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A Multicenter, Randomized, Controlled, Clinical Trial Evaluating Dehydrated Human Amniotic Membrane in the Treatment of Venous Leg Ulcers

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Background: This randomized controlled trial evaluated the safety and effectiveness of weekly and biweekly applications of dehydrated human amnion and chorion allograft (dHACA) plus standard of care compared to standard of care alone on chronic venous leg ulcers.

Methods: This open-label randomized controlled trial included patients with chronic venous leg ulcers at eight wound care centers across the United States. The primary endpoint was the proportion of healed ulcers at 12 weeks. Secondary endpoints included the proportion of ulcers achieving 40 percent closure at 4 weeks and the incidence of adverse events.

Results: Among 101 patients screened for eligibility, 60 were eligible and enrolled. At 12 weeks, significantly more venous leg ulcers healed in the two dHACA-treated groups (75 percent) than in the standard-of-care group (30 percent) ($p = 0.001$) even after adjustment for wound area ($p = 0.002$), with an odds ratio of 8.7 (95 percent CI, 2.2 to 33.6). There were no significant differences in the proportion of wounds with percentage area reduction greater than or equal to 40 percent at 4 weeks among all groups. The adverse event rate was 63.5 percent. Among the 38 adverse events, none were graft or procedure related, and all were resolved with appropriate treatment.

Conclusions: dHACA and standard of care, either applied weekly or biweekly, significantly healed more venous leg ulcers than standard of care alone, suggesting that the use of aseptically processed dHACA is advantageous and a safe and effective treatment option in the healing of chronic venous leg ulcers. (*Plast. Reconstr. Surg.* 150: 1128, 2022.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, I.

Venous leg ulcers are the most common lower extremity ulceration, constituting 60 to 80 percent of all leg ulcers and bearing a tremendous annual cost of $14.9 billion.[1–7] They are caused by venous valve incompetence and calf muscle pump insufficiency, which result in venous hypertension and venous stasis, causing chronic venous insufficiency and tissue ischemia.[8,9] Unfortunately, many venous leg ulcers fail to heal following standard compression therapy, with as many as 50 percent remaining unhealed at 6 months and 20 percent at 2 years.[10–14]

The use of amniotic membrane to help heal chronic venous leg ulcers and other complex, recalcitrant wounds has increased greatly in recent years.[15] Amniotic membranes are structural extracellular

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matrices composed of collagen types IV, V, and VII and wound healing proteins and growth factors that support cellular signaling and migration, facilitating the angiogenesis and reepithelialization processes.[16] Human amniotic membrane has been used in wound care for 110 years, but its clinical use has increased over the past few decades because of improvements in donor screening, processing, and storage techniques to better ensure safety, preserve their biological activity, and allow for a longer shelf-life.[15–19] In particular, dehydrated and cryopreserved human amnion/chorion allografts have been shown to be safe and effective in randomized controlled trials evaluating their use on chronic venous leg ulcers and diabetic foot ulcers.[15–17,20–26] Unlike other commercially available dehydrated amnion and/or chorion allografts that undergo terminal sterilization, this dehydrated human amnion and chorion allograft (dHACA) studied in the current clinical trial is aseptically processed without terminal irradiation, which preserves the natural structure and biological activity.[17,27,28] The objective of this current randomized controlled trial was to evaluate the safety and effectiveness of dHACA plus standard of care compared to standard of care alone in chronic venous leg ulcers.

PATIENTS AND METHODS

Study Design and Population

This multicenter prospective, institutional review board–approved, registered, open-label randomized controlled trial that evaluated weekly and biweekly (every 2 weeks) applications of dHACA with standard of care compared to standard of care alone on patients with chronic venous leg ulcers was performed at eight wound care centers in the United States. (**See Figure, Supplemental Digital Content 1**, which shows the patient flow diagram. *dHACA*, dehydrated human amnion and chorion allograft; *SOC*, standard of care; *AE/SAE*, adverse event/serious adverse event, ***http://links.lww.com/PRS/F437***.) MTF Biologics (Edison, N.J.) provided a research grant to perform this study and donated the allograft to complete the trial. The Western Institutional Review Board approved this study protocol (20151263). The design to study both weekly and biweekly applications stemmed from the focus of providing the most cost-efficient treatment to patients while providing a safe and efficacious option.

The primary study hypothesis was that the healing rate at 12 weeks of dHACA (weekly or biweekly) application plus standard of care versus standard of care alone was equal for both groups. The primary endpoint was the proportion of ulcers achieving complete closure (defined as macroscopic wound closure at 12 weeks) using the Silhouette three-dimensional laser camera system by Aranz Medical (Christchurch, New Zealand). Secondary endpoints included the proportion of ulcers achieving 40 percent area reduction and the incidence of adverse events.

A two-side log-rank test with an overall sample size of 60 subjects (20 in the control group and 40 in the treatment group) achieved 90.6 percent power at a 0.05 significance level to detect a hazard ratio of 3.15 when the proportion surviving in the control group was 0.6. Applying a study duration of 12 weeks, the proportion dropping out of the control group was 0.004 per week, with none dropping out of the treatment group. The proportion switching from the control group to another group with a hazard ratio equal to that of the treatment group was 0. The proportion switching from the treatment group to another group with a hazard ratio equal to that of the control group was 0.

Study Product

The dHACA (AmnioBand Membrane; MTF Biologics) is aseptically processed amnion and chorion donated human tissue that is regulated for use under the U.S. Food and Drug Administration HCT/P, 21 CFR 1271 regulations on homologous use of human tissue and is clinically intended for use as a wound covering in the treatment of diabetic wounds, burns, traumatic wounds, and venous ulcers. (AmnioBand Membrane is a minimally processed human placental allograft containing the amnion and chorion layers and is aseptically processed without terminal irradiation for sterilization, which retains the structural properties of the extracellular matrix. During tissue processing and packaging, this allograft was tested and complied

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with the requirements of U.S. Pharmacopeia <71> Sterility Tests. The resulting dehydrated allograft serves as a wound covering). The placenta with amnion and chorion is generously donated by mothers having full-term, healthy births. Placental donors are rigorously screened before and after their donation through close review of the mother's medical record and personal history to prevent the transmission of infectious diseases to the recipients of the dHACA. All donor procurement and screening comply with the requirements of the U.S. Food and Drug Administration and the American Association of Tissue Banks in addition to even more restrictive criteria used by MTF Biologics. The amnion and chorion human allograft membranes from each donated placenta are minimally processed to retain the structural properties of the extracellular matrix and undergo aseptic processing with chemical disinfection by means of peracetic acid plus ethanol solution, without terminal sterilization, and complies with the requirements of U.S. Pharmacopeia <71>. The dHACA is dehydrated and packaged/sealed into an inner pouch, with a 3-year shelf-life. It is available as a single-use, dry disk or sheet in multiple sizes to ensure optimal sizing to cover a variety of wound sizes.

Screening and Randomization

At the screening visit, patients were screened for eligibility to participate in the study based on the complete inclusion and exclusion criteria listed in Table 1. A physical examination was performed; patient demographics, medical and ulcer history, medications, vital signs, and ulcer assessment using the Silhouette three-dimensional laser camera system by Aranz were recorded for accuracy and consistency in wound measurement; an ankle-brachial index measurement or arterial Doppler study was performed; and the ulcer was débrided, as necessary, and treated with compression therapy. [**See Video 1 (online)**, which illustrates proper surgical débridement of a venous leg wound.] Before study enrollment, eligible patients who provided their written informed consent had to have undergone 14 days of standard compression therapy.

Once the subjects were evaluated and all inclusion and exclusion criteria were confirmed, they were then randomized to one of three study groups: standard of care alone (control), weekly dHACA plus standard of care, or biweekly dHACA plus standard of care. Envelopes were created in blocks of 12 with four patients in each allocation placed in blank envelopes and randomly labeled by separate study staff to ensure a true blinded randomization. This process was repeated five times and envelopes were distributed to the respective sites.

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria
• At least 18 yr old
• ABI >0.75 or SPP >30 mmHg or TCOM >30 mmHg
• VLU extending through the full thickness of the skin but not down to muscle, tendon, or bone
• Only one VLU (the largest) included in the study and was 2 cm apart from other ulcers on the same leg
• Study ulcer had duration of greater than 1 mo before initial screening visit, failed prior standard of care for greater than 1-mo duration, and did not undergo 12 mo of continuous high-strength compression therapy
• Wound area ≥2 cm^2 but <20 cm^2 at randomization visit
• Study ulcer treated with compression therapy for at least 14 days before randomization
• Study ulcer has a clean, granulating base with minimal adherent slough
• Women of childbearing potential were willing to use contraception
• Understood and willing to participate in the trial and could comply with weekly visits and the follow-up regimen
• Read and signed the informed consent form
Exclusion criteria
• Ulcer caused by a medical condition other than venous insufficiency
• Ulcer exhibited signs of infection
• Known allergy to components of or intolerance to multilayer compression therapy
• Ulcer was suspicious for cancer
• History of more than 2 wk treatment with immunosuppressants, cytotoxic chemotherapy, topical steroids applied to study ulcer within 1 mo before initial screening, or during the screening period, or anticipate such medication during study
• On investigational drug(s) or therapeutic device(s) within 30 days of screening
• Ulcer improved >30% during the screening phase if the subject was not in adequate compression 14 days before screening
• Study ulcer was previously treated with CTPs within the past 30 days
• Study ulcer required NPWT or HBOT oxygen therapy during the trial
• Had one or more current medical conditions that, in the opinion of the investigator, made the subject an inappropriate candidate for the study, or had a known history of poor adherence with medical treatment
• Study ulcer was previously treated with CTPs within the past 30 days
• History of HIV/AIDS
• History of drug or alcohol abuse
• History of radiation therapy at the ulcer site
• Ulcers on the dorsum of the foot or with ≥50% of the ulcer below the malleolus
• Pregnant or breastfeeding
• Had diabetes with HbA1c >12.0 within past 90 days
• Had renal dysfunction with serum creatinine levels ≥3.0 mg/dl within the past 90 days
• Used tobacco within the past 30 days
• History of liver disease with active cirrhosis

ABI, ankle brachial index; SPP, skin perfusion pressure; TCOM, transcutaneous oximetry measurement; VLU, venous leg ulcer; CTPs, cellular and/or tissue-based products; NPWT negative-pressure wound therapy; HBOT, hyperbaric oxygen therapy; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; HbA1c, hemoglobin A1c.

Treatment

The investigator applied the dHACA to the study ulcer to subjects allocated to the weekly or biweekly applications of dHACA. [**See Video 2 (online)**, which illustrates proper application of dehydrated amnion and chorion membrane graft.] At each weekly treatment visit regardless of the subject's treatment allocation, the provider assessed each patient and the integrity of the compression bandaging, updated patient medical and medication history, recorded any adverse events or serious adverse events that had occurred, and assessed the ulcer for infection and closure. All subjects underwent standard of care at each weekly treatment visit, which involved cleaning and débriding the study ulcer, as appropriate, applying multilayer compression bandaging to the study ulcer, and instructing the subjects to elevate the study ulcer limb as much as possible while keeping the bandaging dry. For the biweekly group, dHACA was applied only every other visit with a 2-week interval. The steps specifically for dressing application include application of a wound-specific size of dHACA directly over the ulcer for those randomized to weekly or biweekly application, followed by placement of a nonadherent dressing, and then a multilayer compression bandage. [**See Video 3 (online)**, which illustrates proper compression dressing application for a venous leg wound.] Ulcers were digitally photographed to measure wound area after débridement. If the ulcer was observed to be healed, the subjects returned 2 weeks later for a healing confirmation visit, during which the provider assessed vital signs, took a digital photograph of the ulcer site, and confirmed closure. Each wound that was deemed closed by the investigator subsequently underwent a blinded validation by a team of three plastic surgeons, who confirmed closure. The redacted photographs, which were blinded, removing all patient information, site information, and the allocation group, were provided separately to three plastic surgeons, to adjudicate whether the wound was healed. At least two-thirds of plastic surgeons had to deem the wounds as closed during the blinded validation process for the wound to be considered closed.

Subject Withdrawal

A subject could have withdrawn from the study at any time. The investigator could have discontinued the subject if it was medically necessary (such as in the case of an adverse event or serious adverse event that precluded further study treatments) or because the subject did not adhere to the protocol; became pregnant; had revascularization surgery on the study ulcer limb; the study ulcer had exposed bone, tendon, or fascia; multilayer compression was not possible; two separate infections with no treatment response occurred; or the study ulcer merged with an adjacent ulcer. Treatment of a subject with a prohibited medication could also have resulted in study withdrawal.

Data Collection and Statistical Analysis

All sites were monitored for appropriate data completion on the case report form and for good clinical practices. Data were then compiled using a Health Insurance Portability and Accountability Act–compliant Smart Sheet Database. An independent statistician (Strategic Solutions, Inc., Bozeman, Mont.) analyzed the data using SPSS Version 26 (IBM Corp., Armonk, N.Y.).

All statistical testing was two-sided and performed using a significance level of 0.05. Descriptive statistical methods summarized continuous and categorical data. The intent-to-treat population included all subjects who were randomized. The safety population included all subjects who received at least one dHACA treatment or, in the standard-of-care group, completed one visit after randomization. All analyses used the intent-to-treat approach.

To test for statistical differences between study groups at baseline, for categorical variables, chi-square or Fisher exact tests were performed, and for continuous variables, one-way analysis of variance (after contrast: Tukey honestly significant difference if homogeneity of variances was met; Games-Howell if it was not met) was used. Statistical testing of proportion of ulcers healed between groups (primary endpoint) used the chi-square test. Results were adjusted using logistic regression, using whether the wound healed or not after 12 weeks of treatment as the dependent variable, and adjusted for the available independent variables.

Testing of secondary endpoints was carried out if the primary endpoint was statistically significant. The percentage area reduction at 4 or 12 weeks was calculated as $[(A_I - A_{XW})/A_I] * 100$, where A_I was the area of the index wound at randomization and A_{XW} was the area at 4 or 12 weeks.

The proportion of wounds achieving greater than or equal to 40 percent closure was analyzed using the chi-square test at 4 weeks, and the percentage area reduction at 12 weeks was analyzed using the Mann-Whitney test. The proportion of wounds healed at 12 weeks (dHACA groups versus control) was analyzed using chi-square and adjusted using logistic regression, using whether

or not the wound healed after 12 weeks of treatment as the dependent variable, and adjusted for the available independent variables.

The step-down Holm procedure was used for multiplicity adjustment of the secondary endpoints. Missing data were imputed as appropriate, and subjects if lost to follow-up were included in the intent-to-treat analysis using the last observation carried forward principles to impute missing data.

All adverse events were classified using the Medical Dictionary for Regulatory Activities and system organ classification, and their relationship to the study product/procedure was also identified (not related, possibly related, probably related, definitely related, or unknown). Serious adverse events were defined as any adverse event that resulted in death, was life-threatening, required hospitalization or prolonged existing hospitalization, resulted in persistent or significant disability/incapacity, was a congenital anomaly/birth defect, or was considered a serious adverse device effect that jeopardized the subject or required medical or surgical intervention.

RESULTS

This study took place from November of 2015 through January of 2019. There were 101 patients screened for study eligibility; 41 subjects were ineligible to participate. Sixty subjects (53.3 percent male) were enrolled into the trial, with 20 subjects randomized to each group. Table 2 summarizes the patient and wound characteristics. There were no significant differences in baseline variables between groups. However, there was a numerically larger difference in initial wound area in the biweekly dHACA group compared to the other groups, and the wound duration for the standard-of-care group was also somewhat higher than in the other groups. All 60 subjects received the allocated intervention, and none were lost to follow-up or exited because of protocol deviation.

At 12 weeks, a significantly greater number of venous leg ulcers healed in the two groups treated with dHACA [30 of 40 (75 percent)] than in the standard-of-care group [six of 20 (30 percent)] ($p = 0.001$). Six venous leg ulcers (30 percent) healed in the standard-of-care group at 12 weeks compared to 15 venous leg ulcers (75 percent) in the weekly dHACA group ($p = 0.02$) and 15 venous leg ulcers (75 percent) in the biweekly dHACA group ($p = 0.02$) (Fig. 1). The final logistic regression model for up to 12 weeks of treatment included only treatment (standard of care or dHACA) and initial wound area as main effects (for wound area, B = –0.26; $p = 0.005$; OR, 0.77; 95 percent CI, 0.64 to 0.92). Pearson goodness of fit was nonsignificant ($p = 0.38$), Nagelkerke pseudo R-squared was 0.40, and the C statistic was 0.834. Dispersion calculated from deviance and degree of freedom was 1.13. Treatment with dHACA remained statistically significant after adjustment for wound area ($p = 0.002$; OR, 8.7; 95 percent CI, 2.2 to 33.6).

Thirteen venous leg ulcers (65 percent) in the standard of care group had a percentage area reduction greater than or equal to 40 percent at 4 weeks, compared to 16 (80 percent) venous leg ulcers in the weekly dHACA group and 14 (70 percent) venous leg ulcers in the biweekly dHACA group. There were no significant differences in the proportion of wounds with percentage area reduction 40 percent at 4 weeks between all three study groups. At 12 weeks, the standard-of-care group had a median percentage area reduction of 75 percent (interquartile range, 68.7 percent), whereas the dHACA groups had a significantly higher median of 100 percent (interquartile range, 5.3 percent) ($p = 0.012$). A graph of weekly percentage area reduction by study group is shown in Figure 2.

Thirty-eight adverse events occurred during the study, including nine serious adverse events (Table 3). The adverse event incidence rate was 63.5 percent, and the serious adverse event incidence rate was 15 percent. The numbers of adverse events and serious adverse events and

Table 2. Patient and Wound Characteristics

Variable	SOC Group (%)	Weekly dHACA Group (%)	Biweekly dHACA Group (%)
No.	20	20	20
Sex			
Male	13 (65)	10 (50)	9 (45)
Female	7 (35)	10 (50)	11 (55)
Mean age ± SD, yr	70.0 ± 12	70.0 ± 15.6	69.1 ± 12.9
Mean BMI ± SD, kg/m^2	39.2 ± 9.6	37.4 ± 12.7	37.8 ± 10.5
Mean wound duration ± SD, wk	33.0 ± 41.2	22.5 ± 29.8	24.8 ± 37
Mean wound area ± SD, cm^2	6.0 ± 4.1	6.0 ± 4.2	4.7 ± 2.9

SOC, standard of care; dHACA, dehydrated human amnion and chorion allograft; BMI, body mass index.

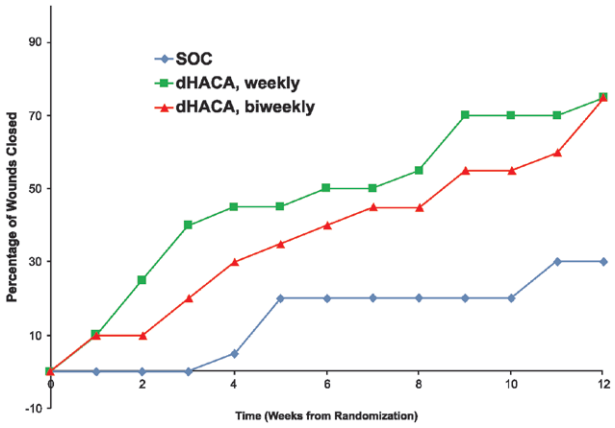

Fig. 1. Graph of weekly wound healing (percentage of wounds healed) for dehydrated human amnion and chorion allograft (*dHACA*) plus standard of care (*SOC*) and standard of care alone.

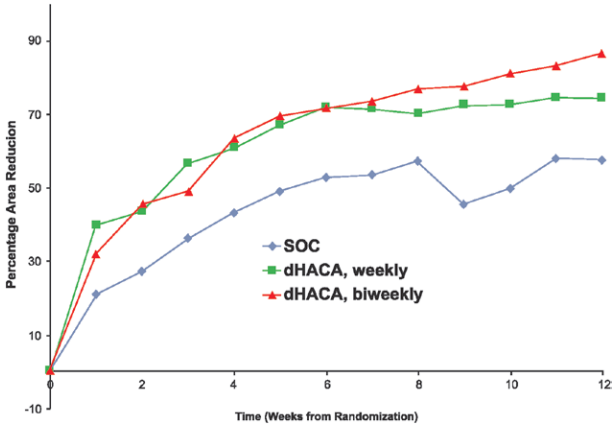

Fig. 2. Graph of weekly percentage area reduction for dehydrated human amnion and chorion allograft (*dHACA*) plus standard of care (*SOC*) and standard of care alone.

their incidence across study groups were similar, although the standard-of-care group had the most adverse events (n = 15; incidence, 75 percent) (Table 3). The most common types of adverse events were wound-related infections [14 of 38 (36.7 percent)], followed by the occurrence of a new ulcer [12 of 38 (31.6 percent)]. None of the events was related to the study allograft or procedure. All adverse events and serious adverse events were resolved with appropriate treatment, and there were no amputations or deaths within any of the groups. An example of a weekly and biweekly application of the dHACA treatment and a standard-of-care patient who did not heal is demonstrated with the following cases and their respective histories. [**See Figure, Supplemental Digital Content 2**, which shows the case of weekly application of dHACA. (*Left*) A 55-year-old White woman (5 feet 6 inches; 310 lb) with a venous leg ulcer for 22 months on right lower leg, anterior shin, at the start of the study (time 0). (*Center*) After the third weekly application, percentage area reduction is 69 percent (time 3 weeks). (*Above, right*) Complete healing was achieved after application of eight weekly grafts (time 8 weeks), ***http://links.lww.com/PRS/F438***. **See Figure, Supplemental Digital Content 3**, which shows the case of biweekly application (every

Table 3. Summary of Adverse Events

Adverse Event	SAE	SOC Group	Weekly dHACA Group	Biweekly dHACA Group	Total No. of Events
New ulcer(s) on study leg	0	4	0	4	8
New ulcer(s) on nonstudy leg	0	1	0	3	4
Infection of study ulcer	0	3	1	0	4
Infection of study leg but not study ulcer					
Requiring hospitalization	2	1		1	2
Not requiring hospitalization			2		2
Infection on nonstudy leg					
Requiring hospitalization	2	1		1	2
Not requiring hospitalization			3		3
Acute increase in edema	0	0	0	1	1
Pruritus of study leg	0	0	1	0	1
Rash to both legs	0	1	0	0	1
Sinus/respiratory infection	0	0	1	0	1
Urinary tract infection/bladder infection	0	1	0	1	2
Chest rash	0	0	1	0	1
Patient fell with laceration on arm, resulting in hospitalization	1	0	0	1	1
Patient fell and hit head	0	1	0	0	1
Pneumonia, resulting in hospitalization	2	1	1	0	2
Cancer, resulting in hospitalization	1	1	0	0	1
Gangrene to toe with osteomyelitis and infection with amputation	1	0	1	0	1
Incidence rate	9/60 (15)	15/20 (75)	11/20 (55)	12/20 (60)	38/60 (63.5)

SAE, serious adverse event; SOC, standard of care; dHACA, dehydrated human amnion and chorion allograft.

2 weeks) of dHACA. (*Left*) A 63-year-old White man (5 feet 6 inches; 220 lb), with a venous leg ulcer open for 22 weeks on the left lateral leg at the start of the study. (*Center*) After application of three biweekly grafts (time 6 weeks). (*Above, right*) Complete healing after 5 biweekly grafts applied (time 10 weeks), ***http://links.lww.com/PRS/F439***. **See Figure, Supplemental Digital Content 4**, which shows the case of standard of care. (*Left*) A 60-year-old White woman (5 feet 3 inches; 201 lb), with a venous leg ulcer open for 18 weeks on the left lateral leg at study start. (*Center*) After 4 weeks of standard-of-care treatment. (*Right*) After 12 weeks of standard-of-care treatment, exiting unhealed at week 13. *PAR*, percentage area reduction; *SOC*, standard of care; *L/W*, length/width, ***http://links.lww.com/PRS/F440***.]

DISCUSSION

The incidence and therapeutic modalities surrounding the treatment of venous leg ulcers is not optimal, with as many as 50 percent remaining unhealed at 6 months, 20 percent remaining unhealed at 2 years,[10–14] and an overall cost of $14.9 billion annually.[1–7] There have been very few peer-reviewed published multicenter, prospective, randomized, controlled trials regarding the use of what are commonly referred to as "skin substitutes" in their treatment.

A 1998 randomized controlled trial by Falanga et al.[10] demonstrated that the treatment of venous leg ulcers with an allogenic human skin equivalent and compression therapy healed 63 percent of the ulcers by 6 months versus 49 percent who were treated with compression therapy alone ($p = 0.02$). A 2005 randomized controlled trial by Mostow et al.[29] found that, after 12 weeks, the use of porcine small-intestine submucosa healed 55 percent of the venous leg ulcers treated with small-intestine submucosa and compression therapy compared to 34 percent of those treated with compression therapy alone ($p = 0.0196$). A 2018 randomized controlled trial by Bianchi et al.[21] found that weekly applications of a terminally sterilized dehydrated human amnion/chorion membrane (dHACA) and compression therapy healed 60 percent of venous leg ulcers versus 35 percent treated with compression therapy alone at 12 weeks ($p = 0.0128$). In this randomized controlled trial, the adjunctive use of dHACA, regardless of treatment frequency (weekly versus biweekly applications), resulted in a statistically significant healing rate at 12 weeks that was more than double the healing rate achieved with standard of care alone (75 percent versus 30 percent; $p = 0.001$).

Most commercially available dehydrated amniotic allografts undergo terminal sterilization processes (such as gamma irradiation or e-beam), which have been reported to disrupt the collagen fibers and basement membrane and result in loss of structural integrity and fragmentation in the amniotic membrane and disintegration of epithelial basement membrane.[30–33] There is extensive literature that amniotic membranes contain extracellular matrices (collagens, fibronectin, hyaluronic acid) and growth factors that support wound healing activities such as reducing inflammation, cell recruitment, granulation, angiogenesis, and epithelization.[34] Therefore, the choice of amniotic tissue processing is important for preserving tissue quality and maintaining tissue integrity and the inherent biological properties compared to the negative impact that arise from terminal sterilization.[32,33] The clinical efficacy of aseptically processed dHACA has been reported in diabetic foot ulcer studies. DiDomenico et al. showed improved healing rates in chronic diabetic foot ulcers at 12 weeks (85 percent dHACA plus standard of care versus 33 percent for standard of care alone), whereas Glat et al. reported a 90 percent healing rate with dHACA versus 40 percent for tissue-engineered skin substitute at 12 weeks.[17,35]

A major strength of this study was the robust statistical analysis applied, including multiplicity adjustment of secondary endpoints. In addition, another strength is the low attrition rate. Historically, the number of adverse events is rather large in any venous leg trial, including our current study, with a rate of 63.5 percent. However, even with a high-risk population and a large number of adverse events, few patients had to exit the clinical trial because of these events and there was no incident of leg amputation or death in this clinical trial. All adverse events were followed to resolution. The main limitation of this study was that blinding of patients and investigators was not possible because of the treatment regimens used in both groups.

An important finding in this study demonstrated that there were no differences in outcomes in terms of weekly versus biweekly application of dHACA. This highlights the flexibility that the wound care specialist has in applying dHACA. Therefore, based on the findings of this trial, clinicians, surgeons, and wound care stakeholders may find that applying dHACA biweekly in venous leg ulcer wounds will allow for fewer graft applications

without affecting the number of patients who progress to complete epithelialization, an important element in a value-based health care delivery model.

CONCLUSIONS

This randomized controlled trial evaluated the application of an aseptically processed dHACA for the adjunctive treatment of chronic moderate sized venous leg ulcers and demonstrated that dHACA, regardless of treatment frequency, was significantly more effective and safer compared to standard of care alone. The results of this trial show that dHACA should be considered as an adjunct to standard of care of nonhealing venous leg ulcers.

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APPENDIX

The authors have the following to disclose: Thomas E. Serena, M.D., is president and chief executive officer of Serena Groups and has received research funds from MTF Biologics to complete this trial, serve as principal investigator, and assist in manuscript preparation. Dennis P Orgill, M.D., Ph.D., is a consultant for PERI and MTF Biologics and has received funds from PERI to assist in completion of this study and of the manuscript. David G Armstrong, D.P.M., M.D., Ph.D., through the Department of Surgery, University of Southern California, has received research funds from PERI provided by MTF Biologics to serve as investigator and assist in completion of this study and manuscript. Robert Galiano, M.D., is a consultant for PERI and MTF Biologics and has received funds from PERI to assist in completion of this study and of the manuscript. Paul M Glat, M.D., owns and is the medical director of Paul Glat, PC, and has received research funds from PERI provided by MTF Biologics to assist in completion of this study and manuscript. Marissa J. Carter, Ph.D., M.A., is a consultant for PERI and MTF Biologics and has received funds from PERI to assist in completion of this study and perform the statistical analysis as well as assist in the completion of the manuscript. Jarrod Kaufman, M.D., is a consultant for PERI and MTF Biologics and has received funds from PERI to assist in completion of this study and of the manuscript. William Li, M.D., is a consultant for PERI and MTF Biologics and has received funds from PERI to assist in completion of this study and of the manuscript. Charles M. Zelen, D.P.M., is medical director and chief executive officer of the Professional Education and Research Institute (PERI), which has received research funds from MTF Biologics to conduct this study and assist in the completion of the manuscript.

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