University of Kansas Medical Center
RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS
TEMPLATE WITH GUIDANCE

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Study Title: An open label trial of cryoneurolysis for improvements in pain, activities of daily living and quality of life in patients with ankle osteoarthritis.

I. Purpose, Background and Rationale

A. Aim and Hypotheses

This will be an open-label trial to describe the effects of cryoneurolysis with iovera on symptom relief in patients with painful Kellgren-Lawrence (KL) grade 2–4 ankle osteoarthritis (OA). The Foot and Ankle Outcome Score (FAOS) subscales will be used to assess outcomes at 6, 12 and 24 weeks after treatment.

The aim of this study is to assess clinically significant long-term symptomatic relief with cryoneurolysis in people with unilateral ankle osteoarthritis (OA).

Depending on the location of pain, we will treat either 1) the Superficial Fibular Nerve (SFN), Sural Nerve (SN) and Saphenous Nerve or 2) the deep fibular nerve with cryoneurolysis using the iovera device. The primary study endpoint, clinically significant improvement in pain 12 weeks after each treatment, will be assessed using the FAOS-Pain subscale.

The secondary outcomes will be improvement in quality of life (FAOS-QoL), activities of daily living (FAOS-ADL) and Numerical Rating Scale (NRS) for pain.

The tertiary outcome will be improvement in physical performance measures (40m fast-paced walking test, Standing Balance Test).

We hypothesize that people with painful ankle OA will experience significant improvement in pain, activities of daily living, quality of life and physical performance for at least 12 weeks following treatment. Based on research done using iovera in patients with knee osteoarthritis, we anticipate that it will have a greater magnitude and duration of analgesic effect along with significantly fewer adverse events compared with the rates reported for standard of care therapy (i.e. corticosteroid injection).[1,2]

Specific Aim 1: To determine the extent of pain relief after cryoneurolysis of nerves innervating the ankle joint in patients with ankle osteoarthritis.

Primary Hypothesis 1: Cryoneurolysis of nerves innervating the ankle joint in patients with ankle OA results in reduction in ankle pain (FAOS-Pain) detectable at the following post-treatment time points:

a) 6 weeks

b) 12 weeks (primary outcome)

c) 24 weeks

Specific Aim 2: To determine the extent of improvement in activities of daily living (ADL) and quality of life (QOL) after cryoneurolysis of nerves innervating the ankle joint in patients with ankle osteoarthritis.

Hypothesis 2a: Cryoneurolysis of nerves innervating the ankle joint in patients with ankle OA results in improved ADL (FAOS-ADL) detectable at the following post-treatment time points:

a) 6 weeks

b) 12 weeks

c) 24-weeks

Hypothesis 2b: Cryoneurolysis of nerves innervating the ankle joint in patients with ankle OA results in improved QOL (FAOS-QOL) detectable at the following post-treatment time points:

a) 6 weeks

b) 12 weeks

c) 24-weeks

Specific Aim 3: To determine the extent of improvement in physical performance measures (40m fast-paced walking test and Standing Balance Test) after cryoneurolysis of nerves innervating the ankle joint in patients with ankle osteoarthritis.

Hypothesis 3a: Each cryoneurolysis treatment results in improved physical performance detectable at the following post-treatment time points:

a) 6 weeks

b) 12 weeks

c) 24-weeks

B. Background and Significance

Approximately 15% of the world's adult population is affected by joint pain and disability resulting from osteoarthritis (OA), and approximately 1% have OA of the ankle. There has been substantial clinical and basic science research related to hip and knee OA. However, research related to ankle OA is sparse.[3] Although the hip and knee joints are most commonly affected by primary OA, at the ankle, OA is most commonly post-traumatic. Traumatic ankle injuries that may result in OA include fractures of the malleoli, tibial plafond, talus, isolated osteochondral damage of the talar dome, and ankle ligament injuries.

OA of the ankle (tibiotalar) joint progresses from early damage to diffuse and severe degeneration and deformity of the joint. The leading causes include trauma, ischemia, abnormal ossification, and genetic predisposition. Although trauma is probably the most common cause of injury and degeneration of the ankle (70% of reported cases), repetitive microtrauma could also be a contributing factor. Despite the high prevalence of ankle trauma and related chondral lesions, the rate of symptomatic and disabling OA is lower than that of the hip and knee, due to the different biologic and biomechanical characteristic of the cartilage and subchondral bone at the ankle[4].

Pharmacologic and surgical therapeutic options for ankle osteoarthritis are limited. Treatments have traditionally consisted of temporizing measures such as physical therapy and topical, oral and intra-articular pharmaceutical therapies until operative treatment with either arthrodesis

or arthroplasty is required. Cryoneurolysis is a specialized technique for providing long-term pain relief in interventional pain management settings[5]. Treated nerves are temporarily blocked from transmitting pain signals, followed by functional recovery after a time period.

Using cryoneurolysis in patients with ankle OA could potentially provide immediate symptomatic relief with a greater duration of analgesia and a lower rate of side effects than other non-operative treatments. These potential benefits could reduce the need for both clinic and injection visits when compared to other treatment modalities, thereby reducing patient and provider burden and health care expenditures.

The iovera° device is 510(k)-cleared (K133453 and K161835) and is used to destroy tissue during surgical procedures by forming a precisely controlled, sub-dermal cold zone. It can also be used to produce lesions in peripheral nervous tissue by forming a precise cold zone of -20 to -88.5 degrees Celsius to temporarily disrupt peripheral nerve function, ultimately blocking pain.[1] It is also indicated for the relief of pain and symptoms associated with osteoarthritis of the knee for up to 90 days. The iovera° system is not indicated for treatment of central nervous system tissue.

The iovera° device uses liquid nitrous oxide (N_2O) that is contained within the handpiece. This pressurized liquid travels from the hand-piece to the closed-end needles of the smart tip, where it undergoes a phase change, forming a precise cold zone. The gaseous nitrous oxide is expelled out of the hand-piece, leaving nothing behind in the body. This treatment causes a temporary nerve block based on a process called Wallerian degeneration or 2nd -degree nerve injury. This degeneration affects the axon and myelin sheath while leaving the surrounding nerve components intact such as the endoneurium, perineurium or epineurium Figure 1. When sensory nerves are treated, their ability to convey sensory signals, such as pain, is immediately interrupted.

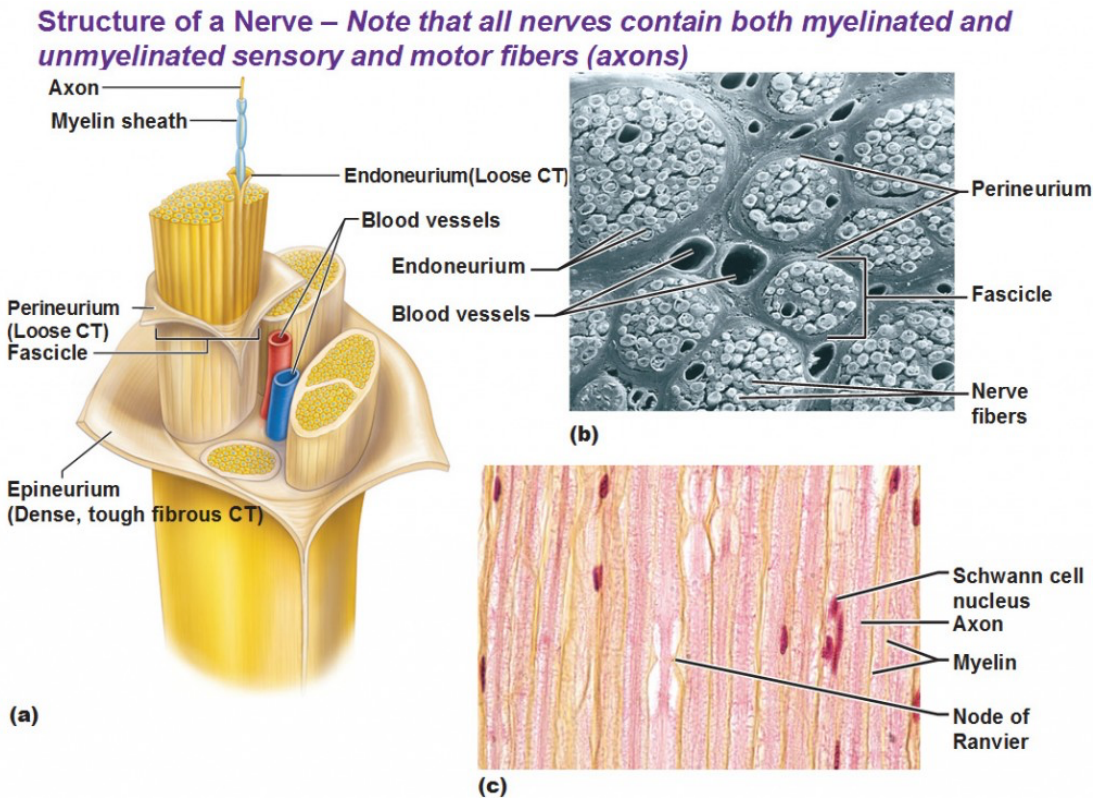

Figure 1

Post-treatment, the axon regenerates until it re-innervates to the sensory receptor for predictable restoration of nerve function. The effect on the nerve is temporary. The duration of the pain relief depends on the individual and the location of the nerve being treated. In general, nerves regenerate at a rate of approximately 1.0-1.5mm per day.[6] Therefore, we hypothesize a duration of relief of ankle pain of approximately 90 days.

Preliminary Data: A recent randomized, double-blind, sham-controlled, multicenter trial (17 sites) with a 6-month follow-up was conducted in 180 patients with mild-to-moderate knee OA. This study demonstrated that a single treatment of the infrapatellar branches of the saphenous nerve with iovera° resulted in a reduction in the pain and symptoms for at least 90 days.[1] Compared to the sham group, patients who received iovera° treatment had a statistically significant greater change from baseline in the WOMAC pain subscale score at Day 30 (p=0.0004), Day 60 (p=0.0176), and Day 90 (p=0.0061) post-treatment. Patients deemed WOMAC pain responders at Day 120 continued to experience a statistically significant treatment effect at Day 150. Most expected side effects were mild in severity and resolved within 30 days. The incidence of device- or procedure-related adverse events were similar in the two treatment groups with no occurrence of serious or unanticipated adverse device effects (ADE).

This clinical trial will be the first to systematically evaluate the symptomatic and functional benefits of cryoneurolysis for patients suffering from painful ankle OA. Patients are more likely to seek medical care when they are experiencing symptoms, have reduced activity and/or functional limitation. Therefore, finding that an affordable, safe treatment could alleviate symptoms and improve activities of daily living while avoiding the side effects of pharmacological and surgical therapies, holds promise to improve quality of life and reduce health care expenditures.

iovera° Device and Treatment Procedure

The iovera° device consists of a reusable, portable hand-piece, along with a single-patient use sterile smart tip, or cryoprobe, and disposable nitrous oxide (N_2O) cartridges. The smart tip contains embedded software that manages procedure parameters and provides physician feedback throughout all stages of device preparation, treatment and post-treatment via communication with the hand-piece. The hand-piece is battery powered and is stored and recharged via the charging dock.

The cryogen is provided in a nitrous oxide cylinder attached to a custom filter, known as the cartridge. To remove contaminants that may be present in the cylinder, a custom filter is added to the cylinder to filter the liquid nitrous oxide before it enters the hand-piece. This ensures optimal performance of the device.

There are 2 types of smart tips (including their respective variants) that we will be utilizing with this study, depending on the depth of the nerves being treated. The shorter smart tip comes in 2 variants - composed of either three 6.9 mm or three 8.9 mm, 27-gauge needles. It has an attached skin warmer to prevent damage to the underlying skin from sub-zero temperatures. The longer smart tip also comes in 2 variants - composed of a 22-gauge single needle measuring 55 mm or a 20G single needle measuring 90 mm and it will be used to treat nerves not accessible by the shorter smart tip. The iovera° device produces the desired effect through the initiation of a cooling cycle that lasts for 60 seconds. Each cooling cycle is initiated by fully inserting the smart tip into the selected procedure site and activating the cryogen flow. As the cryogen gas travels through the length of the needle, an ice ball develops around the needle causing the surrounding tissue to be frozen. Operation instructions and further details on the device are in the *User Guide* provided by Myoscience.

C. Rationale

The cryoneurolysis mechanism of action is well-understood and established in prior studies.[5] Results of a recent study demonstrate that cryoneurolysis can alleviate the pain and symptoms of knee OA for up to 90 days[1]. Pharmacologic and surgical options for ankle OA are limited. Using cryoneurolysis in patients with ankle OA could potentially provide immediate symptomatic relief with a greater duration of analgesia and a lower rate of side effects. These potential benefits could reduce the need for both clinic and injection visits when compared to other available treatment modalities, thereby reducing patient and provider burden and health care expenditures.

II. Research Plan and Design

A. Study Objectives: The goal of this study is to evaluate the efficacy of cryoneurolysis for improving ankle symptoms and function. Specifically, this study will measure changes in pain, activities of daily living, quality of life and physical performance at 6, 12 and 24 weeks after each superficial and deep treatment.

B. Study Type and Design:

This is a single-site, open-label study of participants with painful ankle OA assigned to undergo cryoneurolysis of either the Superficial Fibular Nerve (SFN), Sural Nerve (SN) and Saphenous Nerve, or the Deep Fibular Nerve (DFN) using ultrasound-guided cryoneurolysis. Subjects will initially undergo diagnostic nerve blocks using 1% lidocaine with epinephrine for either the superficial nerves or the DFN to confirm analgesia prior to the cryoneurolysis procedure.

We will evaluate responder status with NRS at clinic visits 6 weeks, 12 weeks and 24 weeks following treatment as well as NRS via web-form or phone calls (per subject preference) at 3, 9 and 18 weeks. "Non-responders" being defined as anyone reporting less than 20% pain relief with respect to their baseline NRS scores. For subjects who become non-responders, we will treat the other nerve group with iovera at their next clinic visit (e.g. if a subject becomes a non-responder 9 weeks after superficial nerves are treated, then at 12 week follow-up, the participant will be offered treatment of the deep fibular nerve).

Figure 2 depicts the flow of how participants will be treated and potentially offered an alternative treatment if their initial treatment fails to provide durable benefit.

FAOS (Pain, ADL, QoL), NRS for pain and physical performance tests (40 m fast paced walking test and, Standing Balance Test) will be assessed at baseline and 6, 12, and 24 weeks post-treatment. Phone calls at 3, 9 and 18 weeks post-treatment will monitor subjects using the NRS pain scale.[7] Electronic data capture will be used for this study. Adverse events will be monitored at each telephone call and follow-up visit. There will be a grace period of 21 days between the screening and treatment visit. There is a +/- 3-day window for follow-up visits. During the time period of March to May, 2020(depending on duration of suspension of non-essential research), we are modifying the protocol to conduct virtual study visits to address the COVID-19 pandemic. The following data points will be or will not be collected during this time as per the individual circumstance: 40mFPWT or Standing Balance test at 6 week, 12 week, and 24 week. In order to ensure subject safety and welfare we will conduct the 6 week, 12 week and 24 week f/u visit remotely for each treatment arm as per KU essential research guidelines. We will also offer participants masks, gloves, and complete the f/u visit at the

earliest to limit any potential exposure to people in the hospital. In order to address data integrity issues, we will try to conduct the 12 week f/u visits in person.

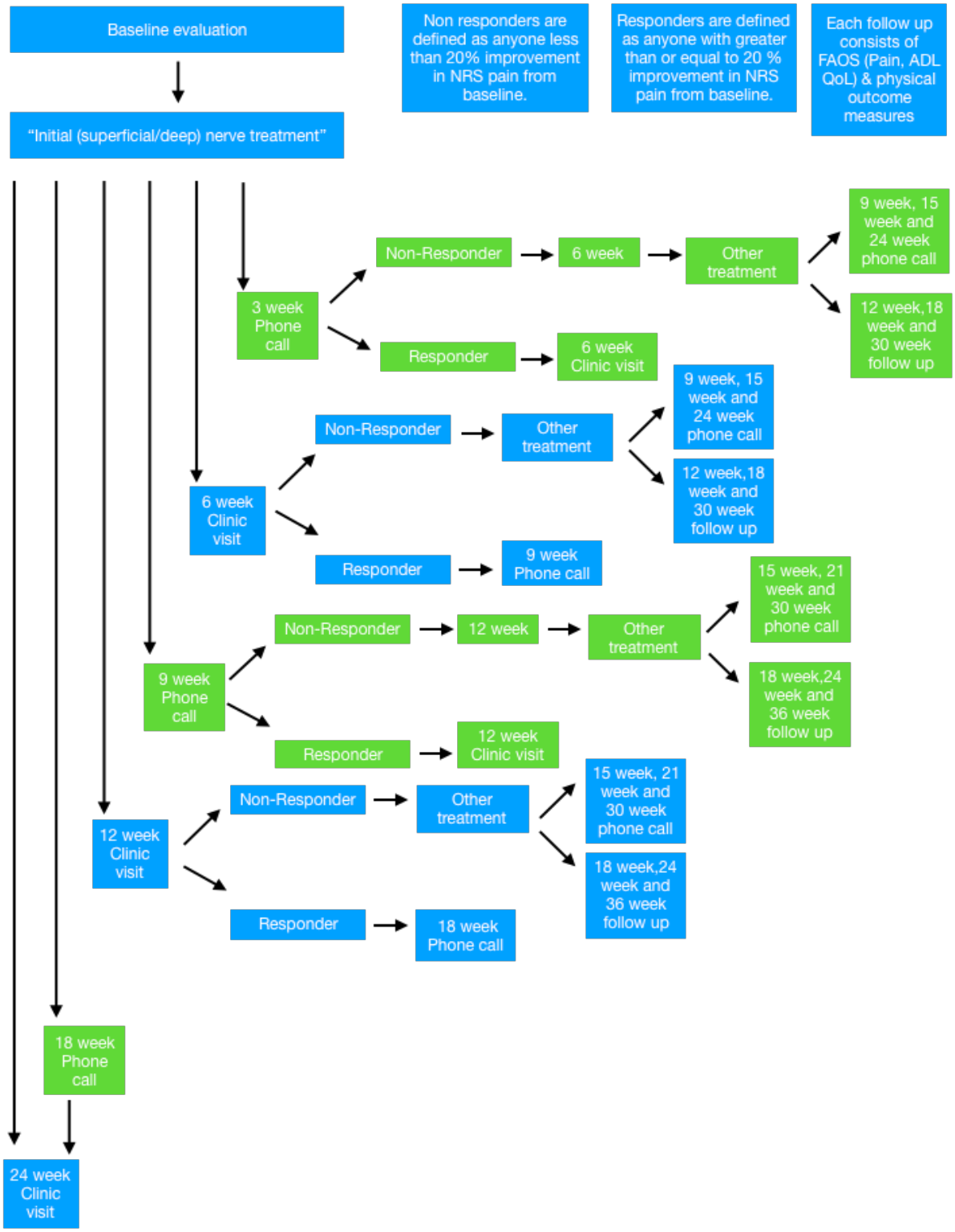

Figure 2 – Protocol flowchart depicting criteria for responders and non-responders at each follow up time point.

C. Sample size and power calculation

In this open-label study, participants will undergo cryoneurolysis using iovera. FAOS (Pain, ADL, QoL), NRS for pain and physical performance tests (40m fast-paced walking test and Standing Balance Test) data will be collected at baseline and 6, 12 and 24 weeks post-treatment. A 24-week follow-up duration was selected to detect meaningful differences from standard of care and long-term effects. A control group will not be included in this initial pilot study. Differences between each of the three follow-up time points and baseline will be assessed using one-sample t-tests or repeated measures analyses, as appropriate, controlling for study-wide alpha level to account for multiple comparisons as well as 6-week and 24-week analyses.

A FAOS validation study, completed by S.B.Mani et al., confirmed that some FAOS sub-scales (i.e. pain, activities of daily living and quality of life) are valid for independent use. According to the study, the effect size for FAOS pain and quality of life is 1.06, whereas for activities of daily living there is an effect size of 0.65. At an effect size of 0.65 and a single-sized alpha of 0.025, a sample size of 32 would provide a power of 90%. While 80% power is customary, it would be desirable to plan for 90% to reduce the probability of missing an effect if one is present and to provide sufficient power for comparisons at 2-time points. Assuming up to 20% dropout, we plan to recruit 40 participants. This should be more than sufficient, considering that the effect size for the other 2 outcomes has been reported to be 1.06.[9]

D. Subject Criteria (See Vulnerable Populations appendix, if applicable):

Enrollment will be comprised of 40 subjects, age 18 and over with symptomatic ankle OA. There will be no exclusions based on sex or ethnic group. Potential subjects will be identified through hospital records, referrals from physicians in the surrounding community, and mass mailings, or advertisements posted in area clinics and businesses.

1. Inclusion criteria

    - Participation in an institutional review board-approved informed consent process, culminating in providing written consent.
    - Willingness and ability to comply with the study procedures, visit schedules and ability to follow verbal and written instructions.
    - Male or female over 18 years of age.
    - Currently Kellgren-Lawrence (KL) Grade 2, 3 or 4 in the ankle based on an X-ray. (weight-bearing mortise views with 20° internal rotation)[10]
    - Limited by unilateral ankle pain, rated on a Numerical Rating Scale for pain severity as ≥5 on most days over the last month.
    - Foot and ankle outcome score (FAOS) of < 75 in at least 1 category.
    - Body mass index (BMI) ≤ 50 kg/m^2
    - Ambulatory.
    - Willingness to abstain from the use of protocol-restricted medications during the study and also willing to abstain from using analgesics other than acetaminophen 1 week prior to the beginning of the study.
    - Has undergone at least one prior conservative osteoarthritis treatment (e.g. physical therapy, analgesics).

2. Exclusion criteria:

- Baseline knee, hip, spine or other limitations that affect the walking ability to a greater extent than their ankle.
- Cryoglobulinemia, paroxysmal cold hemoglobinuria, Raynaud's disease, cold urticaria.
- Clinical signs or symptoms of active or recurrent infection in the index ankle joint or overlying skin.
- IA corticosteroid (investigational or marketed) within 3 months of screening.
- Oral corticosteroids (investigational or marketed) within 2 weeks of screening (unless on a chronic stable dose for ≥3 months prior to enrollment).
- Women who are pregnant (due to potential for the change in body mass and distribution to alter ankle symptoms over the period of follow-up).
- Any condition other than OA of the ankle joint which, in the opinion of the investigators, affects their ability to ambulate to a sufficient degree or interferes with the assessment of the safety and treatment effects of the study injection.
- Arthroscopy or open surgery of the ankle joint within 6 months of screening.
- Planned/anticipated surgery of the index ankle joint during the 6-month study period.
- Any clinically significant degree of cognitive impairment or other condition, finding, or psychiatric illness at screening which, in the opinion of the investigator, could compromise patient safety or interfere with the assessment of the safety and treatment effects of the study injection.
- Skin breakdown at the ankle joint where the injection is planned to take place.
- Participated in any investigational drug or device trial within 30 days prior to screening or concurrent participation in another research study that could complicate interpretation of the findings of either study.
- Current consumption of more than 14 alcoholic drinks per week
- Patients with diffuse pain conditions (Complex pain - diffuse or confounding pain, fibromyalgia, etc).
- Known altered nerve anatomy or physiology (e.g. neuropathy) at the target, such as due to a congenital, traumatic, medical or surgical cause.

3. Withdrawal/Termination criteria:

Subjects may discontinue the study at any time. The investigator may discontinue a subject at any time for subject safety or noncompliance. Subjects who are discontinued for safety will be referred to their primary care physician for follow-up.

The Investigator/Coordinator will complete a study exit form in the CRF for any subject who prematurely discontinues from the study. If discontinuation was the result of an AE, the AE will also be recorded in the CRF.

4. Allowable Medications/Nonpharmacological Therapies

The following medications and nonpharmacological therapies may be taken or used throughout the study:

- Aspirin for cardio-protection at a maximum stable dose of 100 mg per day provided the dose was stabilized over 3 months prior to study entry.
- Physical therapy/bracing for the ankle joint if the program was initiated and consistent prior to study entry.
- Acetaminophen as an occasional rescue medication. Concomitant treatment for OA will be standardized to oral acetaminophen (e.g. Tylenol) for all subjects during the study. During the screening period (i.e., prior to the procedure visit), subjects may take acetaminophen as needed to a maximum of 3000 mg per day. Use of acetaminophen must be discontinued 48 hours prior to the procedure visit and each subsequent scheduled visit.
- The use of acetaminophen for other types of pain or illness during the study (e.g., toothache, headache, fever) should also be recorded.

5. Restricted Medications/Nonpharmacological Therapies

Patients will be asked to abstain from certain medications and therapies. The following medications and nonpharmacological therapies should not be taken or used beginning immediately upon enrollment (after signing informed consent) until the subject reaches the end of the study:

- Oral non-steroidal anti-inflammatory drugs (NSAIDs) (unless on a chronic stable dose for ≥3 months prior to enrollment).
- Topical analgesics applied to the skin over the index ankle (e.g., NSAID's, capsaicin, lidocaine, heat patches).
- Orally administered systemic corticosteroids (unless on a chronic stable dose for ≥3 months prior to enrollment).
- Intra-articular injection into the ankle or subtalar joints.
- Centrally acting pain medications (unless on a chronic stable dose for ≥3 months prior to enrollment).
- Opioid medications (unless on a chronic stable dose for ≥3 months prior to enrollment)

E. Specific methods and techniques used throughout the study

Primary Outcome

The Foot and Ankle Outcome Score (FAOS), derived from the validated Knee Injury and Osteoarthritis Outcome Score (KOOS). It is a region-specific questionnaire that has been well described and validated.[9] [11]

It has been found to be satisfactory in the measurement of many foot and ankle pathologies. The largest study of the FAOS to date is a registry-based study that found high internal consistency across the entire instrument, and high test-retest reliability in three of its five sub scales. It has been specifically evaluated for the assessment of OA in another study. The purpose of this study was to verify the content validity, construct validity and responsiveness of the FAOS in patients with ankle OA.

The FAOS covers pain, other symptoms, activities of daily living (ADL), recreational function and quality of life. This outcome measure comprises of forty-two questions covering pain, disability, self-care and psychological factors. The minimum possible total score for each subscale is 0 points, whereas the score of 100 points represents the maximum score.

The primary endpoint of this study is the FAOS-Pain score compared to baseline at 12 weeks post-treatment.

FAOS - Pain
PAIN = 100 - (Total score P1-P9 x 100/ 36)
FAOS-Pain is a subscale of the Foot and ankle outcome score (FAOS) which is a self-reported outcome score. In our study, it will be the primary outcome measure. It comprises of the following questionnaire. The FAOS-Pain will be completed by the subject and will take approximately 3 minutes to complete. The study coordinator or designee will enter the data into the CRF.

P1. How often do you experience foot/ankle pain?
Never Monthly Weekly Daily Always

What amount of foot/ankle pain have you experienced the last week during the following activities?
P2. Twisting/pivoting on your foot/ankle
None Mild Moderate Severe Extreme

P3. Straightening foot/ankle fully
None Mild Moderate Severe Extreme

P4. Bending foot/ankle fully
None Mild Moderate Severe Extreme

P5. Walking on flat surface
None Mild Moderate Severe Extreme

P6. Going up or down stairs
None Mild Moderate Severe Extreme

P7. At night while in bed
None Mild Moderate Severe Extreme

P8. Sitting or lying
None Mild Moderate Severe Extreme

P9. Standing upright
None Mild Moderate Severe Extreme

Secondary Outcomes
FAOS - ADL

ADL = 100 - (Total score A1-A17 x 100/ 68)

FAOS-ADL is another subscale of the Foot and ankle outcome score (FAOS) which is a self-reported outcome score. It is similar to the KOOS-ADL components and it comprises of the following questionnaire. The FAOS-ADL will be completed by the subject and will take approximately 4 minutes to complete. The study coordinator or designee will enter the data into the CRF.

A1. Descending stairs
None Mild Moderate Severe Extreme

A2. Ascending stairs
None Mild Moderate Severe Extreme

For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your foot/ankle.

A3. Rising from sitting
None Mild Moderate Severe Extreme

A4. Standing
None Mild Moderate Severe Extreme

A5. Bending to floor/pick up an object
None Mild Moderate Severe Extreme

A6. Walking on flat surface
None Mild Moderate Severe Extreme

A7. Getting in/out of car
None Mild Moderate Severe Extreme

A8. Going shopping
None Mild Moderate Severe Extreme

A9. Putting on socks/stockings
None Mild Moderate Severe Extreme

A10. Rising from bed
None Mild Moderate Severe Extreme

A11. Taking off socks/stockings
None Mild Moderate Severe Extreme

A12. Lying in bed (turning over, maintaining foot/ankle position)
None Mild Moderate Severe Extreme

A13. Getting in/out of bath
None Mild Moderate Severe Extreme

A14. Sitting
None Mild Moderate Severe Extreme

A15. Getting on/off toilet
None Mild Moderate Severe Extreme

For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your foot/ankle.
A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc.)
None Mild Moderate Severe Extreme

A17. Light domestic duties (cooking, dusting, etc.)
None Mild Moderate Severe Extreme

Quality of Life
FAOS - QoL
FAOS-QoL is another subscale of the Foot and Ankle Outcome Score (FAOS) which is a self-reported outcome score. It is similar to the KOOS - QoL components and it comprises of the following questionnaire. The FAOS-QoL will be completed by the Subject and will take approximately 2 minutes to complete. The study coordinator or designee will enter the data into the CRF.

Q1. How often are you aware of your foot/ankle problem?
Never Monthly Weekly Daily Constantly

Q2. Have you modified your life style to avoid potentially damaging activities to your foot/ankle?
Not at all Mildly Moderately Severely Totally

Q3. How much are you troubled with lack of confidence in your foot/ankle?
Not at all Mildly Moderately Severely Extremely

Q4. In general, how much difficulty do you have with your foot/ankle?
None Mild Moderate Severe Extreme

Numerical Rating Scale (NRS) for Pain
The Numerical Rating Scale for Pain (NRS for Pain) is a measure of pain intensity. The investigator or designee conducts the 11-point scale verbally with the research subject about the index ankle. The investigator or designee will ask the following questions:

- "On a scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your ankle pain IN THE PAST 7 DAYS?"
- "On a scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your ankle pain RIGHT NOW?"

The subject will respond verbally and the study coordinator or designee will record the response.

The NRS for Pain will be completed by the subject and will take approximately 1 minute to complete. The study coordinator or designee will enter the data into the eCRF.

Tertiary Outcomes

40m fast paced walking test (40m fpwt)

The 40-meter fast paced walking test is one of the three OARSI (Osteoarthritis Research Society International) recommended minimal core set of performance-based outcome measures in OA research and clinical practice.[12] These tests are complementary to patient reported measures. They are recommended for use prospectively as outcome measures in research, and also in clinical practice to make treatment decisions based on the results. The 40m FPWT will take approximately 2 minutes for each subject to complete. The study coordinator or designee will enter the data into the eCRF.

Standing Balance Test

The Standing Balance Test outcome is measured with the feet side by side, then in semi-tandem stance (heel of one foot in front and beside the big toe of the other foot), and then in tandem stance (heel of one foot directly in front of the other foot); each stance was held for up to 10s. These test results are converted to scores (range 0-4). Higher scores represent better function and a score of 0 represents the inability to complete the test. Subjects are given a score of 1 if they can hold a side-by-side standing position for 10 s but are unable to hold a semi-tandem position for 10s, a score of 2 if they can hold a semi-tandem position for 10s but are unable to hold a full tandem position for more than 2s, a score of 3 if they can stand in the full tandem position for 3-9s, and a score of 4 if they can stand in the full tandem position for 10s.[13] The Standing Balance Test will be completed by the Subject and will take approximately 2 minutes to complete. The study coordinator or designee will enter the data into the eCRF.

The tables below depict the schedule of study assessments, or procedures, for each visit for the intervention(s) offered (superficial and/or deep).

Assessment	Visit 1 / Screening	Visit 2 / iovera° Treatment -1	Telephonic 3 Week Follow-up	Visit 3 / 6 Week Follow-up	Telephonic 9 Week Follow-up	Visit 4 / 12 Week Follow-up	Telephonic - 18 Week Follow-up	Visit 5 / 24 Week Follow-up
Informed Consent	X							
Eligibility	X	X						

Radiograph or SCT (if not previously done)	X							
Medical history	X	X						
Concomitant medications	X	X		X		X		X
Prior/Concurrent Therapy	X							
Study Treatment		X						
Physical Exam	X	X						
FAOS Questionnaire	X			X		X		X
NRS for Pain	X	X	X	X	X	X	X	X
Physical Performance Measures	X			X		X		X
AE/SAE Assessment		X	X	X	X	X	X	X

***Each follow up visit will have a window of +/- 3 days as per the schedule**

Assessment	**iovera° Treatment - 2**	**Telephonic 3 Week Follow-up**	**6 Week Follow-up**	**Telephonic 9 Week Follow-up**	**12 Week Follow-up**	**Telephonic - 18 Week Follow-up**	**24 Week Follow-up**
Informed Consent	X						
Eligibility	X						
Radiograph or SCT (if not previously done)							
Medical history	X						
Concomitant medications	X		X		X		X
Prior/Concurrent Therapy							
Study Treatment	X						
Physical Exam	X						

FAOS Questionnaire	X		X		X		X
NRS for Pain	X	X	X	X	X	X	X
Physical Performance Measures	X		X		X		X
AE/SAE Assessment	X	X	X	X	X	X	X

***Each follow up visit will have a window of +/- 3 days as per the schedule**

F. Risk/benefit assessment:

1. Physical risk:
   The iovera° device involves percutaneous access to subcutaneous tissue using a needle and use of dermal anesthesia. Passage of a needle into the skin, cooling of subcutaneous soft tissue and delivery of local anesthesia are known to be associated with the following risks [1]:
   - Bruising (ecchymosis)
   - Swelling (edema)
   - Inflammation and/or redness (erythema)
   - Pain or skin hardening along the treatment line.
   - Tenderness on palpation.
   - Altered sensation (localized anesthesia or dysesthesia such as burning)

   Proper use of the device as described in the iovera° ***User Guide*** can help reduce or prevent the following complications:
   - Injury to the skin related to application of cold or heat
   - Hyper- or hypo-pigmentation at the treatment site
   - Skin dimpling at the treatment site
   - Loss of motor function outside the target area

2. Psychological risk: N/A
3. Social risk: N/A
4. Economic risk: N/A
5. Potential benefit of participating in the study:
   Participation in this study may produce possible benefits for the individual subject. Possible benefits include improved pain, stiffness or function in your ankle.

G. Location where study will be performed: The study will be performed at the University of Kansas Medical Center. All data forms and records will be kept on site at the University of Kansas Medical Center in locked drawers and password-protected computers in a locked office. Only the research team will have access to the data.

H. Collaboration (with another institution, if applicable): N/A

I. Single IRB Review for a Multi-site study (if applicable): N/A

J. Community-Based Participatory Research (if applicable): N/A

K. Personnel who will conduct the study, including:

1. Indicate, by title, who will be present during study procedure(s):
    a. Dr. Neil Segal, Principal Investigator
    b. Dr. Mayank Kothari, Research Assistant
    c. Jennifer Bedard, Research Coordinator
2. Primary responsibility for the following activities, for example:
    a. Determining eligibility: Segal/Kothari/Bedard/Wapp
    b. Obtaining informed consent: Segal/Bedard
    c. Providing on-going information to the study sponsor and the IRB: Bedard/Segal
    d. Maintaining participant's research records: Kothari/Bedard/Segal
    e. Completing physical examination: Segal
    f. Measuring height, weight: Segal/Bedard/Kothari
    g. Drawing / collecting laboratory specimens: N/A
    h. Performing / conducting tests, procedures, interventions, questionnaires: Segal/Kothari/Bedard
    i. Completing study data forms: Segal/Bedard/Kothari
    j. Managing study database: Bedard/Kothari

L. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

Compliance reports will be reviewed by the Principal Investigator on a weekly basis.

Reports containing the proportion of subjects completing the intervention or prematurely terminating participation will be generated monthly.

Adverse event information will be collected during all contacts with subjects. If an adverse event is reported to study staff, data including the type of event, onset/end dates, duration, severity, and outcome will be collected and reported to the Principal Investigator, who will determine severity of the event using CTCAE guidelines. Post-treatment expected symptoms include numbness of the skin over the ankle and foot and possibly redness, swelling, bruising or pain at the site of insertion.

An event that is serious must be recorded on the AE worksheet and requires expeditious handling to comply with regulatory requirements. Any adverse events classified as "serious" by the Investigator must be reported to the IRB within 24 hours of becoming aware of its occurrence.

III. Subject Participation

A. Recruitment:

Potential study participants will be recruited from a HERON generated list, the clinics of the participating investigators, as well as local advertising. Any study-related advertisements will be approved by the governing IRB prior to use. Subjects will be recruited based upon inclusion and exclusion criteria detailed above. Participants will be compensated for their time and effort at follow-up visits.

Each subject will participate for up to 24 weeks following either deep or superficial nerve treatment. Enrollment is expected to take up to 6-12 months following (superficial or deep nerve) treatment. Total study duration is expected to be up to 18 months.

B. Screening Interview/questionnaire: Potential subjects who express interest in the study will be screened by telephone. A script will be used to determine eligibility for the first screening clinic visit by study staff. Please see Appendix II.

C. Informed consent process and timing of obtaining of consent

Subjects who pass the initial screening criteria (telephone screen) will be asked to schedule a screening visit to provide written consent and possibly undergo screening procedures during a screening visit. Subjects will be informed that the study is voluntary and choosing not to participate will not affect their current treatment. They may request the opportunity to review the consent form with their physician and/or family members. Moreover, they will be fully informed of the purpose of the study and of the possible risks involved before providing their consent. Written, informed consent will be obtained and documented by an investigator or a study coordinator prior to initiation of study procedures. All subjects will be provided with a copy of the signed consent form.

D. Alternatives to Participation: The alternative to study participation is choosing not to participate and continue with their current treatment plan.

E. Costs to Subjects: There will be no cost to subjects to participate in the study and no insurance will be billed as a part of the study procedures.

F. How new information will be conveyed to the study subject and how it will be documented: If any new information is to be provided to the study subject the informed consent form (ICF) will be amended and approved by the IRB. Each subject will then be reconsented using the newest revision of the ICF.

G. Payment, including a prorated plan for payment:
Subjects would be provided $25 at each visit that they attend (up to $200 per participant) as compensation for their time. They additionally will be provided with parking validation for in-person visits. Moreover, participants will be provided with encouragement during the study period, especially prior to and after each study visit through letting them know that their contributions are appreciated.

H. Payment for a research-related injury:
If the subject is injured because of study participation, there will be no compensation for physical or non-physical injuries. will be available for any extra expenses that may be the result of Research-related physical injuries include additional hospital bills, lost wages, travel

expenses, etc. No compensation will be available for any non-physical injuries that may occur because of research participation, such as legal problems, problems with your finances or job, or damage to your reputation.

IV. Data Collection and Protection

A. Data Management and Security:

FAOS (Pain, ADL, QoL), NRS for pain and physical performance test (40 m fast paced walking test, Standing Balance Test) data will be collected at baseline and 6, 12 and 24 weeks after cryoneurolysis. All data collected will remain confidential and only accessible to the research team

Data regarding subject demographic, standing ankle radiographs or cone-beam CT., FAOS subscale responses will be obtained directly from subjects. All data will be coded, with no patient identifiers. A master list with patient information and assigned study IDs will be stored in a separate password-protected file on a password-protected research drive available to the study team. Analyses will be performed on coded files. Physical files (e.g. questionnaires) will be stored in locked file cabinets in the research office.

B. Sample / Specimen Collection: N/A

C. Tissue Banking Considerations: N/A

D. Procedures to protect subject confidentiality:

Data regarding subjects' demographic, ankle radiographs or cone-beam CT, FAOS responses will be obtained directly from subjects. All data will be coded, with no information that could identify a subject. A master list with patient information and assigned study IDs will be stored in a separate password-protected file on a password-protected research drive available to the study team. Analyses will be performed on coded files. Physical files (e.g. questionnaires) will be stored in locked file cabinets in the research office.

E. Quality Assurance / Monitoring

Study staff will have proper qualifications for data entry and will receive training in data entry procedures to insure data entered is of highest quality research staff. Data will be entered into two separate databases by two separate members of study staff. The two databases will be reconciled by the database manager and the outcome reported to the Principal Investigator. Data will be backed-up daily.

V. Data Analysis and Reporting

A. Statistical and Data Analysis:

All participants will be assigned to undergo cryoneurolysis using iovera. FAOS (Pain, ADL, QoL), NRS for pain and physical performance test (40 m fast paced walking test, standing balance test) data will be collected at baseline, 6 weeks, 12 weeks and 24 weeks. A 24-week follow-up duration was selected to detect meaningful differences from standard of care and long-term effects, while minimizing the variability that may occur further out from baseline. Differences between each of the three follow-up time points for each of the interventions (superficial and deep) and baseline will be assessed using one-sample t-tests or repeated measures analyses, as appropriate. This will control for study-wide alpha level to account for multiple comparisons as well as 6-week and 24-week analyses. A soft-lock of the database will occur after the 6- and 12-week time-points.

B. Outcome: We expect statistically significant and clinically meaningful improvement in pain, activities of daily living, quality of life and physical performance with iovera° in people with ankle OA at 12 weeks post-treatment.

C. Study results to participants: The study will be registered on www.clinicaltrials.gov and results posted upon completion of the study.

D. Publication Plan: Results will be submitted for presentation at an appropriate conference and for publication in an appropriate journal for the topic of study.

VI. Bibliography / References / Literature Cited

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APPENDIX I: VULNERABLE POPULATIONS

I. N/A
II. Cognitively or decisionally impaired individuals: N/A
III. Children: N/A
IV. Pregnant women: N/A
V. Prisoners: N/A
VI. Students and/or Employees: N/A