Title: Cryoanalgesia to Treat Post-Amputation Phantom Limb Pain: A Multicenter, Randomized, Double-Masked, Placebo-Controlled, Definitive Human Subjects Clinical Trial

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UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN

Instructions for completing the Research Plan are available on the HRPP website.
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General Instructions: Enter a response for all topic headings.
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Version date: 9/30/2013

1. PROJECT TITLE

Cryoanalgesia to Treat Post-Amputation Phantom Limb Pain: A Multicenter, Randomized, Double-Masked, Placebo-Controlled, Definitive Human Subjects Clinical Trial

2. PRINCIPAL INVESTIGATOR

Brian M. Ilfeld, MD, MS

3. FACILITIES

UCSD hospitals and the UCSD CTRI

4. ESTIMATED DURATION OF THE STUDY

Five years (1 year regulatory and preparation, 2 years enrollment, 1 year follow-up, 1 year analysis and publication)

5. LAY LANGUAGE SUMMARY OR SYNOPSIS (no more than one paragraph)

When a limb is severed, pain perceived in the part of the body that no longer exists often develops and is called "phantom limb" pain. Unfortunately, phantom pain goes away in only 16% of afflicted individuals, and there is currently no reliable definitive treatment. The exact reason that phantom limb pain occurs is unclear, but when a nerve is cut—as happens with an amputation—changes occur in the brain and spinal cord that actually increase with worsening phantom pain. These abnormal changes may often be corrected by putting local anesthetic—called a "nerve block"—on the injured nerve, effectively keeping any "bad signals" from reaching the brain with a simultaneous resolution of the phantom limb pain. However, when the nerve block resolves after a few hours, the phantom pain returns. But, this demonstrates that the brain abnormalities—and phantom pain—that occur with an amputation are not necessarily fixed, and may be dependent upon the "bad" signals being sent from the injured nerve(s), suggesting that a very long peripheral nerve block—lasting many months rather than hours—may permanently reverse the abnormal changes in the brain, and provide definitive relief from phantom pain. A prolonged nerve block lasting a few months may be provided by freezing the nerve using a process called "**cryoneurolysis**". ***The ultimate objective of the proposed research study is to determine if cryoanalgesia is an effective treatment for intractable post-amputation phantom limb pain.*** The proposed research study will include subjects with an existing lower extremity amputation who experience intractable daily phantom limb pain. A single ultrasound-guided treatment of cryoneurolysis (or sham block—determined randomly like a flip of a coin) will be applied to the target nerve(s) involved with the phantom pain. Although not required, each subject may return four months later for the alternative treatment (if the first treatment is sham, then the second treatment would be cryoneurolysis) so that all participants have the option of receiving the active treatment. Subjects will be followed for a total of 12 months with data collected by telephone.

6. SPECIFIC AIMS

The ultimate objective of the proposed research is to determine if cryoanalgesia is an effective

treatment for intractable post-amputation phantom limb pain.

Primary Specific Aim: **To test the influence of a cryoanalgesia treatment as compared to sham/placebo on the intensity of existing, intractable phantom limb pain resulting from an amputation.**

Hypothesis 1: Phantom limb pain ***intensity*** will be significantly decreased 4 months following one cryoneurolysis procedure (as measured by the Numeric Rating Scale within the Brief Pain Inventory).

Secondary Specific Aim: **To test the influence of a cryoanalgesia treatment as compared to sham/placebo on the quality of life for individuals with intractable phantom limb pain resulting from an amputation.**

Hypothesis 2a: Perception of **well-being** will be significantly improved 4 months following one cryoneurolysis procedure (as measured with the Patient Global Impression of Change Scale).

Hypothesis 2b: Physical and emotional **functioning** will be significantly improved 4 months following one cryoneurolysis procedure (as measured with the Brief Pain Inventory).

Hypothesis 2c: **Depression** will be significantly decreased 4 months following one cryoneurolysis procedure (as measured with the Beck Depression Inventory).

7. BACKGROUND AND SIGNIFICANCE

Of American veteran and civilian amputees, 35-98% (depending on the study) develop chronic, intractable pain perceived as being from the missing limb, a phenomenon termed "phantom limb pain".[1-3] The pain is usually described as "shooting, stabbing, boring, squeezing, throbbing, and burning".[1,3] Unfortunately, phantom pain resolves in **only 16%** of afflicted individuals (with or without treatment).[4] The rest will experience phantom pain for the remainder of their lives, with most becoming dependent upon chronic opioid use to gain even a small degree of relief. There is currently no reliable, definitive treatment for phantom limb pain.[5]

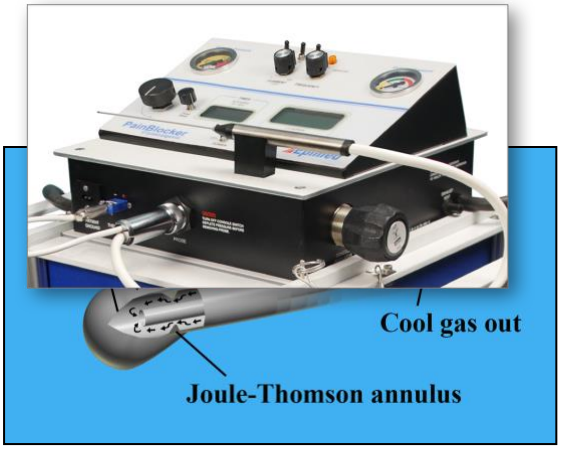
Current evidence suggests that when a nerve is severed, the barrage of nociceptive input triggers a complex interaction between the peripheral and central nervous system. Both systems are dynamic, and injury to peripheral nerves provokes changes in the dorsal horn, thalamus, and cerebral cortex which are referred to as "neuronal plasticity".[6] Reorganization at the level of the spinal cord may result in "sensitization" in which dorsal root ganglion cells become hyperactive, resulting in stump allodynia and hyperalgesia.[7] However, it is the somatosensory cortex within the brain that creates a "map" of the body—each location represented in a specific area of the cortex (i.e. homunculus); and deafferentation of neural pathways often results in changes of the cortical somatotopic map.[8] For example, the zone of the cortex representing the fingers may be invaded by adjacent areas following a hand amputation and subsequent deafferentation.[9]

Imaging techniques such as functional MRI have documented a correlation between phantom limb pain and cortical reorganization—the more intense the phantom pain, the greater the cortical changes.[8] When the neural input from an amputated limb was blocked with a single injection of local anesthetic (a peripheral nerve block) in 6 subjects, 3 had ***immediate, complete resolution of their phantom pain***; and, ***within minutes the cortical abnormalities were corrected for these three individuals***.[10] Unfortunately, when the single-injection nerve block resolved after a few hours, the phantom pain returned. But, this intriguing result suggests that ***a prolonged peripheral nerve block—lasting multiple weeks rather than hours—may permanently reorganize cortical pain***

mapping, thus providing <u>lasting</u> relief from phantom pain.

A "continuous peripheral nerve block"—administration of local anesthetic through a percutaneously-inserted perineural catheter—may provide a prolonged block.[11] In an uncontrolled series of 19 patients with phantom pain treated with continuous blocks, pain intensity was halved at 1 and 6 months.[12] However, the sensory block using this technique is frequently incomplete and/or inconsistent; the infusion duration usually limited to less than one week due to the risk of infection and difficulty of carrying a large bag of local anesthetic; and catheter dislodgement is relatively common.[11] A more reliable, complete block of longer duration would theoretically increase any treatment effects for phantom limb pain, while reducing these inconveniences and complications.

An alternative analgesic technique is <u>cryoneurolysis,</u> consisting of the application of exceptionally low temperatures to *reversibly* ablate peripheral nerves, resulting in temporary pain relief termed "cryoanalgesia".[13] The intense cold temperature at the probe tip produces Wallerian degeneration—a reversible breakdown of the nerve axon—subsequently inhibiting transmission of afferent and efferent signals. Because the nerve endoneurium, perineurium, and epineurium remain intact, the axon regenerates along the exoskeleton at a rate of approximately 1-2 mm/day.[14] While cryoneurolysis of peripheral nerves through surgical incisions has been commonly used to treat pain since 1961,[15] the development of cryo probes that may be inserted percutaneously promise a revolution in the use of this modality. The combination of newly-designed cryoneurolysis devices (above right), the narrow-gauge probes (immediate right), and ultrasound[16,17] now make percutaneous cryoanalgesia as simple as placing a peripheral nerve block: the probe tip is inserted adjacent to the target nerve under ultrasound guidance, and a series of 1-minute freezing cycles are administered followed by probe withdrawal.[13] ***The procedure is essentially the same as placing an ultrasound-guided peripheral nerve block; however, instead of injecting local anesthetic, a gas circulates through the probe, inducing cold at the tip and freezing the target nerve.*** Nothing remains within the patient and there is no external equipment to prepare or manage. Importantly, cryoneurolysis and the cryo probes are already approved by the United States Food and Drug Administration for the treatment of pain, including phantom limb pain, so no additional regulatory approval is required for the proposed clinical trial.



Theoretical benefits of cryoneurolysis include an ultra-long duration of action without opioid involvement, no catheter management/removal, the lack of an infusion pump and anesthetic reservoir to carry, an extraordinarily-low risk of infection (approaching zero), and no risk of local anesthetic toxicity, catheter dislodgement or leakage. With a single 8-minute percutaneous cryoneurolysis procedure consisting of several freeze/defrost cycles, an absolute truncation of nerve conduction is induced for 6-8 weeks with the complete restoration of nerve structure and function following remyelination.[16-18]

Over 200,000 traumatic and surgical amputations occur annually within the United States alone;[19-29] with an estimated 1.6-million people living with an amputation, and this number is expected to double by 2050.[30] A disproportionately large percentage of this population is comprised of active duty personnel and veterans due to combat trauma, peripheral vascular disease, and diabetes.[31,32] The combination of increased munitions force,[33] use of improvised explosive devices,[26,32] and casualty survival rates has resulted in a dramatic increase in the percentage of recently-injured combat

veterans living with a traumatic amputation.[23,27] Additionally, traumatic amputations have occurred in every major military conflict, leaving tens-of-thousands of United States Armed Forces veterans with missing limbs. Furthermore, veterans undergo amputation due to peripheral vascular disease and diabetes at a rate 250% higher than the general population; over 10% of all male amputees within the United States each year are veterans.[31]

Of veteran amputees, 35-98% (depending on the study) develop chronic, intractable phantom limb pain.[1] Similarly, within the civilian population, the published incidence of phantom pain from 16 different studies ranged from 50-95%.[2,3,34] Phantom pain resolves in only 16% of afflicted individuals.[4] Chronic pain greatly decreases quality-of-life and the chances of return to duty or civilian work;[4,35] and the economic toll for chronic nonmalignant pain—including phantom pain—is over $100-billion annually within the United States[29]

There is currently no reliable treatment for phantom limb pain.[5] While more than 43 methods for treating phantom pain have been described,[36] the placebo effect is common,[37] and prolonged relief is experienced by fewer than 10% of treated patients (6% of untreated patients ultimately experience spontaneous resolution).[38] Evidence of the intractable nature of phantom pain may be found in a survey of more than 10,000 amputees which reported a 1% treatment success rate.[39] **Therefore, the overwhelming majority of people suffering from phantom limb pain are dependent upon opioid analgesics to gain even a small degree of relief.** There are few data from randomized trials to guide treatment, leading the authors of a major review to conclude that there remains a substantial "gap between research and practice in the area of phantom limb pain".[5]

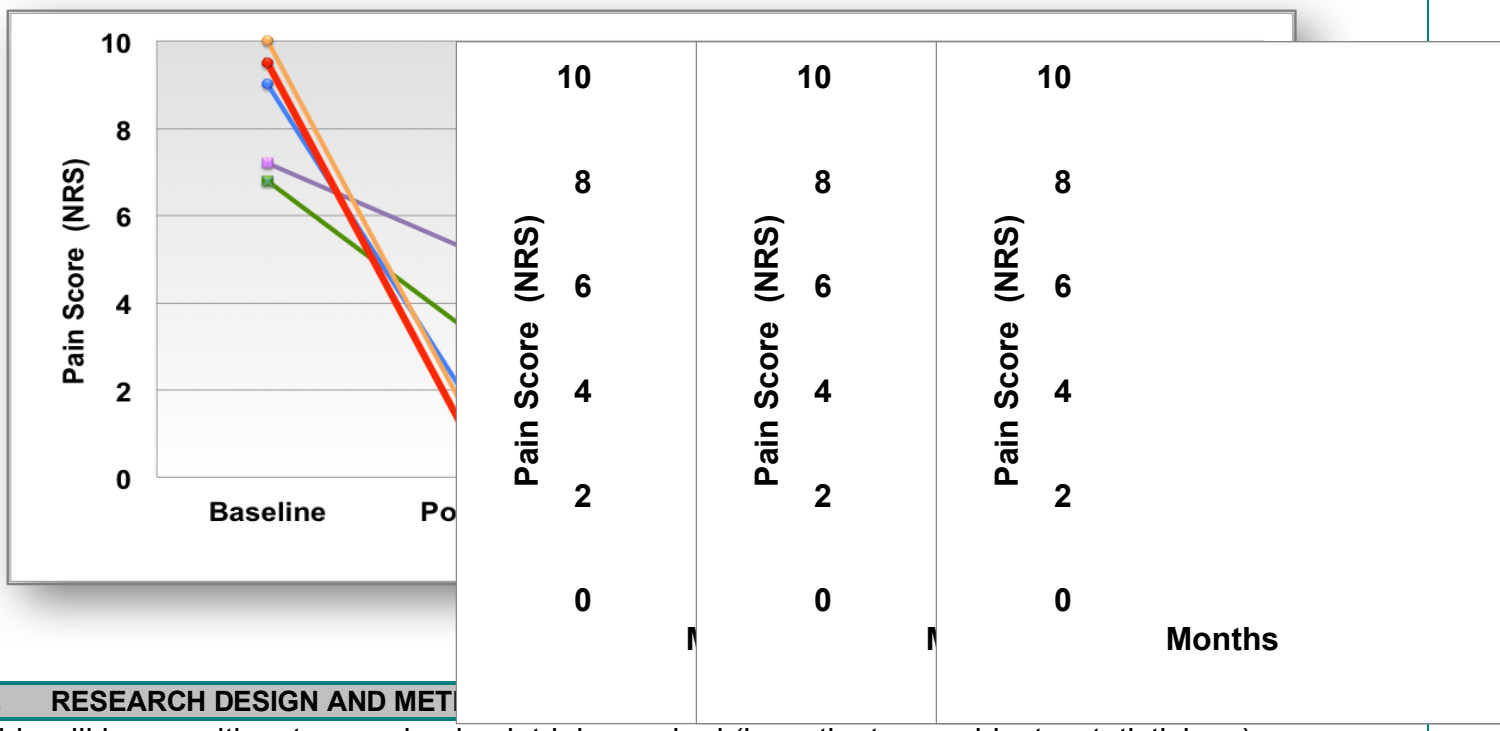
Given chronic pain's enormous costs to individuals and society—and the intractable, currently-untreatable nature of phantom limb pain with concurrent opioid dependence—it is imperative that an effective treatment be developed. If our study demonstrates that ultrasound-guided percutaneous cryoneurolysis is a successful treatment, the resulting impact on the hundreds-of-thousands of Americans suffering from intractable phantom limb pain will be **immediate and profound**, as (1) healthcare providers within the United States Armed Forces, Veterans Affairs Medical Centers, and civilian hospitals already have expertise placing ultrasound-guided peripheral nerve blocks; (2) cryoneurolysis is a relatively inexpensive, single-procedure, outpatient treatment with few complications; and (3) cryoanalgesia is already approved by the United States Food and Drug Administration.

8. PROGRESS REPORT

We have completed a pilot study suggesting that a single percutaneous cryoneurolysis treatment holds great promise to provide very long-term—possibly permanent—phantom limb pain relief. In the residual limb of five subjects, a local anesthetic-based peripheral nerve block was applied to the nerve corresponding to the location of the phantom pain (e.g., the sciatic nerve for phantom foot pain), with percutaneous cryoneurolysis subsequently administered in the same location. Follow-up occurred after 3 and 6 months, and then every 6 months thereafter. For the 3 subjects whose phantom pain resolved following local anesthetic injection (labeled "full responders" as defined with a numeric rating pain score [NRS] ≤ 1 following treatment), **all three reported an NRS ≤ 1 for the 2-5 year follow-up period; and, achieved this level of comfort without requiring any opioids, gabapentin, or additional analgesics (Figure below).** These subjects had upper or lower amputations due to peripheral vascular disease or trauma. The remaining two subjects similarly experienced a significant decrease in their phantom limb pain immediately following cryoneurolysis of at least 2 points on the NRS (29% and 57% decreases), but did not have near-resolution of their phantom pain with the previous local anesthetic injections. These two subjects continued to

experience decreased phantom pain for 5 months until both expired (cause of death unrelated to phantom pain treatment). There were no cryoanalgesia-related complications. ***Importantly, nearly identical results were reported by Prologo, and colleagues, in a similar pilot study involving 21 subjects (currently In Press).***

Figure. *Pilot study results:* phantom limb pain measured on a Numeric Rating Scale (NRS) of 0-10 prior to and following cryoneurolysis. "Partial responders" experienced decreased phantom pain following a local-anesthetic based peripheral nerve block on the NRS, but the NRS did not ultimately fall to ≤ 1.



9. RESEARCH DESIGN AND METH

This will be a multicenter, randomized, triple-masked (investigators, subjects, statisticians), sham/placebo-controlled, parallel (with optional crossover), human-subjects clinical trial to determine if cryoanalgesia is an effective treatment for intractable post-amputation phantom limb pain. We have included a diverse group of recruitment sites that will provide a broad representative patients sample. Study participants will be recruited at 7 centers, including 2 U.S. military, 2 Veterans Affairs, and 3 civilian university medical centers (1 private, 2 public), within a wide geographic range including the South, Midwest, East and West Coasts, providing a study sample with ethnic, racial, geographic and socioeconomic diversity.

U.S. military medical centers:

- Walter Reed National Military Medical Center, Bethesda, Maryland
- Naval Medical Center San Diego, San Diego, California

Veterans Affairs medical centers:

- Palo Alto Veterans Affairs Medical Center, Palo Alto, California
- Pittsburgh Veterans Affairs Healthcare System, Pittsburgh, Pennsylvania

Civilian university medical centers:

- Cleveland Clinic, Cleveland, Ohio
- University of California San Diego, San Diego, California

- University of Florida, Gainesville, Florida

All protocols and study materials will be approved by each center's Institutional Review Board; and, the study will be prospectively registered on the clinicaltrial.gov website. The study will be overseen by both a medical monitor (Salim Hayek, MD, PhD; Case Western Reserve University; Cleveland, Ohio)—in essence a study subject advocate—as well as a Data Safety Monitoring Board comprised of the medical monitor, a physician familiar with the ethical conduct of clinical research, and statistician. The Medical Monitor is responsible to oversee the safety of the research and report observations/findings to the IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the Human Research Protections Office. The medical monitor and DSMB will review enrollment, study data, protocol violations, adverse events, and oversee all aspects of the clinical trial every one and six months, respectively, through data analysis. All procedures are considered experimental given these are volunteers and they would not receive these treatments without study participation—while cleared by the FDA for use in all chronic and acute pain states, cryoneurolysis has not been demonstrated as effective to treat phantom limb pain, and it is not considered "standard-of-care" at either UCSD or other institutions.

Enrollment. Enrolling centers will recruit patients from four sources: (1) surgical and chronic pain *databases*; (2) amputation, surgical, and chronic pain *clinic referrals*; (3) print and internet/web *advertisements*; and (4) *clinicaltrials.gov*. Patients who are interested in the study will be required to give permission for a research coordinator or investigator to contact them to adhere to Health Insurance Portability and Accountability Act (HIPAA) requirements. In addition, with IRB-approval, patients within multiple database types will be informed of the study *via* the United States postal service in the form of an IRB-approved letter. The letters will include research contact information for patients with interest in study participation.

Research coordinators or investigators will both explain the study protocol to interested patients, and subsequently review the inclusion/exclusion criteria. Subjects meeting inclusion/exclusion criteria and desiring study participation will be scheduled for a diagnostic injection and cryoneurolysis procedure at the nearest enrolling center. Written, informed consent will be obtained from each participant prior to any measurements and/or procedures. It is anticipated that this will require 15-30 minutes for each subject.

The method of documenting consent will be using written informed consent forms approved by the local Institutional Review Board. Subjects will be asked to make no changes to their analgesic regimen for at least 1 month prior to the cryoneurolysis procedure and continuing for 4 months until the measurement of the primary end point—for the duration of the study, *all* patients will be allowed to continue their pre-intervention analgesics. In other words, subjects will continue taking the same analgesics during the study period as they were receiving prior to the study period, including their standard rescue analgesics—the study protocol simply freezes the analgesic regimen from 1 month prior to the intervention until the primary end point is measured 4 months later.

Subjects will be asked to not eat or drink after midnight the night before the procedure. For women

of childbearing age with the possibility of pregnancy, a sample of urine will be collected before any study interventions to confirm a non-pregnant state. All subjects will have a peripheral intravenous (IV) catheter inserted, standard noninvasive monitors applied (blood pressure cuff, pulse oximeter, 5-lead ECG), and oxygen administered *via* a facemask or nasal cannula. Oral and intravenous sedatives and analgesics such as Midazolam, valium and fentanyl will be titrated for patient comfort if necessary, while ensuring that patients remained responsive to verbal cues.

The specific nerves targeted will be the sciatic and femoral (or their distal branches). The potential cryoneurolysis sites will be cleansed with chlorhexidine gluconate and isopropyl alcohol. Using the optimal ultrasound transducer for the specific anatomic location and subject anatomy (linear vs curvilinear array), the target nerves will be identified in a transverse cross-sectional (short axis) view. A local anesthetic skin wheal will be raised adjacent to the ultrasound transducer and a Tuohy-tip needle will be inserted through the skin wheal in-plane beneath the ultrasound transducer and directed until the needle tip is immediately adjacent to the target nerve. Local anesthetic (1-3 mL, lidocaine 2%) will be injected in divided doses with frequent aspiration. This will be repeated for the second target nerve (either femoral or sciatic). Within 20 minutes of the second injection, the subject's limb pain level will be evaluated on the 0-10 NRS and if higher than at baseline prior to injection, the subject will NOT continue with treatment and their participation in the study will terminate upon discharge.

Treatment group assignment (randomization). Remaining subjects will be allocated to one of two possible treatments:

1. *cryoneurolysis*
2. *sham cryoneurolysis (placebo control)*

Randomization will be stratified by enrolling institution in randomly chosen block sizes. Randomization lists will be created using computer-generated tables by the Cleveland Clinic. Treatment group assignment will be conveyed to the enrolling sites via the same secure web-based system (RedCap) used to collect and collate all post-intervention endpoints (see "Data Collection" paragraph below). The first digit of the randomization numbers will be a letter denoting the enrolling center (of 7 possible); and, the next two digits will be integers beginning from 1 and increasing for each center. Cryoneurolysis probes are available that either (1) pass nitrous oxide to the tip inducing freezing temperatures; or, (2) vent the nitrous oxide at the base of the probe so that no gas reaches the probe tip, resulting in no temperature change (PainBlocker, Epimed, Farmers Branch, Texas). Importantly, these probes are indistinguishable in appearance, and therefore treating physicians, subjects, and all clinical staff will be masked to treatment group assignment [only one individual at each institution will be unmasked and will provide the treating physician/investigator with the correct probe after opening the randomization envelope]. Unmasking will not occur until statistical analysis is complete (termed "triple masked").

Intervention. The potential cryoneurolysis sites will be again cleansed with chlorhexidine gluconate and isopropyl alcohol. With the same ultrasound transducer used to previously administer local anesthetic, the target nerve will again be identified in a transverse cross-sectional (short axis) view at or distal to the deposition of local anesthetic. A cryoneurolysis device (PainBlocker, Epimed, Farmers Branch, Texas) will be used with the appropriate randomization-designated probe (either active or sham/placebo) and nitrous oxide (**Figured in the Background section**). An angiocatheter will be inserted through a local anesthetic skin wheal in-plane beneath the ultrasound transducer and directed until the probe tip is immediately adjacent to the target nerve (lidocaine 2% will be administered, as needed, to anesthetize the angiocatheter track). The angiocatheter needle will be

removed, leaving the angiocatheter through which the Epimed probe will be inserted until it is adjacent to the target nerve. The cryoneurolysis device will be triggered using 3 cycles of 2-minute gas activation (active or sham) separated by 1-minute defrost periods. For active probes, the nitrous oxide will be deployed to the tip where a drop in temperature to -70°C will result in cryoneurolysis. For the sham probes, the nitrous oxide will be vented prior to reaching the probe shaft, resulting in a lack of perineural temperature change. The process will be repeated with the same treatment probe for the femoral nerve (e.g., both nerves will receive either active cryoneurolysis or sham/placebo, and not a mix of the two possible treatments).

Prior to discharge, subjects will be provided with verbal and written instructions, the telephone and pager numbers of an investigator, and a copy of the Institutional Review Board-approved consent form. Subjects will be provided with crutches if they desire, although we have found that nearly all patients treated with cryoneurolysis continue to ambulate using their prosthesis without difficulty. Subjects will be telephoned to answer any questions they may have the days following the intervention (Days 1-7, as desired by the subject and/or medically indicated). The approximate duration of this visit will total 2-4 hours, from the time the subject enters the treatment facility until the time the depart.

Optional crossover treatment. Four to six months following the initial treatment, subjects ***may*** return for an optional repeated intervention procedure ("crossover") with the alternative treatment (either active cryoneurolysis or sham/placebo), again in a double-masked fashion using the same protocol as described for the initial intervention. **The crossover treatment is not required for study participation, as the primary analyses will include a parallel study design for the initial intervention evaluated prior to any crossover treatment.** However, an optional crossover treatment will be offered to subjects for two reasons: (1) it will ensure that all subjects have access to the proposed treatment, regardless of the treatment they are initially randomized to; and, (2) it will permit intra-subject differences between treatments to be analyzed (e.g., assessing treatment-effect heterogeneity, or the variability of the causal effect across individuals, which will would not be available from the parallel-group portion of the study alone). These intra-subject differences will be secondary analyses, as there may be patient-selection bias regarding which subjects decide to have the crossover treatment (e.g., if the intervention is successful at greatly reducing phantom limb pain, then subjects receiving active cryoneurolysis during their initial treatment will be more likely to forgo the crossover treatment). This crossover will ***not*** affect the primary analyses, which will involve a parallel group study design and investigate the effects of cryoneurolysis within 4 months of the initial intervention. The approximate duration of a crossover visit will total 2-4 hours, from the time the subject enters the treatment facility until the time the depart.

Following study completion, the results will be mailed to all enrolled subjects in written form using non-technical (i.e. "layperson") language.

Outcome measurements (endpoints). We have selected outcome measures that have established reliability and validity, with minimal inter-rater discordance, and are recommended for chronic pain clinical trials by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) consensus statement. ***The primary end point will be the difference in average daily phantom pain intensity at baseline and 4 months following the initial intervention*** *(measured with the NRS as part of the Brief Pain Inventory).* The primary analyses will compare the two treatments (inter-subject comparisons) during the initial treatment period in which half of the subjects will receive active cryoneurolysis and the other half a sham/placebo treatment. Endpoints will be evaluated at baseline and post-treatment (Day 0), Days 1 and 7; and Months 1, 2, 3, 4, and 12,

(Table 1). These same time points through Month 4 will be evaluated following the optional second (crossover) treatment.

The questionnaire will differentiate among multiple dimensions of limb pain:

Residual limb ("stump") pain: painful sensations localized to the portion of limb still present.
Phantom limb sensations: *non*-painful sensations referred to the lost body part.
Phantom limb pain: painful sensations referred to the lost body part.

Each type of pain/sensation will be defined for subjects immediately prior to questionnaire application at each time point, and subjects will be instructed to specifically address phantom limb pain when responding to the various questions. In addition, since there is a strong correlation between phantom and residual limb pain, we will specifically inquire about both types of pain. Each time the questionnaire is applied, subjects will be instructed to respond for the previous 3 days (e.g., worst pain during the previous 3 days) because studies have suggested that patients have "increasing difficulty actually remembering symptom levels beyond the past several days." Exceptions will be on Day 1 for both the initial and crossover treatments because at these time points, the interest is in subjects' experiences subsequent to the treatment and not prior to the intervention. During these two days, subjects will be instructed to respond for the period of time since their treatment.

Table 1. Summary of post-enrollment assessments.

Subjects:	**All**							**Participating in Crossover ***							**All**
Time Point Following:	**Initial** Treatment							**Crossover** Treatment							**Initial**
Time Point (Days):	0	1	7					0	1	7					
Time Point (Months):				1	2	3	4				1	2	3	4	12
Brief Pain Inventory (for Phantom Limb Pain)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Residual Limb Pain (NRS)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Patient Global Impression of Change Scale	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Beck Depression Inventory	•						•							•	•
Non-Painful Phantom Sensations	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Phantom Limb Pain	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Residual Limb Pain	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

* The second—or "crossover"—treatment intervention will occur between 4-6 months following the initial treatment

Demographic and amputation history. Subjects will have demographic data collected, including

age, sex, height, weight, educational level, employment status, marital status, current analgesic regimen (including adjuvants such as acupuncture), and U.S. military service (e.g., none, discharged, active). In addition, amputation-specific data will include date of initial amputation, date(s) of subsequent surgical procedures, amputation etiology, amputation level, other amputations (with dates/etiology/pain), and prosthesis use.

Hypothesis 1: Phantom limb pain ***intensity*** will be significantly decreased 4 months following one cryoneurolysis procedure (as measured by the Numeric Rating Scale within the Brief Pain Inventory).

Pain intensity. Current/present, worst, least, and average phantom pain will be assessed using a Numeric Rating Scale (NRS) as part of the Brief Pain Inventory (short form), with the "average" pain score designated as the primary endpoint. In addition, average and worst residual limb pain NRS will be recorded separately from the phantom pain scores. The NRS is a highly-sensitive measure of pain intensity with numbers ranging from 0 to 10, zero equivalent to no pain and 10 equivalent to the worst imaginable pain. The NRS has been demonstrated to be a valid and reliable measure in multiple pain states—including painful peripheral neuropathy specifically—and following analgesic interventions. In addition, NRS scores correlate well with other measures of pain intensity, and demonstrate high test-retest reliability in chronic nociceptive and neuropathic pain states. These NRS characteristics led to recent IMMPACT consensus recommendations for use of the 10-point NRS of pain intensity for chronic pain trials.

Hypothesis 2a: Perception of **well-being** will be significantly improved 4 months following one cryoneurolysis procedure (as measured with the Patient Global Impression of Change Scale).

Health-related quality of life. While single-item measures of pain level/relief are currently the most reliable and valid options to measure pain intensity, the multidimensional aspect of the pain experience has led consensus recommendations for use of "global" measures of improvement in chronic pain trials. The Patient Global Impression of Change Scale is one such measure allowing patient evaluation of integrated treatment effects. This measure is a 7-point ordinal scale requiring the subject to rate the current severity of their global situation as it relates to phantom limb pain (as defined by each individual) compared to their baseline. This scale has the words "very much worse" to the left by the number one, and "very much improved" to the right, adjacent to the number seven. The words "no change" are in the middle of the scale above the number four. The Patient Global Impression of Change Scale has been validated in over ten prospective trials, including studies specifically involving peripheral neuropathy.

Hypothesis 2b: Physical and emotional **functioning** will be significantly improved 4 months following one cryoneurolysis procedure (as measured with the Brief Pain Inventory).

It is well-recognized that, "pain is a complex, multidimensional, sensory, and emotional experience that is individually perceived and described in many different ways." This observation has led to

consensus recommendations that "multiple core domains and related measures be considered in pain treatment trials," that "tap into a wider experience of pain over time and its impact on functioning and quality of life." Therefore, the proposed trial will include the Brief Pain Inventory, an instrument that includes—in addition to pain intensity scales—seven measures evaluating the pain's interference with physical and emotional functioning, such as sleep, relations with others, and enjoyment of life. The Brief Pain Inventory has been used in countless clinical studies of chronic pain, and validated specifically in neuropathic pain states. This instrument is associated with minimal subject burden and is easily interpreted by patients of all ages and education levels. It has high test-retest reliability and correlates well with much longer questionnaires, including the McGill measures and EuroQol.

Hypothesis 2c: **Depression** will be significantly decreased 4 months following one cryoneurolysis procedure (as measured with the Beck Depression Inventory).

In addition, multiple investigations demonstrate that factors such as anxiety and depression are strong predictors of pain intensity. Therefore, the proposed study will evaluate additional psychosocial factors using the Beck Depression Inventory. This 21-item instrument measures characteristic symptoms and signs of depression, requires only a 5th grade comprehension level to adequately understand the questions, and demonstrates high internal consistency (0.73-0.92, mean of 0.86), reliability and validity. Each of the 21 factors is rated on a 0-3 scale, and then summed to produce the total score of 0-63. Mild, moderate, and severe depression is defined with scores of 10-18, 19-29, and 30-63, respectively. While this instrument requires less than 10 minutes to complete, on average, it will be administered only at the initial baseline and four months following each treatment intervention (initial and crossover) as well as Month 12 to minimize subject burden and fatigue.

Additional pain-related data. Frequency and average duration of non-painful phantom sensations, phantom limb pain, and residual limb pain will be assessed. In addition, supplemental analgesic use will be recorded, and other pain locations/severity will be evaluated using the NRS. Lastly, to investigate masking adequacy, subjects will be queried on Day 0 following the treatment procedure on which treatment they believe they received (active cryoneurolysis vs. sham/placebo).

Data collection. Subject demographic and cryoneurolysis administration data will be uploaded from each enrolling center *via* the Internet to a secure, password-protected, encrypted central server (RedCap, Cleveland Clinic, Cleveland, Ohio). The questionnaires for all subjects—regardless of enrolling center—will be administered by telephone from the University of California San Diego by research coordinators specifically trained in these instruments' application, minimizing inter-rater discordance. Staff masked to treatment group assignment will perform all assessments. This web-based data-collection protocol has been used successfully by the investigators for numerous previously published multicenter clinical trials. Each data collection phone call will require approximately 15 minutes, with any time point including the Beck Depression Inventory requiring an additional 15 minutes.

Statistical Plan and Data Analysis

The randomized groups will be descriptively compared on baseline demographic and pain variables using descriptive statistics. In particular, groups will be considered well-balanced on a particular baseline variable if the standardized difference (difference in means or proportions divided by the pooled standard deviation) is less than $\sqrt{2/n}$, where *n* is the per-group sample size. The primary analysis will be modified intention-to-treat, in which all randomized subjects who received any of the study treatment will be included and retained in their respective treatment groups.

***Aim 1: Primary outcome*.** We will assess the average causal effect of cryoneurolysis versus sham/placebo on phantom limb pain intensity (average pain over past 72 hours) at 4 months after the initial treatment using analysis of covariance to adjust for baseline pain intensity and any imbalanced baseline variables (see above). Results will be summarized as the least squares difference in means at 4 months and 95% confidence interval. Mean and standard deviation change from baseline intensity will also be summarized. Similar analyses will be conducted for the secondary outcomes of current/present, worst and least phantom pain, as well as average and worst residual limb pain.

***Secondary outcomes*.**
Aim 2a. The randomized groups will be compared on the global measure of improvement (Patient Global Impression of Change Scale) at 4 months using the Mann-Whitney test. Proportional odds logistic regression will be used to adjust for any imbalanced baseline variables, as appropriate.
Aim 2b. The randomized groups will also be compared at 4 months on the seven measures of Brief Pain Inventory, which evaluate pain's interference with physical and emotional functioning. We will use a mixed effects multivariate model (random subject term, fixed treatment effect, unstructured correlation matrix) to first assess whether the treatment effect differs across the individual measures (i.e., treatment-measure interaction). In presence of an interaction, each measure will be evaluated univariably. Otherwise, an overall treatment effect will be estimated from the mixed effects model as the primary result for this aim.
Aim 2c. Analysis of covariance adjusting for baseline score will be used to assess the treatment effect of cryoneurolysis versus sham on depression at 4 months after randomization as measured by the Beck Depression Inventory.

***Blinding assessment*.** To assess the quality of the subject blinding as to initial treatment assignment, we will ask each subject on Day 0 to speculate which treatment they received (actual or sham cryo). A Pearson's chi-square test will be used to compare the proportion of correct speculations in each treatment group.

For all analyses, alternative statistical methods will be used if the assumptions of the planned analyses are not met. For instance, t-tests or regression analyses on the change or percent change from baseline (depending on which is less correlated with baseline score) will be used instead of analysis of covariance when comparing groups on the 4-week outcomes if the treatment group-by-baseline interaction is significant. Transformations of the data or Mann-Whitney test or other non-parametric procedures will be used if the assumptions of normality and/or equal variances are not met.

Crossover phase. Beginning 4-6 months after the original randomization, requesting subjects will receive the opposite treatment from that received in their original randomization, and the same measurements will be collected through 4 months (see **Table 1**). This option will allow all subjects the opportunity to receive the study treatment. Although a completely unbiased assessment of the average causal effect will not be possible for this phase because the second treatment will be

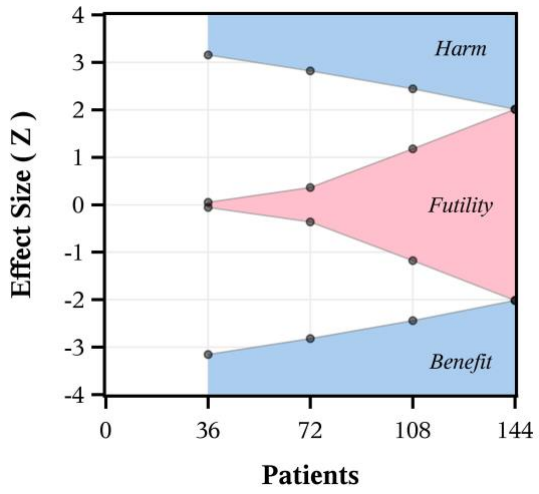
voluntary, and crossover will thus likely be requested more often from those receiving sham/placebo in the first phase, we will still descriptively report the average treatment effect from those choosing to cross over (but no testing will be done; unbiased average causal effect will be obtained from the first phase).

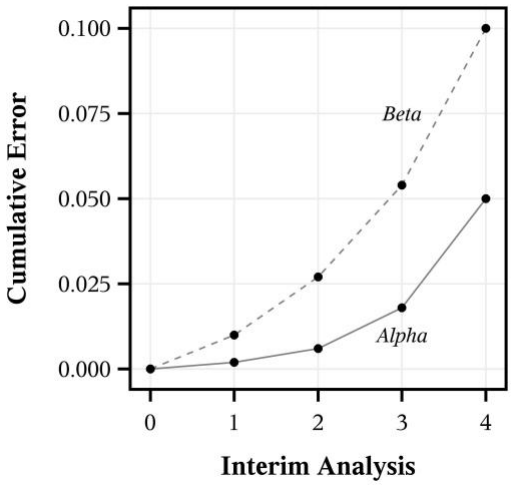
More importantly, we will estimate the variability in the individual causal effects of cryoneurolysis versus sham/placebo using this crossover design. Variability of the individual causal effects cannot be directly estimated in a parallel group study (e.g., from the main portion (Aim 1) of this study we can only directly estimate the average causal effect), since only the outcome for the single treatment received is measurable for each subject. However, estimation of the variability of the individual causal effects from the crossover study, quantified as the standard deviation of within-subject differences on treatment versus sham/placebo, will provide valuable information about the heterogeneity of the treatment effect across subjects associated with cryoneurolysis treatment of phantom limb pain. We will also use regression models to explore whether any baseline factors are associated with higher or lower causal effects of treatment.

Long-term follow-up. Data for all outcomes will also be collected at 12 months post randomization. Due to the crossover design, we will not be able to directly assess the treatment effect of cryoneurolysis versus sham on these outcomes. Rather, we will descriptively assess the change from the initial baseline to 12 months for various groups of subjects: 1) all who received the active treatment either initially or in the crossover, 2) initial control subjects who were not crossed over; 3) initial control subjects who were crossed over; 4) initial treated subjects who were not crossed over; 5) initial treated subjects who were crossed over.

Dropouts. At most, about 7% of subjects in each group are expected to drop out of the study before reaching the 4-month primary outcome assessment (based on an unpublished pilot study of pre-emptive continuous peripheral nerve block use for surgical amputation, for which we observed 1 of 15). Since the current study will consist of volunteers traveling to the centers for enrollment and a treatment procedure, we expect even less. For those missing 4-month data we will use the last-observation-carried-forward method if the brief pain inventory was measured at 3 months. Otherwise, we will use intent-to-treat and conservatively assign the best observed score to the sham/control group and the worst score for the treated group subjects. We do not expect any appreciable effect of dropouts on either the power of the study or the unbiasedness of study results.

Interim analyses. We will conduct interim analyses to assess efficacy (rejecting null) and futility (rejecting alternative) at each 25% of the maximum enrollment using a group sequential procedure. Specifically, a gamma spending function will be used with parameters -4 and -2 for efficacy and futility, respectively. Thus, boundaries at the 1st through 4th analyses for efficacy (futility in parentheses) will be $P \leq 0.0016$ ($P>0.9572$), $P \leq 0.0048$ ($P>0.7186$), $P \leq 0.0147$ ($P>0.2389$) and $P \leq 0.0440$ ($P>0.0440$) (**Figures below**).





Type I error. We will use a parallel gatekeeping procedure to control the study-wide type I error at 0.05. For this procedure, we therefore *a priori* prioritize the study outcomes into ordered sets, as Aim 1, Aim 2a, Aim 2b and then Aim 2c. Analysis will proceed in that order, and testing will proceed through each "gate" to the next set if and only if at least one outcome in the current set reaches significance. The significance level for each set will be 0.05 times a cumulative penalty for non-significant results in previous sets (i.e., a "rejection gain factor" equal to the cumulative product of the proportion of significant tests across the preceding sets). Within a set, a multiple comparison procedure (Bonferroni correction) will be used as appropriate to control the type I error at the appropriate level. SAS statistical software (Carey, North Carolina), R programming language (The R Project for Statistical Computing) and East 5.3 software (Cytel Inc.) will be used for all analyses.

Sample size considerations. Our sample size estimate is based on the primary specific aim of whether the addition of cryoneurolysis decreases phantom limb pain intensity resulting from an amputation compared with current standard-of-care treatment at 4 months following cryoneurolysis. Receiver operating characteristic curve analyses demonstrate that changes from baseline of at least 1.7 along a 10-point NRS accurately identified patients who rated improvements as "much improved" or more, compared with those who perceived no change or worsening following analgesic interventions. Multiple additional studies confirm this degree of reduction as clinically meaningful to individual patients with chronic pain. Meaningful group differences in the mean change would be somewhat smaller than important changes for individuals.

Therefore, we power our study to be able to detect group differences in mean change from baseline of 1.7 points or more on the NRS. Based on a conservative standard deviation estimate for each group of 3.0 at 4 months, a correlation of 0.50 between baseline and follow-up NRS, a two-sided test at the 0.05 significance level, power of 0.90, and 4 equally spaced analyses (3 interim and 1 final, as needed), a maximum of 72 subjects in each group (N=144 total) is required (East 5.3 software, Cytel Inc). The expected sample size for this group sequential design (i.e., average sample size over thousands of such trials, stopping when a boundary is crossed) is a total of 100 under the alternative and 102 under the null hypotheses. **Table 2** reports boundary crossing probabilities at each of the 4 analyses for this design, assuming that either the null or alternative hypotheses were true. For example, there is a cumulative 8%, 37% and 75% chance of crossing a boundary at the 1st, 2nd and 3rd analyses, respectively, if the alternative hypothesis were true. However, if the true standard

deviation at 4 months were smaller, say 2.5 instead of 3.0, then the cumulative probability of stopping for efficacy at the 1st, 2nd and 3rd analyses, respectively, would increase to 16%, 55% and 88% under the alternative hypothesis.

Table 2. P-value boundaries and boundary crossing probabilities for group sequential design

Fraction of Maximum Accrual	Cumulative Accrual	Alpha Spent	Beta Spent	P-value Boundaries		Boundary Crossing Probabilities	
				H0	H1	Under H0	Under H1
0.250	36	0.002	0.010	0.0016	0.9572	0.044	0.083
0.500	72	0.006	0.027	0.0048	0.7186	0.269	0.290
0.750	108	0.018	0.054	0.0147	0.2389	0.485	0.379
1.000	144	0.050	0.100	0.0440	0.0440	0.202	0.248

10. HUMAN SUBJECTS

Approximately 60 subjects will be enrolled at UC San Diego, of approximately 200 participants at all sites.

Inclusion criteria: Adult patients of at least 18 years of age, (1) with a lower limb traumatic or surgical amputation at least 12 weeks prior to enrollment distal to the hip (femoral head remaining); (2) who experience at least moderate phantom limb pain—defined as a 3 or higher on the Numeric Rating Scale (NRS; 0-10, 0= no pain; 10=worst imaginable pain)—at least daily for the previous 2 months. (3) accepting of a cryoneurolysis procedure; and, (4) willing to avoid both changes to their analgesic regimen as well as elective surgical procedures from 1 month prior to and at least 4 months following the initial cryoneurolysis procedure.

Exclusion criteria: (1) allergy to amide local anesthetics; (2) pregnancy; (3) incarceration; (4) inability to communicate with the investigators; (5) morbid obesity (body mass index > 40 kg/m^2); and, (6) possessing any contraindication specific to cryoneurolysis such as a localized infection at the treatment site, cryoglobulinemia, cold urticaria and Reynaud's Syndrome.

11. RECRUITMENT AND PROCEDURES PREPARATORY TO RESEARCH

Study subjects will be identified by each of the enrolling centers from four sources:

1. **Databases.** Databases will be examined by non-investigator database managers following Institutional Review Board approval, and subsequently queried quarterly. The site directors will provide database managers with Institutional Review Board-approved letters describing the study with investigator contact information sealed in envelopes with postage applied. Database managers will then use existing databases to identify individuals with a prior amputation and apply address labels to the supplied letters and mail them—this is to adhere to current HIPAA rules. Individuals who are interested in the study will be directed to contact the study investigators or research coordinators for a thorough description of the study purpose and protocol. Of note, all U.S. service members with a limb amputation during Operation Iraqi Freedom and Operation Enduring Freedom are included in an amputee-specific database, regardless of inclusion in surgical or chronic pain databases; but, the information within this database is not available for clinical investigations to protect patient privacy. We will strive to reach all of these individuals using the additional methods described below to ensure

that all U.S. military personnel are informed of the study and have the opportunity to participate.

2. **Clinics.** Each site director will identify and meet quarterly with clinic (amputee-specific, surgical, and chronic pain) personnel to both inservice them on the study protocol as well as provide Institutional Review Board-approved written information describing the investigation that may be given to prospective subjects during clinic visits. Clinic patients will come into contact with their healthcare providers during regularly-scheduled clinic visits, and these providers will briefly describe the study and offer written information to patients with phantom limb pain who voice interest in the study. Patients who are interested in the study will be required to give permission for a research coordinator to contact them to adhere to Health Insurance Portability and Accountability Act (HIPAA) requirements.

3. **Advertisements.** The Primary Investigator will place Institutional Review Board-approved study advertisements within print and web-based publications that are frequently read by the target population. Additionally, site directors will provide similar Institutional Review Board-approved advertising material to the leaders of local and regional amputee focus/support groups. Individuals who are interested in the study will be directed to contact the study investigators or research coordinators for a thorough description of the study purpose and protocol. In addition, Institutional Review Board-approved advertisements will be placed and a study-specific information page created within Facebook, which has over 1-billion members. Of note, the United States Army, Navy, Air Force, and Marines all have dedicated Facebook information pages. Information on how to volunteer for the study will also be placed in relevant Twitter feeds, such as the Clinical Trial Spotlight San Diego, and Clinical Connection, free portals for finding clinical trials. Twitter users (over 300-million registered users) looking for trials will be able to access contact information to the study, and link to the Facebook page for additional information.

4. **ClinicalTrials.gov.** The trial will be prospectively registered on the ClinicalTrials.gov website. One of the main purposes of trial registries is to provide the general population access to available ongoing investigations. Contact information for the site directors will be included on the website so that prospective subjects may receive a thorough description of the study purpose and protocol. Which site director subjects contact is up to the subjects themselves, because all will be listed on the website. However, for individuals desiring enrollment, site directors will suggest the optimal treatment center based on geographic location and military status (e.g., civilians can be treated exclusively at one of the civilian enrolling centers).

Once a prospective subject contacts a site director or research coordinator by telephone or email, they will be provided information on the study purpose and protocol, as well as have any questions answered. Inclusion/exclusion criteria will be reviewed and documented on a form to identify subject eligibility according to these criteria. If a prospective subject is excluded for any reason, the reason for exclusion will be recorded, but no patient identifying information will be recorded. Candidates who meet inclusion and exclusion criteria and desire study enrollment will be scheduled for their initial study treatment. Written informed consent will be attained prior to any measurements or procedures the morning of the initial treatment (see below for a detailed description of the informed consent process).

Recruitment of Active Duty Military Personnel. The Chain of Command will not be involved in the recruitment of military personnel; and will in no way be asked to encourage or order soldiers to participate in the trial. As per Department of Defense Directive 3216.2, an ombudsman will be present if any group briefings of Active duty personnel are scheduled, to help ensure that those

present understand that study participation is completely voluntary and will not influence their careers or standing within the military.

Study Compensation. To help compensate study subjects for their time and defray travel expenses, participants will receive $500 following each study treatment prior to discharge (initial and the optional crossover). We believe that this compensation amount is both fair and does not provide undue inducement. The University of California San Diego Institutional Review Board approved similar amounts for subjects in clinical trials of similar time, risk and duration. Currently, 24 USC 30 limits payments to Active Duty military personnel for participation in research while on duty to blood donations (which are not required for the proposed investigation). However, military personnel who are on official military leave status *may* receive compensation for study participation, and will do so at the same level and on the same schedule as described for civilians and Veterans.

Recruitment and advertisement materials. These materials are being developed and will be provided to the IRB prior to use. Each of the 7 enrolling centers will include information specific to that center. We believe that these documents accurately reflect the study and are not coercive or offer undue inducements.

12. INFORMED CONSENT

Once a prospective subject contacts a site director or research coordinator by telephone or email, they will be provided information on the study purpose and protocol, as well as have any questions answered. Candidates who meet inclusion and exclusion criteria and desire study enrollment will be scheduled for their initial treatment. Written informed consent will be attained prior to any measurements or procedures the morning of the initial treatment. Each site director is responsible for ensuring that written, informed consent is obtained from every subject at his respective enrolling center. Clinical research coordinators—also one for each enrolling site—will be specifically trained by the site directors to provide informed consent followed by documentation of informed consent using an Institutional Review Board-approved informed consent form. When subjects present for their initial treatment, research coordinators will provide and attain written informed consent. This will occur in private patient care rooms, so that subjects may feel comfortable asking questions of the research coordinator. If a subject desires—or if there is a question that a research coordinator cannot answer—the site director will be called in by the research coordinator to discuss the study directly with the subject.

We do not foresee any issues relevant to the mental capacity of the potential human subjects. Written, informed consent will be attained prior to any study procedures or measurements. Following a history and physical by the site director, subjects will have an intravenous line inserted, external monitors placed, oxygen delivered by face mask, and conscious sedation provided with minimal intravenous fentanyl (opioid) and midazolam (benzodiazepine), when applicable (not all subjects require sedation). This sedation is to increase the comfort of subjects during probe insertion. Therefore, subjects will not be sedated until following the written, informed consent process is completed.

Subjects will be provided privacy and time for decision making both in the study description/explanation telephone call to the site director or research coordinator, as described above; and also the morning of the initial treatment using a private patient care room to again review the study, informed consent form, and answer any remaining subject questions. As noted previously,

subjects may speak with the site director by telephone from initial contact through the morning of treatment; and, will have access during and following the treatment(s) with cellular phone and pager numbers provided upon discharge.

This study protocol has follow-up data-collection telephone calls a maximum of 1 year following the initial study treatment, so repeated informed consent following the initial consent is unnecessary, as opposed to multi-year, longer-term clinical trials.

Surrogate consent will not be accepted; therefore, if human subjects cannot provide consent on their own, they will not be offered study enrollment. Consent by an individual's Legally Authorized Representative is unacceptable for study enrollment. Of note, minors (age < 18 years) will not be offered enrollment, as explained in Section 6b (Inclusion/Exclusion Criteria). Therefore, assent will not be accepted during the informed consent process.

13. ALTERNATIVES TO STUDY PARTICIPATION

Potential study subjects may simply decline enrollment.

14. POTENTIAL RISKS

1. Infection. There is the potential risk of infection since subjects will have a probe inserted through the skin. Since there will be nothing left going through the skin or in the subject after the probe is withdrawn, the risk of infection is very small and there has never been a report of permanent injury due to infection following cryoneurolysis.
2. Bleeding. The probe does not have an open tip and is not particularly sharp, so there is a very low risk of having any type of bleeding as a result of treatment. However, if it was to happen, we would hold pressure until the bleeding stopped.
3. Falling. The risk of falling due to cryoneurolysis in a lower extremity (leg) currently unknown, although it has never been reported in the medical journals. Subjects will be provided with a pair of crutches following treatment if desired, although it is rare that an individual feels the need to use crutches to walk.
4. The skin where the nerve is frozen could lose or gain color if the nerve is particularly close to the surface. However, this has never been reported for deeper nerves and using the probe that will be used for this study.
5. Since a nerve will be frozen, there is the chance of nerve injury. However, in 5 decades of use, only a single case of "neuritis" (nerve irritation) has been reported in medical journals, and this went away after a few months.
6. There is the risk of loss of confidentiality. The following procedures will be done to maintain confidentiality: written, paper forms will be kept in a locked medical office and the locked Investigational Pharmacy's files. Computerized records containing personal health information will be stored on password-protected and encrypted computers.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

During the treatment, subjects will be continuously monitored with pulse oximetry, noninvasive blood pressure cuffs, and EKG (standard for catheter placement). Subjects will receive an IV so that emergency medications could be given, if needed. As described above, probes will be placed under sterile conditions as is standard-of-care for any percutaneous cryoneurolysis.

Following treatment, the subjects will be contacted at least the day following treatment by an

investigator or research coordinator, and longer if the subject desires or if medically indicated (e.g., suspected possible infection). Subjects will have a physicians' pager and cellular phone numbers available to respond 24 hours/day and 7 days/week for at least the first week following treatment.

The risks to confidentiality are the release of names/ telephone numbers/ demographic data (e.g. weight, age, height), which will be minimized by the use of password-protected computers and case report forms that will be stored in locked offices.

Subjects will be given clear instructions to call an investigator with any questions or concerns regarding their study participation. If a patient experiences an injury that is directly caused by this study, only professional medical care that they receive at the medical center. No other compensation is offered. Any adverse events will be reported to the IRB using the standard adverse events reporting and upon continuing review (depending on severity, as defined by the IRB).

The study Data Safety Monitoring Board (DSMB) will be comprised of the Medical Monitor, a physician experienced in both clinical trial management and the ethical conduct of research, and a statistician, also well-experienced in multicenter trials. All three of these individuals will be completely independent of the investigative team. No member of the DSMB will have any financial, proprietary, professional, or other interests that may affect impartial, independent decision-making by the DSMB. The board will comprise individuals with no vested interest in the outcome of the research study. The members will also sign a confidentiality statement. The DSMB will operate from a charter describing its role, membership, reporting procedures, and meeting protocol. The DSMB will decide on its own protocols, set triggers for data review or analyses, and establish guidelines for monitoring the study, stopping the study for safety concerns, and for efficacy based on plans specified in the protocol. Confidentiality will be maintained during all phases of DSMB review and deliberations. DSMB members will maintain strict confidentiality concerning all privileged trial results provided to them. The board will perform the following functions:

- Approval of the trial protocol before enrollment of patients
- Review the data in order to determine efficacy, futility, and safety, and to determine whether the study should continue
- Review data quality and data integrity
- Evaluate risk versus benefit by thorough examination of the data accumulated
- Determine whether the trial is proceeding as planned, the protocol is being followed, the recruitment of patients is on schedule, and data are being collected with the proper accuracy
- Review patient dropouts, if any, and make appropriate recommendations
- Determine whether safety concerns have been raised by the experimental or control treatments

Review Items. Items to be reviewed by the DSMB include:

- Interim/cumulative data for evidence of study-related adverse events
- Interim/cumulative data for evidence of efficacy according to pre-established statistical guidelines in the study protocol
- Data quality, completeness, and timeliness
- Performance of individual centers
- Adequacy of compliance with goals for recruitment and retention, including those related to participation of women and minorities
- Adherence to the protocol

- Factors that might affect the study outcome or compromise the confidentiality of the trial data (such as protocol violations etc.)
- Factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study

Recommendations. The DSMB will conclude each review with their recommendations to the principal investigator and primary site Institutional Review Board (at the University of California San Diego) as to whether the study should continue without change, be modified, or terminated. Recommendations regarding modification of the design and conduct of the study may include:

- Modifications of the study protocol based upon the review of the safety data
- Suspension or early termination of the study or of one or more study arms because of serious concerns about patients' safety, inadequate performance, or rate of enrollment
- Suspension or early termination of the study or of one or more study arms because study objectives have been obtained according to pre-established statistical guidelines
- Optional approaches for executive committee and investigators to consider when the DSMB determines that the incidence of the primary study outcomes is substantially less than expected, such as recommendations to increase the number of trial centers or extend the recruitment period
- Corrective actions regarding a study center whose performance appears unsatisfactory or suspicious

Appropriate reports will be made to the Institutional Review Boards at all enrolling centers and the executive committee, comprised of all co-investigators and site directors

16. PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT

Disposition of data. The original, hard-copy signed informed consent forms and case report forms will be stored within the local site director's locked office, where they will remain for at least 7 years. These hard copies will not be mailed or otherwise transferred. Data will be uploaded and stored in one location: the central servers of Department of Outcomes Research at the Cleveland Clinic, a department dedicated completely to clinical research. This department has a full-time Research Electronic Data Capture (REDCap) programmer dedicated to developing REDCap databases and providing support for clinical trials. REDCap is a relational database for data entry and auditing. This is a web-based application designed exclusively to support data capture for research studies. The Department of Outcomes Research at the Cleveland Clinic web servers are encrypted and password-protected with multiple firewalls to the standards of the National Institutes of Health. Of note, the servers are backed up every night. In the case of a disk failure, only data written to the files since the last backup will be subject to loss and can be easily restored. Databases are protected through electronic measures using a multi-layered, but simple approach: all study related files will reside on the database server rather than on individual hard disk drives and the files will be protected by the operating systems features against general access. User names will be password protected. The electronic data will remain within the Department of Outcomes Research for 7 years following study completion. The UCSD research coordinator may receive training at the UCSD CTRI in REDCap use. With such training, up to six hours of user support is provided without recharge. However, technical and most user support will be provided by the Cleveland Clinic. The USAMRMC (United States Army Medical Research and Materiel Command) is eligible to review study records at any time.

Each local site will transfer certain PHI to the UCSD research coordinator who will make all data

collection phone calls for all subjects. PHI transferred will include the subject's name, phone numbers, and study ID. This information will be transferred via a secure online system known as the Army Missile and Research, Development and Engineering Command Safe Access File Exchange System (AMRDEC SAFE). AMRDEC SAFE is a secure, password-protected, system that the military has approved, and requires, for the transfer of such data. Civilian centers may use this system if access is granted, or fax to a locked office with access restricted only to the UCSD study coordinator and the PI.

Sharing study results. Following study completion, all subjects will be provided with the study results in written form and in language appropriate for non-medical individuals. In addition, the master dataset will be de-identified.

17. POTENTIAL BENEFITS

For subjects randomized to receive a sham treatment first: There will be no difference between being in this study and deciding against participation. Therefore, there is no potential for direct benefits from this sham "treatment". However, all subjects will be offered the option of participating in the cross-over arm of the study, in which case they would receive active treatment (see below).

For subjects randomized to receive active cryoneurolysis first: It is our hope that patients have a permanent decrease in their phantom limb and/or residual limb pain.

Possible benefits to others: Future patients may benefit if it is determined that cryoneurolysis decreases phantom limb and/or residual limb pain. There are millions of individuals world-wide who suffer from these debilitating conditions, and finding an effective treatment would be a tremendous step forward in treating these individuals.

18. RISK/BENEFIT RATIO

Chronic phantom limb and residual limb pain cause significant disability for patients, and there is currently a dearth of reliable treatments for this debilitating pain. Since infection and falling are the largest risks of this intervention, and there have no previous cases of permanent negative sequelae due infection or any falls reported in the literature, we believe the potential risks to be minimal compared to the potential benefits.

Subjects will be given clear verbal and written instructions to call Dr. Ilfeld in the Department of Anesthesia at UCSD, with any questions or concerns regarding their study participation. If a patient experiences an injury that is directly caused by this study, they will receive professional medical care at the University of California, San Diego. No other compensation is offered. Any adverse events will be reported to the UCSD IRB using the standard adverse events reporting website and on continuing review (depending on severity, as defined by the IRB).

19. EXPENSE TO PARTICIPANT

There will be no additional costs to subjects as a result of being in this study, other than travel to and from the medical facility for the study treatment. These expenses may include, but are not limited to, costs for fuel, bus service, parking, bridge tolls, and meals. If a subject is injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those injuries. The University will not provide any other form of compensation for an injury.

20. COMPENSATION FOR PARTICIPATION

To help compensate subjects for their time and to defray travel expenses, they will receive $500 following each visit to the enrolling center, for up to $1,000 for subjects who undergo both an initial treatment and crossover treatment. Currently, 24 USC 30 limits payments to Active Duty military personnel for participation in research while on duty to blood donations (which are not required for the proposed investigation). However, military personnel who are on official military leave status may receive compensation for study participation, and will do so at the same level and on the same schedule as described for civilian and Veterans.

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

Principal Investigator, **Brian M. Ilfeld, MD, MS,** is a board-certified anesthesiologist with fellowship training in and 17 post-training years experience with regional anesthesia and acute pain medicine. Dr. Ilfeld holds a license to practice medicine in California. Dr. Ilfeld has medical privileges at the UC Medical Centers. Dr. Ilfeld, or another investigator, will follow all subjects following their treatment. Dr. Ilfeld will be responsible for the overall management of this study, as well as for the well-being of study subjects.

Co-investigators, **Rodney Gabriel, MD, Matthew Swisher, MD**, and **John Finneran, MD,** are board-certified anesthesiologists with years of experience with regional anesthesia and acute pain medicine. All hold a license to practice medicine in California and has medical privileges at the UC Medical Centers. They will help consent subjects, perform a history and physical exam, perform the treatment on subjects, follow subjects following their treatment, and help manage the study (including regulatory work).

The study will be overseen a medical monitor, **Salim Hayek, MD, PhD**, Case Western Reserve University; Cleveland, Ohio—in essence a study subject advocate. As the Chief of Pain Medicine at Case Western Reserve University, Dr. Hayek is thoroughly experienced in management of clinical trials, the ethical conduct of clinical research, the patient population under investigation (amputees with phantom limb pain), and Pain Medicine interventions for chronic conditions (such as cryoneurolysis). As such, Dr. Hayek is a strong study subject advocate. Dr. Hayek has extensive experience working on committees and authoring/editing peer-reviewed evidence-based publications, and will be an active member of the DSMB. He has been, and will continue to be, completely independent of the investigative team. In addition, Dr. Hayek has no financial, proprietary, professional, or other interests that may affect impartial, independent decision-making by the DSMB. Lastly, he has no vested interest in the outcome of the research study.

Baharin Abdullah is a research coordinator with the UCSD CTRI, with the required training—including up-to-date CITI training—for her position.

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23. FUNDING SUPPORT FOR THIS STUDY

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24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

Not applicable.

25. INVESTIGATIONAL DRUG FACT SHEET AND IND/IDE HOLDER

Not applicable since percutaneous cryoneurolysis and the products used for this protocol are all cleared by the United States Food and Drug Administration for use treating both acute and chronic pain. Therefore, this is an on-label study.

26. IMPACT ON STAFF

Participants will be enrolled by investigators andresearch coordinators specifically hired and trained for the study. Subjects receiving treatment at UCSD will be seen at the CTRI. Since only the research center will be utilized, there will be no impact on hospital clinical staff.

27. CONFLICT OF INTEREST

The Department of Defense is funding this study in its entirety. There is no financial or otherwise conflict of interest for any of the investigators.

28. SUPPLEMENTAL INSTRUCTIONS FOR CANCER-RELATED STUDIES

Not applicable.

29. OTHER APPROVALS/REGULATED MATERIALS

None.

30. PROCEDURES FOR SURROGATE CONSENT AND/OR DECISIONAL CAPACITY ASSESSMENT

Not applicable: surrogate consent will not be accepted.