A POST-MARKET, MULTI-CENTER, PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, CONTROLLED STUDY TO EVALUATE THE IOVERA° DEVICE IN TREATING PAIN ASSOCIATED WITH TOTAL KNEE ARTHROPLASTY

PROTOCOL NUMBER:	MYO-1070 REV 02
VERSION:	MAY 20, 2015
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Revision	Description	DCO#	Release Date
01	• Initial Release.	2473	10/17/2014
02	• Grammar and punctuation corrections • Add Medical Monitor • Add NRS for Pain of Contralateral Knee questions to Screening Visit • Add questions to assess the blind Post-Treatment and Follow-Up Visits • Increase Duration of Study • Specified use of Lidocaine, no epinephrine • Clarification on timing of visit windows and schedule of events • Clarification to Exclusion Criteria #2 and Exclusion Criteria #5 • Updated to reflect staff changes	2589	2/27/2015
02	• Admin fixes to update address, see DCO	2642	5/20/2015

Investigator Protocol Signature Page (REV 02)
Read and initial below.

____ I understand this protocol contains information that is confidential and proprietary to myoscience, Inc.

____ Any additional information added to this protocol is also confidential and proprietary to myoscience, Inc. and must be treated in the same manner as the contents of this protocol.

____ I have read the entire protocol.

____ I understand what the protocol asks me to do as an Investigator.

____ I will conduct this study following this protocol and will make a reasonable effort to complete the study in the time noted.

____ I will provide this protocol to study staff under my direct supervision. My study staff will keep the protocol and associated documents confidential.

____ I will discuss this information with the study staff to ensure they are fully informed about the study and the test articles.

____ I will not start enrolling in this study until it is approved by a governing Institutional Review Board.

____ I understand the study may be terminated or enrollment suspended at any time by myoscience, Inc., with or without cause, or by me if it becomes necessary to protect the interests of the study Subjects.

_____
Name of Investigator

_____ _____
Investigator Signature Date

Protocol Synopsis

Title	A Post-Market, Multi-Center, Prospective, Double-blind, Randomized, Controlled Study to Evaluate the iovera° Device in Treating Pain Associated with Total Knee Arthroplasty
Study Device	iovera° (myoscience Inc., Fremont, CA)
Study Objective	To evaluate the outcomes of patients undergoing iovera° treatment of the ISN and AFCN prior to total knee arthroplasty to provide temporary postoperative pain relief
Study Design	Multi-center, prospective, randomized
Treatment Groups	Pre-operative iovera° treatment vs. pre-operative sham iovera° treatment
Duration of Participation	Up to 16 weeks (12 weeks post-op)
Study Population	Male or female, ages 22 to 79 scheduled to undergo a total knee replacement as a result of osteoarthritis
Total Number of Subjects	150 subjects
Number of Sites	Up to 8 sites
Study Procedures	iovera° treatment introducing temporary nerve conduction block of the ISN and AFCN
Data Collection Tools	• WOMAC • SF-36 • PROMIS • NRS for Pain
Primary Endpoint	Cumulative Opioid Consumption at 6 weeks post-TKA Surgery
Secondary Endpoints	• Length of Hospital Stay • WOMAC Pain • WOMAC Function • Physical Performance (Measured by: Range of Motion, 30 Second Chair Test and 40 Meter Walk Test)

Table of Contents

Abbreviations

ADE	Adverse Device Effect
AE	Adverse Event
AFCN	Anterior Femoral Cutaneous Nerve
AO	Anticipated Observation
ASADE	Anticipated Serious Device Effect
CFR	Code of Federal Regulations
CNS	Central Nervous System
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FCT™	Focused Cold Therapy™
ESE	Expected Side Effect
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IRB	Institutional Review Board
ISN	Infrapatellar branch of the Saphenous nerve
ISO	International Organization for Standardization
MedDRA	Medical Dictionary for Regulatory Activities
NRS Pain	Numeric Rating Scale for Pain
NSAID	Non-Steroidal Anti-Inflammatory Drug
NSR	Non-Significant Risk
OTC	Over-the-Counter
PT	Preferred Terms
PGIC	Patient Global Impression of Change
PROMIS	Patient Reported Outcomes Measurement Information System
QA	Quality Assurance
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SF-36	Medical Outcomes Study 36-Item Short-Form Health Survey
SOC	System Organ Classes
TKA	Total Knee Arthroplasty
UG	User Guide
USADE	Unanticipated Serious Adverse Device Effect
WOMAC	Western Ontario and McMaster Osteoarthritis Index

1. BACKGROUND

Total Knee Arthroplasty (TKA) is a highly effective procedure to relieve symptoms in patients with severe arthritis. Improvements in pain, physical function, and enhanced quality of life in patients who have undergone TKA are well established in the literature[1,2]. However, post-operative pain associated with TKA is severe. Effective pain control following TKA allows for earlier ambulation and initiation of physical therapy, which speeds recovery, reduces hospital length of stay, and decreases the risk of postoperative complications[3]. The VHA/DoD and the American Society of Anesthesiologists have both issued guidelines suggesting that wherever possible, practitioners should use multimodal pain management[4]. Clinical management of post-op TKA pain should include both pharmacologic and non-pharmacologic modalities and minimize preventable postoperative complications[5]. As demand for primary TKAs is projected to grow to 3.48 million procedures per year by 2030 in the United States alone[6], the need for effective and economic multi-modal pain management has never been greater.

Managing pain via multi-modal strategies, including peripheral nerve blocks, in the inpatient postoperative phase has been shown to decrease opioid consumption[7], decrease opioid related side effects, decrease hospital stay, and increase time to ambulation[8,9,10]. Nursing, hospital, and pharmacy utilization in managing PCA, continuous regional nerve blocks, and administration of oral opioid dosing are associated with higher costs of care and introduce sources for staff error[11,12]. Furthermore, the idea of multi-modal pain management extends beyond the in-patient phase of treatment. Decreasing prescription opioid use during outpatient rehabilitation decreases NSAID and opioid related side effects, especially important among the aging population[13,14].

Due to the mechanism of action, iovera° introduces a new mode of pain management delivery in TKA. The iovera° system uses liquid nitrous oxide contained within the device and closed-end needles to create a precise zone of cold at the target nerve sites. This Focused Cold Therapy™ delivery platform causes a temporary peripheral nerve block based on a process called Wallerian degeneration (2nd degree axonotmesis) without disrupting connective nerve tissue. With nerve conduction blocked, pain is relieved in sensory nerves (nerves that pass impulses from receptors toward or to the central nervous system). Degeneration of the nerve axons is followed by a predictable restoration of nerve function involving axon regeneration from the point of treatment to the distal end of the nerve at a rate of 1.0–1.5 mm a day[15,16]. Early clinical study results have demonstrated that the duration of effect of iovera°, when applied to the infrapatellar branch of the saphenous nerve for knee pain, is two to three months; longer than that of other clinically adopted modalities (PCA opioids, single shot regional nerve blocks, continuous regional nerve blocks, extended-release peri-operative local opioids, oral and IV NSAIDs, acetaminophen, and oral opioids). Early clinical data further suggests that iovera°, when applied to the infrapatellar branch of the saphenous nerve (ISN) and anterior femoral cutaneous nerve (AFCN) prior to TKA, reduces the amount of opioids requested by subjects to maintain similar levels of pain relief[17].

This study is designed to investigate whether iovera° treatment prior to TKA decreases cumulative patient opioid use over the course of 6 weeks following TKA while maintaining similar levels of pain relief. The study will also investigate whether there is a relationship between patients treated with iovera° and length of hospital stay; patient reported pain and function as measured by WOMAC; and improved physical rehabilitation as measured by functional performance measures and range of motion.

2. REGULATORY STATUS

The iovera° device is 510(k)-cleared (K133453) for producing lesions in peripheral nervous tissue by application of cold to selected sites for blocking pain. Cleared indications include general tissue destruction during surgical procedures and cryotreatment of nerves to block pain.

Additionally, myoscience and an independent review board have determined that the iovera° device is a non-significant risk device under 21 CFR §812.2(b) as described for use within this protocol. Therefore, an approved Investigational Device Exemption (IDE) from FDA is not required to legally perform the study described herein in the US.

3. STUDY OBJECTIVE

To evaluate the outcomes of patients undergoing iovera° treatment of the ISN and AFCN prior to total knee arthroplasty to provide temporary postoperative pain relief.

4. STUDY DESIGN

This is a multi-center, prospective, randomized, double-blind, sham controlled trial.

5. BLINDING

This is a double-blind study. The treating Investigator and Subject are blinded to study treatment. Investigators and Subjects will be unblinded as medical need arises. Every effort should be made to maintain Subject and Investigator blinding throughout the study. The blind will be assessed immediately following treatment and at the follow-up visits by asking the Subject which treatment they believe he/she received and why.

6. DURATION

Each Subject participates for up to 16 weeks. Enrollment is expected to take up to 10 months. Total study duration is expected to be up to 12 months.

7. INVESTIGATOR QUALIFICATIONS

To participate in this study, an Investigator must have an active medical license and board certification in Orthopedic Surgery, Anesthesia, Pain Management, or Physical Medicine and Rehabilitation. The Investigator or designee must undergo training conducted by myoscience, Inc., on the study device prior to enrolling Subjects in the study.

8. STUDY POPULATION

8.1 Target Patient Population

The target patient population is adult men and women ages 22 to 79 in the United States scheduled to undergo primary unilateral TKA under spinal anesthesia for primary diagnosis of osteoarthritis.

8.2 Subject Eligibility

To be included in the study, Subjects must meet all of the inclusion criteria and none of the exclusion criteria list in **Table 1**.

Table 1. Study Eligibility Criteria.

Inclusion Criteria

1. 22 to 79 years of age
2. Scheduled to undergo primary unilateral TKA under spinal anesthesia for primary diagnosis of osteoarthritis
3. American Society of Anesthesiology (ASA) Physical Classification System classes I-III
4. Anticipation of discharge to home after inpatient acute post-op phase (age, co-morbidities, home environment, and social support are in favor of discharge to home in the opinion of the Investigator)
5. Subject is willing and able to give written informed consent.
6. Subject is fluent in verbal and written English.
7. Subject is willing and able to comply with study instructions and commit to all follow-up visits for the duration of the study.
8. Subject is in good general health and free of any systemic disease state or physical condition that might impair evaluation or which in the Investigator's opinion, exposes the Subject to an unacceptable risk by study participation.

Exclusion Criteria

1. Chronic opioid use (defined as daily or almost daily use of opioids for >3 months).
2. Concurrent painful physical condition, surgery, or musculoskeletal disease that requires analgesic treatment during study follow-up that is not strictly related to the target knee being treated with iovera°, which have the potential to confound the postoperative assessments (e.g., significant pain from other joints, chronic neuropathic pain, concurrent or planned contralateral TKA, concurrent foot, neck, spine, hip, or other musculoskeletal disease, arthritis, or planned surgery, etc.).
3. Greater than 15° malalignment (varus or valgus) on pre-operative radiograph.
4. Previous myoscience FCT™ treatment.
5. Previous Partial or Total Knee Arthroplasty. Partial or Total Knee Arthroplasty of the contralateral knee is permitted if [surgery was completed at least twelve (12) months prior to Screening.]
6. Body Mass Index ≥ 40
7. Prior surgery in the treatment areas that may alter the anatomy of the infrapatellar branch of the saphenous nerve (ISN) or the anterior femoral cutaneous nerve (AFCN) or result in scar tissue in the treatment area.
8. Any clotting disorder and/or use of an anticoagulant (e.g. warfarin, clopidogrel, etc.) within seven (7) days prior to administration of the device. Low dose aspirin (81mg or less daily) for cardiac prophylaxis allowed.
9. Any local skin condition at the treatment sites that in the Investigator's opinion would adversely affect treatment or outcomes.
10. Open and/or infected wound in the treatment areas.
11. Allergy to lidocaine.
12. History of cryoglobulinemia
13. History of paroxysmal cold hemoglobinuria.
14. History of cold urticaria.
15. History of Raynaud's disease.
16. History of opioid or alcohol abuse.
17. Subject is pregnant or planning to become pregnant while enrolled in the study.

18. Current enrollment in any investigational drug or device study or participation within 30 days prior to screening.
19. Currently being treated for related knee injury under worker's compensation claim or equivalent (i.e. legal case).
20. Any chronic medical condition that in the Investigator's opinion would prevent adequate participation.
21. Any chronic medication use (prescription, over-the-counter, etc.) that in the Investigator's opinion would affect study participation or Subject safety.
22. For any reason, in the opinion of the Investigator, the Subject may not be a suitable candidate for study participation (i.e., history of noncompliance, drug dependency, etc.).

9. STUDY DEVICE AND TREATMENT PROCEDURE

The study device and sham treatment are described briefly below.

9.1 Description

The myoscience iovera° device is a next generation device designed to temporarily reduce pain. The device consists of a reusable, portable Handpiece, along with single-patient use sterile Smart Tips (aka cryoprobes) and disposable nitrous oxide (N_2O) cartridges. The Smart Tip contains embedded software that manages procedure parameters and provides physician feedback throughout all states of device preparation, treatment and post-treatment via communication with the Handpiece. The Handpiece is battery powered and is stored and recharged via the Charging Dock.

The iovera° device produces the desired effect through initiation of a cooling cycle. Each cooling cycle is initiated by fully inserting the Smart Tip into the selected procedure site and activating the cryogen flow. A freezing zone forms around the end of the Smart tip affecting the adjacent tissue.

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 Handpiece. This ensures optimal performance of the device.

A specially designed Smart Tip is included. The Smart Tip needles are made of stainless steel and have a closed-tip, fully enclosing the cryogen. 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 provided in the ***User Guide***.

The appearance of the Smart Tips used for the sham treatment is identical to the tips used for an active treatment. The sham Smart Tip does not allow a freezing zone to form therefore it does not provide a therapeutic treatment. External operation of iovera° (status indicator lights and audible cues) is identical for both the active and sham treatment to preserve the double-blind.

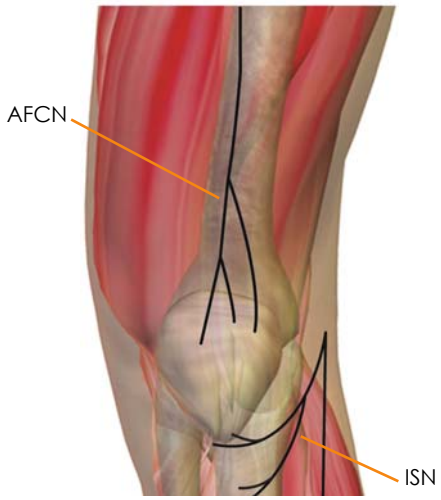
Device training will be provided to the Investigator and study staff prior to the initiation of study enrollment.

9.2 Instructions for Use and Administration

Use of the iovera° device is described briefly herein. For details see the ***User Guide*** as provided by myoscience, Inc.

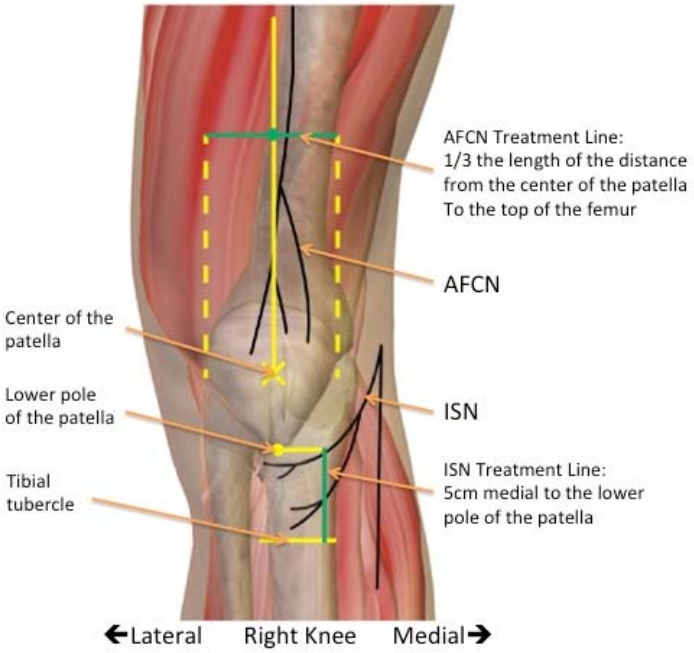
The iovera° device is used on awake Subjects who are prepared with local anesthesia only. The treatment targets are the infrapatellar branch of the saphenous nerve (ISN) and the anterior femoral cutaneous nerve (AFCN) shown in Figure 1. Training for the treatment procedure will be provided to the Investigator and study staff prior to the initiation of study enrollment.

Figure 1. Infrapatellar branch of the Saphenous Nerve and Anterior Femoral Cutaneous Nerve



Treatment will be performed unilaterally and will be guided by visualization and palpation of anatomical landmarks. Landmarks and treatment line will be marked on the skin as shown in Figure 2.

Figure 2. Treatment Lines (Figure not to scale)



Note that the study Sponsor will provide the following supplies:

- iovera° devices, Smart Tips and cryogen cartridges
- Surgical pen or other marking tool
- Measuring tape
- Safety cones (2)
- Goniometers (2)
- Marking Tape
- Stop watch

The investigational site will provide the following:

- Injectable lidocaine (no epinephrine) used for local anesthesia
- Syringes for administration of local anesthesia
- Needles for administration of local anesthesia
- Gauze
- Alcohol wipes
- Wound dressing(s)

9.3 Contraindications

Use of iovera° is contraindicated in the following situations:

- Cryoglobulinemia
- Paroxysmal cold hemoglobinuria
- Cold urticaria
- Raynaud's disease
- Open and/or infected wounds in the target treatment area

Note: physician discretion should be exercised when patient presents with existing neuromuscular disease compromising the regeneration of peripheral nerves that may be involved in the treatment.

9.4 Risks

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:

- Bruising (ecchymosis)
- Swelling (edema)
- Inflammation and/or redness (erythema)
- Pain and/or tenderness
- Altered sensation (localized dysesthesia)

Proper use of the device as described in the ***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

10. STUDY PROCEDURES

10.1 Overview

An Overview of the study visits is shown in **Figure 3**. Eligible Subjects are randomized to iovera° treatment or to sham treatment with the iovera° device. The primary endpoint is cumulative opioid consumption at 6 Weeks Post-TKA.

Figure 3. Overview of Study Visits

*Screening and Treatment can occur on the same day.
**Data collected retrospectively, iovera° Treatment may be same day as TKA.

10.2 Recruitment

Potential study participants will be recruited from clinics of participating Investigators, as well as local advertising. Any study-related advertisements will be approved by the governing IRB prior to use.

10.3 Screening /Visit 1 (-30 to -1 days)

A patient who signs the informed consent document will be considered a study Subject. A Subject who withdraws from the study prior to randomization will not count toward the study's sample size. Potential participants will be screened for eligibility against **Table 1**.

Once the informed consent form is signed, the Subject is assigned a study number. The Subject number is comprised of the 2-digit site number followed by a consecutively assigned 3-digit Subject number that starts with 001. For example, the first screened Subject for site 05 will be assigned study number 05-001. The Subject number is the identification number used on eCRFs and other study documents throughout the study. In the event a Subject withdraws from the study, their Subject number cannot be reassigned to any other Subject.

The Investigator, or designee, will document the Subject's medical history, demographic information, obtain vital signs, complete an assessment of the intended treatment areas and concomitant medications/concurrent procedures.

Once a Subject has been determined to meet all inclusion and none of the exclusion criteria, the Subject will complete the following:

Assessments:

- NRS for Pain when standing from a seated position in target knee
- NRS for Pain when standing from a seated position in the contralateral knee
- PROMIS-29 Profile v2.0 Participant Format
- WOMAC Osteoarthritis Index NRS3.1
- SF-36v2® Health Survey Standard, United States (English)

Physical Performance Measures in the following order:

- Active Range of Motion

- 40 Meter Walk Test
- 30 Second Chair Test

Detailed instructions for the conduct of these performance measures are included in the ***Study Operations Manual***. Assistive/ambulatory device usage and work status will also be assessed.

Subjects may be complete Screening / Visit 1 and iovera° Treatment / Visit 2 on the same day.

10.4 Randomization

After meeting all of the inclusion and none of the exclusion criteria and prior to study treatment, Subjects will be randomized in a 1:1 manner to either:

- **iovera° Treatment**: Subject undergoes treatment with the study device using a functioning Smart Tip.
- **Sham Treatment**: Subject undergoes treatment with the study device using a sham Smart Tip.

Randomization assignments will be stratified by study center with randomly chosen block sizes of 4 or 6. Small but randomly determined block sizes preserve treatment assignment balance within study center while maintaining assignment unpredictability. The Investigator or designee will record the randomization assignment in the source documentation and eCRF. Any Investigator who is discovered to tamper with randomization will be immediately terminated from the study.

10.5 iovera° Treatment/Visit 2 (-07 - 0 Days prior to TKA)

Medical history will be reviewed as well as concomitant medications and procedures. Blood pressure and heart rate will be assessed. The Investigator, or designee, will reconfirm all eligibility criteria are met.

The iovera° device will be prepared by the trained Investigator (or Sponsor designee) according to the ***User Guide***. If at any time the device does not perform as expected the Investigator (or designee) will follow procedures as outlined in the ***User Guide***.

Prior to the initiation of the iovera° treatment, the Investigator, or designee, will mark the treatment lines on the knee to be treated as shown in Figure 2 located in section 9.2. After marking the treatment lines, the study coordinator or designee will measure the knee circumference at the treatment lines per guidance in the ***Study Operations Manual.***

Then the skin along the treatment lines will be cleansed with alcohol. Lidocaine, no epinephrine, will be injected along the treatment lines superficially in order to achieve cutaneous anesthesia and to the bottom of the subcutaneous layer, atop the fascia, to temporarily block conduction of the infrapatellar branch of the saphenous nerve and the anterior femoral cutaneous nerve.

Once localized anesthesia of the treatment lines and the nerves is achieved, the Investigator, or designee, will complete the iovera° treatment along the treatment lines. Regardless of the length of treatment lines (width of patella or from lower pole of the

patella down to tibial tubercle), adjacent insertions are placed along the treatment lines until the entire line has been treated so all Subjects receive the same treatment. Treatment is not to be modified or influenced by any Subject response to changes in knee pain. Representatives of myoscience, Inc. may be present during the treatment. Photos of the treatment area may be taken.

Upon completion of treatment, the treatment areas will be cleansed and the skin will be left undressed. The Investigator or designee will assess the treatment areas for adverse events. Subjects will be asked to assess their treatment assignment (the blind).

The Subject will be instructed to report any adverse events to the Investigator between and at the follow-up visits. Photographs of the treatment area may be obtained post-treatment.

Subjects will be asked to bring all prescription opioid bottles to all study follow-up visits. A pill count will be completed by the study coordinator, or designee.

10.6 Visit 3 / Total Knee Arthroplasty (Day 0)

The Investigator or Designee will confirm their ability to safely perform TKA within the following protocol requirements. All surgical and in-patient data, safety information including adverse event assessments, will be collected retrospectively from hospitalization records:

- Pre-operative History & Physical Exam, including assessment of the target knee, must be documented in the medical record and included as study data.
- Surgery and Implant - Medial parapatellar surgical approach; cruciate-retaining or posterior-stabilized TKA; all surfaces replaced.
- Pre-emptive analgesia – medications administered prior to TKA, orally, intravenously, or otherwise, for the purpose of pain management before, during, or after surgery, are to be recorded in the medical record and included as study data.
- Procedural anesthesia – during Arthroplasty this will be limited to spinal anesthesia, the formulation, or "cocktail," of medications used are to be recorded in the medical record and included as study data.
- Local Infiltration Analgesia – permitted per surgeon or anesthesia preference, the formulation, or "cocktail," of medications used are to be recorded in the medical record and included as study data.
- Regional nerve block for post-operative analgesia – single shot Adductor Canal Block required, the formulation, or "cocktail," of medications used are to be recorded in the medical record and included as study data.
- Post-operative analgesia – opioid or other pain medications used are to be recorded in the medical record and included as study data
- Other data to be collected retrospectively from hospitalization medical records include: Time of admission to and discharge from the hospital, Length of surgery, Method of wound closure, Tourniquet use, Blood loss, Post-operative drain use, and Adverse Events, including Opioid-related adverse events - sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance, and respiratory depression.

Outpatient analgesia – The subject will be required to bring all of their unused prescription narcotic medications dispensed during study participation to all study follow-up visits for

pill count. Any other over-the-counter analgesic medications or other pain relief therapies used (per patient report) will also be recorded by the study coordinator, or designee, at each follow-up visit.

Subjects will be asked to bring all prescription opioid medications to all study follow-up visits. A pill count / accountability will be completed by the study coordinator, or designee.

10.7 Study Follow Up

Study-related follow-up visits occur at the investigational site at Week 2, Week 4, Week 6, and Week 12. The study's schedule of assessments is shown in Table 3.

10.7.1 Visit 4 / 2 Weeks Post-op (14 ±3 Days), Visit 5 / 4 Weeks Post-op (28 ±5 Days), and Visit 6 / 6 Weeks Post-op (42 ±5 Days)

Subjects will be evaluated and data collected per the schedule of events. Any changes in concomitant medications/concurrent procedures will be documented. Any anticipated observations, adverse events, adverse device effects and/or SAE/UADE/USADE will be assessed and documented. Photographs may be taken of the treatment area.

The Subject will complete a WOMAC questionnaire, NRS for Pain when standing from a seated position, PROMIS questionnaire, and SF-36 questionnaire. Subjects will be asked to assess their treatment assignment (the blind).

Subjects will answer Satisfaction questions at Visit 6.

The subject will complete the Physical Performance Measures in the following order under supervision of the investigator or designee:

- Active Range of Motion
- 40 Meter Walk Test
- 30 Second Chair Test

Detailed instructions for the conduct of these performance measures are included in the ***Study Operations Manual***. Assistive/ambulatory device usage and work status will also be assessed.

Utilization of healthcare services post-discharge, or since the previous follow-up visit, will be assessed. Subjects will be asked to report any Secondary Surgical Interventions, Emergency/Urgent care visits, Unscheduled physician office visits, and other healthcare services used since their last study visit. Investigators will also record any additional or unscheduled services performed at follow-up visits (examples include knee aspiration, unscheduled x-ray, other unscheduled diagnostic procedures, etc.).

Subjects will be asked to bring all prescription opioid bottles to all study follow-up visits. A pill count will be completed by the study coordinator, or designee, and opioid medication will be redispensed to subjects.

10.7.2 Visit 7 / 12 Weeks Post-op (84 ±7 days)

Subjects will be evaluated and data collected per the schedule of events. Any changes in concomitant medications/concurrent procedures will be documented. Any anticipated observations, adverse events, adverse device effects and/or SAE/UADE/USADE will be assessed and documented. Photographs may be taken of the treatment area.

The Subject will complete a WOMAC questionnaire, NRS for Pain when standing from a seated position, PROMIS questionnaire, and SF-36 questionnaire. Subjects will answer Satisfaction questions. Subjects will be asked to assess their treatment assignment (the blind).

The subject will complete the Physical Performance Measures in the following order under supervision of the investigator or designee:

- Active Range of Motion
- 40 Meter Walk Test
- 30 Second Chair Test

Detailed instructions for the conduct of these performance measures are included in the ***Study Operations Manual***. Assistive/ambulatory device usage and work status will be assessed. The total number of outpatient Physical Therapy visits conducted between hospital discharge and 12 Weeks post-op will also be obtained.

Utilization of healthcare services since the last follow-up visit will be assessed. Subjects will be asked to report any Secondary Surgical Interventions, Emergency/Urgent care visits, Unscheduled physician office visits, and other healthcare services used since their last study visit. Investigators will also record any additional or unscheduled services performed at follow-up visits (examples include knee aspiration, unscheduled x-ray, other unscheduled diagnostic procedures, etc.).

Subjects will be asked to bring all prescription opioid bottles to all study follow-up visits. A pill count will be completed by the study coordinator, or designee, and opioid medication will be redispensed to subjects.

At the completion of this visit, the Subject will be exited from the study unless the Subject has any ongoing device-related or iovera° treatment-related adverse events as described in section 10.8.

Table 3. Schedule of Assessments

Assessment	Visit 1 / Screening	Visit 2 / iovera° Treatment	Visit 3 / DAY OF TKA (Day 0)	Visit 4 / 2 Weeks Post-op	Visit 5 / 4 Weeks Post-op	Visit 6 / 6 Weeks Post-op	Visit 7 / 12 Weeks Post-op
Informed Consent	X						
Eligibility	X	X					
Medical history	X	X					
Concomitant medication assessment	X	X	X	X	X	X	X
Prior/Concurrent Therapy	X	X		X	X	X	X
Randomization*		X					
Study Treatment		X					
Physical Exam		X					
Vital signs	X	X					
Knee Circumference		X					
PROMIS Questionnaire	X			X	X	X	X
WOMAC Questionnaire	X			X	X	X	X
SF-36	X			X	X	X	X
NRS for Pain	X			X	X	X	X
Work Status Questions	X			X	X	X	X
Subject Satisfaction Questions						X	X
Assessment of Blind		X		X	X	X	X
Physical Performance Measures	X			X	X	X	X
Pain Medication Accountability				X	X	X	X
AE/SAE Assessment		X	X	X	X	X	X
Photographs (if applicable)		X		X	X	X	X

* Randomization occurs after the subject has met eligibility criteria and prior to or at Visit 2

Follow-up visits generally consist of the following:

- The Investigator or designee assesses the occurrence of health status changes and adverse events since last study visit;
- The Investigator or designee determines whether any changes in daily medications have occurred;
- The Investigator or designee performs drug accountability (pill counts) of post-operative outpatient opioid medication and re-dispenses unused opioid medications to subjects after use. The Investigator or designee records other over-the-counter concurrent pain medication use and pain relief therapies used per subject report;
- The Subject completes a WOMAC questionnaire, an SF-36 questionnaire, PROMIS questionnaire, Work Status Questions, Satisfaction questions (Visits 6 and 7 only),

self-assessment of the blind, and reports NRS for Pain when standing from a seated position;
- The Investigator or designee performs Physical Performance Measures (Range of Motion, 30 Second Chair Test and 40 Meter Walk Test).

10.8 Photographs

Photographs may be taken of the treatment area at the treatment visit and at the follow-up visits. Photographs will be labeled appropriately, stored electronically (e.g., JPEG, PNG or other relevant format) and sent to the Sponsor according to Sponsor instructions.

10.9 Study Exit

When the final study visit is complete, the Subject's participation in the study is complete and the Investigator, or designee, will complete the study exit eCRF.

If a Subject is experiencing an unresolved device or iovera° treatment related adverse events at the final study visit, the Subject will be followed by the Investigator until resolution occurs.

10.10 Subject Discontinuation

A Subject may be withdrawn from the study prior to completion for any of the following reasons:

- Voluntary withdrawal of consent
- Adverse event preventing continued study participation
- Investigator believes risk of further subject participation outweighs benefit
- Medical need for prohibited medications or treatment
- Persistent non-compliance or lost to follow-up (A Subject is considered lost to follow-up after the site makes 3 attempts to contact the via email or phone call and a certified letter is sent to the Subject.)

The Investigator/Coordinator will complete a study exit form in the eCRF 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 eCRF.

10.11 Study Termination

The Sponsor may terminate the study as a whole or at individual study sites under the following circumstances:

- Suspicion of risk to subjects, including occurrence of high rate of known AEs or unexpectedly high rate of unexpected AEs
- Poor site compliance with the study protocol
- Inadequate site enrollment
- Obtaining new scientific knowledge that shows that the study is no longer valid or necessary
- Persistent non-compliance with IRB or regulatory requirements
- Persistent failure to comply with obligations arising from the clinical trial agreement

- Other business reasons (e.g., insolvencies or business entity liquidation)

The Sponsor will document reasons for study suspension and notify relevant site Investigators and governing IRBs. If suspension occurred because of a safety issue, all Investigators will be notified. When terminating the study, the Sponsor and Investigator will assure that adequate consideration is given to the protection of the subjects' interests.

10.12 Study-Related Assessments

10.12.1 Western Ontario and McMaster Osteoarthritis Index (WOMAC)

The WOMAC is a tri-dimensional, disease-specific, patient-reported outcome measure. It consists of 24 questions with 5 questions regarding pain, 2 questions regarding stiffness and 17 questions regarding function. A copy of the WOMAC is located in Attachment 1.

Completion of the WOMAC questionnaire will take approximately 15 minutes to complete. After completion by the Subject, the study coordinator or designee will enter into the eCRF.

10.12.2 Numeric Rating Scale for Pain (NRS for Pain)

The Numeric 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 in reference to the target knee. 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 pain RIGHT NOW when standing from a seated position."
- "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 pain IN THE PAST 7 DAYS when standing from a seated position."

The Subject responds 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 2 minutes to complete. The study coordinator or designee will enter the data into the eCRF.

10.12.3 Patient Reported Outcomes Measurement Information System (PROMIS)

PROMIS® instruments use modern measurement theory to assess patient–reported health status for physical, mental, and social well–being to reliably and validly measure patient–reported outcomes (PROs) for clinical research and practice. PROMIS instruments measure concepts such as pain, fatigue, physical function, depression, anxiety and social function. A copy of the PROMIS is located in Attachment 2.

The PROMIS questionnaire will take approximately 10 minutes to complete. After completion by the Subject the study coordinator, or designee, will enter into the eCRF.

10.12.4 36-Item Short Form Health Survey (SF-36)

The SF-36 contains 36 questions assessing the Subject's health-related quality of life. A copy of the SF-36 is located in Attachment 3.

The SF-36 will take approximately 15 minutes to complete. After completion by the Subject, the study coordinator, or designee, will enter into the eCRF

10.12.5 Subject Satisfaction Questions

Subject satisfaction questions will be verbally administered by the study coordinator or designee. Questions will take approximately 3 minutes to complete. The Subject satisfaction questions are located in Attachment 4.

10.12.6 Self-reported Work Status Questions

The Self-reported Work Status Questions will be given to the subject for completion at Visit 1, Visit 4, Visit 5, Visit 6, and Visit 7. At Visit 1 / Screening, the questions are designed to assess the employment status of each subject prior to surgery. At each follow-up visit, the questions will reassess working status. Patients who return to work are asked whether they are doing the same type of work as prior to TKA. For applicable subjects, the self-reported date of Return to Work will be recorded. Subjects who have returned to work will also be asked if there are any limitations in the amount or type of work they are able to perform at their current job. Those subjects who have not returned to work will be asked the reason they have not returned to work.

The Self-reported Work Status Questions will take approximately 5 minutes to complete. The Work Status Questions are located in Attachments 5 and 6.

11. STATISTICAL METHODOLOGY AND ANALYSES

11.1 Introduction

This is a prospective, randomized, sham-controlled, multi-center, post-market trial to evaluate the iovera° device in treating pain associated with total knee arthroplasty (TKA). The study is designed to investigate whether iovera° treatment prior to TKA decreases cumulative patient opioid use over the course of 6 weeks following TKA while maintaining similar levels of pain relief.

The study will also investigate whether there is a relationship between patients treated with iovera° and length of hospital stay; patient reported pain and function as measured by WOMAC; patient satisfaction; and improved physical rehabilitation as measured by functional performance measures and range of motion.

The primary safety endpoint is the onset of device-related adverse events through twelve-weeks after TKA surgery. The primary measure of effectiveness is the Total Daily Morphine Equivalent (TME) of pain medications used from Day of Discharge to the 6-week follow-up visit. In addition, Change from Baseline in WOMAC Pain at the 6-week follow-up visit will be evaluated as a secondary endpoint. A variety of secondary effectiveness endpoints, including performance and pain assessments, will be measured.

Up to 8 study sites within the United States will screen a sufficient number of subjects to ensure that 150 subjects (Randomized 1:1 to approximate 75 Active Treatment subjects plus 75 Sham Treatment subjects) will be randomized and treated.

Subjects will be randomized either to receive therapeutic treatment with the iovera° treatment or to receive a sham treatment with the iovera° treatment, using a balanced 1:1 active-to-sham randomization stratified by Study Site.

11.2 Study Endpoints

11.2.1 Primary Effectiveness Endpoint

The primary effectiveness endpoint is the cumulative consumption of opioids from the time of hospital discharge to 6 weeks post-TKA Surgery. Opioid consumption will be converted to morphine equivalents and subject consumption will be verified by pill count at follow-up visits. The cumulative morphine equivalent will be divided by the number of days to provide the Total Daily Morphine Equivalent (TME) for the subject.

The difference in the average TME between the two treatment groups will be calculated as Active TME average – Sham TME average, such that a positive difference indicates that the Active Treatment Group had a larger average TME than the Sham Treatment group, while a negative value indicates the opposite. A negative difference between treatment groups reflects a positive outcome for the study.

A superiority hypothesis test using the two-independent sample t-test will be used to statistically evaluate the difference in the average TME between the two treatment groups. A confidence interval for the difference in the average TME between the two treatment groups will be provided to assist in the interpretation of the results.

The Primary Effectiveness Study Objective is met when the average TME for the Active Treatment group is less than the average TME for the Sham Treatment group, and the resulting t-test is statistically significant using a one-sided $\alpha = 0.025$ level of statistical significance

11.2.2 Secondary Effectiveness Endpoints

One of the secondary objectives of the study is to show that 6-weeks after treatment the Active Treatment group has a similar amount of pain relief as the Sham Treatment group. Change in WOMAC Pain from the Baseline visit to the six-week visit will be calculated for each subject as:

Change in WOMAC Pain =

6-Week Visit WOMAC Pain – Baseline Visit WOMAC Pain

such that a negative value indicates a decrease in the WOMAC Pain, while a positive value indicates the opposite.

The difference in the average Change in WOMAC Pain between the two treatment groups will be calculated as Active Change in WOMAC Pain average – Sham Change in WOMAC Pain average, such that a positive difference indicates that the Active Treatment Group had a larger average Change in WOMAC Pain than the Sham Treatment group, while a negative value indicates the opposite. Since a negative Change in WOMAC Pain is indicative of pain relief, then a negative difference between treatment groups reflects a positive outcome for the study.

A non-inferiority hypothesis test using the two-independent sample t-test will be used to statistically evaluate the difference in the mean Change in WOMAC Pain between the two treatment groups. A confidence interval for the difference in the average Change in WOMAC Pain between the two treatment groups will be provided to assist in the interpretation of the results.

The objective of this secondary endpoint is to show that the average reduction in WOMAC Pain for the Active Treatment group is not worse than the average reduction for the Sham Treatment group by more than one-half of the common standard deviation for the Change in WOMAC Pain scores. The objective is met when the resulting t-test is statistically significant using a one-sided $\alpha = 0.025$ level of statistical significance.

A variety of additional secondary effectiveness endpoints will be assessed to further characterize pain relief, functional performance, and range of motion. These include:

- Active Range of Motion
- 40 Meter Walk Test
- 30 Second Chair Test
- Length of Hospital Stay
- WOMAC Scores (Pain, Stiffness, and Function)
- SF-36 Scales and Summary Measures
- PROMIS–29 Profile Domains and Pain Intensity Score
- NRS for Pain

For each secondary effectiveness endpoint, the result, as well as the Change from Baseline value, will be summarized at each visit the endpoint is collected. Two sets of hypotheses tests will be performed: the paired t-test will be used to evaluate if the Change from Baseline result is statistically different from no change, while the two-independent sample t-test will be used to statistically evaluate the difference between the two treatment groups for both the original result and for the Change from Baseline result. Confidence intervals will be provided to assist in the interpretation of the results.

11.2.3 Safety

The frequency of each adverse event will be summarized by seriousness, severity and by relationship to the device. Since some subjects may report the same event several times (e.g., headache), the first occurrence of the worst reported case of the event will be used for the purpose of analysis. Refer to the User Guide for full list of expected adverse events.

11.3 Analysis Cohorts

Different groups of subjects, or Cohorts, will be identified depending on the type and extent of analysis being performed. These may include but are not limited to the following:

11.3.1 Screening Cohort

All subjects who are screened for the study will be included in the Screening Cohort. Only an accounting of the numbers of subjects screened in the study, plus the reasons given for subjects not enrolled in the study will be performed on this Cohort.

11.3.2 Safety/ITT (Intent to Treat) Cohort

All subjects who are randomized are included in the Safety/ITT Cohort. Subjects are analyzed in the treatment group they are randomized to.

11.3.3 Per-Protocol Cohort

The Per-Protocol Cohort is defined as the group of subjects who are randomized in the study, who receive the treatment they are randomly assigned to receive, and who complete their 6-week visit without any major protocol deviations.

11.3.4 Cohort for Primary Endpoint

The objective for the primary Effectiveness endpoint needs to be met for the study to be a success. The analysis of the primary Effectiveness endpoint, Total Daily Morphine Equivalent, will be made using the Per-Protocol Cohort.

11.3.5 Cohort for Secondary Endpoint

The Secondary Endpoint, Change in WOMAC Pain, will be made using the Per-Protocol Cohort. Analyses for all other secondary endpoints will be repeated using the Safety/ITT Cohort and the Per-Protocol Cohort.

11.4 Statistical Methods

11.4.1 General

Categorical data will be summarized using frequency tables, presenting the subject counts and relative percentages. McNemar's chi-square may be used to assess within-subject changes in a bivariate response variable.

Continuous variables will be summarized by the mean, standard deviation, median, minimum and maximum. Within-subject changes will be analyzed parametrically using the paired t-test if the differences are normally distributed, or non-parametrically using the sign-rank test if the differences are not normally distributed.

The SAS system or equivalent statistical package will be used to perform all analyses.

Exact confidence intervals will be generated for estimates of proportions. Asymptotic confidence intervals will be generated for estimates of means. Except where otherwise noted, the p-values of all tests will be reported without any correction for the multiplicity of tests performed.

11.4.2 Derived Data – Change from Baseline Parameters

Within-subject change-from-baseline (pre-treatment baseline) values for a parameter are calculated as:

Change-from-Baseline = Follow-Up value – Pre-Treatment value

such that a positive value indicates an increase from the pre-treatment value to the follow-up value, whereas a negative result indicates the opposite.

11.5 Handling of Dropouts or Missing Data

All endpoints will be analyzed "as-is" without any special data imputation methods used to replace missing data. Consequently, subjects missing results for a specific analysis will be excluded from that analyses.

11.6 Poolability

Data will be pooled from multiple study sites for this analysis. The justification for pooling is made on a clinical basis[1]. The basis for pooling comes from three critical factors: the study sites must implement one common protocol; the sponsor must provide monitoring of study site compliance; the study sites must use common data collection procedures.

In addition, poolability across study sites will be assessed by presenting results across study centers and by analyzing treatment-by-site interaction using a two-way analysis of variance with Treatment, Site, and Treatment-by-Site interaction as terms in the model. Assessment of treatment-by-site interaction will be assessed at a 0.15 level of significance. A non-significant interaction effect will support poolability of study sites. A significant treatment-by-site interaction, if deemed to be only quantitative in nature as opposed to qualitative (i.e., if only due to a difference in magnitude of treatment effect across sites as opposed to a difference in direction of treatment effect across sites) will not necessarily disqualify study sites from being pooled. Note that prior to assessing interaction sites with less than 5 subjects will be combined. To avoid the scenario that the data from the combined super-site dominate the trial conclusion, sites with less than 5 enrolled subjects be combined according to their geographical closeness, and once a super-site have five or more enrolled subjects, then the forming of this super-site will stop and the forming of a new super-site will start. Site by treatment interactions of a quantitative nature, (i.e., all sites show the treatment to be beneficial, but perhaps to a different degree by study site) will not be considered to be an impediment to pooling. Site by treatment interactions qualitative in nature (i.e., the vast majority of sites show the treatment to be beneficial, but one or more sites show the treatment to be detrimental) will require extensive evaluation of the sites with contrary results to attempt to determine what factors at those sites led to the result[2].

[1] Meinert, C. (1986). *Clinical Trials: Design, Conduct, and Analysis*. Oxford University Press, New York.
[2] Medical Devices Dispute Resolution Panel Meeting of September 6, 2001 (Panel Transcript)

Lastly, poolability between subjects younger than 65 years old and subjects that are 65 years or older will be assessed using the same methodology described above by replacing Site with Age in the analyses performed. Assessment of treatment-by-age interaction will be assessed at a 0.15 level of significance. A non-significant interaction effect will support poolability of subjects younger than 65 years old and subjects that are 65 years or older.

11.7 Subgroup Analysis

Analyses for the Primary Effectiveness endpoint and the Secondary Effectiveness endpoint will be repeated for subjects younger than 65 years old and for subjects that are 65 years or older.

11.8 Analysis Windows and Definitions

Follow-up visits through 6-weeks should adhere to a window of **±05** days when identifying the analysis visit. The reported-as visit will be used to identify the analysis visit for all follow-up visits after the 6-week visit.

11.9 Sensitivity Analyses

No imputations for missing data will be made for the Primary and Secondary Effectiveness Endpoints. All patients enrolled and randomized in the study will be included in all analyses. Sensitivity analyses may be considered and will be described in the final Statistical Analysis Plan for the study. Nevertheless, the totality of the results will be used when evaluating overall Study Success.

11.10 Sample Size Justification

Ultimately, the objective of this study is two-fold:

1. Primary Effectiveness Endpoint: Show that, on average, the Active iovera° treated subjects used less opioids than the Sham iovera° subjects.

2. Secondary Effectiveness Endpoint: Show that, on average, the Active iovera° treated subjects had similar pain relief as the Sham iovera° subjects.

A feasibility study was conducted to evaluate the safety and effectiveness of iovera°. In that study, the average (standard deviation) Total Daily Morphine Equivalent was 1826 mg (870 mg) among the iovera° subjects, and 2800 mg (1092 mg) among the non-iovera° subjects. All subjects were followed for 12 weeks. Assuming the following for the Primary Effectiveness Endpoint:

Statistical Hypothesis:	H_0: $\mu_{Active} \geq \mu_{Sham}$ vs H_1: $\mu_{Active} < \mu_{Sham}$, where μ_{Active} is the average Total Daily Morphine Equivalent for the Active Treatment group, and μ_{Sham} is the average Total Daily Morphine Equivalent for the Sham Treatment group.
Statistical Test:	two independent sample t-test
Statistical Significance:	one-sided $\alpha = 0.025$
Statistical Power:	$1 - \beta = 0.80$
Minimum Treatment Effect:	$\mu_{Active} - \mu_{Sham} = -7.14$ mg
Common SD:	$\sigma = 13.1$ mg

Minimum Sample Size: n = 54 per treatment group

It is expected that, on average, the Active treatment group will use 7.14 mg less opioids per day after 6-weeks of follow-up than the Sham treatment group. Assuming a common standard deviation of 13.1 mg for the Total Daily Morphine Equivalent and $1 - \beta = 0.80$ statistical power, the two independent sample t-test will be statistically significant at a one-sided $\alpha = 0.025$ level of statistical significance when there are a minimum of 54 analyzable subjects in the Active treatment group and 54 analyzable subjects in the Sham treatment group.

The study is also powered to show that, on average, the pain relief among Active subjects is not worse than the pain relief among the Sham subjects. The non-inferiority margin, Δ, represents how much worse the pain relief among the Active subjects can be than among the Sham subjects, and still be considered "not worse than" the Sham pain relief. In Escobar, et al[3]. the Minimum Detectable Change (MDC_{95}) for WOMAC Pain was calculated as 22.39. Standard deviations for the Change in WOMAC Pain in that paper ranged from 18 to 22, depending on the cohort. In the feasibility study, pain was assessed using the Knee injury and Osteoarthritis Outcome Score (KOOS) instrument. In that study, the average (standard deviation) Change in KOOS Pain at 6-weeks was -24.0 (13.5) among the iovera° subjects, and -18.3 (27.7) among the non-iovera° subjects. In this study, the non-inferiority margin is set at 11, a value roughly one-half the MDC_{95} quoted in Escobar, and that is also equal to roughly one-half of the estimated standard deviation of 22 for the Change from Baseline WOMAC Pain results. That is:

Non-Inferiority Margin (Δ) = 11

$\simeq$ Escobar $MDC_{95} / 2 = 22.39 / 2$

$\simeq$ Change from Baseline WOMAC Pain SD / 2 = 22 / 2

Assuming the following for the Major Secondary Effectiveness Endpoint:

Statistical Hypothesis: H_0: $\mu_{Active} \geq \mu_{Sham} + \Delta$ vs H_1: $\mu_{Active} < \mu_{Sham} + \Delta$,
where μ_{Active} is the average Change from Baseline in WOMAC Pain for the Active Treatment group, μ_{Sham} is the average Change from Baseline in WOMAC Pain for the Sham Treatment group, and Δ is the non-inferiority margin.

Statistical Test: two independent sample t-test

Statistical Significance: one-sided $\alpha = 0.025$

Statistical Power: $1 - \beta = 0.80$

Expected Difference: $\mu_{Active} - \mu_{Sham} = 0$

Common SD: $\sigma = 22$

Non-Inferiority Margin: $\Delta = 22 / 2 = 11$

Minimum Sample Size: n = 64 per treatment group

[3] Escobar A, Quintana JM, Bilbao A, Aróstegui I, Lafuente I, Vidaurreta I. Responsiveness and clinically important differences for the WOMAC and SF-36 after total knee replacement.Osteoarthritis Cartilage. 2007 Mar;15(3):273-80.

It is expected that, on average, the Active treatment group will have the same pain relief after 6-weeks of follow-up as the Sham treatment group. Assuming a common standard deviation of 22 for the Change from Baseline WOMAC Pain, a non-inferiority margin of 11, and $1 - \beta = 0.80$ statistical power, the two independent sample t-test will be statistically significant at a one-sided $\alpha = 0.025$ level of statistical significance when there are a minimum of 64 analyzable subjects in the Active treatment group and 64 analyzable subjects in the Sham treatment group.

Lastly, the study is powered to evaluate Length of Stay, the number of days from surgery to discharge. Assuming a common standard deviation of 1 day for Length of Stay and $1 - \beta = 0.80$ statistical power, the Wilcoxon rank-sum test will be statistically significant at a one-sided $\alpha = 0.025$ level of statistical significance when there are a minimum of 69 analyzable subjects in the Active treatment group and 69 analyzable subjects in the Sham treatment group.

To account for up to 10% of the subjects withdrawing early or not having data available for the analysis of the primary effectiveness endpoint, a total of 150 subjects (Randomized 1:1 to approximate 75 Active treatment subjects plus 75 Sham treatment subjects) will be randomized and treated.

12. ADVERSE EVENTS

The study Investigator and Coordinator will evaluate, characterize and record in the eCRF all adverse events (AEs) occurring in all subjects from the initiation of study treatment to study exit (or premature withdrawal). Device or procedure related AEs will be followed until resolution. AEs may be reported spontaneously by the subject or detected by the Investigator or coordinator. AEs should be evaluated for diagnoses not just symptoms (i.e., "angina", not "chest pain").

AEs may be either spontaneously reported or elicited during questioning and examination of a subject. All AEs must be completely recorded within the Subject's source documentation. All AEs will be evaluated by the Investigator for relationship to the iovera° device and to the treatment.

In addition to verbatim terms, the Sponsor will categorize all AEs using MedDRA preferred terms (PT) and system organ classes (SOC). Analysis will report both verbatim and MedDRA terms.

> Adverse events[4] (AEs) will be assessed continuously from the initiation of study treatment through study exit. Per ISO14155:2011, an AE is any untoward medical occurrence, unintended disease or injury, or untoward clinical signs in subjects, users or other persons, whether or not related to the investigational medical device. An AE can arise from any use of the device (e.g., off-label use, use in combination with any drug) and from any route of administration.
>
> Timely and complete reporting of all AEs assists the Sponsor in identifying any untoward medical occurrence, thereby allowing:
>
> 1) protection of the safety of study subjects;
> 2) a greater understanding of the overall safety profile of the device;
> 3) recognition of device-related ill effects;
> 4) appropriate modification of study protocols;

[4] Definition from ISO14155:2011

5) improvements in study design or procedures; and
6) adherence to worldwide regulatory requirements.

12.1.1 Adverse Device Effect (ADE)

Per ISO14155, an adverse device effect is an adverse event related to the use of an investigational medical device. Adverse events related to the use of iovera° include events resulting from insufficient or inadequate instructions for use, deployment, operation or any malfunction of the device. User error or intentional misuse of the device is also defined as an ADE.

ADEs may be either spontaneously reported or elicited during questioning and examination of a subject. All ADEs must be completely recorded within the subject's source documentation and reported to the Sponsor within 48 hours of Investigator becoming aware of the ADE.

12.1.2 Serious Adverse Event (SAE)/ Serious Adverse Device Effect (SADE)/Anticipated Serious Device Effect (ASADE)/ Unanticipated Serious Adverse Device Effect (USADE)

Per ISO14155, an international clinical trial standard, an SAE is an AE that:

1. Led to a death,
2. Led to a serious deterioration in the health of the subject that
    a. Resulted in a life-threatening illness or injury,
    b. Resulted in a permanent impairment of a body structure or a body function,
    c. Required in-patient hospitalization or prolongation of existing hospitalization,
    d. Resulted in medical or surgical intervention to prevent permanent impairment to body structure or a body function.
3. Led to fetal distress, fetal death or a congenital abnormality or birth defect

An event that is serious must be recorded on the AE worksheet and requires expeditious handling to comply with regulatory requirements.

Events NOT considered to be serious adverse events are:

- Hospitalizations for the treatment, which was elective or pre-planned, of a pre-existing condition that did not worsen, and

- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of "serious" given above and not resulting in hospital admission.

A serious adverse device effect (SADE) is an adverse device effect that has resulted in any of the consequences characteristic of an SAE per the ISO definition.

An SADE which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report is defined by ISO as an unanticipated serious adverse device effect (USADE). Accordingly, an SADE which by its nature, incidence, or severity has been previously been identified in

the current version of the risk analysis report is considered an anticipated serious adverse device effect (ASADE).

Any adverse events classified as "serious" by the Investigator or the Sponsor require expeditious handling and reporting to the Sponsor. All SAEs, whether or not the event was related to the study device or anticipated, must be immediately (within 24 hours of becoming aware of the SAE) reported to the sponsor by telephone, email, or confirmed facsimile transmission:

Tracey Henry, and/or Study Manager
myoscience, Inc.
510-933-1500 (office)
510-933-1501 (fax)
THenry@myoscience.com

12.2 AE Severity and Relatedness

Each AE occurring in the study will be characterized by the study Investigator as to severity (Table 4) and relatedness (Table 5).

Table 4. AE Severity Grading System.

Severity Grade	AE Description
Mild	AE is transient and easily tolerated by the subject, even if it causes discomfort
Moderate	AE causes the discomfort and interrupts usual activities
Severe	AE causes considerable interference with usual activities and may be incapacitating or life-threatening

Table 5. AE Relatedness Grading System.*

Grade	Relationship of AE to study device or procedure	Description
5	Definite	An event that follows a reasonable temporal sequence from administration of the study device; that follows a known or expected response pattern to the study device; and that is confirmed by improvement on stopping.
4	Probable	An event that follows a reasonable temporal sequence from administration of the study device; that follows a known or expected response pattern to the study device; and that is unlikely to have been caused by concurrent/underlying illness or other drugs, procedures, or other causes.
3	Possible	An event that follows a reasonable temporal sequence from administration of the study device; that follows a known or expected response pattern to the study device; but may have been caused by concurrent/underlying illness, drugs, procedure, or other causes.
2	Unlikely	An event that does not follow a reasonable temporal sequence from administration of the study device; that does not follow a known or expected response pattern to the study device, or most likely was caused by concurrent/underlying illness, drugs, procedure, or other causes, because of their known effects.
1	Not related	An event almost certainly caused by concurrent/underlying illness, drugs, procedure, or other causes.

*Note that change in medical condition occurring between Screening and the initiation of Study Treatment will be reported as a change in Medical History.

13. DEVICE TRACKING

The Sponsor will send the investigational devices to study sites. The Investigator must house study devices in a secure location. The Investigator must carefully and completely track receipt, use and disposition of all investigational devices. The Sponsor will track sending and receiving of devices. The Sponsor will monitor site device accountability periodically.

If a Sponsor representative or designee is present at the time of use, he/she may directly take possession of used device(s). All devices will be returned to the Sponsor after the study is complete.

14. DEVICE DEFICIENCIES AND MALFUNCTIONS

Throughout the study, the Investigator and study staff will report and document all device deficiencies and malfunctions related to the identity, quality, durability, reliability, safety or performance of the device. This includes reporting of device deficiencies/malfunctions that did not lead to an AE but could have if: 1) suitable action had not been taken, 2) intervention had not been made, or 3) circumstances had been less fortunate. If possible, the Investigator should return devices suspected of deficiency or malfunction to the Sponsor for analysis.

15. ETHICAL AND REGULATORY CONSIDERATIONS

15.1 Compliance with Good Clinical Research Practice

This study will be conducted in compliance with the principles of the Declaration of Helsinki, with the current Good Clinical Practice (GCP) guidelines and with other applicable regulations. The Investigator and all study staff will conduct the study in compliance with this protocol. Voluntary informed consent will be given by every subject prior to the initiation of any study-related procedures. The rights, safety and well-being of the study subjects are the most important considerations and prevail over the interests of science and society. All personnel involved in the conduct of this study must be qualified by education, training and experience to perform their assigned responsibilities.

15.2 Institutional Review Board (IRB) and Informed Consent

Before study initiation, the Investigator must have written and dated approval from the IRB for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The Investigator should also provide the IRB with a copy of the product labeling, information to be provided to subjects and any updates. The Investigator will submit documentation of the IRB approval to the Sponsor. Copies of all correspondence with the IRB regarding this study must be sent to the Sponsor.

The IRB-approved consent form must include all elements required by FDA, state, and local regulations, and may include appropriate additional elements.

The Investigator/designee will explain the study to each potential subject and the subject must indicate voluntary consent by signing and dating the approved informed consent form. The Investigator must provide the subject with a copy of the consent form in a language the subject understands. The Investigator will maintain documentation that informed consent was obtained prior to the initiation of any study-specific procedures.

Withdrawal of IRB approval of the Investigator's part in the investigation must be reported to the Sponsor within 5 working days.

15.3 Protocol Compliance

The Investigator will comply to the extent possible with the IRB-approved protocol. All deviations from the protocol must be documented. The Investigator will notify the Sponsor immediately if a deviation from the protocol was required to protect patient safety.

15.4 Protocol Revisions

Revisions to the study protocol can be made only by the study Sponsor. A revised protocol can be put into place only after governing IRB approval. All administrative letters must be submitted to the IRB for their information.

New or altered consent forms required by the IRB due to a protocol change must be signed by all subjects currently enrolled in the study and must be used for any subsequent subject enrollment.

15.5 Study Monitoring

Representatives of the Sponsor will visit all study sites intermittently to review study records and to directly compare them with source documents (including, but not limited to patient and hospital records), to discuss the study conduct with the Investigator and study staff and to verify that the Investigator, study staff and facilities remain acceptable for the conduct of the study.

Representatives of government regulatory authorities may also evaluate the study records, source documents, Investigator, study staff and facilities.

The Investigator should immediately notify the Sponsor of any audits of this study by any regulatory agency, and must promptly provide copies of any audit reports.

15.6 Safety Reporting

The Sponsor is responsible for performing ongoing safety evaluation in this study protocol. Sponsor activities regarding safety include:

- classification of all AEs,
- review of all AEs reported in the study,
- confirm site's classification of AEs in terms of severity and relatedness to the study device and/or study procedure,
- review of severity and relatedness with the study Investigator, especially when there is disagreement between the Investigator and the Sponsor,
- review of device deficiencies and malfunctions, including determination and documentation of whether deficiencies/malfunctions could have led to an AE or SAE,
- ensuring the reporting of all SAEs and device deficiencies/malfunctions that could have led to an AE or SAE to the IRB and, if required, regulatory authorities in a timely fashion,
- informing all site Investigators in writing of all SAEs at all sites in a timely fashion and
- updating the risk analysis and assessment of corrective or preventive actions potentially required as a result of new information obtained in the investigation

The Sponsor will evaluate all serious adverse events against US reporting requirements (Medical Device Reporting, 21 CFR 812) and Medical Device Directive (vigilance incident reporting) as per its standard operating procedures. The Sponsor will investigate each SAE to determine whether the event represents an unanticipated serious adverse device effect (USADE, see Section 12.1.2). The Sponsor will report any event to regulatory authorities, Investigators and reviewing IRBs/ECs as necessary. If an investigation shows that a USADE presents an unreasonable risk to subjects, the Sponsor will terminate all investigations or parts of investigations presenting that risk as soon as possible. The Sponsor will only resume a terminated investigation after corrective actions have taken place, site Investigators are informed and IRBs/ECs have been notified and given approval to resume the study.

15.7 Electronic Case Report Forms/Electronic Data Capture

The study will use an electronic data capture (EDC) system to implement electronic case report forms (eCRF). The system will allow compliance with 21 CFR 11 Electronic Signatures. All CRFs are housed in the EDC system. The Investigator and Coordinator will be trained in use of the eCRF prior to study initiation. Retraining in use of EDC can occur at any time. The EDC system will be validated prior to use.

An eCRF is required and should be completed for each randomized Subject. The Investigator has ultimate responsibility for the collection and reporting of all data entered on the eCRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring and available when required. The eCRFs must be signed by the Investigator to attest that the data contained therein are true.

The site will be provided with eCRF Completion Guidelines which will assist in data entry and data issues/questions. All persons allowed to enter or change eCRF data must appear on the Delegation of Responsibilities Log.

The Sponsor will remotely monitor eCRFs to identify possible data errors. The system will have query mechanism whereby the site Coordinator can respond to Sponsor queries. All data discrepancies will be resolved prior to database lock.

15.8 Quality Assurance Audits

Sponsor representatives or designees may conduct site quality assurance (QA) audits during the study. The Investigator must agree to provide the auditor with direct access to all relevant documents and discuss any findings with the auditor.

In the event of an inspection by the FDA or other regulatory authorities, the Investigator must give the inspector direct access to relevant documents and to discuss any findings with the inspector. The Investigator must notify myoscience, Inc. in the event of a FDA site audit.

15.9 Confidentiality

The Investigator is responsible for ensuring the confidentiality of subjects throughout the trial. A unique identification code will be assigned to each Subject participating in this trial. Any data that may be published in abstracts, scientific journals, or presented at medical meetings will reference a unique subject code and will not reveal the subject's identity.

15.10 Records Retention

The Investigator must maintain all study records (including device disposition, informed consents, source documents, correspondence, regulatory documents, contracts etc.) for at least 2 years after study completion. At the Investigator's discretion, all records may be sent to the Sponsor for permanent storage.

The Investigator must contact the Sponsor or designee prior to destroying any records associated with this study. If the Investigator withdraws from the study, all study-associated records must be transferred to a mutually agreed upon designee. Written notification of such a transfer must be given to the Sponsor or designee.

15.11 Publication and Reporting of Study Results

The study will be registered with clinicaltrials.gov before the first patient is treated. Study results will be documented in a study report that will be signed by myoscience representatives and by the Principal Investigator of the entire Study. Individual site Principal Investigators will not be required to sign this report.

The results of this myoscience sponsored study will be published in accordance with standard editorial and ethical practices. Results from multi-center studies must be published or presented at congresses only in their entirety with data pooled from all centers. Individual Investigators may not publish data from individual centers, unless granted specific written permission by myoscience to do so.

The list of authors of any formal publication or presentation of study results may include, as appropriate, representatives of myoscience.

16. SELECTED REFERENCES

[1] Ethgen, O., Bruyere, O., Richy, F., Dardennes, C., Reginster, J. Y. 2004. Health-related quality of life in total hip and total knee arthroplastyA qualitative and systematic review of the literature. *The Journal of Bone & Joint Surgery*,*86*(5), 963-974.

[2] Jones, C. A., Beaupre, L. A., Johnston, D. W. C., Suarez-Almazor, M. E. 2005. Total joint arthroplasties: current concepts of patient outcomes after surgery. *Clinics in geriatric medicine*, *21*(3), 527-541.

[3]Paul, J. E., Arya, A., Hurlburt, L., Cheng, J., Thabane, L., Tidy, A., Murthy, Y. 2010. Femoral nerve block improves analgesia outcomes after total knee arthroplasty: a meta-analysis of randomized controlled trials. *Anesthesiology*,*113*(5), 1144-1162.

[4]American Society of Anesthesiologists Task Force on Acute Pain Management. 2004. Practice guidelines for acute pain management in the perioperative setting: an updated report by the American Society of Anesthesiologists Task Force on Acute Pain Management. *Anesthesiology*,*100*, 1573-1581.

[5] Veterans Health Administration. VHA/DoD clinical practice guideline for the management of postoperative pain.Washington, DC 2002.

[6] Kurtz, S., Ong, K., Lau, E., Mowat, F., Halpern, M. 2007. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *The Journal of Bone & Joint Surgery*, *89*(4), 780-785.

[7] Allen, H. W., Liu, S. S., Ware, P. D., Nairn, C. S., Owens, B. D. 1998. Peripheral nerve blocks improve analgesia after total knee replacement surgery.*Anesthesia & Analgesia*, *87*(1), 93-97.

[8] Wang, H., Boctor, B., Verner, J. 2002. The effect of single-injection femoral nerve block on rehabilitation and length of hospital stay after total knee replacement. *Regional anesthesia and pain medicine*, *27*(2), 139-144.

[9] Goyal, N., McKenzie, J., Sharkey, P. F., Parvizi, J., Hozack, W. J., Austin, M. S. 2013. The 2012 Chitranjan Ranawat Award: intraarticular analgesia after TKA reduces pain: a randomized, double-blinded, placebo-controlled, prospective study. *Clinical Orthopaedics and Related* 2013, Volume 471, Issue 1, pp 64-75

[10] Hebl, J. R., Dilger, J. A., Byer, D. E., Kopp, S. L., Stevens, S. R., Pagnano, M. W., Hanssen, A.D., Horlocker, T. T. 2008. A pre-emptive multimodal pathway featuring peripheral nerve block improves perioperative outcomes after major orthopedic surgery. *Regional anesthesia and pain medicine*, *33*(6), 510-517
[11] Meissner, B., Nelson, W., Hicks, R., Sikirica, V., Gagne, J., Schein, J. 2009. The rate and costs attributable to intravenous patient-controlled analgesia errors. *Hospital Pharmacy*, *44*(4), 312-324.
[12] Strassels, S. A., Chen, C., Carr, D. B. 2002. Postoperative analgesia: economics, resource use, and patient satisfaction in an urban teaching hospital. *Anesthesia & Analgesia*, *94*(1), 130-137.
[13] McCartney, C. J., Nelligan, K. 2014. Postoperative Pain Management After Total Knee Arthroplasty in Elderly Patients: Treatment Options. *Drugs & aging*,*31*(2), 83-91.
[14] White, P. F. 2002. The role of non-opioid analgesic techniques in the management of pain after ambulatory surgery. *Anesthesia & Analgesia*, *94*(3), 577-585.
[15] Evans (1981). "Cryoanalgesia: the response to alterations in freeze cycle and temperature". *British Journal of Anesthesiology* **53** (11): 1121–1127
[16] Tetzlaff W, Bisby MA (1989) Neurofilament elongation into regenerating facial nerve axons. Neuroscience 29: 659-666.
[17] Data on File, Vinod Dasa, MD, 2014. Retrospective chart review of patients treated with iovera° prior to TKA. *Unpublished data.*