1 Institutional Review Board (IRB) approval

The Sponsor-Investigator will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the Sponsor-Investigator will promptly notify the University of Pittsburgh IRB of the deviation.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).

In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of the Sponsor-Investigator's decision to modify the previously accepted clinical protocol:

- For a Phase 1 clinical study: The Sponsor-Investigator will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to the Phase 1 clinical protocol that significantly affects the safety of the subjects. For changes that do not affect critical safety assessments, the revisions to the clinical protocol will be addressed in the Annual Report to the IND.

- For Phase 2 and 3 clinical studies: The Sponsor-Investigator will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to a Phase 2 or Phase 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of Phase 2 and 3 clinical protocol changes requiring the submission of a Protocol Amendment include:

- Any increase in drug dosage or duration of exposure of individual subjects to the investigational drug beyond that described in the current protocol, or any significant increase in the number of subjects under study.

- Any significant change in the design of the protocol (such as the addition or deletion of a control group).

- The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor the safety of the investigational drug.

12.2 Ethical and scientific conduct of the clinical research study

The clinical research study will be conducted in accordance with the current IRB-approved clinical protocol; ICH GCP Guidelines adopted by the FDA; and relevant policies, requirements, and regulations of the University of Pittsburgh IRB, University of Pittsburgh and UPMC, Commonwealth of Pennsylvania, and applicable federal agencies.

12.3 Subject informed consent

The Sponsor-Investigator will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The Sponsor-Investigator, or a sub-investigator(s) designated by the Sponsor-Investigator, will obtain the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The date and time that the subject, or the subject's authorized representative, signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The Sponsor-Investigator will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.

The Sponsor-Investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the

Sponsor-Investigator will obtain the informed consent of enrolled subjects for continued participation in the clinical study

13. Study Discontinuation Criteria:

13.1 Discontinuation of individual research subjects (refer also to sections 5.1.1 and 8.4)

Because we are interested in evaluating efficacy, we will use a per-protocol approach, as opposed to an intention-to-treat approach for the conduct of this study. As such, subjects that are non-compliant with the medication regimen (defined as taking less than 80% of the medication doses) will be withdrawn from the study and additional subjects will be recruited to replace subjects that have been withdrawn due to non-compliance. Subjects that are withdrawn for non-compliance will be instructed to terminate administration of the study medication without tapering down the dose and to return unused medication to the investigators.

As specified in section 8.4 subjects with symptomatic hypotension or abnormal lab values will be withdrawn from study participation. Additionally, females that become pregnant while taking the study medication will immediately stop the medication and will be withdrawn from the study. These withdrawn subjects will be followed clinically based on standards of care until the adverse event or abnormal test findings felt to be associated with the study drug resolve or stabilize at a level satisfactory to the Sponsor-Investigator, however no further research data will be collected once the subject has been withdrawn.

Subjects that are withdrawn due to non-compliance with the study medication, symptomatic hypotension, abnormal lab values or pregnancy will be reported as an adverse event. Withdrawn subjects will be replaced by recruiting another subject to take the place of the withdrawn subject.

13.2 Sponsor-Investigator discontinuation of the clinical research study

Adverse events will be continuously monitored by the Data and Safety Monitoring Committee. If the number or severity adverse events are greater than the expected adverse events associated with Losartan, the DSMC will make the recommendation for termination or modification of study.

Any modifications in the study protocol will be submitted for review and approval by the IRB before the modification is implemented. Modifications in the study protocol will be communicated to all study sites in writing. Subjects will be notified of modifications in the study protocol and if appropriate will be re-consented for their continued participation in the modified study.

If the DSMC recommends termination of the study due to safety concerns, the IRB and all study sites will be notified immediately by the study principal investigator, followed by notification in writing. Enrolled subjects will be notified of the study termination by the site principal investigator by phone, and also in writing. If subjects are taking study medication at the time of study termination, they will be instructed to discontinue the study medication as clinically appropriate.

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