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ORIGINAL ARTICLE

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A multicentre, randomised controlled clinical trial evaluating the effects of a novel autologous, heterogeneous skin construct in the treatment of Wagner one diabetic foot ulcers: Interim analysis

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Abstract

We desired to carefully evaluate a novel autologous heterogeneous skin construct in a prospective randomised clinical trial comparing this to a standard-of-care treatment in diabetic foot ulcers (DFUs). This study reports the interim analysis after the first half of the subjects have been analysed. Fifty patients (25 per group) with Wagner 1 ulcers were enrolled at 13 wound centres in the United States. Twenty-three subjects underwent the autologous heterogeneous skin construct harvest and application procedure once; two subjects required two applications due to loss of the first application. The primary endpoint was the proportion of wounds closed at 12 weeks. There were significantly more wounds closed in the treatment group (18/25; 72%) vs controls (8/25; 32%) at 12 weeks. The treatment group achieved significantly greater percent area reduction compared to the control group at every prespecified timepoint of 4, 6, 8, and 12 weeks. Thirty-eight adverse events occurred in 11 subjects (44%) in the treatment group vs 48 in 14 controls (56%), 6 of which required study removal. In the treatment group, there were no serious adverse events related to the index ulcer. Two adverse events (index ulcer cellulitis and bleeding) were possibly related to the autologous heterogeneous skin construct. Data from this planned interim analysis support that application of autologous heterogeneous skin construct may be potentially effective therapy for DFUs and provide supportive data to complete the planned study.

KEYWORDS

biological products, diabetic foot, randomised controlled trial, ulcer, wound healing

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1 | INTRODUCTION

Diabetic foot ulcers (DFUs) cost Medicare $6.2–18.7 billion each year and have a devastating annual impact on the economy of United States, with an annual burden of over $50 billion.[1,2] Approximately 1.5 million Americans have DFUs, which contribute to 130 000 annual lower-extremity amputations.[3,4] A real-world analysis of 62 964 DFUs registered in the US Wound Registry found that their healing rate at 12 weeks was only 30.5%.[5] A meta-analysis of DFUs treated in trials with standard of care revealed a 12-week closure rate of 24%.[6] Biological skin substitutes are commonly used as adjunctive therapy to improve wound closure.[7,8] However, most products are quite costly and require multiple applications. Split-thickness skin grafting (STSG) can contribute new healthy tissue to the wound bed but has a failure rate of approximately 30% when applied to DFUs as a consequence of poor graft take by the chronic wound bed, the presence of diabetes, vascular insufficiency, other comorbidities, and/or bacterial contamination.[9-14] As many DFUs are treated in the outpatient setting, another disadvantage of skin grafting is that it involves a surgical procedure in the operating room.

A novel autologous heterogeneous skin construct (AHSC) created from a small harvest of full thickness, healthy skin may be safe and effective as adjunctive therapy in treating complex and refractory wounds.[15-24] AHSC is composed of small multicellular segments and contains the endogenous regenerative cellular populations of healthy skin that promote wound closure, so that a single application can regenerate full-thickness, functionally polarised skin on the wound bed.[20-25] The manufacturing process of the AHSC retains the endogenous regenerative cellular populations associated with wound healing present within hair follicles, glands, and the interfollicular epidermis, facilitating engraftment optimisation and wound closure.[24] AHSC is not cultured ex vivo, but rather it is expeditiously returned to the provider to be administered topically over a clean, debrided, viable wound bed and covered with common nonadherent, nonabsorbent dressings in the outpatient setting. The AHSC conforms nicely to the wound and over days forms small skin islands that expand and coalesce across the entire wound bed to close the wound, rather than initiating epithelialisation solely from the wound margin.[20,21] In a pilot study of 11 patients with DFUs extending up to the tendon, bone, or capsule, 10 patients closed within 8 weeks of a single application of AHSC, with the mean percent area reduction (PAR) for all wounds at 4 weeks at 83%.[24] A larger, controlled trial was needed to confirm these initial findings in DFUs. A planned interim analysis of the first 50 of the 100 patients of a randomised controlled trial (RCT) was performed to compare the effects of AHSC to standard of care in the treatment of Wagner 1 DFUs.

Key Messages

- This interim analysis of an ongoing, randomised controlled trial evaluated a single application of autologous heterogeneous skin construct (AHSC) as adjunctive therapy to standard of care in Wagner 1 diabetic foot ulcers compared to standard of care alone in 50 initial subjects.
- There were significantly more wounds closed in the AHSC group (18/25; 72%) compared to the control group (8/25; 32%) ($P = .005$) and a significantly greater percent area reduction in the AHSC group compared to the control group at each prespecified timepoint of 4 weeks (79% vs 24%, $P = .0002$), 6 weeks (83% vs 44%, $P = .004$), 8 weeks (87% vs 47%, $P = .002$), and 12 weeks (88% vs 50%, $P = .012$), respectively.
- In the AHSC group, there were no serious adverse events related to the index ulcer or determined to be related to AHSC treatment.
- These data support continuation of the planned study

2 | METHODS

2.1 | Study design and population

This was a planned interim analysis of the first 50 patients of a prospective, multicentre, RCT evaluating wound closure rates of DFUs treated in an outpatient setting. Thirteen wound care centres in the United States participated in this study. The null hypothesis was the proportion of wounds closed at 12 weeks, after up to 12 weeks of AHSC and standard of care or standard of care alone, would be equal for groups 1 (AHSC + standard of care) and 2 (control). Formally, H0: I1–I2 = 0; HA: I1–I2 = D1 ≠ 0, where I1 was the proportion of wounds closed in group 1, I2 was the same metric for group 2, D1 was the difference (I1–I2); assuming the alternative hypothesis and statistical test used was chi square/Fisher exact test. The primary endpoint was the percentage of index ulcers closed at 12 weeks. Complete closure was defined when 100% epithelialisation without drainage was first observed, followed by a closure confirmation visit

2 weeks later. Secondary endpoints included the PAR at 4, 6, 8, and 12 weeks; changes in wound quality-of-life (W-QOL short questionnaire, with each question scored on a scale of 0 = "not at all" to 4 = "very much"); reduced pain (based on the Visual Analogue Scale [VAS], with 0 = no pain and 10 = worst possible pain); improvements in peripheral neuropathy by Semmes Weinstein monofilament test; and incidence of adverse events (AEs) and complications.

The sample size was determined to be 102 (51 in each group) to achieve 89% power to detect a difference between the group proportions of 0.3. The proportion in the AHSC group was assumed to be 0.3 under the null hypothesis and 0.6 under the alternative hypothesis. The proportion in the control group was 0.3. The test statistic used was the two-sided Z test with pooled variance. The significance level of the test was targeted at 0.05. The significance level actually achieved by this design was 0.05. Unblinded interim analysis was performed after 50 subjects completed the study in order to assess subject outcomes between the groups and to recalculate the sample size for the primary endpoint. This study was conducted according to the principles expressed in the Declaration of Helsinki, and the Institutional Review Board Advarra (Columbia, MD) approved the study protocol. The study protocol was registered on clinicaltrials.gov (NCT03881254).

Adult patients with a Wagner 1 DFU that did not involve the tendon, muscle, or bone, provided that it was below the aspect of the medial malleolus, were screened for study participation. Table 1 details complete inclusion and exclusion criteria. Eligible patients provided their written informed consent and were enrolled into the study. During their first screening visit, their demographics and medical history were recorded; a complete physical examination was performed; laboratory tests were taken; the index ulcer was assessed for infection and pain; adequate perfusion was confirmed; Semmes Weinstein monofilament test for peripheral neuropathy was performed; subjects answered the W-QOL short questionnaire; sharp debridement of the index ulcer was performed as needed; the wounds were dressed with standard of care; and offloading was initiated.

Two weeks after the initial screening visit, subjects returned to undergo the same assessments to check for any changes in their health, ulcer healing status, and eligibility. Randomisation occurred if the ulcer did not change in size greater than 30% and still met eligibility. The Organisation1 (City2, State2) used a block size of 10 for randomisation (5 sheets of paper with a standard-of-care assignment and 5 with an AHSC assignment). Each sheet was inserted into an opaque envelope that was sealed. The study coordinator shuffled the envelopes, while under observation by the principal investigator and staff. After repeating the process 10 times, the envelopes were sent to the study sites, ensuring that site investigators were blinded to the randomisation method and treatment assignment. The site investigators enrolled the subjects into the study and were aware of the study group following randomisation.

2.2 | AHSC preparation, application, and follow-up

Following randomisation, standard of care was applied to both groups, and the AHSC group underwent the skin harvest procedure. Standard of care included offloading of the DFU (CAM boots or total contact casting, if the subject's foot was too large for a CAM boot, or per the provider's discretion), appropriate sharp or surgical debridement, collagen alginate and appropriate wound care covering, including 4×4 gauze pads, foam, and a multilayer compression bandaging system comprised a soft roll layer, an elastic layer, and a cohesive bandage layer (Dyna-Flex, KCI, St. Paul, MN).In the AHSC group, a 1×2 cm full-thickness harvest of healthy skin was excised from the index limb of each subject using sterile technique and local. The provider sutured closed the harvest site. The harvest was shipped overnight to a Food and Drug Administration–registered biomedical manufacturing facility (PolarityTE, Salt Lake City, UT) and used to manufacture the AHSC (Product, Organisation3). The AHSC was returned to the provider within 48 hours of tissue harvest and applied to the wound within 4 days after the harvesting procedure. The AHSC was shipped and stored at 4°C before application.

On the day of the application procedure, the wound was cleaned and sharply debrided, if required. The AHSC was spread evenly across the wound bed. Next, the wound was dressed with a silicone dressing covered by an absorbent foam dressing (DermaFoam, DermaRite Industries, North Bergen, NJ). A three-layer compression bolster was then applied. Dressings were changed weekly, and wounds continued to be offloaded. At the third follow-up visit, a nonadherent contact layer (Adaptic Touch, KCI) replaced the silicone dressing. After the AHSC was applied and the wound was addressed, a time-out procedure undertaken by the on-site study team confirmed the application of the subject's own harvested construct to the index ulcer.

Subjects in both groups had weekly follow-up visits and dressing changes with standard of care for up to 12 weeks. At each visit, wound sites (including the harvest sites in the AHSC group) were assessed for healing

TABLE 1 Patient inclusion and exclusion criteria

Inclusion criteria
• At least 18 years old
• Presence of a Wagner 1 DFU that did not extend through the dermis or subcutaneous tissue and did not involve the tendon, muscle, or bone, provided that it was below the aspect of the medial malleolus
• If two or more Wagner 1 DFUs were present, then the index ulcer was the largest ulcer and the only one evaluated in the study. Any other ulceration must have been 2 cm distant from the index ulcer.
• Index ulcer was present for at least 4 weeks
• Index ulcer was a minimum of 1.0 cm^2 and a maximum of 25 cm^2 at screening visit and did not reduce/increase in area by 30% or more after 14 days of standard of care prior to first treatment visit
• Index ulcer had been offloaded for ≥14 days prior to randomisation
• Index ulcer had a clean granular base, was free of necrotic debris, and appeared to healthy, vascularised tissue at time of AHSC placement
• Affected foot had adequate circulation as documented by a dorsal transcutaneous oxygen measurement or a skin perfusion pressure measurement of ≥30 mmHg, or an ankle brachial index of ≥0.7 and ≤1.2, or arterial Doppler with a minimum of biphasic flow within 3 months of treatment
• Women of childbearing age were willing to use contraception during the study and undergo pregnancy tests
• Patient understood and was willing to participate in the study, could comply with the weekly visits and follow-up, and provided written informed consent.
Exclusion criteria
• Active osteomyelitis, cellulitis, soft tissue infection, or active Charcot's arthropathy of the affected foot involving or near the index ulcer site, or on the same limb as the index ulcer within 30 days prior to randomisation
• Index ulcer was suspicious of cancer
• History of radiation at the index ulcer site
• History of >2 weeks treatment with immunosuppressants (including systemic corticosteroids), cytotoxic chemotherapy, or application of topical steroids to the index ulcer surface within 1 month prior to screening, or who were anticipated to require such medications during the study
• Evidence of unstable HIV, hepatitis B, or hepatitis C
• On an investigational drug or therapeutic device within 30 days of screening
• Index ulcer was previously treated or needed to be treated with any prohibited therapies such as chlorhexidine or collagenase
• Presence of any condition which seriously compromised the patient's ability to complete the study or had a known history of poor adherence with medical treatment
• In the opinion of the investigator, the patient was noncompliance with offloading or index ulcer dressing prior to randomisation
• Pregnant or breastfeeding
• Presence of diabetes with poor metabolic control as documented with an HbA1c ≥12.0 within 30 days of randomisation
• Presence of end-stage renal disease as evidenced by serum creatinine of greater than 3.0 mg/dL within 120 days of randomisation

Abbreviations: AHSC, autologous homologous skin construct; DFU, diabetic foot ulcer.

status, pain, and infection; the index ulcer was measured and assessed for graft take; and AEs were reported. A licensed provider who did not treat the index ulcer first performed an initial, blinded wound closure assessment of the wound in-person. Once considered healed by the blinded investigator, the wound images were forwarded to a group of university plastic surgeon adjudicators who determined if the wound was healed within 24 hours of receiving the photographs. If two-thirds of the adjudicators agreed that the wound had closed, then the subject returned for a closure confirmation visit 2 weeks later. At the end-of-study visit, W-QOL was also assessed, and a Semmes-Weinstein monofilament test was administered for peripheral neuropathy.

2.3 | Data collection and analysis

Data were stored in an Excel database. The statistical analysis was performed using PASW 27 (IBM, Chicago, IL). Blinded, interim analysis was first performed, and coding for treatment was then applied to the analysis involving comparison of groups.

The intent-to-treat (ITT) and safety populations comprised randomised subjects who received at least 1 treatment. All analyses used the ITT approach. The last observation carried forward principle that was used with regard to missing area data at study visits. Study variables were summarised as means and SDs for continuous variables as well as medians for nonnormal data. Categorical

variables were presented as counts and proportions or percentages. Statistical testing between groups at baseline was carried out to examine the success of randomisation. For categorical variables, chi-squared or Fisher exact tests were performed, and for continuous variables independent *t* tests or Mann-Whitney tests were used (depending on variable normality) to test for statistical differences.

The PAR for the index ulcer at X weeks was calculated as $([A_I - A_{XW}]/A_I)\times 100$, where A_I is the area of the index wound at randomisation and A_{XW} is the area at *X* weeks. When AHSC was applied twice, area data by week was based on data associated with the first AHSC application, followed by the second AHSC application, and then follow-up.

The primary endpoint (proportion of wounds closed at 12 weeks) between study groups was analysed using chi square.

Secondary endpoints between study groups were analysed by chi-squared or Fisher exact tests for categorical variables, while independent *t* tests or Mann–Whitney tests were used to test for statistical differences for continuous variables depending on outcome variable normality, which was examined using the Wilks-Shapiro test. The exception was PAR at 2, 4, 6, 8, and 12 weeks, which was analysed using general linear mixed modelling (GLMM) with repeated measures (no random effects). Two-sided *P* values <.05 were considered significant.

Summary statistics were used as inputs to calculate the conditional statistical power for all endpoints based on a final N of 100 using PASS13 software (NCSS, LLC, Kaysville, UT).

All AEs were categorised as "serious" or "not serious" and assessed for severity (mild, moderate, severe, or life-threatening) and relationship to the AHSC product and harvesting and placement procedures (not related, possibly related, probably related, or definitely related).

3 | RESULTS

Study recruitment began on April 2, 2019, and all subjects exited the trial by June 20, 2020. This interim analysis covers the 79 patients screened for eligibility and the 50 subjects (63%) who were enrolled (Figure 1). One subject (4%) was withdrawn from the AHSC group due to development of respiratory illness and sepsis, whereas 6 subjects (24%) were withdrawn in the control group due to 1 subject being incarcerated and 5 having AEs occur that required study removal (Figure 1). Table 2 summarises patient demographics and medical history with no significant differences between groups. Three subjects in the AHSC and 6 in the control group had missing HbA1c data. The index ulcer was treated with multiple therapies prior to study enrolment, with similar treatments applied to both groups, except for antibiotics, which were administered significantly more to the control group ($P = .023$) (Table 2).

All 25 subjects in the AHSC group underwent the AHSC harvest and application procedure, but 2 subjects required a second AHSC application due to loss of the first application requiring a second tissue harvest. The proximal medial calf was the most common harvest site (17/27, 63%). Upper medial thigh and proximal lateral leg were harvested for the remainder of the cases. Nine AHSC constructs (33%) were applied 2 days after harvest, 17 (63%) after 3 days, and 1 (4%) after 5 days.

3.1 | Closure rates

There were statistically significantly more wounds closed in the AHSC group (18/25; 72%) compared to the control group (8/25; 32%) at 12 weeks ($P = .005$). Closure rates through week 12 are shown in Figure 2. Based on these data and using the 2-side Z test with pooled variance, the projected statistical power for 100 subjects was 98.8%.

Table 3 and Figure 3 summarise the PAR data through 12 weeks. The GLMM model would not always converge when a random intercept model was incorporated into a factorial fixed effects model with 4 levels (PAR at the 4 time periods) no matter what covariance matrix was selected. Removing the random effects and using the simpler model with an unstructured correlations covariance matrix resulted in a worse fit but similar to other covariance matrices (−2LL or BIC); however, for treatment, a significant effect was observed ($P = .013$). Based on these data, the projected statistical power for 100 subjects was 90+%. Representative images of wound closure are shown in Figure 4.

All harvest sites remained closed following primary closure and fully healed within 12 weeks except for in 1 subject who was withdrawn from the trial before healing could be confirmed.

3.2 | Safety analysis

There were 86 AEs allocated to 25 subjects. The AHSC group had 38 AEs allocated to 11 subjects (44%), while the control group had 48 AEs allocated to 14 subjects (56%). The overall AE rate was 1.5 for the AHSC group and 1.9 for the control group.

There were 13 SAEs, 7 in the AHSC group and 6 in the Control group. In the AHSC group, 1 subject had 3 SAEs (congestive heart failure, dyspnea episode that was a symptom of SARS-CoV-2 infection, and his index

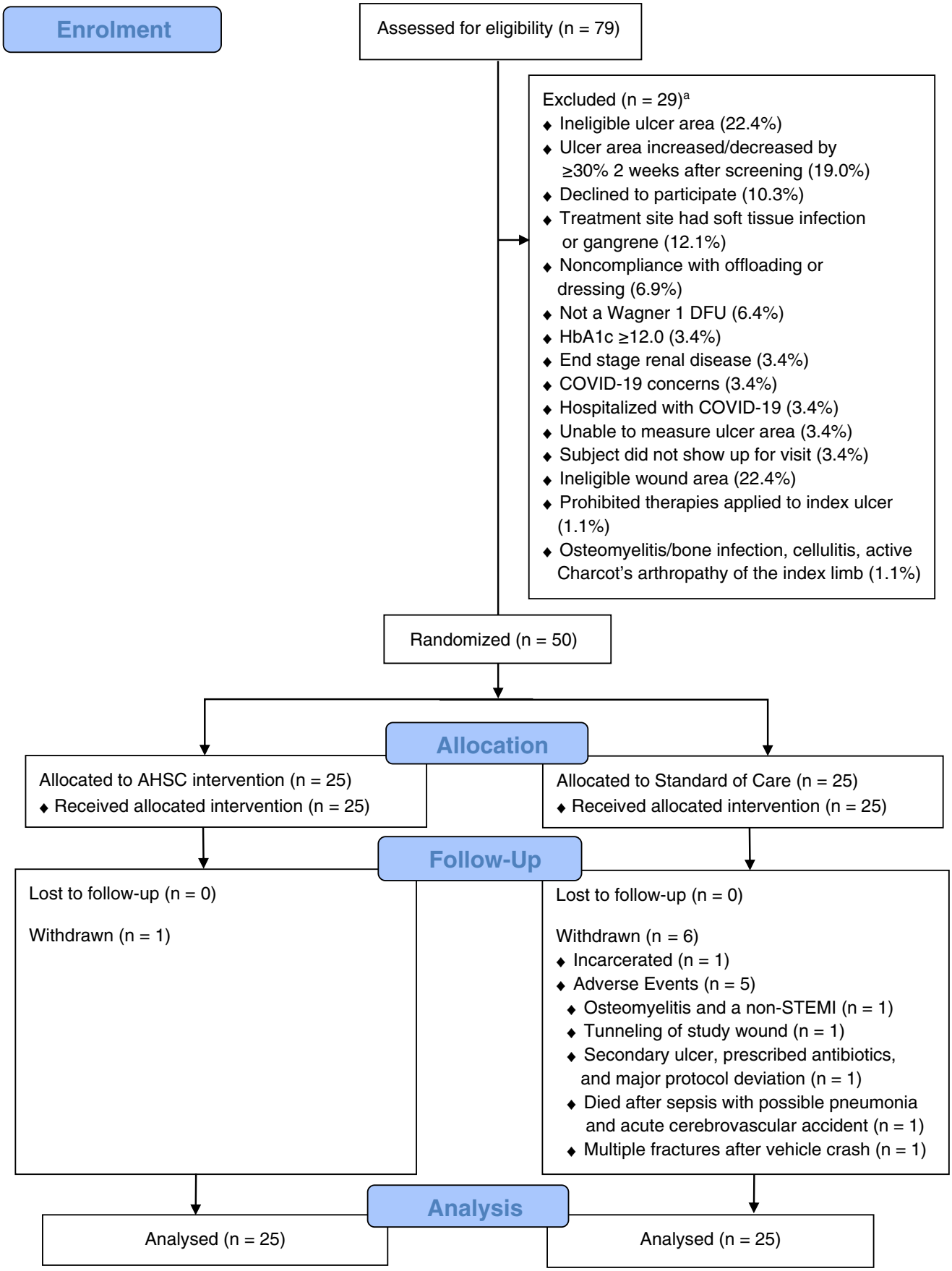

FIGURE 1 Legend on next page.

wound required cauterisation following admission for his congestive heart failure and during the admission, his wound was debrided against protocol by the non-trail site-admitting service, while the patient was on anti-coagulation), while another subject had 2 SAEs (sepsis, related to a hepatitis A infection, and cellulitis of the right leg, which was not related to the index ulcer). Two other subjects had 1 SAE each: an upper gastrointestinal bleed and an acute kidney injury. In the control group, 1 subject had 4 SAEs over a 3-week period, beginning with the development of left foot cellulitis related to the index ulcer, followed by acute osteomyelitis, which required surgery; severe sepsis occurred after the surgical procedure, but it quickly resolved. A separate SAE also occurred in a control group subject during this time period (non–ST-segment elevation myocardial infarction). Another control group subject developed a soft tissue infection related to the index ulcer, which was treated with sharp debridement and antibiotics and resolved after 7 weeks.

In the AHSC group, there were no product-related SAEs. There were two AEs that were possibly related to the treatment of the index ulcer: 1 infection of the study right heel DFU and a bleeding episode of the study ulcer located on the plantar aspect of the 5th metatarsal head, right foot. Only 2 AEs (pain and cellulitis) occurred in the harvest site, both in the AHSC group. There were 7 index ulcer infections (including cellulitis) in the control group compared to 1 in the AHSC group. There were 4 non-index ulcer infections in both groups. The AHSC group had 7 other complications reported, including 1 for the index ulcer, while the Control group had 17 other complications, including 4 for the index ulcer. There were 24 other causes of AEs in the AHSC group versus 20 in the Control Group.

3.3 | Other secondary endpoints

The mean (SD) difference in the W-QOL scores between week 1 and week 12 visits was 0.1 (0.8) in the AHSC group vs 0.6 (1.2) in the control group ($P = .09$).

The mean (SD) difference in pain scores between week 1 and week 12 visits was 0.7 (1.6) in the AHSC group and 0.5 (1.6) in the control group ($P = .48$).

The mean (SD) difference in Semmes-Weinstein scores between week 1 and week 12 visits was 0.1 (1.4) in the AHSC group and 0.4 (2) in the control group ($P = .16$).

4 | DISCUSSION

A traditional method of tissue reconstruction for Wagner 1 ulcers is a skin graft once the wound has been cleaned and granulating.[20] However, a skin graft requires technically demanding surgical procedure with careful postoperative care, which is not easily available in many wound care centres. It is further complicated because neuropathy increased the risk of infection, endothelial dysfunction, and overall higher graft failure rate compared to other wound types and locations..[9-14,26,27] There are many investigators developing biological ulcer products with the goal of creating an ideal cost effective wound dressing that when applied to wounds will assist with healing without the complexities of surgical intervention.[26,28] In a small pilot study, AHSC applied just once to DFUs in the outpatient setting was able to close 10/11 (91%) of index ulcers by 12 weeks.[24] In our current study, we analysed the outcome data of the initial 50 patients as part of a planned interim analysis of a larger, ongoing RCT. These data support that adjunctive AHSC appears to facilitate greater DFUs closure compared to standard of care alone. The AHSC 12-week closure rates were significantly superior to the controls (72% vs 32%, $P = .005$) and allow us to project statistical power for 100 subjects in this ongoing trial at 99%. The AHSC 12-week DFU closure rate of 72% in this interim analysis is a stark contrast to the mean closure rate reported in an analysis of 26 DFU RCTs, whereby only 38% of wounds healed at 12 weeks.[5] In our study, 92% of subjects required only 1 application of AHSC. Additionally, all harvest sites remained closed following primary closure at the time of harvest and the harvest procedure was tolerated well by all participants. The occurrence of AEs and SAEs was similar between the AHSC and control groups, and only 2 AEs were possibly related to the study product. Notably, in the AHSC group, there were no SAEs related to the index ulcer, whereas 2 subjects in the control group had 4 SAEs related to the index ulcer, including 1 subject that developed cellulitis followed by acute osteomyelitis requiring surgical incision and drainage. Statistical significance between groups for AEs and SAEs was not included in the interim analysis predefined statistical analysis plan, but the occurrence of more index ulcer-

FIGURE 1 Patient flow diagram. The superscript letter "a" indicates that when multiple exclusion criteria applied, a weighted figure was applied so that percentages for each criterion added up to 100%. AHSC, autologous homologous skin construct, DFU, diabetic foot ulcer; non-STEMI, non–ST-segment elevation myocardial infarction

TABLE 2 Patient demographics and medical history

Variable	AHSC group (n = 25)	Control group (n = 25)	*P*
Patient age (years)	61.6 (10.3)	59.3 (13.5)	.51
BMI	32.3 (7.6)	33.4 (7.5)	.59
Sex			
Male	18 (72)	17 (68)	.76
Female	7 (28)	8 (32)	
No. of comorbidities	9.6 (3.3)	10.8 (6.2)	.40
Creatinine	1.4 (0.6)	1.3 (0.5)	.37
HbA1c			
Baseline	7.1 (1.4)	7.7 (1.7)	.16
End of study	7.1 (1.6)[a]	8.0 (1.3)[b]	.059
Wound area (cm^2)	4.3 (4.2); median: 3.6; IQR: 3.2	3.3 (4.3); median: 1.8; IQR: 1.4	.19
Wound age (weeks)	25.3 (31.4); median: 15.3; IQR: 19	22.1 (22.6); median: 14.0; IQR: 20	.57
DFU location			
Plantar	21 (84)	21 (84)	1.00
Dorsal	4 (16)	4 (16)	
DFU location			
Toe	4 (16)	5 (20)	
Forefoot	10 (40)	13 (52)	.16
Midfoot	9 (38)	2 (8)	
Heel	2 (8)	4 (16)	
Ankle	0 (0)	1 (4)	
No. of debridements prior to enrolment	9.0 (3.8); median: 9; IQR: 6	10.6 (4); median: 10; IQR: 8	.17
Frequency of comorbidities			
Hypertension	23 (92)	22 (88)	.64
Peripheral arterial/vascular disease	3 (12)	4 (16)	1.00
Heart disease (any type)	3 (12)	6 (24)	.46
Gastroesophageal reflux disease	6 (24)	3 (12)	.46
Hyperlipidaemia	15 (60)	14 (56)	.77
Renal disease	3 (12)	3 (12)	1.00
Venous insufficiency	3 (12)	1 (4)	.61
Prior lower extremity amputation (any kind)	10 (40)	10 (40)	1.00
Mental disorder (any)	7(28)	10 (40)	.37
Treatments up to 1 year prior			
Debridements	14 (56)	16 (64)	.56
Wraps or offloading	12 (48)	10 (40)	.57
Negative pressure wound therapy	0 (0)	2 (8)	.49
Cellular and/or tissue-based product	1 (4)	2 (8)	.55
Collagen or oxidised regenerated cellulose	8 (320	6 (24)	.53
Antibacterial dressing	4 (16)	3 (12)	.68
Nonactive dressing	8 (32)	14 (56)	.087
Antibiotics (any route)	1 (4)	8 (32)	.023

Note: Continuous variables are reported as means (SD) and categorical variables as counts (percentage).

Abbreviations: AHSC, autologous homologous skin construct; BMI, body mass index; DFU, diabetic foot ulcer; IQR, interquartile range.

[a]n = 22.

[b]n = 19.

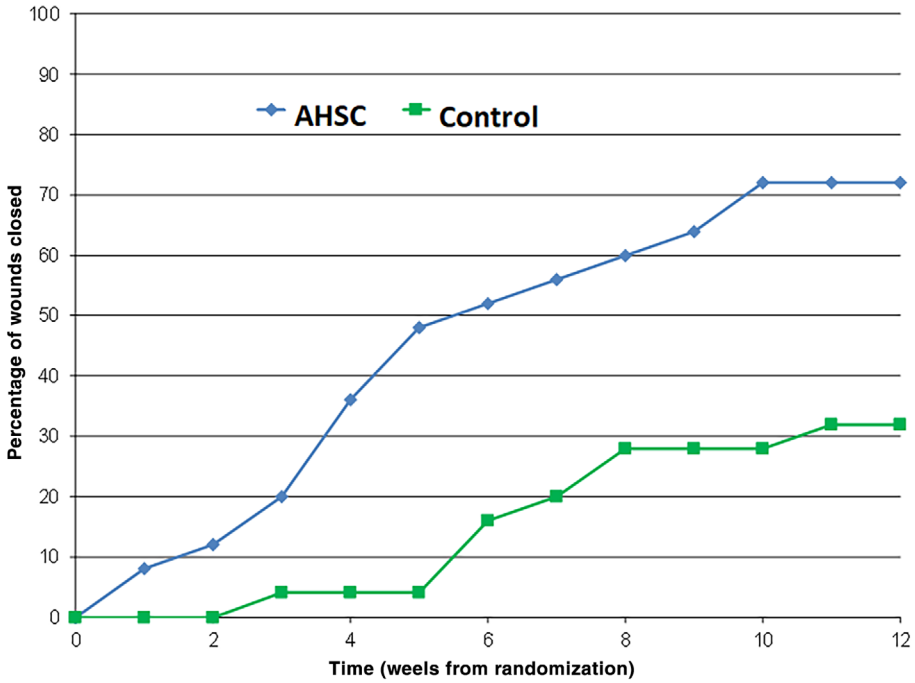

FIGURE 2 Weekly closure rates. AHSC, autologous homologous skin construct

TABLE 3 Mean (SD) percentage area reduction at weeks 4, 6, 8, and 12

Week	AHSC group	Control group
4	78.6 (35.6)	24.0 (106.5)
6	83.2 (40.9)	43.8 (102)
8	86.6 (39.6)	47.2 (89.9)
12	88.2 (39.1)	49.6 (101.4)

related SAEs and index ulcer infections in the control group suggests that earlier wound closure and a higher rate of wound closure with AHSC adjunctive treatment may avoid wound-related complications.

The manufacturing process of the AHSC retains the endogenous regenerative cellular populations associated with wound healing present within hair follicles, glands, and the interfollicular epidermis, facilitating engraftment optimisation and wound closure.[24] The resulting

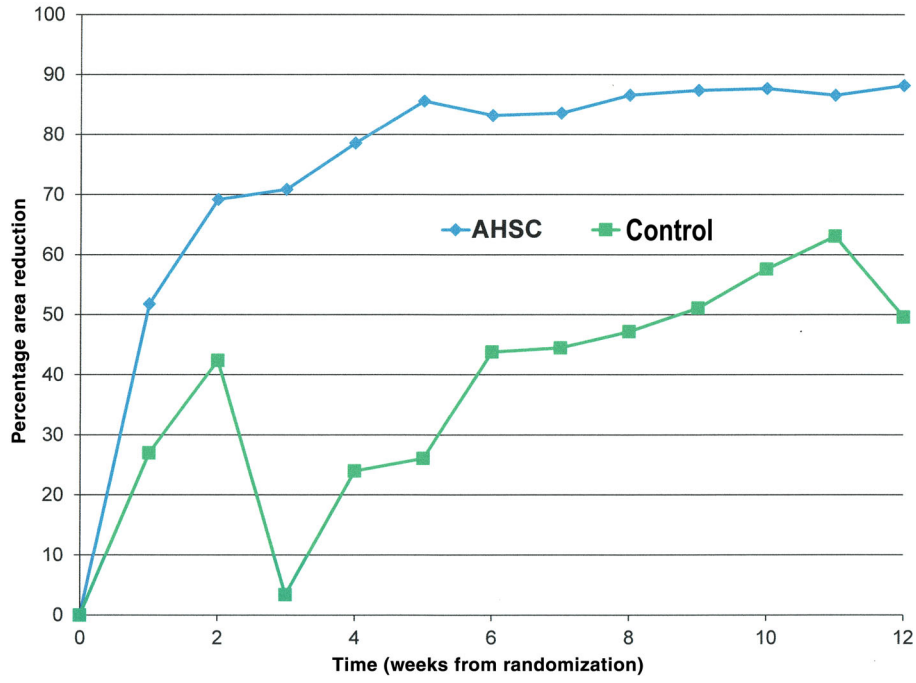

FIGURE 3 Weekly percentage area reduction values. AHSC, autologous homologous skin construct

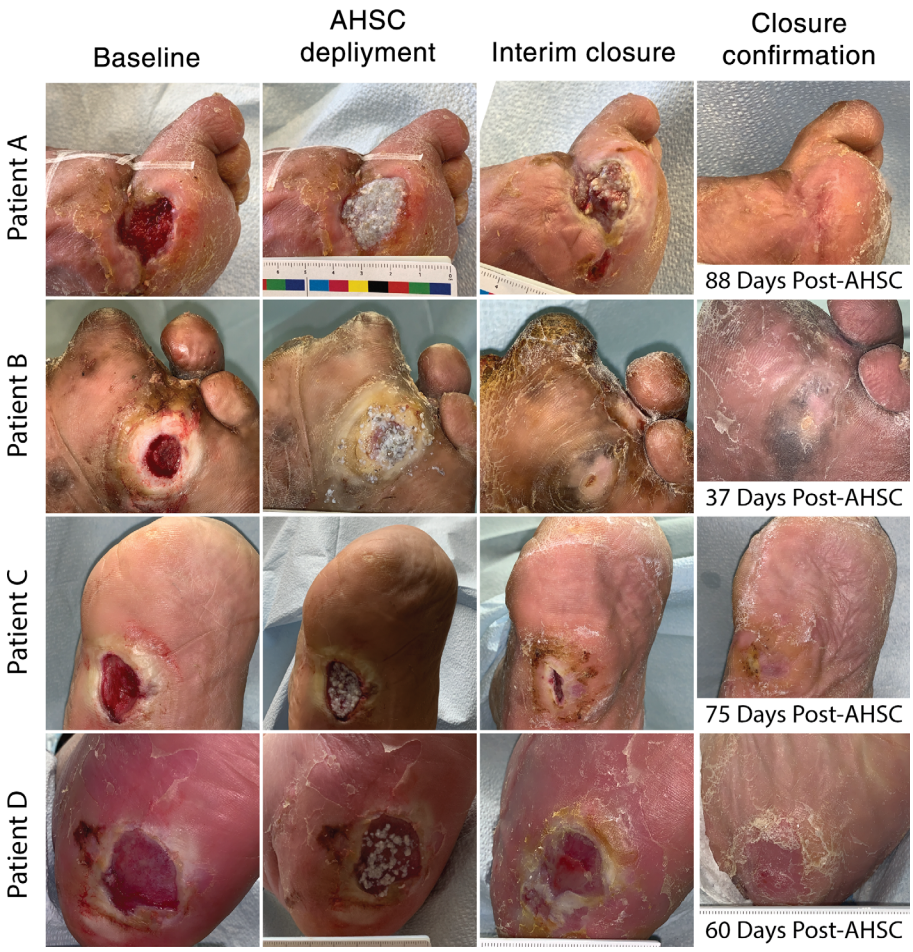

FIGURE 4 Representative images of AHSC-treated patients, at the time of randomisation (baseline), AHSC deployment, during follow-up (interim closure), and at closure confirmation visit. AHSC, autologous homologous skin construct

construct has a high surface area-to-volume ratio, facilitating cellular sustenance from plasmatic imbibition in the DFU wound bed during the first 48 hours prior to inosculation and vascularisation.[24,29,30] Consequently, a single application of AHSC can quickly regenerate healthy tissue and close DFUs, which has significant cost implications (to be further explored in the final trial analysis).

There were no significant differences in W-QOL or the Semmes-Weinstein test between groups. This is notable as patients were required to undergo a small tissue harvest for the AHSC treatment. The harvest site procedure did not significantly negatively impact their W-QOL scores, which may have been balanced by faster wound closure with AHSC treatment. The lack of significant difference in the Semmes-Weinstein test may be due to the prevalence and severity of neuropathy present in both patient groups that cannot be corrected with topical treatments alone.

The results of this interim analysis are limited by the ongoing nature of the trial. However, the purpose of this interim analysis was to determine conditional statistical power for all study endpoints. A further study limitation is that there was no follow-up period after 12 weeks or following wound closure beyond 2 weeks. This RCT is also limited by its lack of blinding, which, given the intervention, was not possible. For blinding to have occurred, all patients would have had to undergo the harvest site procedure, which would not be ethically justified in the control group. However, wound closure was assessed in person by nontreating blinded study personnel and further confirmed by a blinded adjudication panel of three plastic surgeons using high-resolution digital photography.

This interim analysis of data from 50 patients enrolled in a larger, ongoing RCT demonstrated that a single, topical application of the AHSC facilitated DFU closure. The results of this analysis confirm our previous power analysis and are encouraging to complete the planned study.

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CONFLICT OF INTEREST

This study was funded through a research grant from PolarityTE; provided to the Professional Education and Research Institute (PERI), which Charles M Zelen, DPM, is medical director. David Armstrong, DPM, MD, PhD, received research funds from PERI to serve as Principal Investigator for this trial and to design and administrate

the trial and also assist with the writing and review of the manuscript. Dennis Orgill, MD, PhD, received research funds to serve as a validating/adjudicating plastic surgeon to review study photos and assist with the writing and review of the manuscript. Robert Galiano, MD, received research funds to serve as a validating/adjudicating plastic surgeon to review study photos and assist with the writing and review of the manuscript. Paul Glat, MD, received research funds to serve as a validating/adjudicating plastic surgeon to review study photos and assist with the writing and review of the manuscript. Lawrence Didomenico, DPM, received research funds and served a site investigator for this trial and assisted with the writing and review of the manuscript. Alexander Reyzelman, DPM, received research funds and served a site investigator for this trial and assisted with the writing and review of the manuscript. Robert Snyder, DPM, received research funds and served a site investigator for this trial and assisted with the writing and review of the manuscript. Marissa Carter, PhD, received research funds to provide the statistical analysis plan and provide the statistical analysis for this trial and assist with writing of the result section of the manuscript. William W Li, MD, received research funds to serve as the medical monitor and assisted with the writing and review of the manuscript. Charles M. Zelen, DPM, is the medical director of the PERI and his company received research funds to administrate the clinical trial and write the paper for publication. There are no other conflicts of interest with any of the authors in relationship to this study or with regard to PolarityTE. IRB conflict of interest statements are on file with PERI.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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