A Prospective, Randomized, Controlled Trial Comparing the Effects of Noncontact, Low-frequency Ultrasound to Standard Care in Healing Venous Leg Ulcers

Gary W. Gibbons, MD, FACS; Dennis P. Orgill, MD, PhD; Thomas E. Serena, MD, FACS, MAPWCA, FACHM; Aksone Novoung, DPM, FACFAS; Jessica B. O'Connell, MD, FACS; William W. Li, MD; and Vickie R. Driver, DPM, MS, FACFAS

Abstract

Current scientific evidence suggests venous leg ulcers (VLUs) that do not respond to guideline-defined care may have a wound microenvironment that is out of physiological balance. A prospective, randomized, controlled, multicenter trial was conducted to compare percent wound size reduction, proportions healed, pain, and quality-of-life (QOL) outcomes in patients randomized to standard care (SC) alone or SC and 40 kHz noncontact, low-frequency ultrasound (NLFU) treatments 3 times per week for 4 weeks. One hundred, twelve (112) eligible participants with documented venous stasis, a VLU >30 days' duration, measuring 4 cm^2 to 50 cm^2, and demonstrated arterial flow were enrolled. Of these, 81 reduced <30% in size during the 2-week run-in study phase and were randomized (SC, n = 40; NLFU+SC, n = 41). Median age of participants was 59 years; 83% had multiple complex comorbidities. Index ulcers were 56% recurrent, with a median duration of 10.3 months (range 1 month to 204.5 months) and median ulcer area of 11.0 cm^2 (range 3.7 cm^2–41.3 cm^2). All participants received protocol-defined SC compression (30–40 mm Hg), dressings to promote a moist wound environment, and sharp debridement at the bedside for a minimum of 1 time per week. Ulcer measurements were obtained weekly using digital planimetry. Pain and QOL scores were assessed at baseline and after 4 weeks of treatment using the Visual Analog Scale and the Short Form-36 Health Survey. After 4 weeks of treatment, average wound size reduction was 61.6% ± 28.9 in the NLFU+SC compared to 45% ± 32.5 in the SC group (P = 0.02). Reductions in median (65.7% versus 44.4%, P = 0.02) and absolute wound area (9.0 cm^2 versus 4.1 cm^2, P = 0.003) as well as pain scores (from 3.0 to 0.6 versus 3.0 to 2.4, P = 0.01) were also significant. NLFU therapy with guideline-defined standard VLU care should be considered for healing VLUs not responding to SC alone. The results of this study warrant further research on barriers to healing and the changes occurring in the tissue of the wound to explore theories that the microenvironment impacts wounds that do not heal despite provision of guideline-defined care.

Keywords: randomized controlled trial, leg ulcer, ultrasound therapy, wound healing, pain

Index: *Ostomy Wound Management* 2015;61(1):16–29

Potential Conflicts of Interest: This study was sponsored by Celleration, Inc (Eden Prairie, MN). The authors consulted on study design, protocol development, and study oversight. The authors remained blinded throughout the study.

Dr. Gibbons is Director, South Shore Hospital Center for Wound Healing, Weymouth, MA; and Professor of Surgery, Boston University School of Medicine, Boston, MA. Dr. Orgill is Vice Chairman for Quality Improvement, Department of Surgery, Brigham and Women's Hospital, Boston, MA; and Professor of Surgery, Harvard Medical School, Boston, MA. Dr. Serena is CEO and Medical Director, SerenaGroup™ wound care centers, Cambridge, MA. Dr. Novoung is Chief, Department of Podiatric Surgery, Department of Veteran Affairs Greater Los Angeles; and Associate Clinical Professor, Department of Vascular Surgery, David Geffen School of Medicine at the University of California-Los Angeles, Los Angeles, CA. Dr. O'Connell is Associate Director, Surgical and Perioperative Care, and Co-Chief, Vascular Surgery Service, Greater Los Angeles Veterans Administration Medical Center; and Assistant Clinical Professor, Gonda (Goldschmied) Vascular Center, UCLA Department of Surgery, Los Angeles, CA. Dr. Li is President and Medical Director, The Angiogenesis Foundation, Cambridge, MA. Dr. Driver is Chief of Podiatric Surgery, VA New England Health Care Division; Professor, Brown University School of Medicine (clinical); and Director, Clinical Research, HBO and Wound Healing Center-Rhode Island Hospital, Providence, RI. Please address correspondence to: Gary W. Gibbons, MD, FACS, SSH Center for Wound Healing, South Shore Hospital, 90 Libbey Industrial Parkway, Suite 100, Weymouth, MA 02189; email: dr.garygibb@gmail.com.

Ostomy Wound Management 2015;61(1):16–29

Key Points

- The authors conducted a carefully controlled clinical study to compare the effect of adding noncontact, low-frequency ultrasound (NLFU) to venous leg ulcer (VLU) standard care procedures on healing.
- After 4 weeks of care, percent reduction in wound area and pain reduction was significantly greater in the NLFU than in the control group.
- During the study and the follow-up period (total 11 weeks), a higher proportion of NLFU than control treated ulcers healed.
- More indepth analysis is needed regarding the role of wound microenvironment on healing.

Venous leg ulcers (VLUs) are under-recognized, undertreated, and hard-to-heal wounds. More than 2.5 million individuals in the United States have a VLU and experience significant morbidity, loss of productivity, and high recidivism rates, resulting in reduced quality of life (QOL).[1-5] The annual economic burden of VLUs in the US is estimated at up to $18 billion.[6,7] A retrospective cohort study[8] of 78 VLU patients at the Cleveland Clinic demonstrated timely intervention is paramount to reducing these costs; the study found the first 90 days following onset of ulcer were the most resource-intensive, with ulcers >2.8 cm^2 incurring nearly twice the cost of smaller VLUs.

Normal venous blood flow depends on healthy cardiac function, an effective venous pressure gradient and calf muscle pump, and veins with functional and intact valves. VLUs occur when valve incompetence and venous reflux lead to ambulatory venous hypertension and a potentiated inflammatory response, with hyperpigmentation, clotting, and tissue injury and/or necrosis. The Society for Vascular Surgeons (SVS) and The American Venous Forum (AVF), as well as the Association for the Advancement of Wound Care (AAWC), have published clinical practice guidelines for the treatment of VLUs.[9,10] These guidelines focus on diagnosing and addressing the underlying condition of venous disease, especially insufficiency and reflux, as well as promoting a local environment that facilitates new tissue growth and prevents further tissue injury. The guidelines recommend standard treatment include: 1) obtaining an appropriate history and accurate diagnosis; 2) removing nonvital tissue utilizing debridement; 3) providing and maintaining a clean, moist wound environment; and 4) applying rigid or elastic compression therapy to compensate for increased ambulatory hypertension and reflux to promote venous return.[9,10]

Standard care (SC) in a chronic VLU is often insufficient, evidenced by stalled healing and high recidivism. Nonhealing VLUs may have a tissue microenvironment characterized by sustained inflammation, high bioburden, biofilm presence, and impaired microcirculation. A pilot study[11] of 4 participants with long-standing VLUs that failed to heal despite standard wound care showed the chronic venous ulcer bed in these patients was rich in active proteases, highly inflammatory, and exhibited measurable destruction of the extracellular matrix. The study found multiple cytokines and proteases in both the wound bed and wound exudate of these recalcitrant venous ulcers. A prospective study[12] of tissue from 30 previously untreated VLUs demonstrated both significantly higher concentrations of inflammatory cytokines in VLUs compared to healthy tissue and differences between rapid (ulcers that reduced by >40% in 4 weeks of compression) and delayed healers. Initial pro-inflammatory protein levels were elevated to a higher degree in rapid healers compared to delayed healers. Additionally, pro-inflammatory levels in the rapid healers dropped significantly with 4 weeks of compression as compared to the delayed healers. Various theories exist of inflammation in chronic venous ulcers. A review of the literature[13] notes a general trend of overactivity of metalloproteinases (MMPs) and underactivity of tissue inhibitors of metalloproteinases in nonhealing ulcers.

A noncontact, low-frequency ultrasound (NLFU) therapy system (Celleration MIST® Therapy [Celleration, Inc, Eden Prairie, MN]) received US Food and Drug Administration (FDA) clearance in May 2005 with an indication for promoting wound healing.[14] The system delivers low-frequency (40 kHz) ultrasound energy into and below the surface of the wound using sterile saline as a conduit and without direct contact with the patient (see Figure 1).

Preclinical work has been performed to assess the effects of NLFU on the wound bed and the microenvironment. The effect of NLFU on *Pseudomonas*-generated biofilm was studied in a well-defined rabbit ear model.[15] Tissue analysis of 78 wounds receiving 3 NLFU treatments over 6 days showed breakdown of the biofilm, a significant reduction in the epithelial gap, and an increase in total granulation area compared to the 78 control wounds. NLFU also significantly decreased inflammatory cytokine expression, including interleukin (IL)-1b and tissue necrosis factor (TNFα). NLFU also has been shown to stimulate wound angiogenesis *in vivo*.[16] Nine (9) diabetic mice (db/db) were wounded and treated with NLFU 3 times per week. Wounded tissues were analyzed for expression of stromal derived factor (SDF-1), a proangiogenic protein involved in cell signaling and regeneration, and platelet endothelial cell adhesion molecule (CD-31), a marker of neovascularization. Both markers were found to be significantly higher in the NLFU arm compared to the control arm. Statistically significant accelerated wound healing was observed in NLFU-treated mice.[16]

Pilot clinical studies also have evaluated the effects of NLFU on the wound bed microenvironment. The bacteria load from punch biopsies of 11 noninfected Stage III pressure ulcers was examined and found to contain pretreatment bacterial loads

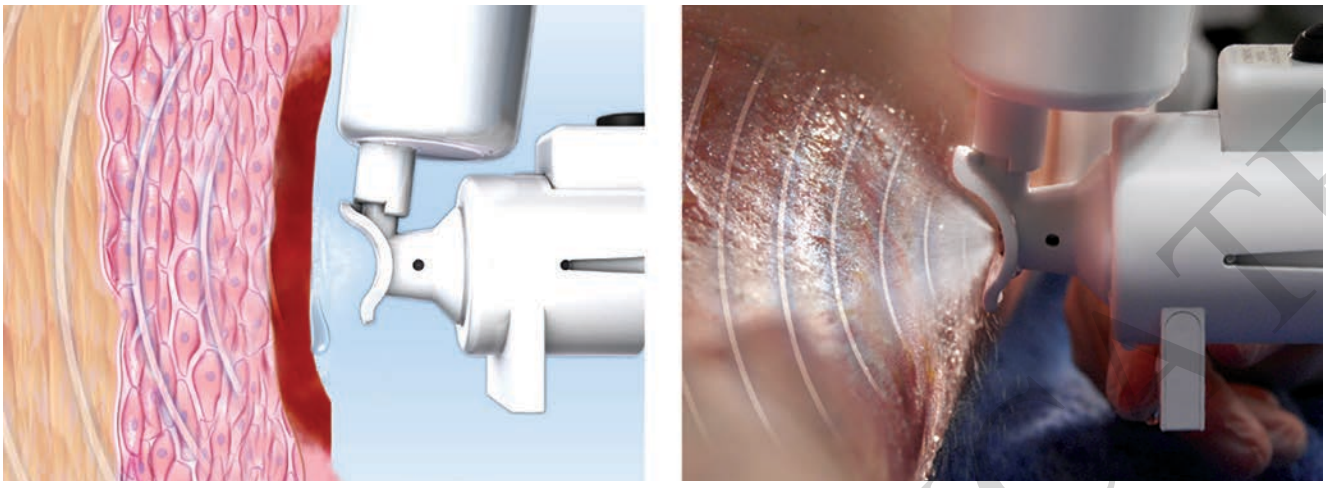

Figure 1. Illustration of MIST® Therapy delivery and penetration.

of >10^6 CFU/g tissue [17]; after 6 NLFU therapy treatments delivered over 2 weeks, the load of common wound bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Acinetobacter baumannii*) was reduced by more than 90%. The average reduction in wound size after 2 weeks was 24%.

A 3-arm, prospective randomized study[18] of 12 nonhealing diabetic foot ulcers (DFUs) found participants who received NLFU 3 times a week for 4 weeks, in addition to standard wound care and offloading, had decreased proinflammatory cytokines IL-1b, IL-6, IL-8, TNFα, MMP-9, and granulocyte-macrophage colony-stimulating factor (gm-CSF) at 4 weeks compared to the control group of standard DFU care alone. MMP-9 levels were positively related to reduction in wound area (P <0.05). The study found an initial increase of vascular endothelial growth factor (VEGF) expression in the first 2 weeks of NLFU treatment followed by a decrease in VEGF consistent with wound area. However, VEGF was not shown to be significantly correlated to healing. Histological analyses showed a reduction of inflammation concomitant with healing. An 86% wound area reduction was observed in the group that received NLFU 3 times per week group, compared to 25% reduction in participants who received NLFU 1 time per week and 39% in the control group (P = <0.05).

In a single-arm, prospective pilot study[19] of 9 VLUs of >6 months' duration that failed to improve with supervised multilayer compression, NLFU was administered 3 times per week over the course of 4 weeks. Wound fluid and tissue analyses showed a decline in cytokine levels from baseline to 4 weeks of treatment for TNFα, IL-1a, IL-6, and IL-8. Ulcer healing significantly correlated with a decrease in TNFα and IL-1a cytokine expression (P = 0.04). Although the association for the other cytokines was not significant, it showed a trend towards significance (P values of 0.05 and <0.1).

Taken together, the preclinical and human studies suggest a dysfunctional microenvironment exists in nonresponding VLU wounds and a potential effect of NLFU on this abnormal microenvironment may facilitate healing of the nonresponding wound. A large, randomized controlled trial (RCT) was warranted to expand upon these findings. The purpose of this RCT was to compare the effects of NLFU+SC to SC alone on percent ulcer area reduction after 4 weeks of treatment in chronic VLUs.

Methods and Procedures

Study overview and conduct. IN BALANCE VLU was a prospective, randomized, controlled, parallel-group, multicenter clinical trial conducted between April 2012 and March 2014. The trial was performed in 22 facilities across the US with multispecialty and multidisciplinary teams, including 8 community hospital outpatient wound centers, 5 private wound clinics, 6 university outpatient wound centers, and 3 Veterans Administration outpatient wound centers.

The trial is registered on clinical trials.gov as NCT01549860 and was sponsored by Celleration, Inc (Eden Prairie, MN). Institutional Board Review approval was obtained in accordance with the principles of the Declaration of Helsinki and recognized Good Clinical Practices were followed, including participant informed consent and third-party monitoring. A third-party statistician prepared the randomization program and performed the data analysis for this study. Randomization was computer-generated using a 6-block permuted randomization scheme stratified by study site and automated through the clinical database.

Unique participant IDs were assigned by the clinical database. Study sites labeled all data with the participant ID and redacted any personal health information before submitting any diagnostic tests, ulcer images, or source documents. Data were entered by the study sites using an electronic data capture system (Clindex® EDC Fortress Medical, Minneapolis, MN). Study site personnel received training and were tested on digital wound image

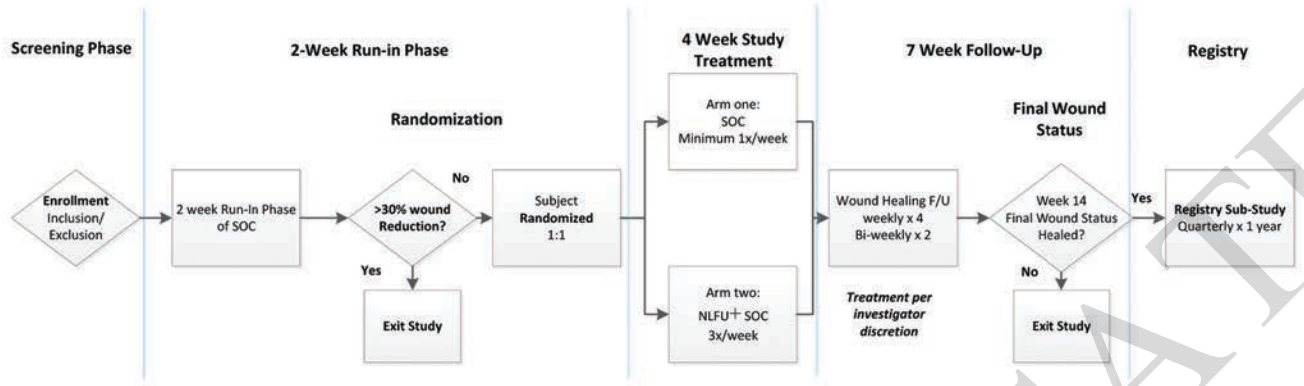

Figure 2. Study design flow diagram.

procurement, drawing of epithelial boundaries for area measurements, NLFU therapy application, and 4-layer compression wrap application. Study conduct, data management, adjudication practices, and participant safety were overseen by a third-party Data Safety Management Board (DSMB).

Ulcer area measurements. Study endpoints for healing were based on weekly measurements of the nonepithelialized area of the index ulcer using a digital camera (SilhouetteStar® Aranz Medical, Christchurch, NZ). Images were taken by the study site, and epithelial boundaries were electronically drawn using the associated software (SilhoutteConnect® Aranz Medical, Christchurch, NZ). The software performed a calculation of the nonepithelialized area based on the boundaries drawn. Study sites uploaded each deidentified image (labeled with the participant ID) and entered the calculated ulcer area measurement into the clinical database. A third-party, blinded wound expert was sent an automated message when an image was uploaded to the database. The adjudicator was blinded to the study treatment, participant history, and visit sequence and reviewed each individual image to validate the wound boundaries were drawn correctly and to ensure intrareliability. Images collected at enrollment, randomization, after 4 weeks of study treatment, and final study visit were validated for all participants. The adjudicator confirmed that ulcer images were indicated as healed or recidivated.

Inclusion/Exclusion. Eligible participants ranged from 18 to 90 years of age, had a VLU >30 days' duration, with a size between 4 cm^2 to 50 cm^2, and a documented etiology of venous stasis with reflux. Participants were required to have demonstrated adequate arterial flow in their index extremity as documented by an ankle brachial index (ABI) 0.8 to 1.2, a biphasic or triphasic Doppler reading, or a $TcPO_2$ or toe pressure >40 mm Hg.

Participants who had received other treatment modalities, including cellular-based tissue products, growth factor therapies, negative pressure therapy, hyperbaric oxygen, or ultrasound therapy, were required to have a minimum of 14 days since the last treatment. Participants were required to be a minimum of 6 weeks post vascular or skin graft procedure. If an ulcer was present for >6 months, a biopsy was required to rule out a malignancy. For participants who presented with multiple ulcers, the largest ulcer that met all other inclusion criteria was selected to be the index ulcer in this study.

Participants were excluded if the index ulcer's primary etiology was other than venous disease; if the index ulcer was located within 1 cm of another ulcer; if the patient had cellulitis, osteomyelitis, or gangrene; or if there was exposed tendon, muscle, or bone. Participants with more than 5 ulcers on the index limb, an amputation of the study limb above the transmetatarsal region, or with confounding treatments or comorbidities were excluded. Confirmation of participant inclusion/exclusion eligibility was performed by a blinded Physician Advisory Board.

Study phases. The clinical trial was comprised of 4 phases: 1) screening and enrollment; 2) 2-week run-in; 3) 4-week treatment; and 4) 7-week follow-up. Participants whose VLU healed during the trial period were eligible to participate in a 12-month ulcer recidivism registry substudy (see Figure 2).

Screening and enrollment. Participants were evaluated for eligibility based on medical records, including documentation of venous stasis and reflux within the past 12 months and/or performance of a venous duplex scan at enrollment. Participants were screened for arterial blood flow by conducting a bedside or vascular lab ABI, Doppler study, $TcPO_2$, or toe pressure on day of enrollment. If a wound was of >6 months' duration, a biopsy was performed to rule out other conditions. Upon confirmation of all eligibility criteria, a participant entered the run-in phase of the study.

Run-in. Eligible participants completed a 2-week run-in of standardized treatment. The protocol for SC treatment was based on societal guidelines for VLU[9,10] and consisted of compression (30–40 mm Hg), moist wound environment dressings, and debridement at the investigator's discretion. A 4-layer compression wrap (Profore™ Smith & Nephew, Hull, UK) was provided for the study. If a participant could not tolerate this compression, other forms that met a minimum level of 30 mm Hg were allowed. All participants were seen a

minimum of once a week during the run-in phase. Per protocol, participants could be seen up to 3 times per week for ulcer cleansing and debridement, dressing changes, or compression reapplication as deemed medically necessary by the study site investigator.

Randomization. Participants that exhibited an index ulcer area reduction <30% during the 2-week run-in were randomized. Participants whose ulcers reduced >30% during the run-in were deemed nonstalled VLUs and were exited before randomization. Randomization eligibility was based on a calculation of percent area reduction made by the clinical database using the enrollment and randomization visit ulcer sizes entered by the study site. Participants meeting the randomization criteria were issued a 1:1 randomization assignment by the clinical database to either SC or NLFU+SC. Study personnel and participants were not blinded to the study treatment.

Study treatment. Participants received their assigned study treatment for 4 weeks. Participants randomized to the control group (SC) continued with the protocol-required SC treatment (wound cleansing, dressing change, compression, debridement as needed) for a minimum of 1 and up to 3 times per week as determined clinically appropriate by the investigator. Participants randomized to the intervention group (NLFU+SC) received the protocol-required SC plus NLFU 3 times per week for a total of 12 treatments. NLFU treatment therapy duration was delivered based on the ulcer size determined at each treatment visit, per manufacturer's recommendations, starting at 3 minutes for wound areas >10 cm^2, with increases of 1 minute for every 10 cm^2 increment.[20]

Follow-up. Upon completion of the treatment phase and evaluation for the primary endpoint, participants were followed for an additional 7 weeks to evaluate secondary endpoints of ulcer closure. The protocol-required SC treatment continued for all participants throughout the follow-up phase. Additional modalities, including crossover to NLFU therapy for SC participants, could be introduced at the discretion of the investigator. A final ulcer measurement was performed at the last study visit, 11 weeks post randomization.

Study variables, data collection, and management. Baseline patient demographics, comorbidities, index limb and compression history, and index ulcer and treatment history were collected from the medical record and entered by the study sites into the clinical database using the EDC system. Clinical characteristics and duplex results documenting venous insufficiency, arterial blood flow measurements, and ulcer biopsies were recorded. At each weekly visit, a single trained research coordinator at each study site performed a digital ulcer measurement and drew the wound epithelial border that was then confirmed by the investigator and adjudicated by a single third-party blinded wound expert. The investigator assessed the ulcer bed tissue and exudate, periwound characteristics, and edema level of the index limb each week. Participant compression adherence and the ulcer treatments performed were entered into the record at each study visit.

Participants completed pain and QOL questionnaires at time of randomization and at the completion of 4 weeks of study treatment. Pain assessments were completed using the Visual Analog Scale (VAS) and recorded in the database via the EDC system.[21] VAS measurements were verified for accuracy by the study monitor. QOL was measured using the Short Form-36 Health Survey (SF-36).[22] Participant-completed SF-36 forms were collected by the study monitor, and responses were entered into the QualityMetric Health Outcomes™ Scoring Software 4.5. The software-calculated scores were transferred to the SAS version 9.4 software for analysis by the third-party statistician.

Endpoints. The primary trial endpoint was mean percent ulcer area reduction, calculated as the ulcer size differences from randomization to the 4-week, post treatment measurement, for each treatment group.

Secondary endpoints included actual area reduction from randomization to 4 weeks of study treatment, weekly percentage and actual ulcer area reduction, mean ulcer area reduction from randomization to 4 weeks of study treatment stratified by ulcer size and age, ulcer area reduction in ulcers treated with bioengineered skin equivalents in the follow-up, ulcer healing rates and times to healing defined as full epithelialization of the ulcer, ulcer recidivism rates and times, pain score comparison between randomization and 4 weeks of study treatment, and QOL score comparison between randomization and 4 weeks of study treatment.

Statistical analysis. Statistical analysis was performed with SAS version 9.4 software (SAS Institute Inc, Cary, NC), and Fisher's Exact test or Student's *t*-test and the Wilcoxon Rank Sum tests were used to compare the treatment arms with both nonadjusted and adjusted results. A multivariate model was used to test the primary endpoint. The model included published covariates of wound size and wound age[23] as well as variables of diabetes mellitus, coronary artery disease, hypertension, anemia, wound location, and compression adequacy. The study had a group-sequential design with one planned interim analysis for superiority at 87 randomized participants. The full study was 80% powered to detect a 15% difference (SD 33%) with a 2-sided overall alpha level of 0.05, requiring 156 randomized participants (78 participants per arm). Statistical significance was defined as $P < 0.05$. Statistical differences between the treatment arms were evaluated for impact to the primary endpoint.

Study revisions. Two revisions to the study protocol occurred during the trial. The first revision occurred at the beginning of enrollment and included removal of the maximum ulcer duration and expanded the ulcer size lower range from 10 cm^2 to 4 cm^2. All enrolled participants met these criteria. The DSMB guided the second protocol revision in response to eligibility violations and randomization

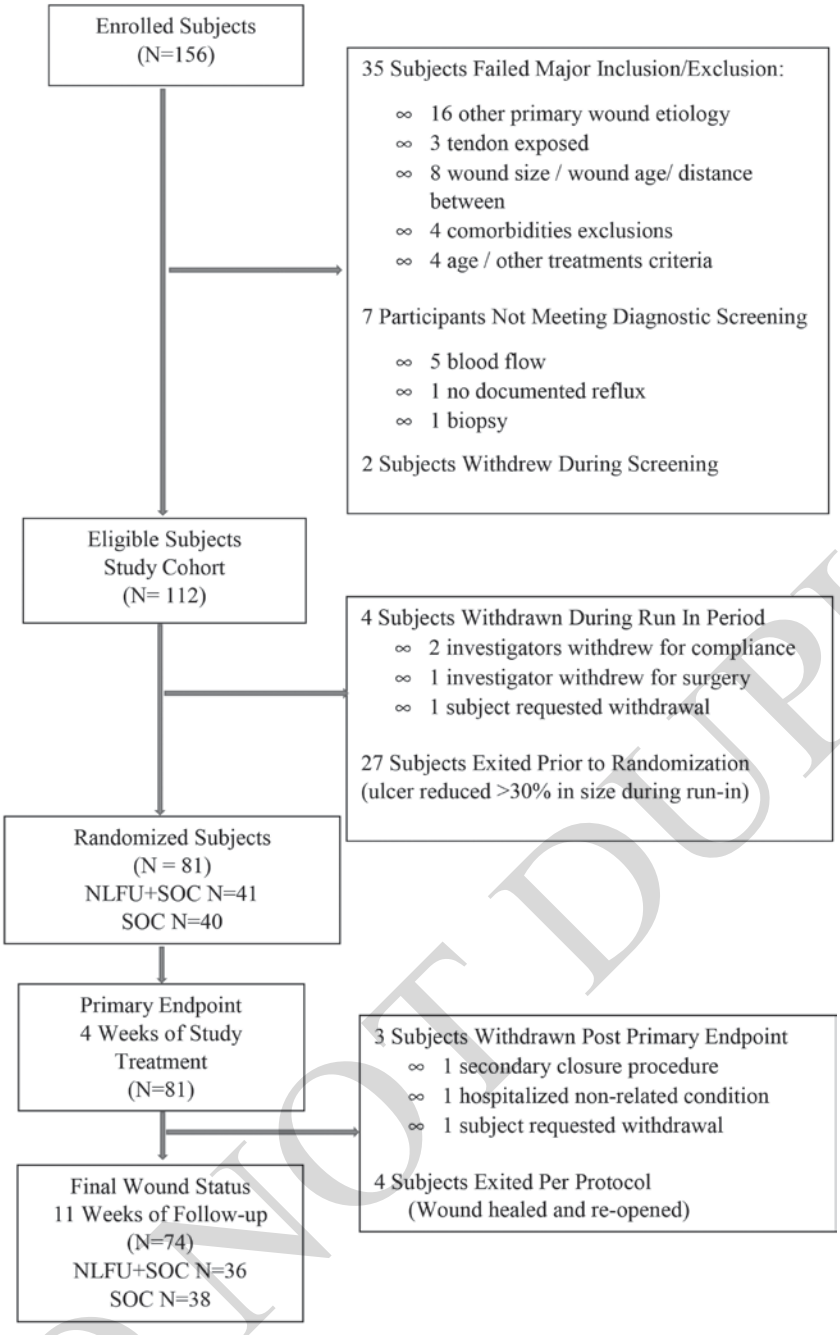

Figure 3. Study participants and flow diagram.

failure rates. Compression requirements per SVS and AVF guidelines were added for the 30-day chronic ulcer period. Documentation requirements of venous stasis and reflux were clarified. New criteria of a biopsy to rule out malignancy for wounds >6 months' duration and a maximum of 5 wounds on the index limb were added. An evaluation of the impact of protocol revisions to the primary endpoint was conducted with no significant differences detected, and no adjustment for differences was required.

Due to slower-than-anticipated enrollments and randomization rates, the study sponsor decided to stop enrolling, forego the prespecified interim analysis, and report current results as final. No alpha penalty was taken because the data were only given a single look. The data presented in this report meet intent-to-treat principles per International Conference on Harmonization and Food and Drug Administration Guidelines for Statistical Principles.[24,25]

Results

Enrollments. A total of 156 persons were enrolled, of which 112 met the major inclusion and exclusion criteria of venous or mixed venous etiology with documented venous stasis; adequate arterial blood flow to the lower index limb; negative for ulcer infection and depth to the tendon, muscle, or bone; or major confounding treatments or comorbidities. Of the 112 eligible persons, 81 (72%) experienced <30% reduction in ulcer area during the run-in phase and were randomized (SC, n = 40; NLFU+SC, n = 41). Participants were distributed evenly across the study sites with no more than 11% of participants from a single study site (see Figure 3).

Demographics and history at baseline. The proportion of participants with comorbidities that may impact wound healing was high. The median body mass index (BMI) was 35, 67% had hypertension (HTN), 35% had type 2 diabetes mellitus (DM), 61% had lower extremity edema, and 83% had multiple associated comorbid conditions. In randomized participants for whom ABI was measured, the median ABI was 1.08. The mean number of active ulcers on the index limb was 1.6 ± 1.0; 45 (56%) of the index ulcers were recurrent. The median ulcer duration at randomization was 10.3 months (range 1 month to 204.5 months) and median ulcer area was 11.0 cm^2 (range 3.7 cm^2 –41.3 cm^2). No statistically significant differences were observed between the treatment groups in arterial blood flow, comorbidities, or reduction in ulcer size during the run-in phase. Statistically significant differences were noted between study arms for index ulcer location: the NLFU+SC treatment had more lateral ulcers and the SC treatment arm ulcers

Table 1. Patient demographics, baseline history, and ulcer characteristics

	Mean ± SD (N), Median [Interquartile Range] Range, or % (n/N)			*P* value Mean (Median)
Characteristic	**Randomized (N=81)**	**SC[a] (N=40)**	**NLFU[b]+SC (N=41)**	**SC versus NLFU+SC**
Age (years)	60.1±12.1 (81) 59.0 [52.0, 68.0] 33.0–86.0	60.0±11.9 (40) 60.0 [52.0, 67.5] 34.0–81.0	60.3±12.3 (41) 58.0 [51.0, 68.0] 33.0–86.0	0.92 (0.84)
Gender				0.81
Male	70% (57/81)	72% (29/40)	68% (28/41)	
Female	30% (24/81)	28% (11/40)	32% (13/41)	
Body mass index (kg/m²)	36.6±10.7 (81) 34.7 [28.6, 43.4] 17.2–69.7	35.5±9.8 (40) 33.2 [28.2, 42.2] 21.2–55.7	37.7±11.5 (41) 37.4 [29.3, 45.2] 17.2–69.7	0.37 (0.44)
Ankle brachial index	1.07±0.12 (60) 1.08 [0.99, 1.18] 0.80–1.39	1.08±0.11 (29) 1.08 [1.00, 1.17] 0.90–1.39	1.06±0.12 (31) 1.07 [0.96, 1.18] 0.80–1.20	0.58 (0.84)
Coronary artery disease	7% (6/81)	10% (4/40)	5% (2/41)	0.43
Hypertension	67% (54/81)	65% (26/40)	68% (28/41)	0.82
Diabetes mellitus	35% (28/81)	28% (11/40)	42% (17/41)	0.24
Anemia	14% (11/81)	8% (3/40)	20% (8/41)	0.19
Pulmonary disease	6% (5/81)	5% (2/40)	7% (3/41)	1.00
Auto-immune disease	6% (5/81)	8% (3/40)	5% (2/41)	0.68
Arthritis	26% (21/81)	25% (10/40)	27% (11/41)	1.00
Kidney disease	5% (4/81)	0%	10% (4/41)	0.12
Current tobacco use	20% (16/81)	28% (11/40)	12% (5/41)	0.10
Edema	61% (49/81)	62% (25/40)	58% (24/41)	0.82
Prior ulcers	82% (66/81)	85% (34/40)	78% (32/41)	0.57
Number of active ulcers	1.6±1.0 (81) 1.0 [1.0, 2.0] 1.0–7.0	1.4±0.7 (40) 1.0 [1.0, 2.0] 1.0–4.0	1.7±1.2 (41) 1.0 [1.0, 2.0] 1.0–7.0	0.24 (0.20)
Index ulcer location				0.03
Lateral	38% (31/81)	30% (12/40)	46% (19/41)	
Posterior	2% (2/81)	0%	5% (2/41)	
Medial	50% (40/81)	52% (21/40)	46% (19/41)	
Anterior	10% (8/81)	18% (7/40)	2% (1/41)	
Recurrent ulcer	56% (45/81)	60% (24/40)	51% (21/41)	0.58
Ulcer size at randomization (cm²)	13.5±10.4 (81) 11.0 [5.4, 17.4] 2.5–53.3	12.1±9.6 (40) 9.8 [4.6, 16.8] 2.5–36.9	14.8±11.1 (41) 12.4 [6.5, 19.2] 3.1–53.3	0.29 (0.15)
Ulcer age at randomization (months)	24.4±37.1 (81) 10.3 [3.9, 25.4] 1.5–204.5	27.0±44.8 (40) 8.9 [3.6, 29.3] 1.7–204.5	21.8±27.9 (41) 10.3 [3.9, 24.1] 1.5–114.0	0.53 (0.85)
Ulcer area reduction in the run-in phase (%)	1.6±31.3 (81) 9.2 [-7.9, 20.9] -144.2–47.9	7.0±19.3 (40) 9.6 [-2.0, 18.7] -51.9–47.9	-3.6±39.3 (41) 3.8 [-27.9, 23.2] -144.2–45.6	0.12 (0.63)

[a] *SC = Standard care*
[b] *NLFU= Noncontact, low-frequency ultrasound*

Table 2. Index ulcer treatment before enrollment

	Mean ± SD (N), Median [Interquartile Range] Range, or % (n/N)			*P* value Mean (Median)
Characteristic	**Randomized (N=81)**	**SC (N=40)**	**NLFU+SC (N=41)**	**SC versus NLFU+SC 3**
0-day compression:				0.24
Adequate (>30 mm Hg)[c]	67% (54/81)	60% (24/40)	73% (30/41)	
Inadequate (<30 mm Hg)[d]	33% (27/81)	40% (16/40)	27% (11/41)	
Debridement (any type)	65% (53/81)	72% (29/40)	58% (24/41)	0.24
NLFU[b]	7% (6/81)	10% (4/40)	5% (2/41)	0.43
Pulse electromagnetic induction	1% (1/81)	0%	2% (1/41)	1.00
Electrical stimulation	4% (3/81)	5% (2/40)	2% (1/41)	0.62
Negative pressure ulcer therapy	2% (2/81)	0%	5% (2/41)	0.49
Cellular-based tissue products	22% (18/81)	25% (10/40)	20% (8/41)	0.60
Number of cellular-based tissue products	3.7±2.6 (18) 3.0 [1.0, 5.0] 1.0–9.0	5.1±2.4 (10) 5.0 [3.0, 7.0] 1.0–9.0	2.0±1.8 (8) 1.0 [1.0, 2.5] 1.0–6.0	0.01 (0.01)
Skin graft or flap	9% (7/81)	5% (2/40)	12% (5/41)	0.43
Other	12% (10/81)	8% (3/40)	17% (7/41)	0.31

[a] *SC = Standard care*
[b] *NLFU = Noncontact, low-frequency ultrasound*
[c] *Adequate compression defined as a level >30 mm of mercury*
[d] *Inadequate compression defined as <30 mm of mercury*

were located more on medial and anterior surfaces of the lower extremity (see Table 1).

VLU treatments performed within 12 months before enrollment included any debridement (53 [65%] of randomized participants), skin graft and/or flap (7 [9%]), and use of 1 or more cellular-based tissue products (18 [22%]), with a median of 3 applications per wound (range 1–9 applications). Of the randomized participants, 54 (67%) had compression levels >30 mm Hg applied in the 30 days before enrollment. Statistically significant differences (P <0.05) between treatment groups included the number of cellular-based tissue product applications before enrollment; participants in the SC arm received more applications (see Table 2).

Protocol adherence. Adherence to required study protocol clinic visits and treatments was high. Only 1% of visits were documented as missed, and 3% occurred outside the treatment window. Participants were cooperative with the requirement for 30–40 mm Hg compression in 98% of the study visits. Monitors confirmed all participants were treated per randomization assignment and met the minimum number of treatments per protocol (a minimum of 4 weekly treatments in SC and a minimum of 8 NLFU therapy treatments in NLFU+SC).

Primary endpoint. After 4 weeks of study treatment, participants in the NLFU+SC arm had a mean area percent reduction of 61.6%, compared to 45.0% for participants in the SC arm (P = 0.02). This significance remained after adjusting for the covariates of ulcer size and ulcer age (see Table 3).

Secondary endpoints. In ulcers <6 months' duration, the NLFU+SC treatment arm exhibited a mean 79.4 ± 18.9 reduction in ulcer area, compared to 49.2 ± 29.3 in SC participants (P = 0.002). Clinically relevant differences in mean area reductions were observed in ulcers >6 months with differences of up to 31% for ulcers >60 months of age (NLFU+SC at 37.6 ± 16.9 compared to SC at 6.4 ± 36.4). The differences were not statistically significant (P = 0.12). In wounds >10 cm^2, NLFU+SC-managed wounds had a mean 60.0 ± 32.2 versus mean 31.9 ± 32.8 in the SC group (P = 0.01). In ulcers <10 cm^2, differences were not significant. A negative correlation between mean area reduction and ulcer size at randomization was observed in the SC treatment group (P = 0.03). By contrast, in the NLFU+SC treatment group, the ulcer area reduction was more consistent regardless of the initial size and not significantly correlated (see Figure 4).

Participants who received NLFU+SC reported an 80% reduction of VAS pain scores (median 0.6) after 4 weeks of study treatment, compared to a 20% reduction (median 2.4) reported by the SC treatment arm (P = 0.01) (see Table 4.)

Table 3. Ulcer reductions after 4 weeks of study treatment

	Mean ± SD (N), Median [Interquartile Range] Range, or % (n/N)			SC versus NLFU+SC	
Outcomes	**Randomized (N=81)**	**SC[a] (N=40)**	**NLFU+SC[b] (N=41)**	***P* value Mean (Median)**	***P* value (adjusted for ulcer size and age)**
Percent area reduction (cm^2) at 4 weeks post randomization	53.4±31.7 (81) 61.0 [28.0, 80.7] -23.6–100.0	45.0±32.5 (40) 44.4 [20.9, 68.1] -23.6–100.0	61.6±28.9 (41) 65.7 [48.4, 83.9] 2.0–99.2	0.02 (0.02)	0.01
Absolute area reduction (cm^2) at 4 weeks post randomization	6.6±7.4 (81) 4.4 [2.1, 8.6] -4.1–43.8	4.1±4.1 (40) 3.1 [1.5, 6.2] -4.1–17.2	9.0±9.0 (41) 5.5 [3.3, 11.3] 0.8–43.8	0.003 (0.01)	0.02

[a] *SC = Standard care*
[b] *NLFU = Noncontact low frequency ultrasound*
Primary endpoint of mean percent area reduction. Secondary endpoints of median and absolute area reductions

Table 4. Visual Analog Scale (VAS) pain scores at randomization and after 4 weeks of study treatment

	Mean ± SD (N), Median [Interquartile Range], Range, or % (n/N)			SC versus NLFU+SC
Outcomes	**Randomized (N=81)**	**SC[a] (N=40)**	**NLFU[b]+SC (N=41)**	***P* value Mean (Median)**
VAS pain score at randomization	3.5±3.0 (80) 3.0 [0.7, 5.7] 0.0–10.0	3.3±2.6 (39) 3.0 [0.6, 5.2] 0.0–9.0	3.7±3.3 (41) 3.0 [0.8, 6.1] 0.0–10.0	0.57 (0.73)
VAS pain score after 4 weeks of study treatment	2.6±2.9 (80) 1.2 [0.2, 4.7] 0.0–10.0	3.4±3.2 (39) 2.4 [0.4, 5.9] 0.0–10.0	2.0±2.4 (41) 0.6 [0.1, 3.8] 0.0–9.1	0.03 (0.04)
Reduction in VAS pain score after 4 weeks of study treatment	0.9±2.8 (79) 0.3 [-0.4, 2.2] -7.3–9.8	0.0±2.3 (38) 0.0 [-0.8, 1.3] -7.3–4.4	1.7±3.0 (41) 0.5 [0.0, 2.9] -3.2–9.8	0.01 (0.01)

[a] *SC = Standard care*
[b] *NLFU = Noncontact low frequency ultrasound*

Randomized participants reported baseline median QOL scores of 38.9 for the physical component and 48.2 for the mental component. In general, QOL scores among participants in both treatment arms improved after 4 weeks of treatment; no statistical differences were noted except for bodily pain scores, where participants in the NLFU+SC treatment group reported median bodily pain scores of 45.9 compared to 41.5 for SC alone ($P = 0.04$).

The NLFU+SC arm had nearly twice the number of ulcers that achieved complete closure by 7 weeks post-randomization compared to those in the SC arm with 11(28%) and 6 (15%) ulcers healed, respectively. By the final study visit at 11 weeks post randomization, 16 (39%) participants in NLFU+SC had ulcers that achieved complete closure versus 17 (42.5%) participants randomized to SC. However, 7 (37%) of these SC participants received NLFU in the follow-up phase and healed within 7 weeks of initiating NLFU.

Additional analyses. Participants that received compression at a level of 30–40 mm Hg in the 30 days before enrollment showed NLFU+SC resulted in a 67.0% median ulcer area reduction, compared to 40.5% in SC alone ($P = 0.0005$). Additionally, ulcers with a lateral location also exhibited a higher rate of area reduction (81.3%) for NLFU+SC than for SC alone (31.4%, $P = 0.01$).

Discussion

IN BALANCE VLU is the most comprehensive, randomized, controlled clinical trial to date of NLFU treatment of VLU. This trial employed a 4-week primary endpoint for healing based on previously validated surrogate markers of healing rate, area ratio, and percent change in wound area as predictors of healing at 12 and 24 weeks.[26-28] The study was tightly conducted and adherence to the protocol was high, including an extremely low rate of missed visits and withdrawals after randomization, as well as a high degree of adherence to the required compression.

NLFU+SC demonstrated a clinically and statistically significant higher rate of percent wound size reduction within

Table 5. Noncontact low-frequency ultrasound (NLFU) studies performed to date

DATE	N	AUTHOR	RESULTS
2011	444	Driver et al[29]	Meta-analysis of 8 NLFU studies on chronic wounds (87 venous leg ulcers [VLU]); 85.2% reduction at 7 weeks (n=188); 32.7% healing by 6 weeks;41.7% healing by 12 weeks; 79% pain reduction
2013	90	Olyaie et al[30]	Randomized controlled study of VLUs; 72.8% NLFU versus 55.4% standard care (SC) wound area reduction at 20 weeks; 47.1% NLFU versus 17.7% SC pain reduction
2007	70	Kavros et al[31]	Randomized controlled study of critical limb ischemia; >50% decrease in wound size at 12 weeks; 63% NLFU versus 29% SC healed
2005	55	Ennis et al[32]	Randomized controlled trial of diabetic foot ulcers (DFU); 40.7% NLFU versus 14.3% sham NLFU healed at 12 weeks
2012	12	Yao et al[18]	Prospective randomized controlled pilot of DFU; 85% NLFU versus 34% SC wound area reduction at 4 weeks
2013	85	Honaker et al[33]	Retrospective study of deep tissue injury (DTI); 127 DTIs (63 NLFU, 62 SC); 80% NLFU at Stage II or resolved versus 22% SC
2007	51	Kavros et al[34]	Observational study of chronic foot and leg ulcerations (4 VLU); baseline control SC 94.9%; NLFU wound volume reduction at 5.5 weeks versus 37.3% SC wound volume reduction at 9.8 weeks
2012	10	Escandon et al[19]	Prospective, pilot, single-arm study of VLU;45% reduction in wound area at 4 weeks
2009	11	Serena et al[17]	Prospective, single-arm study on pressure ulcers; 50% reduction in bacteria at 2 weeks, 26% reduction in wound size at 2 weeks
2008	210	Kavros et al[35]	Retrospective, observational study of chronic wounds (31 VLU) (163 NLFU, 47 SC);53% NLFU versus 32% SC in wound healing at an average of 140 days
2006	23	Ennis et al[36]	Observational study of lower extremity ulcers (3 VLU); 69% NLFU healed at 7 weeks
2009	41	Cole et al[37]	Retrospective, single-arm study of chronic wounds (15 VLU); 88% median wound area reduction at 7.6 weeks, 38% healed in 6.8 weeks
2009	48	Haan and Lucich[38]	Retrospective, single-arm study of chronic wounds (26 VLU); 92% median wound area reduction in 5.5 weeks; 24% healed in mean of 4.3 weeks
2008	76	Bell et al[39]	Retrospective, single-arm study of chronic wounds (37 VLU); 79% reduction in wound area at 4.3 weeks
2007	15	Gehling et al[40]	Retrospective, single-arm study of ischemic wounds; 80% mean pain reduction at 2–4 weeks

4 weeks of treatment compared to SC alone. Absolute ulcer area reduction differences were significant as well. The study showed the larger and older the initial ulcer, the less likely SC treatment alone was able to achieve healing. By contrast, participants that received NLFU+SC responded favorably, with a reduction in ulcer area regardless of the initial ulcer size or duration. Additionally, NLFU+SC achieved significantly higher ulcer area reduction rates than SC alone in complicated ulcers that often are located laterally. These findings suggest NLFU may be able to catalyze healing even in complex wounds.

By 7 weeks post randomization, the NLFU+SC group had almost twice the percentage of wounds closed compared to SC. In participants who did not respond to SC alone, an improved response was noted following the introduction of NLFU (7 of 19 [37%] healed); interestingly, all 4 of the SC participants who crossed over and received NLFU per the recommended treatment frequency of 3 times per week healed within the 7 weeks.

To date, 15 peer-reviewed articles on the use of NLFU for wound healing have been published. Multiple wound types have been studied, including arterial, burn, deep tissue injury, diabetic, pressure, and venous ulceration. These studies have shown consistent wound area reduction levels ranging from 45% to 92% in 4 to 8 weeks of NLFU treatment. Complete healing rates in these studies are reported at a range from 33% at 6 weeks to 69% at 12 weeks. In comparing this RCT to the meta-analysis on NLFU that included 87 VLUs, this study had similar rates of ulcer area reduction at 67.7% in 4 weeks compared to 85% in 7 weeks and similar healing rates of 39% in 11 weeks compared to 41.7% at 12 weeks[17-19, 29-40] (see Table 5).

A 1-arm RCT[30] compared low-frequency versus high-frequency ultrasound to SC in 90 participants. Participants were treated for 3 months and clinically observed for 1 additional month. Ulcer areas reductions were 5.32 cm^2 (55.4%) for SC, 6.6 cm^2 (66.9%) for high-frequency ultrasound, and 7.29 cm^2

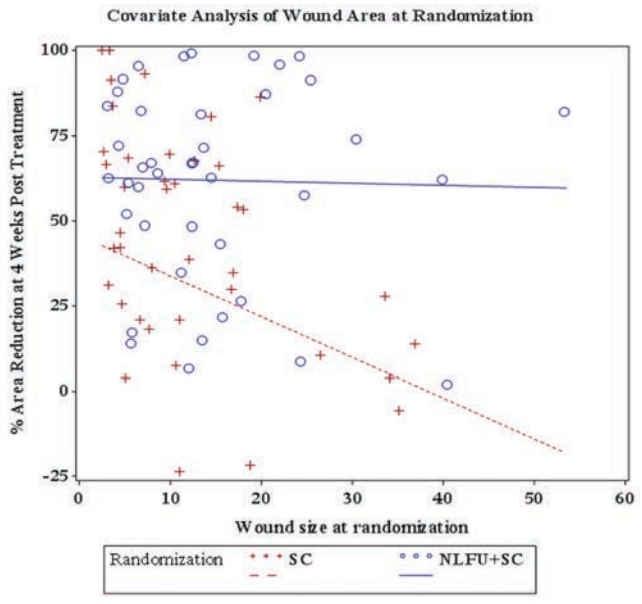

Figure 4. Wound size reduction (%) by wound size at randomization (baseline).
SC=standard care
NLFU=noncontact, low-frequency ultrasound

(72.9%) for NLFU. IN BALANCE VLU demonstrated similar percentages of reduction after 4 weeks of treatment. Patients with diabetes, rheumatoid arthritis, and ulcers with a component of arterial disease were excluded in the trial, and the mean age of the cohort was 38.2 years, which is much younger than the current study.

A limited number of VLU RCTs has been published on other commercially available therapies in the past 3 years. One RCT[41] of a biologic cell-based therapy on 182 participants demonstrated 12-week heal rates of 34% in the treatment group and 31% in the SC group, but these differences were not statistically significant. A higher rate of healing of 39% was noted in this study at 11 weeks for the NLFU arm, while the SC alone arms were similar in both studies. The study populations were similar for ulcer age and rate of recurrence. Ulcer size was slightly larger in the current study at 11.0 cm^2 compared to 7.4 cm^2. Comorbid conditions were not reported for comparison.

Another recent 4-arm RCT[42] evaluated the safety, efficacy, and dosing of a polymer wound healing technology in 82 participants with VLUs.[42] The trial reported 86% ulcers healed in the treatment arm with an every-other-week application for the 20-week study. Comorbidities were similar to the current trial. Ulcer duration was shorter at a mean of 3.6 months compared to a median of 10.3 months in the current study. The endpoint was measured at 5 months, which is nearly twice the healing time. Results for 11 weeks of treatment were not reported.

Even fewer QOL data have been published for patients with VLUs to compare with current trial results. When the IN BALANCE VLU QOL results are compared to a large population study[43] of 2,404 individuals with chronic venous disease, the current cohort had a much lower QOL with baseline SF-36 QOL scores 19% lower for physical functioning and 5% lower for mental health than corresponding patients with deep venous ulcers. A limited number of VLUs in the population study may explain the lower QOL results in the current study.

Looking at other disease states, the IN BALANCE VLU cohort suggests VLU have a higher impact on QOL than colorectal and breast cancer patients with scores that ranged from 7% to 41% lower for physical functioning; 7% to 31% lower in mental health; and 28% to 31% lower for bodily pain in the current study.[44-46]

A report[5] of 547 patients with venous stasis ulcers noted 55% required assistance with activities of daily living, including issues with ambulation, dressing, and toileting. The lack of independence may contribute to a poorer overall QOL for VLU patients than chronic venous disease and cancer patients.

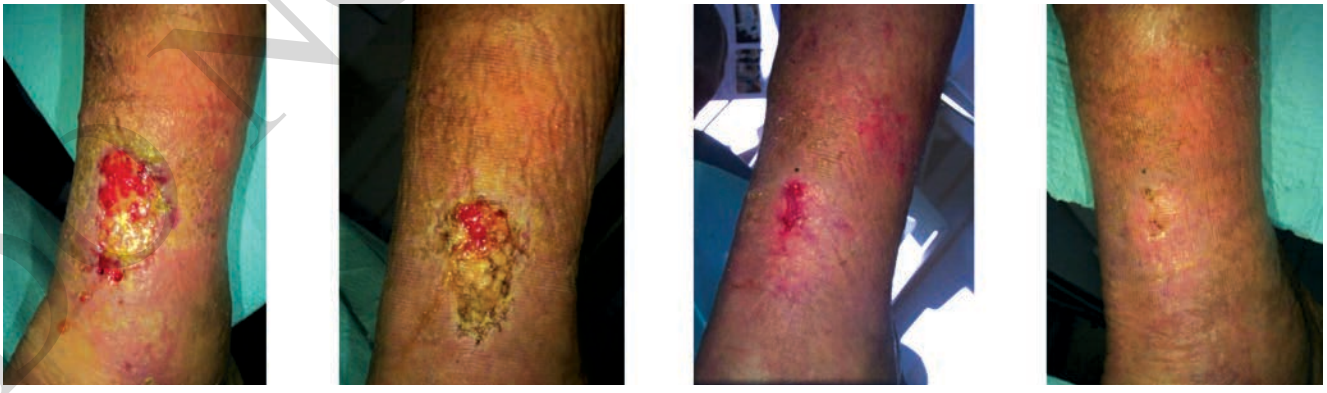

Figure 5. Example of weekly wound progression in a participant randomized to noncontact, low-frequency ultrasound (NLFU) plus standard care from baseline (randomization) to ulcer closure (5 weeks) after a total of 12 NFLU treatments. The index ulcer was 14 months' duration, 14.4 cm^2 in size, and located laterally. The participant received a minimum of 30 mm Hg compression for the 30 days before enrollment.

Table 6. List of participating investigators

PI	Institution	Location
Robert Galiano, MD	Northwestern University Division of Plastic Surgery	Chicago, IL, USA
Gary Gibbons, MD	South Shore Hospital Center for Wound Healing	Weymouth, MA, USA
Robert Kirsner, MD	University of Miami Department of Dermatology and Cutaneous Surgery	Miami, FL, USA
Mark Melin, MD	Methodist Park Nicollet Wound Care Clinic	Saint Louis Park, MN, USA
Dennis Orgill, MD	Brigham and Women's Hospital Division of Plastic Surgery	Boston, MA, USA
Christopher Attinger, MD	Georgetown University Hospital Department of Plastic Surgery	Washington, DC, USA
H. John Samies, MD	Regional Medical Center, The Wound Center	Orangeburg, SC, USA
Alik Farber, MD	Boston Medical Center Vascular and Endovascular Surgery	Boston, MA, USA
Randy Wolcott, MD	Southwest Regional Wound Care Center	Lubbock, TX, USA
Pathanjali Sharma, MD	St. Joseph Medical Center Vascular Center	Reading, PA , USA
David Eisenbud, MD	Overlook Hospital, The Wound Healing Program	Summit, NJ, USA
Thomas Serena, MD	Snyder Institute for Vascular Health & Research	Kittanning, PA, USA
Alexander Reyzelman, DPM	Sacramento Foot and Ankle Center	Fair Oaks, CA, USA
Mher Vartivarian, DPM	Bay Area Foot Care	Castro Valley, CA, USA
Roslyn Isseroff, MD	Sacramento VA Medical Center Dermatology Department	Mather, CA, USA
Shawn Cazzell, DPM	Valley Vascular Surgery Associates	Fresno, CA, USA
Aksone Nouvong, DPM	Olive View Hospital- UCLA Medical Center Surgery Department	Sylmar, CA, USA
Jessica O'Connell, MD	Greater Los Angeles VA Medical Center Vascular Surgery Department	Los Angeles, CA, USA
Ian Gordon, MD	Long Beach VA Medical Center Vascular Surgery Department	Long Beach, CA, USA
Mitch Silver, DO	OhioHealth Research Institute Heart and Vascular Center	Columbus, OH, USA
Steven Farley, MD	UCLA Gonda (Goldschmied) Vascular Center	Los Angeles, CA, USA
David Smith, MD	Tampa General Hospital	Tampa, FL USA

In the current study, NLFU reduced pain levels and improved QOL scores, even in a short-time frame of 4 weeks. Bodily pain was reduced significantly in the NLFU+SC treatment group; participants reported nearly zero pain (0.6 VAS) within 4 weeks of treatment. This reduction in pain score is consistent with previous findings for NLFU. A meta-analysis on multiple types of chronic wounds by Driver et al[29] reported pain reduction of 79% after use of NLFU treatment. This cohort reported a similar 83% median reduction in pain after 4 weeks of NLFU treatment.

No reported debridement (35%), a lack of adequate compression (33%), use of advanced therapies (48%), and a history of anatomical correction (>25%) was common in this study group with a median ulcer age of 9.9 months (mean age 23.9 ± 37.1 months) at the time of study entry. These practices may have perpetuated a dysfunctional microenvironment that resulted in failed therapies, as well as a high recurrence (56%) and an overall lower QOL noted in these participants. This study, which required an aggressive level of compression (30–40 mm Hg) during the trial, demonstrated a highly significant difference in mean area reduction between NLFU over SC randomized participants who entered the study with a minimum of 30 days of 30–40 mm Hg compression ($P = 0.0005$).

Because the incidence of VLUs is rising and afflicting younger patients who are living longer and with multiple associated comorbidities, early and appropriate intervention to promote ulcer healing is paramount to limiting the impact of VLUs on patients and to reducing costs of care. The statistically significant differences in wound area reduction after 4 weeks of treatment between the NLFU+SC and SC alone treatment group as well as the significant differences observed between the treatment groups in participants who were compressed at 30–40 mm Hg for a minimum of 10 weeks (30 days before entering the study, during the 2 week run-in, and 4 week treatment phase) suggest NLFU will facilitate VLU healing in wounds where guideline defined SC has failed. Therefore, the authors recommend if an ulcer has been adequately treated with SC — including high-level compression — and has not reduced a minimum of 30% after 2 weeks, interventions that address the ulcer at a microenvironment level, such as NLFU, should be introduced.

This approach may result in successful healing, without the need for other advanced wound therapies.

Limitations

A limitation of this study was that the investigators and participants were not blinded to treatment group assignment. This was limited to participant-reported measures such as QOL and VAS pain scores. A second limitation was the treatment groups did not have the same required frequency of treatment visits. The protocol required participants randomized to NLFU to have 3 clinic visits per week during the treatment phase, while SC participants had a minimum of 1 visit per week, reflective of current wound care clinical practice. These differences in visit frequency could have both a positive and a negative impact. For example, achieving a more consistent level of compression in participants with edema may be an advantage. However, increased pain levels and potential disruption of newly epithelialized tissue may be increased with more frequent dressing changes. This limitation was mitigated by allowing investigators to follow up with SC participants up to 3 times per week, per their discretion, for medically necessary treatment.

Conclusion

In this study, percent reduction in ulcer area was statistically significantly greater in patients randomized to NLFU therapy plus SC compared to SC alone after 4 weeks of treatment. At the end of the 11 week follow-up, the number of healed ulcers was also higher in the treatment than in the control group. The improvement was observed regardless of the age of the wound, the size of the wound, or compounding comorbidities reported in this cohort. Participants treated with NLFU reported statistically significant reductions in pain within 4 weeks of treatment and improved QOL scores, though not significant, over those treated with SC alone. These outcomes confirm results of previously published NLFU studies and suggest NLFU should be considered simultaneously with guideline defined standard care for VLU, particularly in complex patients.

More indepth analysis on barriers to healing and the changes occurring in the tissue of the wound are needed to explore theories that the microenvironment is impacting wounds that do not heal despite provision of guideline-defined care. ■

Acknowledgments

The authors thank the patients and their caregivers for their dedication in participating in this study. The authors acknowledge the contributions of the IN BALANCE VLU study investigators and research coordinators (see Table 6).

References

1. Brem H, Kirsner RS, Falanga V. Protocol for the successful treatment of venous ulcers. *Am J Surg*. 2004;188(1A suppl):1S–8S.
2. Margolis DJ, Bilker W, Santanna J, Baumgarten M. Venous leg ulcer: incidence and prevalence in the elderly. *J Am Acad Dermatol*. 2002;46(3):381–386.
3. McGuckin M, Waterman R, Brooks J, Cherry G, Porten L, Hurley S, et al. Validation of venous leg ulcer guidelines in the United States and the United Kingdom. *Am J Surg*. 2002;183(2):132–137.
4. Franks PJ, Moffatt CJ. Health related quality of life in patients with venous ulceration use of the Nottingham Health Profile. *Qual Life Res*. 2001;10(8):693–700.
5. Fife C, Walker D, Thomson B, Carter M. Limitations of daily living activities in patients with venous stasis ulcers undergoing compression bandaging: problems with the concept of self-bandaging. *WOUNDS*. 2007;19(10):255–257.
6. Kom P, Patel ST, Heller JA, Deitch JS, Krishnasastry KV, Bush HL et al. Why insurers should reimburse for compression stockings in patients with chronic venous stasis. *J Vasc Surg*. 2002;35(5):950–957.
7. Rice JB, Desai U, Cummings AKG, Birnbaum HG, Skornicki M, Parsons N. Medical, drug, and work-loss costs of venous leg ulcers. Poster presentation at the Symposium on Advanced Wound Care. New Orleans, LA. May 18–22, 2013.
8. Olin JW, Beusterien KM, Childs MB, Seavey C, McHugh L, Griffiths RI. Costs in the management of venous stasis ulcers: implications for health care providers and payers. *Vasc Med*. 1999;4(1):1–7.
9. Gloviczki P, Comerota AJ, Dalsing MC, Eklof BG, Gillespie DL, Gloviczki ML, et al. The care of patients with varicose veins and associated chronic venous disease: clinical practice guideline of the Society for Vascular Surgery and The American Venous Forum. *J Vasc Surg.* 2011;53(5 suppl):2S–48S.
10. Association for the Advancement of Wound Care (AAWC) Venous Ulcer Guideline. Malvern, Pennsylvania: Association for the Advancement of Wound Care (AAWC). December 2010. Available at: http://aawconline.org/professional-resources/resources/. Accessed on December 1, 2014.
11. Moor AN, Vachon DJ, Gould LJ. Proteolytic activity in wound fluids and tissues derived from chronic venous leg ulcers. *Wound Repair Regen.* 2009;17(6):832–839.
12. Beidler S, Douillet C, Berndt D, Keagy B, Rich P, Marston W. Inflammatory cytokine levels in chronic venous insufficiency ulcer tissue before and after compression therapy. *J Vasc Surg*. 2009;9(4):1013–1020.
13. Liu YC, Margolis DJ, Isseroff RR. Does inflammation have a role in the pathogenesis of venous ulcers? A critical review of the evidence. *J Invest Dermatol*. 2011;131(4):818–827.
14. US Food and Drug Administration, Center for Devices and Radiological Health. Celleration MIST Therapy System 5.1 K140782 clearance letter, May 17, 2005.
15. Seth AK, Nguyen KT, Geringer MR, Hong SJ, Leung KP, Mustoe, TA, et al. Noncontact, low-frequency ultrasound as an effective therapy against *Pseudmonas aeruginosa* infected biofilm wounds. *Wound Repair Regen.* 2013;21(2):266–274.
16. Maan ZN, Januszyk, M, Rennert, RC, Duscher D, Rodrigues M, Fujiwara, T, et al. Noncontact low frequency ultrasound therapy enhances neovascularization and wound healing in diabetic mice. *Plast Reconstr Surg*. 2014;134(3):402e–411e.
17. Serena T, Lee SK, Lam K, Attar P, Meneses P, Ennis W. The impact of noncontact, nonthermal, low-frequency ultrasound on bacterial counts in experimental and chronic wounds. *Ostomy Wound Manage*. 2009;55(1):22–30.
18. Yao M, Hasturk H, Kantarci A, Gu G, Garcia-Lavin S, Fabbi M, et al. A pilot study evaluating noncontact low frequency ultrasound and underlying molecular mechanism on diabetic foot ulcers. *Int Wound J.* 2014;11(6):586–593.
19. Escandon J, Vivas AC, Perez R, Kirsner R, Davis S. A prospective pilot study of ultrasound therapy effectiveness in refractory venous leg ulcers. *Int Wound J*. 2012;9(5):570–578.
20. MIST Therapy™ Instructions For Use, PL-61000 rev. E, June 17, 2011.
21. Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ration scale measures for chronic and experimental pain. *Pain*. 1983;17(1):45–56.
22. Ware JE, Kosinski M, Bjorner JB, Turner-Bowker DM, Gandek B, Maruish ME. *SF-36v2© Health Survey: Administration Guide for Clinical Trial Investigators*. Lincoln, RI. QualityMetric Incorporated;2008.
23. Gelfand JM, Hoffstad O, Margolis DJ. Surrogate endpoints for the treatment of venous leg ulcers. *J Invest Dermatol*. 2002;119(6)1420–1425.
24. International Conference on Harmonization. ICH harmonized tripartite guideline: statistical principles for clinical trial E-9 Feb 5, 1998;ver 4. Available at: http: www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E9/Step4/E9_Guideline.pdf. Accessed December 12, 2013.
25. US Food and Drug Administration. Guidance for industry: E9 statistical principles for clinical trials. US Dept of Health & Human Services. Sept. 1998. Available at: http: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073137.pdf. Accessed December 12, 2013.
26. Margolis DJ, Berlin JA, Strom BL. Risk factors associated with the failure

of a venous leg ulcer to heal. *Arch Dermatol.* 1999;1(35):920–926.
27. Donahue K, Falanga V. Healing rate as a prognostic indicator of complete healing: a re-appraisal. *WOUNDS*. 2003;15(3):71–76.
28. Gottrup F, Apelqvist J, Price P. Outcomes in controlled and comparative studies on non-healing wounds: recommendations to improve the quality of evidence in wound management. *J Wound Care*. 2010;19(6):237–268.
29. Driver VR, Yao M, Miller CJ. Noncontact low-frequency ultrasound therapy in the treatment of chronic wounds: a meta-analysis. *Wound Repair Regen*. 2011;19(4):475–480.
30. Olyaie M, Rad FS, Elahifar MA, Garkaz A, Mahsa G. High-frequency and noncontact low-frequency ultrasound therapy for venous leg ulcer treatment: a randomized, controlled study. *Ostomy Wound Manage*. 2013:59(8):14–20.
31. Kavros SJ, Miller JL, Hanna SW. Treatment of ischemic wounds with noncontact, low frequency ultrasound: the Mayo Clinic experience, 2004-2006. *Adv Skin Wound Care*. 2007;20(4):221–226.
32. Ennis WJ, Formann P, Mozen N, Massey J, Conner-Kerr T, Meneses P, et al. Ultrasound therapy for recalcitrant diabetic foot ulcers: results of a randomized, double-blind, controlled, multicenter study. *Ostomy Wound Manage*. 2005;51(8):24–39.
33. Honaker JS, Forston MR, Davis EA, Wiesner MM, Morgan JA. Effects of non contact low-frequency ultrasound on healing of suspected deep tissue injury: a retrospective analysis. *Int Wound J*. 2013;10(1):65–72.
34. Kavros SJ, Schenck EC. Use of noncontact low frequency ultrasound in the treatment of chronic foot and leg ulcerations: a 51 patient analysis. *J Am Pod Med Assoc*. 2007;97(2):95–101.
35. Kavros SJ, Liedl DA, Boon AJ, Miller JL, Hobbs JA, Andrews KL. Expedited wound healing with noncontact, low-frequency ultrasound therapy in chronic wounds: a retrospective analysis. *Adv Skin Wound Care*. 2008:21(9):416–423.
36. Ennis WJ, Valdes W, Gainer M, Meneses P. Evaluation of clinical effectiveness of MIST ultrasound therapy for the healing of chronic wounds. *Adv Skin Wound Care*. 2006;19(8):437-446.
37. Cole PS, Quisberg J, Melin MM. Adjuvant use of acoustic pressure wound therapy for treatment of chronic wounds: a retrospective analysis. *J Wound Ostomy Continence Nurs*. 2009;36(2):171–177.
38. Haan J, Lucich S. A retrospective analysis of acoustic pressure wound therapy: effects on the healing progression of chronic wounds. *J Am Col Certif Wound Spec*. 2009;1(1):28-34.
39. Bell AL, Cavorsi J. Noncontact ultrasound therapy for adjunctive treatment of nonhealing wounds: retrospective analysis. *Phys Ther.* 2008;88(12):1517-1524.
40. Gehling ML, Samies JH. The effect of noncontact, low-intensity, low-frequency therapeutic ultrasound on lower-extremity chronic wound pain: a retrospective chart review. *Ostomy Wound Manage*. 2007;53(3):44–50.
41. Harding K, Sumner M, Cardinal M. A prospective, multicenter, randomized controlled study of human fibroblast-derived dermal substitute (Dermagraft) in patients with venous leg ulcers. *Int Wound J.* 2013;10(2):132–137.
42. Kelechi TJ, Mueller M, Hankin CS, Bronstone A, Samies J, Bonham PA. A randomized, investigator-blinded, controlled pilot study to evaluate the safety and efficacy of a poly-N-acetyl glucosamine-derived membrane material in patients with venous leg ulcers. *J Am Acad Dermatol*. 2012;66(6):e209–e215.
43. Kaplan RM, Criqui MH, Denenberg JO, Bergan J, Fronek A. Quality of life in patients with chronic venous disease: San Diego population study. *J Vasc Surg*. 2003:37(5):1047–1053.
44. Ganz PA, Desmond KA, Leedham B, Rowland JH, Meyerowitz BE, Belin TR. Quality of life in long-term, disease-free survivors of breast cancer: a follow-up study. *J Natl Cancer Inst*. 2002;94(1):39–49.
45. Sampaio E, Albuquerque I, Linhares J. Assessing the quality of life in women with breast cancer through the questionnaire SF-36. *Appl Clin Res*. 2008;28(3):93–98.
46. Trentham-Dietz A, Remington, PL, Moinpour CM, Hampton JM, Sapp AL, Newcomb PA. Health related quality of life in female long-term colorectal cancer survivors. *Oncologist*. 2003;8(4):342–349.