

## ANESTHESIOLOGY

# Ultrasound-guided Percutaneous Cryoneurolysis to Treat Chronic Postamputation Phantom Limb Pain: A Multicenter Randomized Controlled Trial

Brian M. Ilfeld, M.D., M.S., Cameron R. Smith, M.D., Ph.D., Alparslan Turan, M.D., Edward R. Mariano, M.D., M.A.S., F.A.S.A., Matthew E. Miller, M.D., Rick L. Fisher, D.O., M.B.A., F.A.S.A., Andrea M. Trescot, M.D., Steven P. Cohen, M.D., James C. Eisenach, M.D., Daniel I. Sessler, M.D., J. David Prologo, M.D., Edward J. Mascha, Ph.D., Liu Liu, M.S., Rodney A. Gabriel, M.D., M.A.S., and the PAINfRE Investigators\*

*ANESTHESIOLOGY* 2023; 138:82–97

## EDITOR'S PERSPECTIVE

### What We Already Know about This Topic

- Chronic postamputation pain is relatively common and difficult to treat once established
- Continuous neural blockade with local anesthetic may reduce symptoms of persistent postamputation pain, even beyond the off-set of local anesthetic effect
- Cryoneurolysis can reduce nociceptive transmission for a longer period of time by ablating peripheral nerves

### What This Article Tells Us That Is New

- In patients with established chronic postamputation pain, cryoneurolysis of distal sciatic and femoral nerves did not reduce pain scores at 4 months of follow-up compared to sham
- Exploratory analysis suggested cryoneurolysis was associated with more pain among patients with transfemoral and ankle/foot amputations, but less pain among patients with transtibial amputation

## ABSTRACT

**Background:** Postamputation phantom pain is notoriously persistent with few validated treatments. Cryoneurolysis involves the application of low temperatures to reversibly ablate peripheral nerves. The authors tested the hypothesis that a single cryoneurolysis treatment would decrease phantom pain 4 months later.

**Methods:** The authors enrolled patients with a lower-limb amputation and established phantom pain. Each received a single-injection femoral and sciatic nerve block with lidocaine and was subsequently randomized to receive either ultrasound-guided percutaneous cryoneurolysis or sham treatment at these same locations. The primary outcome was the change in average phantom pain intensity between baseline and 4 months as measured with a numeric rating scale (0 to 10), after which an optional crossover treatment was offered. Investigators, participants, and clinical staff were masked to treatment group assignment with the exception of the treating physician performing the cryoneurolysis, who had no subsequent participant interaction.

**Results:** Pretreatment phantom pain scores were similar in both groups, with a median [quartiles] of 5.0 [4.0, 6.0] for active treatment and 5.0 [4.0, 7.0] for sham. After 4 months, pain intensity decreased by 0.5 [−0.5, 3.0] in patients given cryoneurolysis ( $n = 71$ ) versus 0 [0, 3] in patients given sham ( $n = 73$ ), with an estimated difference (95% CI) of −0.1 (−1.0 to 0.7),  $P = 0.759$ . Following their statistical gatekeeping protocol, the authors did not make inferences or draw conclusions on secondary endpoints. One serious adverse event occurred after a protocol deviation in which a femoral nerve cryolesion was induced just below the inguinal ligament—instead of the sensory-only saphenous nerve—which resulted in quadriceps weakness, and possibly a fall and clavicle fracture.

**Conclusions:** Percutaneous cryoneurolysis did not decrease chronic lower extremity phantom limb pain 4 months after treatment. However, these results were based upon the authors' specific study protocol, and since the optimal cryoneurolysis treatment parameters such as freeze duration and anatomic treatment location remain unknown, further research is warranted.

(*ANESTHESIOLOGY* 2023; 138:82–97)

Tens of millions of people are living with a lower limb amputation,<sup>1</sup> with up to 50 to 85% developing chronic, intractable pain perceived as originating from the missing limb, often described as “phantom limb pain.”<sup>2</sup> Phantom pain is notoriously persistent,<sup>3</sup> with few adequately powered randomized controlled trials to guide treatment.<sup>4</sup> The precise etiology of phantom pain is unclear. However, neural restructuring frequently occurs after limb amputation, and the degree of cortical reorganization is associated with phantom pain intensity.<sup>5</sup>

This article is featured in “This Month in Anesthesiology,” page A1. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site ([www.anesthesiology.org](http://www.anesthesiology.org)). This article has a visual abstract available in the online version.

Submitted for publication June 23, 2022. Accepted for publication October 20, 2022. Published online first on November 10, 2022.

Brian M. Ilfeld, M.D., M.S.: Department of Anesthesiology, University of California San Diego, San Diego, California.

Copyright © 2022, the American Society of Anesthesiologists. All Rights Reserved. *Anesthesiology* 2023; 138:82–97. DOI: 10.1097/ALN.0000000000004429

One study suggested that a single-injection local anesthetic peripheral nerve block can resolve both phantom pain and cortical abnormalities, although the improvements disappeared after block resolution.<sup>6</sup> Nevertheless, this demonstrated that at least in some cases, persistent cortical abnormalities and phantom pain may be maintained from abnormal input from the peripheral nervous system.<sup>7</sup> A recent randomized controlled trial reported that prolonging a peripheral nerve block using a 6-day continuous perineural local anesthetic infusion extended limb analgesia for at least 1 month.<sup>8</sup> These findings suggest that a peripheral nerve block of extended duration—lasting weeks or months rather than days—may allow prolonged cortical reorganization and provide lasting relief from phantom pain.

A prolonged neural block is provided with cryoneurolysis, which entails the application of very low temperatures (approximately  $-70^{\circ}\text{C}$  using nitrous oxide) to reversibly ablate peripheral nerves.<sup>9</sup> Guided using real-time imaging, a percutaneously inserted probe has gas circulated throughout its length, inducing cold at the distal end and freezing the target nerve.<sup>10</sup> There is no implanted device, and there is no external equipment to prepare, manage, or malfunction—a single administration results in effects measured in weeks to months without any subsequent patient or clinician interventions. While multiple uncontrolled case series suggest a possible analgesic benefit in treating phantom and residual limb pain with percutaneous cryoneurolysis,<sup>11–13</sup> the technique has not been validated for postamputation pain in a randomized controlled study.

Cameron R. Smith, M.D., Ph.D.: Department of Anesthesiology, University of Florida, Gainesville, Florida.

Alparslan Turan, M.D.: Departments of General Anesthesia and Outcomes Research, Cleveland Clinic, Cleveland, Ohio.

Edward R. Mariano, M.D., M.A.S., F.A.S.A.: Department of Anesthesiology, Perioperative and Pain Medicine, Palo Alto Veterans Affairs, Palo Alto, California.

Matthew E. Miller, M.D.: Department of Anesthesiology, Walter Reed National Military Medical Center, Bethesda, Maryland.

Rick L. Fisher, D.O., M.B.A., F.A.S.A.: Department of Anesthesiology, Naval Medical Center San Diego, San Diego, California.

Andrea M. Trescot, M.D.: Florida Pain Relief Group, Tampa, Florida.

Steven P. Cohen, M.D.: Department of Anesthesiology, Johns Hopkins, Baltimore, Maryland.

James C. Eisenach, M.D.: Department of Anesthesiology, Wake Forest School of Medicine, Winston-Salem, North Carolina.

Daniel I. Sessler, M.D.: Department of Outcomes Research, Cleveland Clinic, Cleveland, Ohio.

J. David Prologo, M.D.: Department of Radiology, Emory University, Atlanta, Georgia.

Edward J. Mascha, Ph.D.: Departments of Quantitative Health Sciences and Outcomes Research, Cleveland Clinic, Cleveland, Ohio.

Liu Liu, M.S.: Departments of Quantitative Health Sciences and Outcomes Research, Cleveland Clinic, Cleveland, Ohio.

Rodney A. Gabriel, M.D., M.A.S.: Department of Anesthesiology, University of California San Diego, San Diego, California.

\*A list of the Perineural Anesthesia Infusion Network for Research and Education (PAINIRE) Investigators is provided in the appendix.

We therefore designed this multicenter, randomized, observer- and participant-masked, sham-controlled, parallel-arm, partial crossover clinical trial to determine if a single treatment of ultrasound-guided percutaneous cryoneurolysis would provide effective and lasting analgesia for established lower extremity phantom limb pain. Specifically, we tested the primary hypothesis that the change in average phantom limb pain intensity between baseline and 4 months would be greater after cryoneurolysis *versus* sham treatment (as measured with the Numeric Rating Scale of the Brief Pain Inventory).

## Materials and Methods

This study was conducted within the ethical guidelines outlined in the Declaration of Helsinki and followed Good Clinical Practice. The trial was prospectively registered at clinicaltrials.gov (NCT03449667; Principal Investigator: Brian M. Ilfeld, M.D., M.S.; initial posting: February 28, 2018). The protocol was approved by the Institutional Review Board at each of the 6 enrolling centers as well as the United States Army Medical Research and Development Command Human Research Protection Office (Fort Detrick, Maryland). Responsible for the oversight and conduct of the investigation was an independent Data Safety Monitoring Board (appendix). Written, informed consent was obtained from all participants.

## Participants

Six medical centers enrolled patients, including public and private civilian, Veterans Affairs, and military treatment facilities. Potential participants were presented with the study in chronic pain clinics, and advertisements were posted in amputee-centered national print and web-based publications. Enrollment was offered to adult patients of at least 18 yr of age with a lower limb traumatic or surgical amputation at least 12 weeks before enrollment. The amputation had to be distal to the hip (femoral head remaining), and patients had to experience at least moderate phantom limb pain defined as a 3 or higher on the Numeric Rating Scale (0 to 10; 0 = no pain; 10 = worst imaginable pain) at least daily for the previous 2 months. Patients had to agree to avoid both changes to their analgesic regimen as well as elective surgical procedures from 1 month before, and at least 4 months after, the initial study intervention. Patients were excluded for an amide local anesthetic allergy, pregnancy, incarceration, inability to communicate with the investigators, morbid obesity (body mass index greater than  $40\text{ kg/m}^2$ ), and possessing any contraindication specific to cryoneurolysis such as a localized infection at the treatment site, cryoglobulinemia, cold urticaria, and Raynaud's syndrome.

## Intervention

Participants were asked to not eat or drink after midnight before the procedure. For women of childbearing age with the possibility of pregnancy, a sample of urine was collected

before any study interventions to rule out pregnancy. All participants had a peripheral intravenous catheter inserted, standard noninvasive monitors applied (blood pressure cuff, pulse oximeter, five-lead electrocardiogram), and oxygen administered *via* a facemask or nasal cannula. Oral and/or intravenous sedatives and analgesics such as midazolam, diazepam, and fentanyl were titrated for patient comfort, if necessary, while ensuring that patients remained responsive to verbal cues.

The specific nerves targeted were the sciatic and femoral (or their distal branches), with the most distal location clearly visualized with ultrasound treated (but before the sciatic bifurcation and at the level of the medial epicondyle for the saphenous nerve). The potential cryoneurolysis entry sites were prepared with chlorhexidine gluconate and isopropyl alcohol and a sterile, fenestrated drape. Using the appropriate ultrasound transducer for the specific anatomic location and subject anatomy (linear *vs.* curvilinear array), the target nerves were identified in a transverse cross-sectional (short axis) view. A local anesthetic skin wheal was raised adjacent to the ultrasound transducer, and a Tuohy-tip needle was inserted through the skin wheal in plane beneath the ultrasound transducer and directed until the needle tip was immediately adjacent to the target nerve. Local anesthetic (1 to 3 ml, lidocaine 2%) was injected in divided doses with frequent aspiration. This was repeated for the additional target nerve(s). Within 20 min of the last injection, the subject's limb pain level was evaluated on the 0 to 10 Numeric Rating Scale, and if higher than at baseline before injection, the subject did not continue with treatment, and their participation in the study ended.

### Treatment Group Assignment (Randomization)

Remaining participants were allocated to one of two possible treatments: *active* cryoneurolysis or *sham* (*placebo*). Randomization was stratified by institution in randomly chosen block sizes using computer-generated lists by the informatics group of the Department of Outcomes Research at the Cleveland Clinic (Cleveland, Ohio). Treatment group assignment was conveyed to the enrolling sites *via* the same secure web-based system used to collect and collate all postintervention outcomes (Research Electronic Data Capture, Cleveland Clinic).<sup>14</sup>

A cryoneurolysis console device was used for all participants (PainBlocker, Epimed International, USA). Cryoneurolysis probes are available that either (1) pass nitrous oxide to the tip inducing freezing temperatures (approximately  $-70^{\circ}\text{C}$ ); 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. Importantly, these 16-gauge, trocar-tipped probes are indistinguishable in appearance and audible cues, and therefore, investigators, participants, and all clinical staff were masked to treatment group assignment (with the exception of the treating physician performing the cryoneurolysis). After repeated sterile preparation and draping, an angiocatheter-like introducer was inserted beneath the ultrasound transducer and

directed until immediately adjacent to the target nerve. The appropriate probe (active *vs.* sham) was inserted through the introducer, and the cryoneurolysis device was triggered using three cycles of 2-min gas activation separated by 1-min defrost periods.<sup>15</sup> The process was repeated for each treated nerve using the same probe for all applications (*e.g.*, all nerves received either active cryoneurolysis or sham/placebo, and not a mix of the two possible treatments).

Of note, the treating physician was not masked to treatment group assignment during the cryoneurolysis procedure. This was because the ice ball forming at the distal end of the probe—with active treatment—is clearly visible by ultrasound, and the lack of an ice ball for placebo participants is equally clear.<sup>16</sup> We believe it is essential to continuously visualize the probe and target nerve throughout the freeze and thaw cycles to ensure (1) the entire nerve diameter is fully encompassed by the sphere of ice and (2) the ice ball remains relatively motionless to prevent it from tearing surrounding tissue. This cannot be achieved if the ultrasound is turned off during nitrous oxide administration to mask the provider, and we prioritized patient safety over provider masking. Treating physicians did not have subsequent contact with study participants, or data collection, management, and analysis.

Before discharge, participants and their caretakers were provided with verbal and written instructions as well as the contact information for an investigator. Patients were informed that any sensory deficits from the short-acting lidocaine bolus that they may be experiencing would regress, and that they should not be alarmed by any subsequent increase in pain. Participants were provided with crutches if they so desired, although previous experience suggested that nearly all patients treated with cryoneurolysis continue to ambulate using their prosthesis without difficulty.

### Optional Crossover Treatment

Up to 2 months after the primary outcome measurement at month 4, participants could return for an optional repeated intervention procedure (“crossover”) with the alternative treatment (either active cryoneurolysis or sham/placebo), using the same protocol as described for the initial intervention. The crossover treatment was not required for study participation, as the primary analysis included a parallel study design for the initial intervention evaluated before any crossover treatment. However, the optional crossover treatment was offered for two reasons: (1) to ensure that all participants had access to the proposed treatment, regardless of the treatment to which they were initially assigned; and (2) to permit intrasubject differences between treatments to be analyzed (*e.g.*, assessing treatment-effect heterogeneity, or the variability of the causal effect across individuals, which will not be available from the parallel-group portion of the study alone). These intrasubject differences were secondary analyses, as there would be patient selection bias regarding which participants decided to have the crossover treatment.

The main results of the study were provided to all participants after final analysis.

## Outcome Measurements

We selected outcome measures that have established reliability and validity, with minimal interrater discordance, and are recommended for chronic pain clinical trials by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials consensus statement.<sup>17</sup> Outcomes were evaluated at baseline (before intervention), on days 1 and 7, as well as months 1, 2, 3, and 4, relative to the initial and optional crossover treatment(s). In addition, outcomes were evaluated 12 months after the initial intervention. Outcome measures were collected in person for the baseline measurements immediately before the initial intervention as well as the crossover treatments. All other outcomes were collected by investigators at the University of California–San Diego (San Diego, California) by telephone regardless of enrolling center.

The questionnaires differentiated multiple dimensions of limb sensations or pain. *Residual limb* (“stump”) pain indicated painful sensations localized to the portion of limb still physically present.<sup>18</sup> *Phantom limb pain* indicated painful sensations referred to the lost body part.<sup>18</sup> *Phantom limb sensations* indicated non-painful sensations referred to the lost body part.<sup>18</sup>

Each type of pain or sensation was defined for patients immediately before questionnaire application at each time point, and patients were instructed to address phantom limb pain when responding to questions unless otherwise specified. Each time the questionnaire was applied, participants were instructed to respond for the previous 3 days.<sup>19</sup> Exceptions included day 1 for both the initial and crossover treatments, because at these time points, the interest was in participants’ experiences subsequent to the intervention. At these time points, participants were instructed to respond for the period since the intervention the previous day.

The primary instrument was the Brief Pain Inventory (short form), which assesses pain and its interference with physical and emotional functioning.<sup>20</sup> The form includes three domains: (1) *pain*, with four questions using an Numeric Rating Scale to evaluate 4 pain levels: “current,” “least,” “worst,” and “average”; (2) percentage of *relief* provided by pain treatments with one question; and (3) *interference* with physical and emotional functioning using a 0 to 10 scale (0 = no interference; 10 = complete interference). The seven interference questions involve general activity, mood, walking ability, normal work activities (both inside and outside of the home), relationships, sleep, and enjoyment of life.<sup>20</sup> The seven functioning questions can be combined to produce an interference subscale (0 to 70). The use of both single items (e.g., mood) and the composite scores are supported by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials recommendations for assessing pain in clinical trials.<sup>17,21</sup> Because phantom limb and residual limb (“stump”) pain have been correlated, the latter was assessed with the same four pain intensity questions.

To provide a global measure of worsening or improvement, the Patient Global Impression of Change was administered, allowing patient evaluation of integrated treatment effects.<sup>17</sup> This measure is a seven-point ordinal scale requiring the patient to rate the current intensity of phantom limb pain compared to their pretreatment baseline: 1 for “very much worse” to 7 for “very much improved” (4 is “no change”). Additional psychosocial factors were evaluated using the Beck Depression Inventory, a 21-item instrument measuring characteristic symptoms and signs of depression.<sup>22</sup> Each of the 21 factors is rated on a 0 to 3 scale, and then summed to produce the total score of 0 to 63. Mild, moderate, and severe depression are defined with scores of 10 to 18, 19 to 29, and 30 to 63, respectively.<sup>23</sup> Last, the frequency and average duration of nonpainful phantom sensations as well as phantom and residual limb pain were assessed.

## Statistical Analysis

Treatment group assignment was unmasked only after completion of the statistical analysis. We used descriptive statistics to compare the treatment groups for baseline variables. Groups were considered well balanced on a particular baseline variable if the absolute standardized difference (difference in means, mean ranks or proportions divided by the pooled SD) was less than  $1.96\sqrt{(n_1 + n_2)/(n_1 n_2)} = 0.46$ , where  $n_1$  and  $n_2$  are the per-group sample sizes.<sup>24</sup> All analyses were modified intention-to-treat, in which all randomized participants who received any of the study treatment were included and retained in their respective treatment groups.<sup>25</sup> CIs were adjusted for the group sequential design with overall alpha of 0.05, such that 95.6% CIs are reported throughout (referred to as “95% CI”). The study was designed with 90% power to detect a mean change of 1.7 or more on the Numeric Rating Scale for “average phantom pain” while adjusting for three interim analyses. Missing data were imputed using last observation carried forward for the primary outcome and using multiple imputation for secondary outcomes and sensitivity analysis on the primary outcome.

**Aim 1: Primary Outcome.** We assessed the average causal effect of cryoneurolysis (active) *versus* sham or placebo on phantom limb pain intensity (average pain during previous 72 h) at 4 months after the initial treatment using analysis of covariance to adjust for clinical site, baseline average pain intensity, clinical site, and any imbalanced baseline variable. We also assessed the treatment effect on the change from baseline average pain intensity (instead of adjusting for baseline pain score) in an analogous linear regression model. As a sensitivity analysis, we assessed the median difference (95% CI) of active *versus* placebo using the Hodges–Lehmann estimator of location shift and compared groups with a Wilcoxon rank sum test stratified by study site. We also assessed the treatment-by-clinical site interaction in the linear regression models.

**Assessing Treatment Effect Heterogeneity.** We assessed whether the treatment effect on the primary outcome



(phantom limb pain intensity during the past 72 h) varied across levels of specific baseline variables (besides clinical site) using linear regression as in the primary analysis and testing the treatment-by-covariate interaction. We assessed treatment effect heterogeneity across level of sex, body mass index, amputation level, phantom pain 20 min after the second lidocaine injection, and baseline average phantom and residual limb pain, with a predetermined significance criterion of  $P < 0.10$  for the interaction, without correction made for these multiple covariate analyses. For the last three variables, which are continuous/ordinal pain scores, the interaction was assessed on a continuous scale, although the results are shown in the forest plot dichotomizing pain into mild (Numeric Rating Scale 3 or less) versus moderate to severe (Numeric Rating Scale greater than 3).

**Secondary Outcomes (at 4 Months).** Randomized groups were compared at 4 months on the global measure of improvement (Patient Global Impression of Change scale; Aim 2a) using the Wilcoxon rank-sum test and Hodges–Lehman estimation of location shift, stratified by study site. We used a mixed-effects regression model with a fixed effect for treatment and an unstructured correlation matrix adjusted for study site and baseline pain interference components to assess the treatment effect across the seven components of the Brief Pain Inventory pain interference (Aim 2b). Randomized groups were compared on the Beck Depression Inventory using the Wilcoxon rank-sum test and estimating the treatment effect using the Hodges–Lehmann estimator of location shift, stratified by study site (Aim 2c).

The crossover treatment 4 to 6 months after the initial intervention allowed all participants the opportunity to receive the study treatment, but because it was optional also introduced selection bias from this time point forward. For crossover patients, we assessed the treatment effect using a linear mixed effects regression model with a fixed effect for treatment and random effect for patient, adjusted for treatment sequence and period. We tested for evidence of differential carryover effect with the treatment-by-period interaction. We also descriptively assessed (no treatment effects were estimated) the change from the initial baseline to 12 months for the initial active and sham participants for those who both did and did not receive the crossover treatment.

**Interim Analyses.** We conducted 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 was used with parameters  $-4$  and  $-2$  for efficacy and futility, respectively.<sup>26</sup> Thus, boundaries at the first through fourth analyses for efficacy (futility in parentheses) were  $P \leq 0.0016$  ( $P > 0.9572$ ),  $P \leq 0.0048$  ( $P > 0.7186$ ),  $P \leq 0.0147$  ( $P > 0.2389$ ), and  $P \leq 0.0440$  ( $P > 0.0440$ ) (Supplemental Table A, <http://links.lww.com/ALN/C958>, and Supplemental Figure A, <http://links.lww.com/ALN/C957>).

## Type I Error and Gatekeeping

We designed the study to use a parallel gatekeeping procedure to control the study-wide type I error at 0.05.<sup>27</sup> For this procedure, we therefore *a priori* prioritized the study outcomes into ordered sets, as Aim 1, Aim 2a, Aim 2b, and then Aim 2c. Analysis would proceed in that order, and testing would proceed through each “gate” to the next set if and only if at least one outcome in the current set reached significance. The significance level for each set would be 0.05 times a cumulative penalty for nonsignificant 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) was planned to control the type I error at the appropriate level.

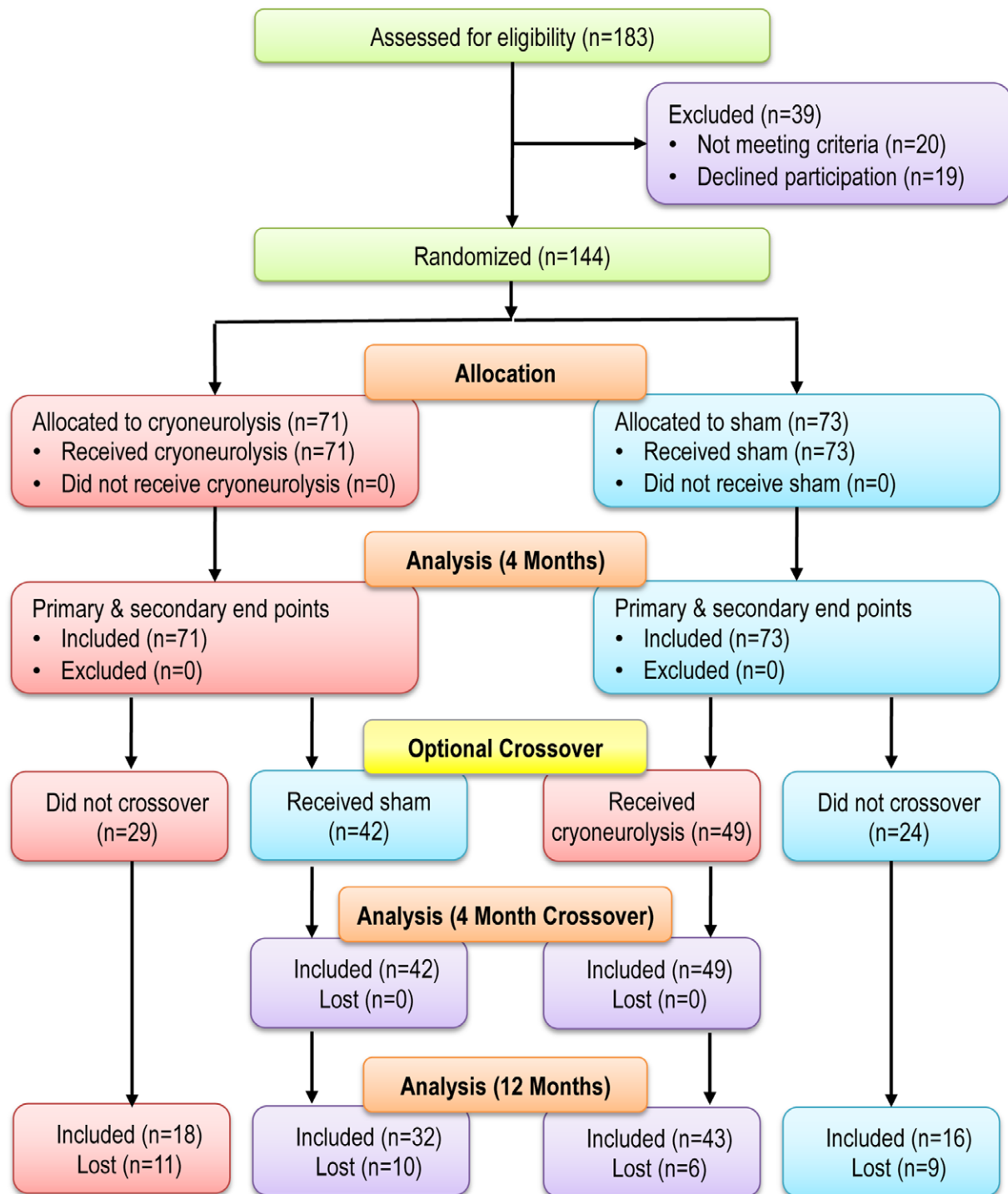
## Sample Size Considerations

Our sample size estimate was 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 after cryoneurolysis. Receiver operating characteristic curve analyses demonstrated that changes from baseline of at least 1.7 along a 10-point Numeric Rating Scale accurately identified patients who rated improvements as “much improved” or more, compared with those who perceived no change or worsening after analgesic interventions.<sup>28</sup> Multiple additional studies confirm this degree of reduction as clinically meaningful to individual patients with chronic pain.<sup>29–31</sup> Although meaningful group differences in the mean change would be somewhat smaller than important changes for individuals, we took a very conservative approach and powered our study to be able to detect group differences in mean change from baseline of 1.7 points or more on the Numeric Rating Scale.

Based on a conservative SD estimate for each group of 3.0 at 4 months, a correlation of 0.50 between baseline and follow-up Numeric Rating Scale, a two-sided test at the 0.05 significance level, power of 0.90, and four equally spaced analyses (three interim and one final, as needed), a maximum of 72 participants in each group ( $n = 144$  total) was required. 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) was a total of 100 under the alternative and 102 under the null hypotheses. East 5.3 software (Cytel Inc., USA) was used for sample size calculations and all analyses.

## Results

Between March 2018 and March 2021, a total of 144 patients were enrolled at six medical centers (fig. 1). Phantom limb pain fell from a median [quartiles] of 4.0 [2.0, 6.0] immediately before the initial single-injection lidocaine bolus to 0 [0, 3.0] for the active group and 0 [0, 2.0] for the placebo



**Fig. 1.** Consolidated Standards of Reporting Trials diagram.

group 20 min after the bolus. No participant experienced an increase in limb pain in the 20 min after the lidocaine injections. Therefore, all participants were randomized to either active treatment with cryoneurolysis ( $n = 71$ ) or

sham/placebo ( $n = 73$ ). Regarding baseline characteristics, all the variables were balanced between the two randomized groups with absolute standardized difference 0.33 or less (table 1).

**Table 1.** Patient Characteristics by Treatment Group (n = 144)

|   | Active<br>(n = 71) | Placebo<br>(n = 73) | Absolute Standardized<br>Difference |
|---|--------------------|---------------------|-------------------------------------|
| <b>Anthropometrics and demographics</b>         |                    |                     |                                     |
| Age (yr)  | 58 ± 13            | 58 ± 13             | 0.020                               |
| Female (%)                                      | 18 (25)            | 29 (40)             | 0.310                               |
| Body mass index (kg/m <sup>2</sup> )            | 29 ± 5.8           | 28 ± 5.3            | 0.049                               |
| Marital status (%)                              |                    |                     | 0.028                               |
| Single (never married)                          | 16 (23)            | 16 (22)             |                                     |
| Single (divorced)                               | 18 (25)            | 12 (16)             |                                     |
| Currently married                               | 33 (47)            | 36 (49)             |                                     |
| Others (separated and widowed)                  | 4 (5)              | 9 (13)              |                                     |
| Military status (%)                             |                    |                     | 0.182                               |
| Civilian (never in military)                    | 59 (83)            | 55 (75)             |                                     |
| Veteran   | 11 (16)            | 18 (25)             |                                     |
| Active duty                                     | 1 (1)              | 0 (0)               |                                     |
| Years of education                              | 14 [12, 16]        | 14 [12, 16]         | 0.075                               |
| <b>Study limb information</b>                   |                    |                     |                                     |
| Right (vs. left) side (%)                       | 32 (45)            | 32 (44)             | 0.025                               |
| Level of amputation (%)                         |                    |                     | 0.100                               |
| Transfemoral                                    | 22 (31)            | 21 (29)             |                                     |
| Transtibial                                     | 46 (65)            | 46 (63)             |                                     |
| Foot/ankle                                      | 3 (4)              | 6 (8)               |                                     |
| History of residual limb pain (%)               | 55 (78)            | 55 (75)             | 0.050                               |
| Current residual limb pain (%)                  | 44 (62)            | 45 (62)             | 0.007                               |
| Current prosthesis use (%)                      | 66 (93)            | 67 (92)             | 0.044                               |
| <b>Intervention information</b>                 |                    |                     |                                     |
| Pain score in limb                              |                    |                     |                                     |
| After premedication but before procedure        | 4 [2, 6]           | 4 [2, 6]            | 0.034                               |
| 20 min after lidocaine injections               | 0 [0, 3]           | 0 [0, 2]            | 0.005                               |
| Phantom limb pain before discharge              | 0 [0, 1]           | 0 [0, 0]            | 0.064                               |
| Residual limb pain before discharge             | 0 [0, 0]           | 0 [0, 0]            | 0.041                               |
| Distance of treatment from end of residual limb |                    |                     |                                     |
| Sciatic nerve (cm)                              | 15 [11, 23]        | 17 [12, 23]         | 0.132                               |
| Femoral nerve (cm)*                             | 22 [15, 31]        | 22 [16, 33]         | 0.021                               |
| <b>Enrollment center</b>                        |                    |                     |                                     |
| Cleveland Clinic                                | 20 (50%)           | 20 (50%)            |                                     |
| Naval Medical Center San Diego                  | 0 (0%)             | 1 (100%)            |                                     |
| Palo Alto Veterans Affairs                      | 1 (50%)            | 1 (50%)             |                                     |
| University of California-San Diego              | 25 (50%)           | 25 (50%)            |                                     |
| University of Florida                           | 24 (49%)           | 25 (51%)            |                                     |
| Walter Reed National Military Medical Center    | 1 (50%)            | 1 (50%)             |                                     |

Any variable with an absolute standardized difference 0.33 or greater was considered unbalanced. Statistics presented as mean ± SD, median [interquartile range] or N (column %).

Some groups do not total 100% due to rounding error.

\*One missing value from the sham treatment group.

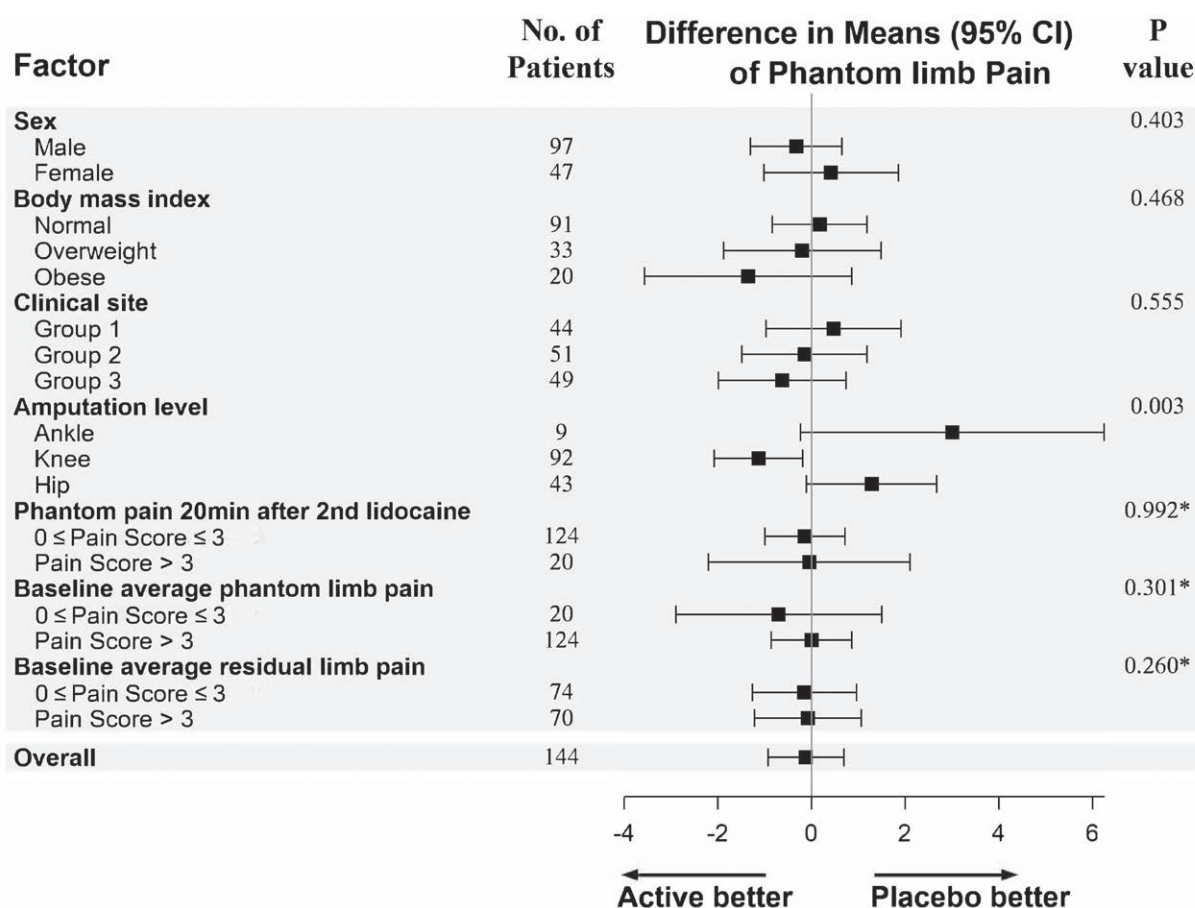
## Primary Outcome

Pretreatment phantom pain scores were a median [quartiles] of 5.0 [4.0, 6.0] for active treatment (cryoneurolysis) and 5.0 [4.0, 7.0] for sham or placebo. At 4 months, average phantom limb pain scores were 4.3 [1.5, 6] for active and 4.5 [2, 6] for placebo, with estimated difference in means (95% CI) of −0.1 (−1.0 to 0.7),  $P = 0.759$ , adjusting for baseline pain score and clinical site while using last-observation-carried-forward (for  $n = 1$  cryoneurolysis and  $n = 7$  placebo patients); the futility boundary was crossed (Supplemental Figure A, <http://links.lww.com/ALN/C957>). We also assessed change from baseline: pain intensity decreased by 0.5 [−0.5, 3.0] in patients given cryoneurolysis ( $n = 71$ ) versus 0 [0, 3] in patients given sham

( $n = 73$ ): estimated difference (95% CI) −0.1 (−1.0 to 0.7),  $P = 0.759$ . Finally, the nonparametric Hodges–Lehman estimator comparing active and placebo on 4-month average phantom limb pain scores gave a similar result, with median difference (95% CI) of −0.25 (−1, 0.5),  $P = 0.565$ .

**Treatment Effect Heterogeneity.** There was little notable evidence of treatment effect heterogeneity across levels of most of the selected baseline (prerandomization) variables, except for amputation level (interaction  $P = 0.003$ , fig. 2). Active cryoneurolysis was better for a transtibial amputation level, but worse for transfemoral and ankle/foot amputations (table 2).

**Gatekeeping Rules.** Since the primary outcome was not significant, based on our *a priori* statistical plan to use a parallel



**Fig. 2.** Forest plot assessing interactions between prespecified baseline factors and the effect of ultrasound-guided percutaneous cryoneurolysis on phantom limb pain at 4 months. \**P* value was estimated from continuous pain score by multivariable linear regression adjusted for study site and day 0 average phantom limb pain.

gatekeeping approach to control study-wide type I error at 5%, we cannot make an inference on any of the secondary endpoints. Therefore, secondary outcome results are given in the form of estimated difference and CI (not *P* value), but we do not make any formal inference or conclusions on them—only on the primary outcome.

## Secondary Endpoints

Using the 1 to 7 Global Impression of Change Scale at month 4, participants who received active treatment rated their phantom pain as a median of 4 (“no change”) [4, 7] versus 4 (“no change”) [4, 6] for placebo participants with an estimated median difference (95% CI) of 0 (0 to 0) at 4 months (Aim 2A). Using the Brief Pain Inventory interference subscale to measure pain’s interference with physical and emotional functioning at month 4, patients who received active cryoneurolysis scored 23 [0, 39] versus 22 [3, 34] for sham, with a median difference (95% CI) of 0 (–5 to 6; Aim 2B, table 3, fig. 3). The

mixed effects model suggested no treatment-by-component interaction, and the estimated difference in means (95% CI) [scale 0 to 10] was 0.2 (–0.5 to 0.9). Using the Beck Depression Inventory (Aim 2C), participants receiving active treatment reported a median change from baseline of –2 [–7, 0] versus –2 [–5, 0] for sham, with a difference (95% CI) of 1 (–1 to 3). Descriptively, cryoneurolysis did not demonstrably improve phantom and residual limb pain outcomes at any time point compared with the sham treatment (table 3, figs. 4 and 5).

## Crossover Treatments

The crossover treatment administered 0 to 2 months after the measurement of the primary outcome was optional, resulting in selection bias on patients who did not cross over and, on those who did cross over, potential interference with the longer-term effects of the initial treatment. Therefore, outcomes after the 4-month time point are reported descriptively only. Ninety-one patients



**Table 2.** Assessing Interactions between Treatment and Specified Baseline Factors on Primary Outcome of Month 4 Average Phantom Limb Pain

| Factor                                     | Active*   | Placebo*  | Difference in Means†<br>Active - Placebo (95% CI) | P Value‡ | P Value§ |
|--|-----------|-----------|---|----------|----------|
| Sex  |           |           |   |          | 0.403    |
| Male                                       | 3.5 ± 2.7 | 3.9 ± 2.7 | -0.33 (-1.32 to 0.65)                             | 0.506    |          |
| Female                                     | 4.6 ± 2.6 | 4.3 ± 3.2 | 0.42 (-1.04 to 1.87)                              | 0.573    |          |
| Body mass index                            |           |           |   |          | 0.468    |
| Normal                                     | 3.7 ± 2.8 | 4.0 ± 2.9 | 0.17 (-0.85 to 1.19)                              | 0.742    |          |
| Overweight                                 | 4.2 ± 2.4 | 3.7 ± 2.4 | -0.20 (-1.89 to 1.49)                             | 0.815    |          |
| Obese                                      | 3.7 ± 2.7 | 5.1 ± 3.7 | -1.35 (-3.58 to 0.87)                             | 0.230    |          |
| Clinical site                              |           |           |   |          | 0.555    |
| Group 1                                    | 3.9 ± 2.2 | 3.7 ± 3.1 | 0.47 (-0.98 to 1.92)                              | 0.525    |          |
| Group 2                                    | 3.8 ± 2.7 | 3.9 ± 2.7 | -0.15 (-1.50 to 1.20)                             | 0.827    |          |
| Group 3                                    | 3.7 ± 3.1 | 4.6 ± 2.9 | -0.63 (-2.01 to 0.75)                             | 0.367    |          |
| Amputation level                           |           |           |   |          | 0.003§   |
| Ankle                                      | 4.3 ± 3.8 | 2.4 ± 3.8 | 3.00 (-0.25 to 6.26)                              | 0.070    |          |
| Knee                                       | 3.1 ± 2.5 | 4.4 ± 2.7 | -1.14 (-2.09 to -0.18)                            | 0.020    |          |
| Hip  | 5.3 ± 2.3 | 3.9 ± 2.9 | 1.28 (-0.13 to 2.68)                              | 0.074    |          |
| Phantom pain 20 min after second lidocaine |           |           |   |          | 0.992    |
| Pain score in [0,3]                        | 3.5 ± 2.6 | 3.9 ± 2.8 | -0.14 (-1.01 to 0.72)                             | 0.744    |          |
| Pain score > 3                             | 5.5 ± 2.8 | 5.1 ± 3.3 | -0.05 (-2.21 to 2.11)                             | 0.961    |          |
| Baseline average phantom limb pain         |           |           |   |          | 0.301    |
| Pain score in [0,3]                        | 1.5 ± 1.8 | 2.5 ± 1.4 | -0.7 (-2.91 to 1.5)                               | 0.529    |          |
| Pain score > 3                             | 4.1 ± 2.6 | 4.4 ± 3.0 | 0 (-0.88 to 0.87)                                 | 0.992    |          |
| Baseline average residual limb pain        |           |           |   |          | 0.260    |
| Pain score in [0,3]                        | 3.5 ± 2.6 | 3.9 ± 2.6 | -0.16 (-1.28 to 0.97)                             | 0.784    |          |
| Pain score > 3                             | 4.1 ± 2.7 | 4.3 ± 3.2 | -0.08 (-1.24 to 1.07)                             | 0.887    |          |
| Overall                                    | 3.8 ± 2.7 | 4.1 ± 2.9 | -0.12 (-0.95 to 0.70)                             |          | 0.759    |

\*Mean ± SD for month 4 average phantom limb pain.

†Difference in means of active *versus* placebo and *P* value (significant if *P* < 0.05) estimated using a linear mixed effects regression model adjusted for study site, factor, and baseline pain interference components. ‡Interaction *P* value from same linear model assessing treatment-by-covariate interaction. §Since the overall interaction was significant, we report pairwise interactions here as well: ankle *versus* hip (*P* = 0.34), knee *versus* ankle (*P* = 0.017), and knee *versus* hip (*P* = 0.006). In summary, the treatment effect for knee was statistically different from ankle and hip.

participated in the crossover phase, receiving either an active (*n* = 49) or sham (*n* = 42) treatment (Supplemental Table B, <http://links.lww.com/ALN/C959>). Active treatment appeared to be similar to sham on 4 months average phantom limb pain intensity, pain's interference on physical and psychologic functioning, and Patient Global Impression of Change (Supplemental Table C, <http://links.lww.com/ALN/C960>). The period-by-treatment interaction *P* value of 0.04 suggested that there was some evidence of differential carryover effect between the first and second periods. These results would be generalizable to patients like those who chose to receive the crossover, which may differ from the main trial population. As well, active treatment had a larger reduction from baseline in average phantom limb pain intensity compared to placebo, with a mean difference (95% CI) of -1 (-2 to -0.5). The variability in the individual causal effects of active *versus* placebo as measured by the SD of the individual treatment effects was 1.3. Outcomes at 12 months after randomization did not appear to differ between treatment groups (table 4).

## Serious Adverse Events and Major Protocol Deviations

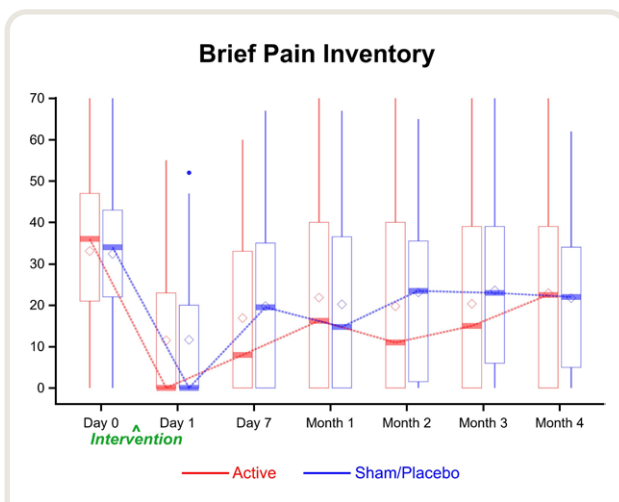
There were two deaths within the year after treatment, neither determined to be related to study participation: one myocardial infarction and one related to COVID-19 infection with severe acute respiratory syndrome. One participant developed dementia of unknown etiology within the 6 months after his initial treatment per an adult child's report. The only adverse event deemed related to study participation was a woman with a transtibial amputation who first received a sham treatment and subsequently crossed over with an active treatment that was performed just distal to the inguinal ligament for the femoral nerve. This protocol deviation resulted in profound quadriceps femoris weakness and some insensate areas of skin on the medial thigh. These deficits resolved slowly until complete resolution after 12 to 15 months. However, 3 months after the active crossover treatment, she fell while climbing stairs and fractured a clavicle, which required three subsequent surgical fixation procedures.

**Table 3.** Effect of Treatment Group on Secondary Outcomes (N = 144)

|  | Active<br>(n = 71) | Placebo<br>(n = 73) | Difference in Means* or Median†<br>Active - Placebo (95% CI)‡ |
|--|--------------------|---------------------|---|
| Patient Global Impression of Change scale (month 4)§ |                    |                     |   |
| Score (1–7)  | 4 [4, 7]           | 4 [4, 6]            | 0 (0 to 0)†   |
| Score ≤ 4 (worse or no change)                       | 47 (66%)           | 42 (58%)            |   |
| Score > 4 (improved)                                 | 23 (32%)           | 24 (33%)            |   |
| Brief Pain Inventory (Interference Subscale)§        |                    |                     |   |
| Total score  | 23 [0, 39]         | 22 [3, 34]          | 0 (–5 to 6)†  |
| Overall treatment effect                             | 3.4 (0.4)          | 3.0 (0.4)           | 0.2 (–0.5 to 0.9)*  |
| General Activity                                     | 2.5 [0, 6]         | 3.5 [0, 6]          |   |
| Mood   | 3.0 [0, 6]         | 1.5 [0, 5]          |   |
| Walking ability                                      | 2.5 [0, 6]         | 2.5 [0, 5]          |   |
| Normal work  | 2.0 [0, 6]         | 2.0 [0, 5]          |   |
| Relations with other people                          | 1.5 [0, 4]         | 0 [0, 4]            |   |
| Sleep  | 3.0 [0, 7]         | 5.0 [0, 8]          |   |
| Enjoyment of life                                    | 3.0 [0, 7]         | 3.5 [0, 6]          |   |
| Beck Depression Inventory                            |                    |                     |   |
| Total score  | 4 [0, 14]          | 2 [0, 9]            |   |
| Change from baseline                                 | –2 [–7, 0]         | –2 [–5, 0]          | 1 (–1 to 3)†  |

Summary statistics presented as median [interquartile] or mean ± SD with complete dataset. Last-observation-carried-forward method was applied for all analyses, if month 3 measurements were available.

\*Overall treatment effect: difference in means between two groups across the seven components was estimated from a linear mixed-effects regression model. The model adjusted for study site and baseline pain interference components. Treatment by component interaction was nonsignificant ( $P = 0.202$ ). Per-group mean (standard error) across components is also reported. †Median difference (CI) of placebo *versus* active was estimated with the Hodges–Lehmann estimator of location shift between groups stratified by study site;  $P$  value from Wilcoxon–Mann–Whitney test. ‡CIs adjusted for group sequential design to maintain overall study alpha of 0.05.  $P$  value of 0.044 or less was considered significant for treatment effect on all outcomes. §One and seven missing values from the active and sham treatment groups, respectively. ||Estimates (standard error) were reported for each group.



**Fig. 3.** Effects of ultrasound-guided percutaneous cryoneurolysis (denoted in green) on the Brief Pain Inventory interference domain over time. Data expressed as pain's interference on each of seven components (higher scores indicate more interference) demarked as median (dark horizontal bars) with 25th to 75th percentiles (box), 10th to 90th percentiles (whiskers), mean (diamonds), and outliers (circles). Following our statistical gate-keeping protocol, we do not make inferences or draw conclusions on the secondary endpoints, since no difference was found on the primary endpoint.

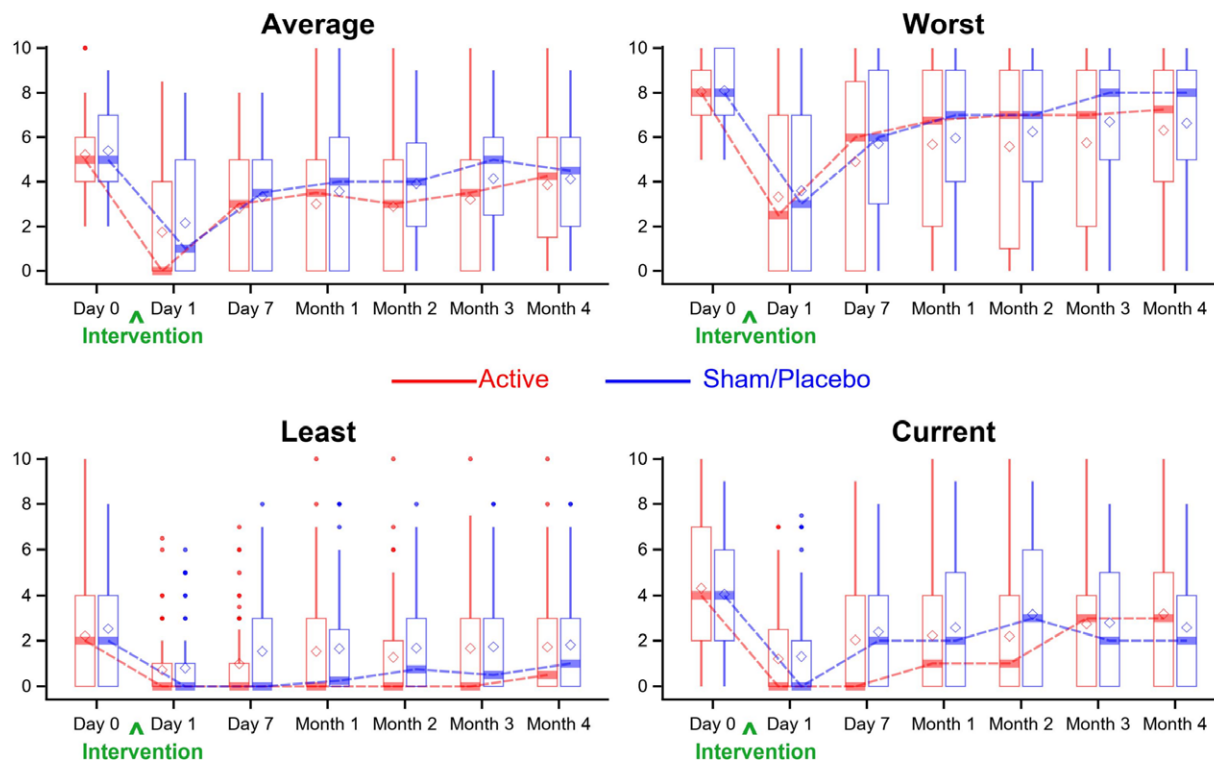
## Discussion

This multicenter, randomized, sham-controlled trial failed to identify a benefit in treating established postamputation phantom limb pain with ultrasound-guided percutaneous cryoneurolysis. This is a somewhat surprising and disappointing finding considering that cryoneurolysis has been used to treat postamputation pain for decades with favorable outcomes reported in *uncontrolled* case series.<sup>11–13</sup> We can only speculate on the reasons for these contrasting findings.

The most obvious potential explanation is that cryoneurolysis does not, in fact, result in lasting, measurable analgesic benefits, and previous reports of posttreatment improvement in uncontrolled series are due to a placebo effect, selective reporting, and/or natural resolution of pain over time.<sup>11–13</sup> As possible evidence of a placebo effect, 29% of the sham group experienced a decrease in pain score of at least 1.7, the threshold we prospectively defined as the smallest clinically relevant improvement for individuals based on previously published data (similar to the 36% who had received active cryoneurolysis).<sup>28</sup>

It is also possible that the improvement in about one third of all patients was not a placebo response to the study intervention, but rather a consequence of the single injection of local anesthetic administered to all participants immediately before the study intervention.<sup>6</sup> This would help to explain why participants who chose to cross over did not

## Phantom Limb Pain



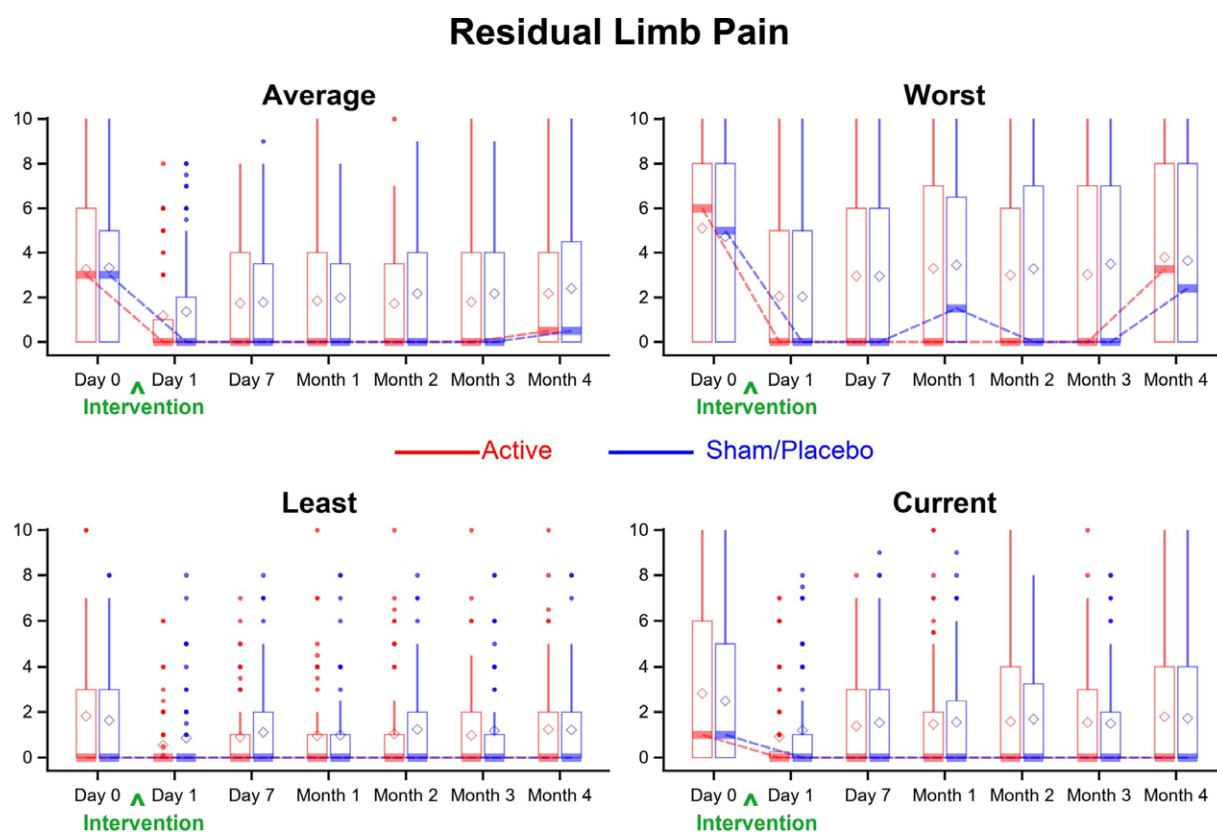
**Fig. 4.** Effects of ultrasound-guided percutaneous cryoneurolysis (denoted in green) on worst, average, least, and current *phantom limb pain* over time (primary outcome: *average phantom limb pain* at 4 months). Pain intensity indicated using a numeric rating scale of 0 to 10, with 0 equal to no pain and 10 being the worst imaginable pain. Data expressed as median (dark horizontal bars) with 25th to 75th percentiles (box), 10th to 90th percentiles (whiskers), mean (diamonds), and outliers (circles). Following our statistical gatekeeping protocol, we do not make inference or conclusions on the secondary endpoints, since no difference was found on the primary endpoint.

experience the analgesic benefits of those who responded to the initial treatment and who therefore, presumably, chose not to undergo the crossover treatment. While a possible explanation for our results during the first few months, it is doubtful that a single injection of lidocaine is responsible for the finding that most of these “responders” reported continued improvement after 12 months.<sup>6,8</sup>

Alternatively, our negative results may be explained by the locations where we applied cryoneurolysis. In the subset of patients with a transtibial amputation—a majority of participants ( $n = 92$ )—cryoneurolysis was associated with an improved outcome compared with sham at 4 months ( $P = 0.003$  overall; pairwise comparisons: transtibial *vs.* ankle,  $P = 0.017$ ; transtibial *vs.* transfemoral,  $P = 0.006$ ). Conversely, patients with a transfemoral or foot/ankle amputation who received active treatment fared worse than their sham counterparts. These could be spurious findings (type I error), but it is worth exploring given that medical progress is usually iterative, and three major differences between transtibial and transfemoral amputations may help inform future research: (1) duration of effect, (2) impact

on abnormal input from the peripheral to central nervous system, and (3) target nerve cross-sectional area.

Regarding the first—a reduced duration of a treatment effect for transfemoral amputations—it is important to note that the optimal point for cryoneurolysis along a target nerve remains unknown. We chose to treat both the sciatic and femoral nerves at the most distal locations clearly visualized with ultrasound as low as the bifurcation of the sciatic nerve and medial femoral epicondyle for the saphenous nerve. Our reasoning was that a more proximal lesion could increase sensory, motor, and proprioception deficits in the residual limb, increasing the risk of falls when using a prosthesis for the entire treatment effect duration, usually measured in months. Of 144 participants, the only serious adverse event deemed related to study participation may be seen as supporting this decision: an investigator chose to treat the femoral nerve at the inguinal ligament for a patient with a transtibial amputation instead of more distally at the medial femoral epicondyle, resulting in profound quadriceps femoris muscle weakness lasting more than 1 yr and possibly contributing to a fall 3 months after treatment.



**Fig. 5.** Effects of ultrasound-guided percutaneous cryoneurolysis (denoted in green) on worst, average, least, and current residual limb pain over time. Pain intensity indicated using a numeric rating scale of 0 to 10, with 0 equal to no pain and 10 being the worst imaginable pain. Data expressed as median (dark horizontal bars) with 25th to 75th percentiles (box), 10th to 90th percentiles (whiskers), mean (diamonds), and outliers (circles). Following our statistical gatekeeping protocol, we do not make inference or conclusions on the secondary endpoints, since no difference was found on the primary endpoint.

However, using a distal cryoneurolysis location for the remaining participants likely decreased the duration of effect for transfemoral amputations. The primary determinant of cryoneurolysis duration is a function of the distance between the cryolesion and nerve endings, with nerves regrowing at approximately 1 to 2 mm/day.<sup>32</sup> Therefore, if our theory that block duration and analgesic benefits are correlated, the short cryolesion–nerve ending distance for transfemoral amputations would greatly decrease both the duration of cryoneurolysis effects and the impact on phantom limb pain. In contrast, because we did not apply cryoneurolysis distal to the medial femoral epicondyle, for transtibial amputations there was a greater length of remaining nerve distal to the cryolesion, resulting in an increased treatment effect duration and therefore possibly analgesic effects.<sup>12</sup>

The second difference between the two amputation locations—reduced impact on abnormal input from the peripheral to central nervous systems—is also based on our chosen protocol. We did not treat the obturator or posterior

femoral cutaneous nerves, which contribute to the innervation at the level of transfemoral amputations, so the coverage provided by the cryoneurolysis intervention was inherently incomplete. In contrast, afferent sensory input for transtibial amputations is carried by the two nerves we did treat—the sciatic nerve and saphenous branch of the femoral nerve. Effective treatment for transfemoral amputations may require the administration of cryoneurolysis to all nerves innervating the lower extremity.

The third difference between the two amputation locations is the target nerve cross-sectional area, which is larger the more proximal within the lower extremity. The premise of our study hypothesis is that phantom limb pain is at least partially sustained by abnormal input from the peripheral to the central nervous systems. Therefore, interrupting the abnormal input with cryoneurolysis requires a thorough neural lesion with a prolonged duration. Reducing the temperature of a nerve below  $-20^{\circ}\text{C}$  (but not colder than  $-100^{\circ}\text{C}$ ) results in a Sunderland second-degree nerve injury characterized by a reversible degeneration of axons known



**Table 4.** Long-term Follow-up at 12 Months Postrandomization

| Initial Treatment               | Active         | Sham           | Active           | Sham          |
|---------------------------------|----------------|----------------|------------------|---------------|
|                                 | No Crossover   |                | Had Crossover    |               |
|                                 | (n = 29)       | (n = 24)       | (n = 42)         | (n = 49)      |
| Phantom pain                    |                |                |                  |               |
| Worst pain                      | -6 [-7, 0]*    | -4 [-6, 1]†    | -1 [-5, 0]‡      | -1 [-4.3, 0]  |
| Average pain                    | -4 [-5, 0]*    | -4 [-5, -1.5]† | -3.5 [-5, -1]‡   | -1.3 [-5, 0]  |
| Residual limb pain              |                |                |                  |               |
| Worst pain                      | 0 [-4, 0]*     | -2 [-5, 0]†    | -0.3 [-5.5, 0]‡  | 0 [-2, 0]     |
| Average pain                    | 0 [-3, 0]*     | -2 [-5, 0]†    | -1.5 [-3.5, 0]‡  | 0 [-2, 0]     |
| Brief Pain Inventory Components |                |                |                  |               |
| General activity                | -4 [-7, -1]*   | -3 [-4, 0]†    | -2 [-5, 0]‡      | -1 [-5, 0]#   |
| Mood                            | -2 [-5, 0]*    | -3 [-6, 0]†    | -2 [-5, 0]‡      | 0 [-3, 0]#    |
| Walking ability                 | -2 [-5, -1]*   | -3 [-10, -2]†  | -1.5 [-5, -0.5]‡ | -1 [-5, 0]#   |
| Normal work                     | -2 [-7, -1]*   | -3 [-7, -1]†   | -2 [-5, 0]‡      | 0 [-3, 0]#    |
| Relations with others           | -1 [-4, 0]§    | -1 [-5, 0]†    | -1 [-1.5, 0]‡    | 0 [-2, 0]#    |
| Sleep                           | -3.5 [-8, -1]* | -5 [-9, 0]†    | -2 [-4, -0.5]‡   | -2.5 [-7, 0]# |
| Enjoyment of life               | -2 [-6, 0]§    | -1 [-7, 1]†    | -2.5 [-5, 0]‡    | -1 [-4, 0]#   |
| Global Impression of Change     | 7 [4, 7]*      | 7 [4, 7]†      | 5 [4, 7]‡        | 4 [4, 7]#     |
| Beck Depression Inventory       | -7 [-11, -5]*  | -4 [-10, -1]†  | -7 [-10, -1]‡    | -6 [-9.5, -3] |

Values represent the change from initial baseline with the exception of the Patient Global Impression of Change, which are presented as raw values (n = 144). Data presented as median [interquartile range].

\*11 missing values. †9 missing values. ‡10 missing values. §12 missing values. ||5 missing values. #6 missing values.

as Wallerian degeneration.<sup>33</sup> In contrast, temperatures warmer than  $-20^{\circ}\text{C}$  result in a first-degree nerve injury,<sup>34</sup> which induces a shorter, unpredictable neuropraxia that can itself result in dysesthesias.<sup>35</sup> In other words, an inadequate freeze can actually induce pain. In our study, while treating physicians visualized the ice ball with ultrasound to ensure it encompassed the entire nerve, there is no guarantee that the entire sphere of ice cooled below  $-20^{\circ}\text{C}$ , possibly inducing a variable duration neuropraxia that could result in analgesia initially but increase pain subsequently.<sup>36</sup> In addition, without Wallerian degeneration, the entire nerve could have remained functional since myelinated fibers can conduct “over” small lesions: while a lesion length of 3 to 6 mm is adequate to severe conduction in laboratory animals, the length in humans remains unknown.<sup>37</sup> Since the sciatic nerve is the largest in the human body, cryoneurolysis of more proximal application for the transfemoral amputations may have resulted in incomplete cryolesions. Supporting this theory is our seemingly counterintuitive finding that patients with a transfemoral amputation who received active treatment fared *worse* than their sham counterparts.

Evidence contradicting these last two interpretations is that amputations at or below the ankle had the same cryoneurolysis administration levels as transtibial yet were *not* associated with improved outcomes. Possibly explaining this apparent contradiction is that there were only nine ankle cases, and therefore this finding may itself be spurious, with confidence in the result far lower than for the transfemoral (n = 43) and transtibial (n = 92) subgroups.

## Limitations

The major limitation of our trial is the reality that the optimal cryoneurolysis treatment parameters such as duration of freeze, duration of thaw, number of freeze and thaw cycles, freeze temperature, probe design, and anatomic treatment location all remain unknown.<sup>10</sup> The specific technique used in our trial was based on published (successful) pilot studies and decades of previous clinical experience,<sup>9,11,12</sup> but whether other techniques might have different effects is unknown.

A second limitation is the local anesthetic administered before the study intervention for both active cryoneurolysis and sham treatment groups. As such, even participants who underwent the sham procedure had a single-injection peripheral nerve block, which may decrease postamputation pain for up to a few weeks.<sup>38</sup> We provided local anesthetic to participants who would receive the active treatment because (1) it negates the discomfort experienced by many patients undergoing cryoneurolysis; and (2) we wanted to confirm that a peripheral nerve block would not induce paradoxical pain: a rare response, but one that could result in months of increased pain after a cryoneurolysis procedure (which we did not observe).<sup>39</sup> We provided the local anesthetic block to patients who would receive sham to retain masking of treatment group assignment: we presumed that patients who experienced absolutely no sensory changes during the (sham) study intervention without a peripheral nerve block would assume they had received the sham. While

this protocol does not decrease confidence in our primary outcome—we can conclude that the addition of cryoneurolysis failed to improve pain outcomes 4 months after treatment—it does make interpretation of our negative results and designing subsequent research more challenging.

In summary, ultrasound-guided percutaneous cryoneurolysis did not reduce phantom limb pain 4 months after treatment. Although we do not make inferences or draw conclusions on the secondary endpoints due to our gatekeeping procedure and negative primary outcome, assessment of treatment effect heterogeneity remains important. Exploratory *post hoc* analysis revealed that treatment effect after 4 months was associated with the level of amputation, with transtibial amputation responsive to cryoneurolysis as opposed to ankle/foot and transfemoral, which fared worse than sham. The reasons for this difference remain unclear, and future research is warranted.

## Acknowledgments

The authors appreciate the invaluable assistance of the members of the uncompensated Data Safety Monitoring Board, without whom this study would not have been possible: Salim M. Hayek, M.D., Ph.D. (Department of Anesthesiology, Case Western Reserve University, Cleveland, Ohio); Anupama N. Wadhwa, M.B.B.S., M.Sc., F.A.S.A. (Department of Anesthesiology, University of Texas Southwestern, Dallas, Texas); and Gerald Beck, Ph.D. (Department of Quantitative Health Sciences, Cleveland Clinic, Cleveland, Ohio).

## Research Support

The U.S. Army Medical Research Acquisition Activity (Fort Detrick, Maryland) is the awarding and administering acquisition office. This work was supported by the Assistant Secretary of Defense for Health Affairs endorsed by the Department of Defense (Washington, D.C.), through the Congressionally Directed Medical Research Program under award No. W81XWH-17-2-0051 (PR160263). The project was also partially supported by the National Institutes of Health (Bethesda, Maryland), grant UL1TR001442. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the funding entities. None of the authors has a personal financial interest in this research.

## Competing Interests

Drs. Ilfeld and Gabriel report that the University of California San Diego (San Diego, California) has received funding and/or equipment for other research projects from Myoscience (Fremont, California), Epimed International (Farmers Branch, Texas), Infutronics (Natick, Massachusetts), Avanos (Irvine, California), and SPR Therapeutics (Cleveland, Ohio). Dr. Trescot has served on an advisory board for Atricure (Mason, Ohio) and is the Chief Medical Officer for Stimwave Technologies (Pompano, Florida). Dr.

Cohen receives research funding (paid to the institution) from Avanos, and research funding (paid to the institution) from Scilex (Palo Alto, California) for a randomized controlled trial evaluating a local anesthetic patch for neck pain, and as a consultant for a randomized trial evaluating a steroid for epidural injection. He serves as a consultant for SPR (Cleveland, Ohio), and is involved in research projects with their peripheral nerve stimulation system at Walter Reed National Military Medical Center (Bethesda, Maryland). Dr. Sessler is the Chair, Data and Safety Monitoring Board, Neuros Medical Quest (Land O' Lakes, Florida) trial. He is a consultant for Pacira Pharmaceuticals (Parsippany-Troy Hills, New Jersey), and this company funds trials in the Department of Outcomes Research, The Cleveland Clinic (Cleveland, Ohio). Dr. Prologo is a consultant and research grant recipient for Boston Scientific (Natick, Massachusetts), a consultant for Varian Medical Systems (Crawley, United Kingdom), and cofounder, Focused Cryo, Inc. (Smyrna, Georgia). The other authors declare no competing interests.

## Reproducible Science

Deidentified patient-level data will be shared for collaborative analyses on request to Dr. Ilfeld (bilfeld@health.ucsd.edu) shortly after publication. The data dictionary and statistical tables and code will be provided as appropriate; a data-sharing contract will be required. The protocol is available by request.

## Correspondence

Address correspondence to Dr. Brian M. Ilfeld, M.D., M.S., Department of Anesthesiology, 9500 Gilman Drive, MC 0898, La Jolla, California 92093-0898. bilfeld@health.ucsd.edu. This article may be accessed for personal use at no charge through the Journal Web site, [www.anesthesiology.org](http://www.anesthesiology.org).

## Supplemental Digital Content

Supplemental Figure A, <http://links.lww.com/ALN/C957>  
Supplemental Table A, <http://links.lww.com/ALN/C958>  
Supplemental Table B, <http://links.lww.com/ALN/C959>  
Supplemental Table C, <http://links.lww.com/ALN/C960>

## References

1. Ziegler-Graham K, MacKenzie EJ, Ephraim PL, Travison TG, Brookmeyer R: Estimating the prevalence of limb loss in the United States: 2005 to 2050. *Arch Phys Med Rehabil* 2008; 89:422–9
2. Hsu E, Cohen SP: Postamputation pain: Epidemiology, mechanisms, and treatment. *J Pain Res* 2013; 6:121–36
3. Wartan SW, Hamann W, Wedley JR, McColl I: Phantom pain and sensation among British veteran amputees. *Br J Anaesth* 1997; 78:652–9
4. Richardson C, Kulkarni J: A review of the management of phantom limb pain: Challenges and solutions. *J Pain Res* 2017; 10:1861–70

5. Flor H, Elbert T, Knecht S, Wienbruch C, Pantev C, Birbaumer N, Larbig W, Taub E: Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. *Nature* 1995; 375:482–4
6. Birbaumer N, Lutzenberger W, Montoya P, Larbig W, Unertl K, Topfner S, Grodd W, Taub E, Flor H: Effects of regional anesthesia on phantom limb pain are mirrored in changes in cortical reorganization. *J Neurosci* 1997; 17:5503–8
7. Silva S, Loubinoux I, Olivier M, Bataille B, Fourcade O, Samii K, Jeannerod M, Demonet JF: Impaired visual hand recognition in preoperative patients during brachial plexus anesthesia: Importance of peripheral neural input for mental representation of the hand. *ANESTHESIOLOGY* 2011; 114:126–34
8. Ilfeld BM, Khatibi B, Maheshwari K, Madison SJ, Esa WAS, Mariano ER, Kent ML, Hanling S, Sessler DI, Eisenach JC, Cohen SP, Mascha EJ, Ma C, Padwal JA, Turan A, Investigators PA: Ambulatory continuous peripheral nerve blocks to treat postamputation phantom limb pain: A multicenter, randomized, quadruple-masked, placebo-controlled clinical trial. *Pain* 2021; 162:938–55
9. Trescot AM: Cryoanalgesia in interventional pain management. *Pain Physician* 2003; 6:345–60
10. Ilfeld BM, Finneran JJ: Cryoneurolysis and percutaneous peripheral nerve stimulation to treat acute pain. *ANESTHESIOLOGY* 2020; 133:1127–49
11. Moesker AA, Karl HW, Trescot AM: Treatment of phantom limb pain by cryoneurolysis of the amputated nerve. *Pain Pract* 2014; 14:52–6
12. Prologo JD, Gilliland CA, Miller M, Harkey P, Knight J, Kies D, Hawkins CM, Corn D, Monson DK, Edalat F, Dariushnia S, Brewster L: Percutaneous image-guided cryoablation for the treatment of phantom limb pain in amputees: A pilot study. *J Vasc Interv Radiol* 2017; 28:24–34.e4
13. Neumann V, O'Connor RJ, Bush D: Cryoprobe treatment: An alternative to phenol injections for painful neuromas after amputation. *AJR Am J Roentgenol* 2008; 191:W313; author reply W314
14. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG: Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; 42:377–81
15. Gill W, Fraser J, Carter DC: Repeated freeze-thaw cycles in cryosurgery. *Nature* 1968; 219:410–3
16. Kastler A, Gruber H, Gizewski E, Loizides A: Ultrasound assessment of ice-ball formation by cryoneurolysis device in an *ex vivo* model. *Reg Anesth Pain Med* 2018; 43:631–3
17. Dworkin RH, Turk DC, Peirce-Sandner S, Baron R, Bellamy N, Burke LB, Chappell A, Chartier K, Cleeland CS, Costello A, Cowan P, Dimitrova R, Ellenberg S, Farrar JT, French JA, Gilron I, Hertz S, Jadad AR, Jay GW, Kalliomaki J, Katz NP, Kerns RD, Manning DC, McDermott MP, McGrath PJ, Narayana A, Porter L, Quessy S, Rappaport BA, Rauschkolb C, Reeve BB, Rhodes T, Sampaio C, Simpson DM, Stauffer JW, Stucki G, Tobias J, White RE, Witter J: Research design considerations for confirmatory chronic pain clinical trials: IMMPACT recommendations. *Pain* 2010; 149:177–93
18. Manchikanti L, Singh V: Managing phantom pain. *Pain Physician* 2004; 7:365–75
19. Broderick JE, Schwartz JE, Vikingstad G, Pribbenow M, Grossman S, Stone AA: The accuracy of pain and fatigue items across different reporting periods. *Pain* 2008; 139:146–57
20. Cleeland CS, Ryan KM: Pain assessment: Global use of the Brief Pain Inventory. *Ann Acad Med Singapore* 1994; 23:129–38
21. Turk DC, Dworkin RH, Burke LB, Gershon R, Rothman M, Scott J, Allen RR, Atkinson JH, Chandler J, Cleeland C, Cowan P, Dimitrova R, Dionne R, Farrar JT, Haythornthwaite JA, Hertz S, Jadad AR, Jensen MP, Kellstein D, Kerns RD, Manning DC, Martin S, Max MB, McDermott MP, McGrath P, Moulin DE, Nurmikko T, Quessy S, Raja S, Rappaport BA, Rauschkolb C, Robinson JP, Royal MA, Simon L, Stauffer JW, Stucki G, Tollett J, von Stein T, Wallace MS, Wernicke J, White RE, Williams AC, Witter J, Wyrwich KW: Developing patient-reported outcome measures for pain clinical trials: IMMPACT recommendations. *Pain* 2006; 125:208–15
22. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J: An inventory for measuring depression. *Arch Gen Psychiatry* 1961; 4:561–71
23. Dworkin RH, Turk DC, Wyrwich KW, Beaton D, Cleeland CS, Farrar JT, Haythornthwaite JA, Jensen MP, Kerns RD, Ader DN, Brandenburg N, Burke LB, Cella D, Chandler J, Cowan P, Dimitrova R, Dionne R, Hertz S, Jadad AR, Katz NP, Kehlet H, Kramer LD, Manning DC, McCormick C, McDermott MP, McQuay HJ, Patel S, Porter L, Quessy S, Rappaport BA, Rauschkolb C, Revicki DA, Rothman M, Schumacher KE, Stacey BR, Stauffer JW, von Stein T, White RE, Witter J, Zavisic S: Interpreting the clinical importance of treatment outcomes in chronic pain clinical trials: IMMPACT recommendations. *J Pain* 2008; 9:105–21
24. Austin PC: Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. *Stat Med* 2009; 28:3083–107
25. Moher D, Schulz KF, Altman D; CONSORT Group (Consolidated Standards of Reporting Trials): The CONSORT statement: Revised recommendations for improving the quality of reports of parallel-group randomized trials. *JAMA* 2001; 285:1987–91

26. Hwang IK, Shih WJ, De Cani JS: Group sequential designs using a family of type I error probability spending functions. *Stat Med* 1990; 9:1439–45
27. Dmitrienko A, Tamhane AC: Gatekeeping procedures with clinical trial applications. *Pharm Stat* 2007; 6:171–80
28. Farrar JT, Young JP Jr., LaMoreaux L, Werth JL, Poole RM: Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain* 2001; 94:149–58
29. Farrar JT, Portenoy RK, Berlin JA, Kinman JL, Strom BL: Defining the clinically important difference in pain outcome measures. *Pain* 2000; 88:287–94
30. Dworkin RH, Turk DC, McDermott MP, Peirce-Sandner S, Burke LB, Cowan P, Farrar JT, Hertz S, Raja SN, Rappaport BA, Rauschkolb C, Sampaio C: Interpreting the clinical importance of group differences in chronic pain clinical trials: IMMPACT recommendations. *Pain* 2009; 146:238–44
31. Gilon I, Jensen MP: Clinical trial methodology of pain treatment studies: Selection and measurement of self-report primary outcomes for efficacy. *Reg Anesth Pain Med* 2011; 36:374–81
32. Trojaborg W: Rate of recovery in motor and sensory fibres of the radial nerve: Clinical and electrophysiological aspects. *J Neurol Neurosurg Psychiatry* 1970; 33:625–38
33. Sunderland S: A classification of peripheral nerve injuries producing loss of function. *Brain* 1951; 74:491–516
34. Evans PJ, Lloyd JW, Green CJ: Cryoanalgesia: the response to alterations in freeze cycle and temperature. *Br J Anaesth* 1981; 53:1121–7
35. Goulding K, Beaulé PE, Kim PR, Fazekas A: Incidence of lateral femoral cutaneous nerve neuropraxia after anterior approach hip arthroplasty. *Clin Orthop Relat Res* 2010; 468:2397–404
36. Young S, Sharma S, Rubin N, Hall D, Anderson JK: Ablation zone considerations in cryoablation of RCC: Accuracy of manufacturer provided model and change from first to second freeze cycle. *Clin Imaging* 2022; 89:10–5
37. Douglas WW, Malcolm JL: The effect of localized cooling on conduction in cat nerves. *J Physiol* 1955; 130:53–71
38. Ilfeld BM, Khatibi B, Maheshwari K, Madison SJ, Ali Sakr Esa W, Mariano ER, Kent ML, Hanling S, Sessler DI, Eisenach JC, Cohen SP, Mascha EJ, Yang D, Padwal JA, Turan A, Investigators PA: Immediate effects of a continuous peripheral nerve block on postamputation phantom and residual limb pain: Secondary outcomes from a multicenter randomized controlled clinical trial. *Anesth Analg* 2021; 133:1019–27
39. Martin G, Grant SA, MacLeod DB, Breslin DS, Brewer RP: Severe phantom leg pain in an amputee after lumbar plexus block. *Reg Anesth Pain Med* 2003; 28:475–8

## Appendix. Data Safety Monitoring Board and Recruiting Site Investigators

Data Safety Monitoring Board  
 Salim M. Hayek, M.D., Ph.D. (Chair, Medical Monitor and Patient Advocate)  
 Case Western Reserve  
 Cleveland, Ohio  
 Anupama N. Wadhwa, M.D.  
 University of Texas, Southwestern  
 Dallas, Texas  
 Gerald J. Beck, Ph.D. (statistician)  
 Cleveland Clinic  
 Cleveland, Ohio  
 Recruiting Site Investigators (Perineural Anesthesia Infusion Network for Research and Education Investigators)  
 No conflicts to report unless otherwise noted.  
 Baharin Abdullah, M.D. (National Program Manager)  
 University of California–San Diego  
 San Diego, California  
 Conflicts of interest: The University of California has received funding and/or equipment for other research projects from Myoscience (Fremont, California), Epimed (Farmers Branch, Texas), Infutronics (Natick, Massachusetts), Avanos (Irvine, California), and SPR Therapeutics (Cleveland, Ohio).

Cleveland Clinic  
 Cleveland, Ohio  
 Wael Ali Sakr Esa, M.D., Ph.D., M.B.A.  
 Ece Yamak Altinpulluk  
 Omer Bakal  
 Mauro Bravo  
 Qiliang Jiang  
 Zafeer Khan  
 Kamal Maheshwari, M.D., M.P.H.  
 Loran Mounir-Soliman, M.D.  
 Ahmed Salih  
 Naval Medical Center San Diego  
 San Diego, California  
 Robert J. Hackworth, M.D.  
 Carter Sigmon, M.D.  
 Stanford University  
 Stanford, California  
 Jennifer Padwal, M.D., M.S.  
 University of Florida  
 Gainesville, Florida  
 Amy M. Gunnnett, R.N., C.C.R.C.  
 Amanda Slater  
 Walter Reed National Military Medical Center  
 Bethesda, Maryland  
 Akshay Thaper, B.S.  
 Morgan Hunt, B.S.