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

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Background: Standard care for venous leg ulcers (VLUs) has remained unchanged over several decades despite high rates of initial treatment failure and ulcer recurrence.

Objective: We sought to evaluate the efficacy, safety, and tolerability of an advanced, poly-N-acetyl glucosamine (pGlcNAc), nanofiber-derived, wound-healing technology among patients with VLUs (Talymed, Marine Polymer Technologies Inc, Danvers, MA).

Methods: In this randomized, investigator-blinded, parallel-group, controlled study, eligible patients were randomized to treatment with standard care plus pGlcNAc (applied only once, every other week, or every 3 weeks) or to standard care alone. The primary end point was the proportion of patients with complete wound healing at week 20 in the intent-to-treat population (all randomized subjects), with last observation carried forward.

Results: Among 82 randomized patients, 71 completed the study with 7 lost to follow-up and 4 discontinued because of systemic infection. There were no significant group differences with regard to baseline demographic, illness, and VLU characteristics. At 20 weeks, the proportion of patients with completely healed VLUs was 45.0% (n = 9 of 20), 86.4% (n = 19 of 22), and 65.0% (n = 13 of 20) for groups receiving standard care plus pGlcNAc only once, every other week, and every 3 weeks, respectively, versus 45.0% (n = 9 of 20) for those receiving standard care alone (\(P < .01\) for pGlcNAc every other week vs standard care). The novel pGlcNAc advanced wound-healing technology was well tolerated and safe.

Limitations: Limitations were small sample size and patients unblinded to treatment allocation.

Conclusion: These pilot study results suggest that the pGlcNAc advanced wound-healing technology is well tolerated and effective. (J Am Acad Dermatol 2012;66:e209-15.)

Key words: membrane; poly-N-acetyl glucosamine; therapy; venous leg ulcer; wound healing.

Chronic venous insufficiency of the lower extremity affects an estimated 2.5 million adults in the United States,1 and underlies the onset of approximately 600,000 new cases of venous leg ulcers (VLUs) each year.2 At an estimated annual direct cost of $3 billion,3 VLUs are associated with chronic venous insufficiency of the lower extremity.

Abbreviations used:
FDA: Food and Drug Administration
pGlcNAc: poly-N-acetyl glucosamine
VLU: venous leg ulcer

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Supported by Marine Polymer Technologies Inc.
Drs Kelechi, Mueller, and Bonham have received grant support from, and Drs Hankin, Bronstone, and Samies are consultants to, Marine Polymer Technologies Inc.
Accepted for publication January 29, 2011.

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Published online May 27, 2011.
0190-9622/$36.00
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doi:10.1016/j.jaad.2011.01.031
with substantial morbidity and decrements in quality of life. The current standard of care for VLUs includes cleaning, debridement, application of low-adherent dressings that promote moist wound healing and absorb exudate, multilayer elastic high compression bandaging, leg elevation, and lower extremity exercise. When treated with the current standard of care, about 30% of VLUs remain unhealed at 6 months. Even if healed, approximately 26% to 34% of VLUs recur within 1 year and 56% within 4 years. Given these results, there is a compelling need for new therapies that offer improved efficacy for the management of VLUs.

A new polymer, poly-N-acetyl glucosamine (pGlcNAc), nanofiber-derived, wound-healing technology (Talymed, Marine Polymer Technologies Inc, Danvers, MA) has been shown to initiate the wound-healing process in animal studies. Research in leptin receptor minus mice (db/db diabetic mice) demonstrates that the application of pGlcNAc to full-thickness wounds significantly increases the rate of wound healing compared with control materials. Increased wound-healing rates may occur in response to enhanced angiogenesis and granulation tissue formation, in concert with rapid epithelialization. We describe a randomized, investigator-blinded, parallel-group, controlled pilot study to investigate the tolerability, safety, and efficacy of pGlcNAc advanced wound technology in patients with VLUs.

**METHODS**

**pGlcNAc nanofibers and membranes**

pGlcNAc is a wafer-thin matrix consisting of nanofibers approved by the US Food and Drug Administration (FDA) for the management of wounds, including diabetic ulcers, venous ulcers, pressure ulcers, ulcers of mixed vascular causes, partial- and full-thickness wounds, second-degree burns, surgical wounds, and other bleeding surface wounds and abrasions. Native diatom-derived pGlcNAc fibers have average length dimensions of approximately 80 to 100 μm and mean molecular weight of the internal polysaccharide of approximately 3,000,000 Da. Gamma radiation of pGlcNAc fibers results in shorter length, biodegradable pGlcNAc nanofibers with an average length of 4 to 7 μm, width of 100 to 150 nm, and thickness of 40 to 60 nm, as measured by electron microscopy. Although the average molecular weight of the pGlcNAc nanofiber-containing polymers is decreased to approximately 60,000 Da, the 3-dimensional semi-crystalline structure is maintained. pGlcNAc is provided in a sterile peel-back container.

**CAPSULE SUMMARY**

- Standard care for venous leg ulcers has remained largely unchanged over the past 4 decades, yet is often ineffective.
- This randomized, investigator-blinded, parallel-group, controlled pilot study involving patients with venous leg ulcers unhealed after a mean 3 months showed superior healing rates among patients receiving standard care plus a novel wound-healing technology applied every other week, compared with standard care alone, at 20 weeks.
- Results warrant larger clinical trials of this technology.

**Setting and procedures**

After institutional review board approval, patients from 3 wound centers in the Southeastern United States (St Francis Hospital, Charleston, SC; Regional Medical Center of Orangeburg, Orangeburg, SC; and ESU Inc, Pooler, GA) were recruited from October 2008 through December 2009 for this randomized, investigator-blinded, parallel-group, controlled trial (ClinicalTrials.gov identifier: NCT00720239; study registration date: February 7, 2008).

Eligible subjects were adults aged 21 years or older with a partial-thickness venous ulcer (extending through epidermis and into the dermis) diagnosed within the past 4 weeks without recent skin grafts or use of growth factors; a viable and clean wound bed with granulation tissue and greater than or equal to 90% free of necrotic debris; and wound size between 2 and 20 cm². Excluded were patients with full-thickness ulcers extending beyond the dermis; current wound, skin, or systemic infection; insufficient blood supply to wound (ankle-brachial index <0.8 or >1.3); wound duration more than 6 months; or a history of collagen vascular disease, severe arterial disease, organ transplant, Charcot disease, or sickle cell disease. In addition, patients were excluded if they received previous radiation therapy to the wound site, current hemodialysis, or treatment with another investigational drug or device within 30 days of study initiation. Those currently pregnant or unable or unwilling to comply with the study protocol were also excluded.

**Randomization to treatment groups**

After informed consent, eligible patients were randomly assigned to 1 of 4 study arms using computer-generated, stratified, permuted block randomization. Randomization was stratified by site to
ensure equal subject allocation across the 4 treatment arms. Block size was randomly varied to minimize the likelihood that study nurses could guess the next allocation on the basis of previous allocations. Patients and certified wound care nurses, who provided wound treatment and applied the wound-healing product, were not blinded to subject group assignment. Certified wound care nurses at all study sites received standardized training from the principal investigator regarding application of the investigational wound-healing therapy. This training consisted of a demonstration on wound bed preparation, application of the wound technology product, and the standard dressing, compression, and patient instruction. After demonstration by the principal investigator, the certified wound care nurses also demonstrated the wound care procedures and received feedback.

Study nurses obtained informed consent, assigned patients to groups based on the randomization sequence, and collected data; they also entered the data into the electronic study database. The principal investigator and participating physicians who provided medical oversight were blinded to subject group assignment.

Patients began therapy with a viable and clean wound bed greater than or equal to 90% free of necrotic debris. All patients received VLU standard of care, which included cleaning the wound with saline; patting the wound dry with gauze; applying a moisture-barrier product, such as a nonsting thin saline; performing wound drainage from damaging the skin; and applying a nonadherent absorptive primary dressing (Mepilex, Mölnlycke Health Care, Norcross, GA). The leg was then wrapped from the base of the toes to below the fulcrum of the knee with a multilayer compression system consisting of a zinc oxide-impregnated bandage (Viscopaste PB7, Smith and Nephew, Hull, United Kingdom), cotton padding wrap, and a self-adherent elastic wrap (Coban, 3M, St Paul, MN). The compression method used in the study protocol was already part of standard wound care in each of the study sites. Standard care also included providing patients with instructions for daily, at-home self-care (elevating the affected leg for at least 30 minutes and performing 3 to 4 lower-extremity exercise sets daily). Surgical, autolytic, or chemical debridement was not performed for any wounds during the course of the study.

Patients randomized to receive pGlcNAc advanced wound technology received standard care, as described above, except that the pGlcNAc wound-healing product (a tissue paper-thin matrix material) was applied directly to the wound immediately before administration of the primary nonadherent absorptive dressing. This advanced wound technology was supplied in a sterile container that could be opened by peeling back the cover. Once removed from the container this was applied directly to the wound bed for 100% (complete) wound coverage. Overlap onto the periwound skin was permitted. There were 3 active treatment groups: group A received the pGlcNAc advanced wound technology only once every week (biweekly); and group C received this active treatment technology once every third week. The control group (group D) received standard care only.

Assessments
Patients were seen twice weekly for the first 3 weeks and then weekly until wound closure. Study nurses measured wound length and width at each visit. Study nurses recorded adverse events, including deterioration of the wound bed (color, debris, pain, exudate, or inflammation) or changes in the periwound skin.

Statistical analysis
All statistical calculations were performed using software (SAS, Version 6.10 for Windows, SAS Inc, Cary, NC). The primary goal of this pilot study was to obtain estimates of variances and group differences to determine the effect size to power future studies. Comparisons between treatment groups for baseline demographic, illness, and ulcer characteristics were performed using \( \chi^2 \) tests or \( t \) tests. The primary efficacy end point was the proportion of patients with complete wound healing (defined as complete wound epithelialization and closure) at week 20 in the intent-to-treat population (all randomized subjects). Patients lost to follow-up or withdrawn by investigators because of adverse events before week 20 were assumed not to have achieved complete wound healing, and data from the last observation were carried forward. Group differences in achieving the primary end point (complete wound healing) were examined by \( \chi^2 \) tests.

RESULTS
Demographics and clinical characteristics
In all, 82 subjects were randomly assigned to one of the 4 study groups, and 71 completed the study (Fig 1). Seven subjects were lost to follow-up and 4 subjects who developed systemic infections were withdrawn from the study by the investigator. Demographic, illness, and ulcer characteristics are shown in Table 1. The overall mean (SD) age of the sample was 61.5 (13.8) years, ulcer size at baseline.
was 11.2 (10.2) cm², and age of ulcer was 3.2 (1.8) months. There were no significant differences among groups in terms of baseline variables except for a trend toward fewer men assigned to group A (pGlcNAc applied once only at week 1) than to the other 3 groups (\( P = .06 \)). The 5 most common comorbid conditions across the entire sample were hypertension (\( n = 61, 74.4\% \)), diabetes (\( n = 50, 61.0\% \)), class III obesity (body mass index \( \geq 40 \) kg/m²) (\( n = 37, 45.1\% \)), arthritis (\( n = 38, 46.3\% \)), and blood clotting disorders (\( n = 19, 23.2\% \)).

### Wound healing at 20 weeks

The primary analysis used the intent-to-treat sample (imputing missing values with last observation carried forward), which included all 82 patients who were randomized. At 20 weeks, the proportion of patients with completely healed wounds was 45.0% (\( n = 9 \) of 20), 86.4% (\( n = 19 \) of 22), and 65.0% (\( n = 13 \) of 20) for subjects in groups A, B, and C, respectively, compared with 45.0% (\( n = 9 \) of 20) for those receiving standard care. Only group B (pGlcNAc applied every other week) had a significantly greater proportion of patients with completely healed wounds compared with standard care (\( P = .005 \)). Representative photographs of a VLU at baseline, 3 weeks, and 6 weeks for a patient in group C are shown in Fig 2.

### Safety and tolerability

Four patients developed systemic infections that were determined to be unrelated to their wounds or study treatment. Specifically, 3 patients who had been given the diagnosis of and treated for *Clostridium difficile* several months before study enrollment developed a recurrence, and one patient developed methicillin-resistant *Staphylococcus aureus* after a previous surgical procedure. No significant treatment-related adverse events or reactions occurred during the study and none of the subjects experienced increased pain or edema.

### DISCUSSION

Although many wound care products have been introduced over recent years, the standard of care for...
VLUs has long remained graduated compression therapy. Current evidence indicates that multilayered compression systems are more effective than single-layered systems, and that multilayered systems with an elastic component are better than those without. Although compression therapy can be effective in many cases, patients with chronic VLUs (those that fail to heal after 3 months of optimal wound care) pose serious challenges for clinicians, and there is a need for new approaches and products to stimulate the healing process. In this randomized, controlled trial, 86% of patients with VLUs (mean VLU duration of 3 months at study entry) who received pGlcNAc every other week plus standard care had completely healed wounds at 5 months compared with 45% of patients who received standard care alone (P = .005). This statistically significant result in wound-healing efficacy was unexpected, given the small sample size. By comparison, in a review of 8 VLU clinical trials, only 60% of patients (n = 232) who received standard care, or standard care plus various therapeutic wound-healing agents, had completely healed wounds at 5 months compared with 45% of patients who received standard care alone (P = .005). The statistically significant result in wound-healing efficacy was unexpected, given the small sample size. By comparison, in a review of 8 VLU clinical trials, only 60% of patients (n = 232) who received standard care, or standard care plus various therapeutic wound-healing agents, had completely healed wounds at 5 months compared with 45% of patients who received standard care alone (P = .005). The improved rate of wound-healing efficacy achieved with pGlcNAc applied every other week may confer important additional benefits, such as decreased potential for infection, fever, lost work days, and reduced costs of care.

Currently available advanced treatments for difficult-to-heal VLUs include bioengineered skin substitutes, vacuum-assisted closure devices/negative pressure wound therapy, autologous skin grafting, and surgery. When used as adjuncts to standard compression therapy, FDA-approved bioengineered skin substitutes Apligraf (Organogenesis, Canton, MA) and Oasis Wound Matrix (Cook Biotech Inc, West Lafayette, IN) are associated with VLU healing rates, respectively, of 65% at 6 months and 55% at 3 months. A randomized trial involving 60 hospitalized patients with recalcitrant, chronic leg ulcers (venous, arteriosclerotic, and combined) compared the wound bed preparation efficacy (defined by 100% granulation and minimal wound secretion) achieved with a vacuum-assisted closure device (V.A.C., Kinetic Concepts Inc, San Antonio, TX) versus standard wound care, preceding autologous punch skin-graft transplantation. Although the vacuum-assisted closure device resulted in significantly faster (about 10 days, on average) wound preparation compared with standard care, time to complete wound healing after skin grafting was not significantly different between groups, nor was there a significant reduction in recurrence rates. Despite the common use of autologous skin grafting to treat difficult-to-heal ulcers, no randomized clinical trial of this treatment has demonstrated increased healing of VLUs or compared its wound-healing efficacy with other wound care strategies. Although venous surgery with compression bandaging reduces ulcer recurrence, it does not improve healing, and carries the usual risks of surgery and anesthesia; further, up to 35% of eligible patients refuse surgical intervention. Given the benefits and risks associated with currently available treatments

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A (n = 20)</th>
<th>B (n = 22)</th>
<th>C (n = 20)</th>
<th>D (n = 20)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Race, n (%)</td>
<td></td>
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<tr>
<td>Non-Hispanic white</td>
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<td>14 (70)</td>
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<td></td>
<td>15 (75)</td>
<td>9 (40.9)</td>
<td>7 (35)</td>
<td>10 (50)</td>
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<td>Edema, n (%)</td>
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<tr>
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<td>3 (13.6)</td>
<td>1 (5.9)</td>
<td>2 (10)</td>
<td>.80</td>
</tr>
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<td>8 (36.4)</td>
<td>5 (29.4)</td>
<td>6 (30)</td>
<td></td>
</tr>
<tr>
<td>2 Small amount</td>
<td>3 (25)</td>
<td>5 (22.7)</td>
<td>9 (52.9)</td>
<td>7 (35)</td>
<td></td>
</tr>
<tr>
<td>3 Moderate</td>
<td>3 (25)</td>
<td>3 (13.6)</td>
<td>2 (11.8)</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>4 Large amount</td>
<td>1 (8.3)</td>
<td>3 (13.6)</td>
<td>0</td>
<td>3 (15)</td>
<td></td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>59 (13.5)</td>
<td>63.2 (14.8)</td>
<td>60.8 (12.2)</td>
<td>63.0 (15.3)</td>
<td>.74</td>
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<td>Ulcer age, mo, mean (SD)</td>
<td>3.4 (1.5)</td>
<td>3.6 (1.8)</td>
<td>2.7 (2.1)</td>
<td>2.7 (1.6)</td>
<td>.78</td>
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<tr>
<td>Ulcer size, cm², mean (SD)</td>
<td>12.1 (11.3)</td>
<td>9.8 (7.3)</td>
<td>10.5 (10.3)</td>
<td>12.8 (12.0)</td>
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<tr>
<td>Hypertension, n (%)</td>
<td>14 (70)</td>
<td>16 (72.7)</td>
<td>15 (75.0)</td>
<td>16 (80.0)</td>
<td>.78</td>
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<td>Diabetes, n (%)</td>
<td>12 (60)</td>
<td>12 (54.5)</td>
<td>14 (70.0)</td>
<td>12 (60.0)</td>
<td>.78</td>
</tr>
<tr>
<td>Class III obesity (BMI ≥ 40 kg/m²), n (%)</td>
<td>8 (40.0)</td>
<td>12 (54.5)</td>
<td>10 (50.0)</td>
<td>7 (35.0)</td>
<td>.57</td>
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<td>6 (30.0)</td>
<td>12 (54.6)</td>
<td>10 (50.0)</td>
<td>10 (50.0)</td>
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<td>Blood clotting disorders, n (%)</td>
<td>4 (20.0)</td>
<td>9 (40.9)</td>
<td>2 (10.0)</td>
<td>4 (20.0)</td>
<td>.14</td>
</tr>
</tbody>
</table>

BMI, Body mass index.
for difficult-to-heal VLUs, data from this pilot study suggest that pGlcNAc wound-healing technology is effective and results in no significant treatment-related adverse effects.

The mechanism of action of pGlcNAc nanofiber-based membrane has been investigated in vitro and in vivo. pGlcNAc nanofiber membranes increase cell metabolism and activate in vitro migration of endothelial and fibroblast cells. In diabetic mouse studies, treatment of full-thickness wounds with pGlcNAc nanofiber membranes enhanced wound healing mainly by re-epithelialization and stimulation of angiogenesis, tissue remodeling, and endothelial cell proliferation.

**Study limitations and strengths**

This pilot study is limited by its small sample size, and findings should be replicated in a larger trial. A strength of this study was the use of complete wound healing, the FDA-recommended outcome for evaluating new wound-healing agents, as the primary end point. Unlike wound size, which was measured with manual techniques that tend to overestimate the extent of the wound, complete wound healing remains unaffected by potential measurement error. Other study strengths included a randomized, investigator-blinded, controlled design and an intent-to-treat analysis based on data from all randomized subjects. On the other hand, patients were not blinded to group assignment and knowledge of group assignment may have differentially motivated patients to adhere to recommended at-home, self-care.

**Conclusions**

This randomized, controlled, investigator-blinded, intent-to-treat pilot study demonstrated that pGlcNAc advanced wound-healing technology was effective in the treatment of patients with VLUs of approximately 3 months’ duration, and that its application was not associated with significant adverse effects.

**REFERENCES**