Safety and Efficacy of an Autologous Blood Clot Product in the Management of Texas 1A or 2A Neuropathic Diabetic Foot Ulcers: A Prospective, Multicenter, Open Label Pilot Study

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ABSTRACT

Objective. This pilot study evaluates safety in terms of the occurrence of adverse events (AEs) as well as the efficacy in terms of complete wound healing rates of a blood clot product when applied to chronic neuropathic diabetic foot ulcers (DFUs). **Materials and Methods.** Participants were chosen from patients with DFUs visiting the wound care clinic. Up to 10 mL of blood drawn from each participant was injected into the product's clotting tray. Within 12 minutes, the blood clot product was formed, applied to the single DFU of each participant, and covered with primary and secondary dressings. Patients received up to 12 blood clot product applications every 5 to 9 days for up to 12 weeks. **Results.** Twenty patients were enrolled; 20 were analyzed in the intent-to-treat (ITT) population and 18 were in the per-protocol (PP) population. Thirty-two AEs occurred (only 2 were possibly device related). The mean AE rate for both the ITT and PP populations was 1.6. The proportion of wounds healed in the ITT and PP populations was 13 out of 20 (65%) and 13 out of 18 (72.2%), respectively. Percentage area reduction (PAR) for the ITT population at 4 and 12 weeks was 61.6% and 67.1%, respectively; the PARs for the PP population were 60.3% and 76.2% at 4 and 12 weeks, respectively. Mean times to wound healing were 59 days and 56 days in the ITT and PP populations, respectively. **Conclusions.** This study demonstrates that the blood clot product is safe and efficacious for treating DFUs.

KEY WORDS

RedDress 1, blood clot product, safety, efficacy, pilot, diabetic foot ulcers

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Up to 4% of patients with diabetes develop diabetic foot ulcers (DFUs) each year,[1] the chronic state of which results in 80 000 annual lower extremity amputations.[2] The economic burden of DFUs is very steep, with the annual treatment of a single patient with a DFU costing an average of $31 419 in the United States.[3] Consequently, researchers are scrambling to develop effective advanced interventions that can improve DFU healing when standard of care fails, but systematic reviews repeatedly find little evidence to support the use of most advanced modalities.[4]

The extracellular matrix (ECM) is an emerging therapeutic target for DFUs and other chronic wounds, as its components are crucial to the entire wound healing process by enabling cellular adhesion, chemotaxis, and migration.[5,6] During early wound repair, a provisional fibrin clot is formed, whose surface serves for fibroblast migration and tissue remodeling.[6,7] As hemostasis transitions to the proliferative healing phase, fibroblasts produce vital ECM proteins such as collagen to develop a permanent granulation ECM.[6,8] There are commercially available matrix- and cellular-based products to treat DFUs that use materials such as platelet-rich plasma, collagen, or human cells or tissue to comprise a whole or partial provisional ECM or contribute to a hybrid scaffold made of biologic and synthetic materials.[9-16] However, there are doubts over their long-term efficacy,[14] and these products consist of foreign-derived materials that risk immunologic rejection and/or bear high costs.[16]

The risk of immunologic rejection is decreased by an autologous, biodegradable scaffold that attempts to mimic the body's healing mechanisms by replacing

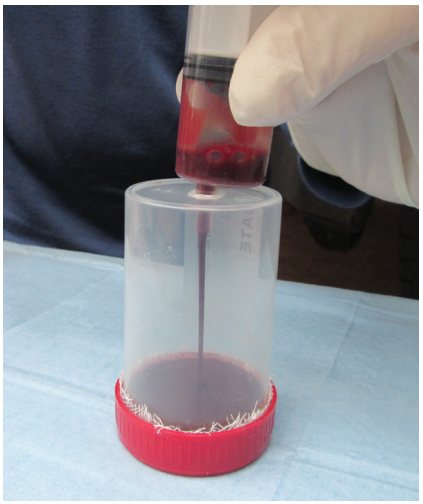

Figure 1. Coagulating blood is injected into the clotting tray using moderate pressure.

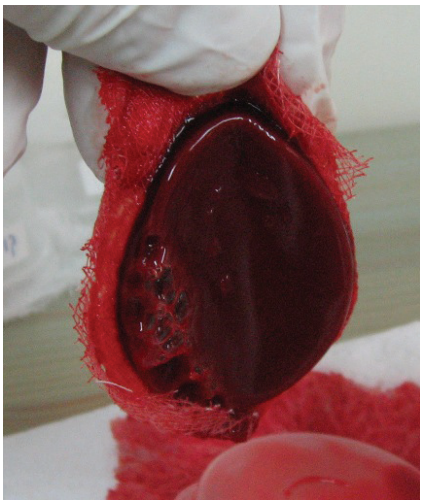

Figure 2. The blood clot product is formed after 12 minutes in the clotting tray and removed by gently grabbing it from its rim using both hands.

the ECM missing in chronic DFUs with a fibrin-based matrix.[17] The RD1 (RedDress 1; RedDress Ltd, Pardes-Hanna, Israel) is created in vitro by drawing the patient's blood at the point of care, with the use of citrate anticoagulant. The clotting cascade is reinitiated and promoted by mixing the blood with calcium gluconate plus kaolin powder (insoluble aluminum silicate) suspension. Within minutes, the blood clot product is formed, applied to the wound, and covered with primary and secondary dressings to serve as a functional, natural ECM for the wound healing process. This blood clot product was recently found to be safe and effective in a small pilot study of patients with multiple serious comorbidities and chronic wounds of various etiologies, excluding DFUs.[17] Until now, evidence on the safety of the blood clot product applied to DFUs was still needed.

The objective of this pilot study was to evaluate safety in terms of the occurrence of adverse events (AEs) as well as the efficacy of complete wound healing of the blood clot product when applied to chronic neuropathic DFUs.

MATERIALS AND METHODS

This was a multicenter, prospective, open label pilot study on patients treated for DFUs with the blood clot product at 3 study sites: ACMH Hospital - Snyder Institute for Vascular Health and Research, Kittanning, PA; Barry University Clinical Research, North Miami Beach, FL; and Martin Foot and Ankle, York, PA. The primary objective was to determine the safety of the blood clot product based on the incidence of all AEs, which also included serious AEs (SAEs), device-related AEs (DRAEs), and any AEs related to lack of venous access. The secondary objective was to determine the efficacy of the blood clot product, measured as complete healing (defined as skin reepithelialization without drainage or dressing requirements confirmed at 2 consecutive study visits 2 weeks apart) and also as percentage area reduction (PAR) from baseline. The Chesapeake Institutional Review Board (Columbia, MD) approved the study protocol, and the study complied with the Declaration of Helsinki and Good Clinical Practices.

Patient eligibility and enrollment

The target number of participants to be enrolled in this study was 20; no formal sample size calculations were made. Patients with DFUs at any of the 3 study sites were screened for their participation. Eligible patients were aged 18 years and older with a University of Texas (UT) grade 1A or 2A[18] neuropathic DFU that probed to the bone, tendon, or capsule and had a life expectancy of >12 months. Patients with a DFU wound duration longer than 1 year also were included. Diabetic foot ulcers with exposed bone, capsule, or tendon were excluded. The complete inclusion and exclusion criteria are listed in **eTable 1**. The Semmes-Weinstein monofilament test was used to diagnose diabetic neuropathy.[19,20] Patients with multiple DFUs were eligible to participate in the study; the largest ulcer was chosen as the study ulcer.

Eligible patients provided written informed consent to participate in the study prior to inclusion. During a 2-week screening and standard of care phase, all wounds were debrided, cleansed, and assessed via digital photography and subsequently were treated with standard moist wound therapy and assessed for infection (using the STONEES method),[21] for the use of an active offloading walker (boot and/or shoe), and for adequate perfusion. Any patient whose DFU area decreased or increased by at least 30% during this period was excluded from the treatment phase.

Preparation, application, and removal of the blood clot product

During this study, the investigators used single-use, disposable, sterile blood clot product kits containing a blood withdrawal kit, which included a citrate phosphate dextrose adenine (CPDA-1) blood collection bag; a coagulation initiator and accelerator kit with 10 mL sterile ampoule of 10% calcium gluconate injection (APP Pharmaceuticals, Schaumburg, IL), a 10 mL sterile syringe, and 30 mg of pharmaceutical-grade kaolin powder (Charles B. Chrystal Co, Inc, Larchmont, NY) sterilized in a vial; and 3 sizes of clotting trays (small: 14.5 cm^2; medium: 26.4 cm^2; and large: 64 cm^2) with cotton gauze. Five nurses handled all the preparation procedures of the blood clot product, and 3 physicians performed all the applications.

The preparation and application procedures have previously been described in detail.[17] In brief, the appropriately sized clotting tray was first chosen. Then, the wound was debrided with sterile sa-

line. The nurse documented the patient and wound current and past clinical status, performed a comprehensive wound assessment, and digitally photographed the wound. Next, the nurse filled a syringe with the CPDA-1 and drew 10 mL of blood from the patient. The calcium gluconate plus kaolin suspension was mixed and extracted using the syringe containing the citrated blood. Using moderate pressure, the nurse injected a specified amount of the coagulating blood, based on the size of the clotting tray, into the tray (**Figure 1**). After 10 to 12 minutes, the blood clot product was created (**Figure 2**). The physician gently placed the whole blood clot on the wound, with the embedded gauze pad facing upward, and anchored it by its rim with Steri-Strips (3M, St Paul, MN). Finally, primary and secondary dressings were placed over the blood clot product.

The blood clot product was gently pulled off the wound using gloves after 5 to 9 days. In case of adhesions, the blood clot product was wetted prior to removal.

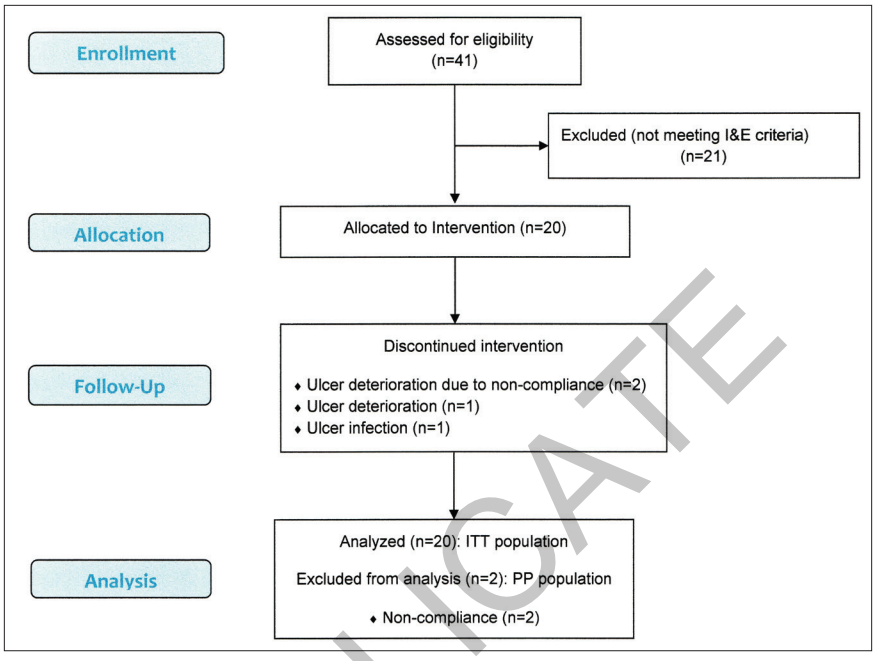

Figure 3. Participant flow diagram.
I&E: inclusion and exclusion; ITT: intent-to-treat; PP: per protocol

Follow-up and reapplication

The treatment phase lasted up to 12 weeks, during which time the blood clot product was applied for up to a total of 12 times at the clinic and 20 dressing changes were performed twice weekly at the clinic or at the home. Every 5 to 9 days, the blood clot product was removed and a new one was created and applied as previously described as necessary. During each clinic visit, concomitant medication was reviewed, and offloading compliance, moisture control, and AEs were assessed. After blood clot product removal, wound assessment occurred, and 2 digital photographs of the wound were taken as described below. If the wound was closed, then a confirmatory visit took place 2 weeks after complete healing. At the confirmatory visit or at the end of study week 12 visit, laboratory analyses also were performed to determine the complete blood count, prothrombin time, partial thromboplastin time, and glycated hemoglobin. After the study terminated, participants were treated with standard of care at each study site as necessary.

Wound photographic assessment

A nurse took 2 digital wound photographs of each wound and measured the wound at each visit before and after debridement and cleansing using a wound imaging, measurement, and documentation system (Silhouette; ARANZ Medical, Christchurch, New Zealand). The wound images and related data were emailed to an independent central reviewer, who assessed for wound closure and communicated the status to the monitor as part of an online central review process.

Safety assessment

The safety of the blood clot product was assessed at each visit during the treatment phase based on the occurrence of AEs, which were defined and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4 scale.[22] Examples of anticipated AEs included infection not related to the device application, increase in UT grade of wound, sudden increase in ulcer size, peripheral edema or localized swelling, a new ulcer, systemic fever, and maceration unrelated to edema, swelling, or an allergic reaction. Examples of anticipated DRAEs included complications related to venipuncture (but not including lack of venous access), infections appearing within 2 to 4 days of device application, bleeding at the wound site unrelated to any debridement, any allergic reactions, and high pain related to product application or removal. A SAE was any event that resulted in death, was life-threatening, required hospitalization or prolongation of existing hospitalization, and/or resulted in persistent or significant disability/incapacity. All AEs, SAEs, and DRAEs were recorded on the participant's case report form (CRF) and, within 24 hours of the initial discovery of the event, were reported to the ethics committee and the sponsor.

Participants could be withdrawn if they desired to do so at any time for any reason, the investigator thought it was in the participant's best interest, the participant missed more than 2 consecutive visits during the treatment phase, or if

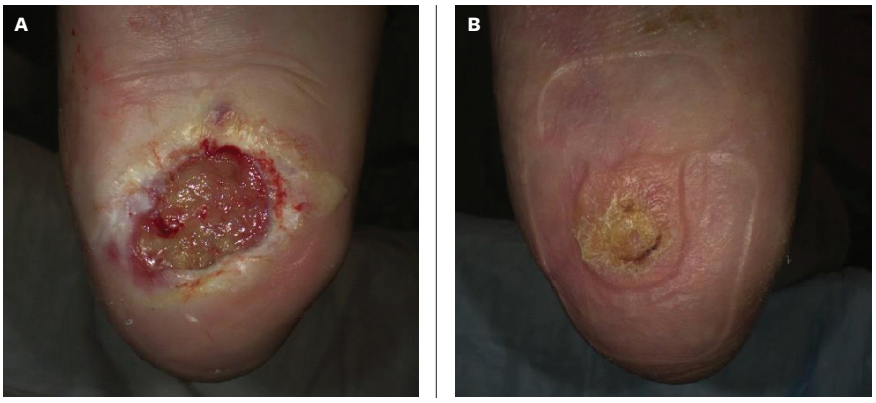

Figure 4. (A) Texas grade 2A diabetic foot ulcer with a duration of 1.8 years measuring 5.7 cm² on the heel of a 62-year-old man on day 1 of treatment; and (B) the same ulcer completely closed on day 78.

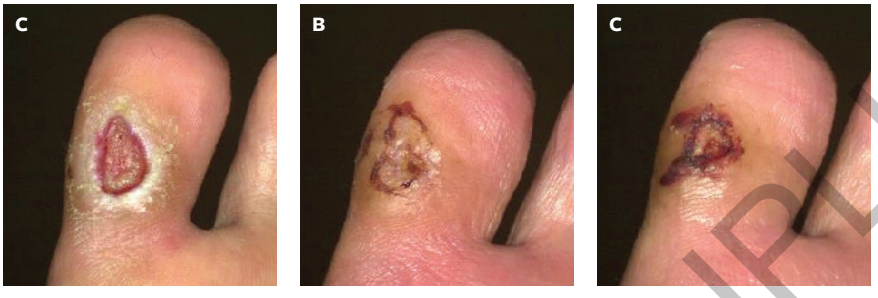

Figure 5. (A) Texas grade 1A diabetic foot ulcer measuring 1 cm² on the hallux of a 51-year-old woman on day 0; (B) the same ulcer completely closed on day 62; and (C) the end-of-study size of the ulcer measured 0.1 cm², as the ulcer failed to remain healed during the confirmatory visit on day 83.

any of the following occurred that interfered with the treatment or risked the participant's health: infection, lack of venous access (due to inability to complete a venipuncture), ulcer deterioration (defined as at least a 50% increase in area or an increase in UT grade), and any health deterioration requiring hospitalization or likely to interfere with treatment and result in treatment failure.

Data collection and statistical analysis

The study coordinators recorded all participant data on CRFs, which were kept in a secure location in accordance with Health Insurance Portability and Accountability Act of 1996 regulations. Data from the CRFs were transmitted manually or electronically to the clinical database.

Statistical analysis was performed by Strategic Solutions, Inc, (Cody, WY) using SAP version 4 of January 11, 2017, software. Three study populations were analyzed. The intent-to-treat (ITT) and safety populations included all participants who received at least 1 treatment. The per-protocol (PP) population permitted only those participants who had no major protocol violations (in addition to the ITT requirements). Participants who were lost to follow-up were included in the safety analysis but excluded from the analysis of secondary objectives. For missing data, imputation using last observation carried forward was used.

Descriptive statistical methods were used to analyze the study data and included the number of participants, mean, median, standard deviation (SD), and range for continuous data. Frequencies and percentages were used for categorical data. No adjustment for multiplicity was made of any initial *P* values, as comparisons between groups were not made. Unless otherwise specified, all statistical testing was 2-sided and performed using a significance (alpha) level of 0.5

The primary endpoint was the AE rate, which was calculated based on all SAEs, AEs, and DRAEs for both the safety and ITT population. Secondary endpoints were calculated for the ITT population and included incidence of complete wound closure at 12 weeks, PAR over 12 weeks, and complication rates due to lack of venous access involving the blood clot product procedures as well as for other study procedures. In addition, associations between lack of healing and patient/wound parameters were found by using a simple Cox regression (due to the very small sample size).

RESULTS

Demographics

From June 2014 to August 2016, 41 patients were screened for study eligibility, of whom 20 (48.8%) met the inclusion criteria and were enrolled (**Figure 3**). The mean age of study participants was 58.6 years (SD, 10.5), and they were overwhelmingly male (n = 16; 80%), white (n = 17; 85%), and fully ambulatory (n = 16; 80%) (**eTable 2**). The mean number of serious comorbidities per participant was 8.8 (SD, 3.7) (**eTable 3**). Half of the patients (n = 10; 50%) were on anticoagulants, 40% (n = 8) took proton pump inhibitors, and 45% (n = 9) were taking selective serotonin releasing agents or selective serotonin reuptake inhibitors.

Study wound characteristics are provided in **eTable 4**. The majority of study DFUs were a UT grade 1 (n = 15; 75%) located on the foot (n = 12; 60%) and new wounds (n = 14; 70%). The majority of wounds were debrided at the initial screening visit (n = 17; 85%), with surgical debridement used in all but 1 wound that underwent sharp debridement; mean debridement count during treatment was 4.9 (SD, 3.7).

All 20 wounds received at least 1 application of the blood clot product and were included in the safety and ITT analysis. Two patients and their respective wounds were excluded from the PP population due to 1 participant developing an infection and being excluded from the ITT population after extreme physical exertion resulting in nonadherence to the offloading requirement, and the other for nonadherence to the protocol

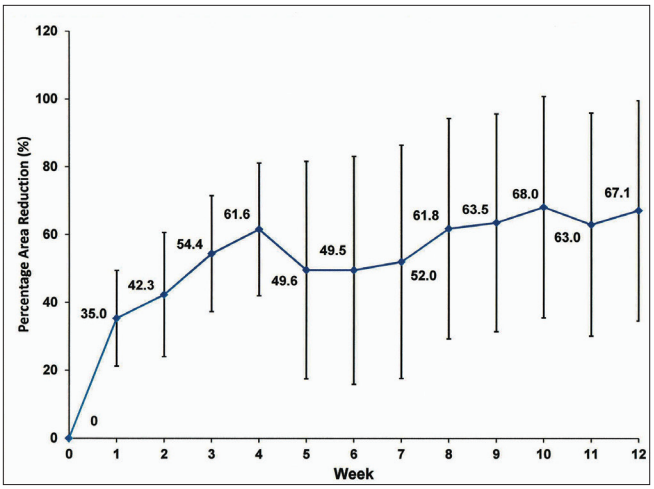

Figure 6. Percentage area reduction for the intent-to-treat population, showing mean reduction per week with associated 95% confidence interval error bars.

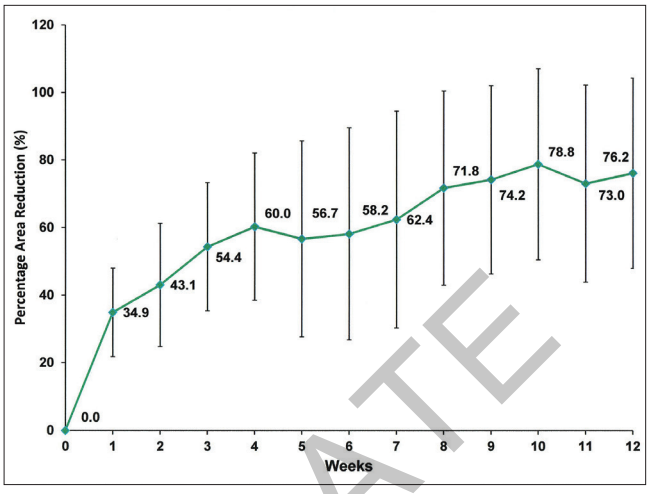

Figure 7. Percentage area reduction for the per-protocol population, showing mean reduction per week with associated 95% confidence interval error bars.

after going on vacation without notice and consequently missing a visit.

Safety analysis

Thirty-two AEs occurred during this study, of which 2 were SAEs and 2 were possible DRAEs (**eTable 5**). Twenty-one (65.6%) AEs were classified as mild, 9 (28.1%) as moderate, and 2 (6.3%) as severe. The 2 DRAEs were defined as possibly related to the blood clot product because of the AE location and occurrence in the same participant, who experienced a left hallux infection treated with medication and felt subsequent increased pain around the hallux and foot. Blood clot product treatment was interrupted for 2 weeks and continued after the possible DRAEs were resolved. Two participants experienced a SAE, which included a nervous breakdown and a pulmonary embolism caused by a deep vein thrombosis in the nontreated leg. None of the SAEs were related to the blood clot product or study wounds. The nervous breakdown was resolved after 2 days of hospitalization and medication. The pulmonary embolism was treated with hospitalization and medication and was still ongoing after the participant exited the study.

The mean AE rates for the ITT and PP populations were 1.6 (SD, 1.50; 95% confidence interval [CI], 0.90–2.30) and 1.7 (SD, 1.53; 95% CI, 0.90–2.43), respectively. The proportion of participants experiencing any type of AE and venous access complications for the ITT and PP populations is shown in **eTable 6**. Adverse events impacted the use of the blood clot product in 5 of 20 participants (25%) in the ITT population; treatment was discontinued in 4 participants while 1 participant had treatment interrupted at 1 visit (visit 6).

There were no complications involving venous access whatsoever, and there were no delays in coagulation during the preparation of the blood clot product. The total count for blood clot product preparations was 153, with a mean count of 7.6 applications (SD, 2.91) per participant. During 5 preparations, coagulation time slightly exceeded 12 minutes, 4 instances of which the time was 14 minutes and 1 took 14 minutes 30 seconds.

Healing rates and outcomes

In the ITT population, 13 of 20 (65%) wounds completely healed and 13 of 18 (72.2%) PP population wounds completely healed. Among the participants with healed DFUs, there was a 62-year-old man with a DFU on the right heel measuring 5.7 cm^2 on day 0 that had a duration of 1.8 years after failing to heal following multiple treatments (**Figure 4A**). The following products and procedures were previously applied to this wound without success: gauze, absorption foam, calcium alginate, silver alginate, saline irrigation, surgical debridement, sharp debridement, autologous skin graft (CELLUTOME; Acelity, San Antonio, TX), collagen dressing, and hyperbaric oxygen therapy (HBOT). After the blood clot product was applied to the ulcer, it was completely healed at day 78 (**Figure 4B**).

Following initial healing, there were 4 ulcer recurrences, with 2 occurrences resulting in unhealed wounds; the ITT and PP populations had 1 nonhealing and 1 healing recurrence each. **Figure 5** provides an example of a DFU on the left hallux of a 51-year-old woman that initially measured 1 cm^2, healed on day 62, and later recurred with an end-of-study area of 0.1 cm^2 during the confirmatory visit on day 83. Three participants (15%) experienced infection episodes in the ITT population, while 2 participants (11.1%) experienced infection episodes in the PP population.

There was no difference in the proportion of wounds healed based on wound age. For wounds with a duration of at least 26 weeks, 6 of 9 (66.7%) healed. For wounds with a duration less than 26 weeks, 7 of 11 (63.6%) healed.

In the ITT population, there was a substantial mean PAR after 1 week of

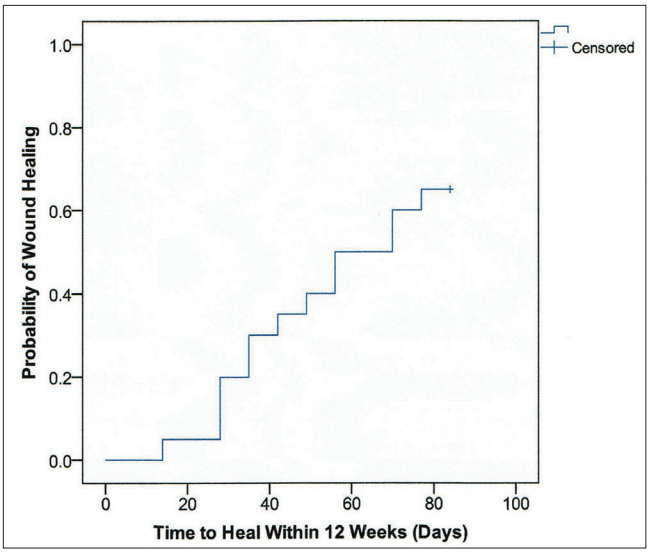

Figure 8. Kaplan-Meier plot of time to heal within 12 weeks for the intent-to-treat population.

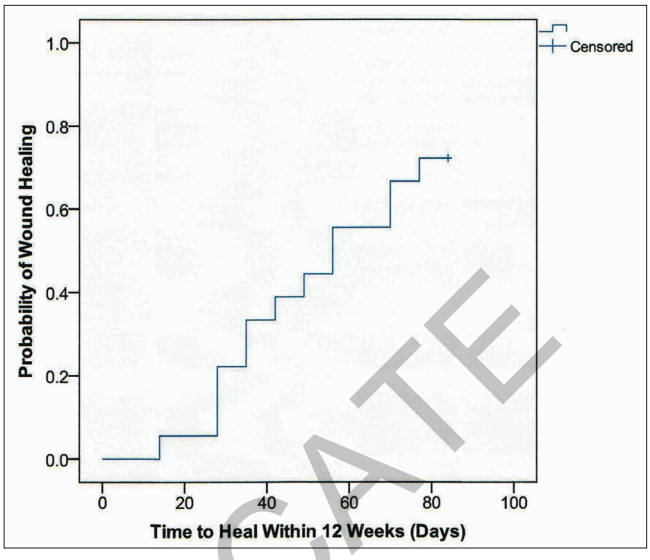

Figure 9. Kaplan-Meier plot of time to heal within 12 weeks for the per-protocol population.

treatment, which was followed by a slower increase until week 5 when the effect of several stalled wounds lowered the mean value (**Figure 6**). The mean PAR increased by week 8, and a slight further increase is demonstrated by week 12. A similar healing pattern is shown for the PP population in **Figure 7**, except the mean PAR decrease is less between weeks 5 and 8. The mean PAR at 4 and 12 weeks for the ITT population was 61.6% and 67.1%, respectively. The mean PAR for the PP population was comparable at 4 weeks (60.3%), but it was better than the ITT population at 12 weeks (76.2%). Among the 7 wounds that did not heal in the ITT population, their final PAR values were 90.9%, 90.0%, 74.5%, 60.0%, -69.6%, -83.3%, and -120.0%. In 3 of the participants, the blood clot product treatment was discontinued, while it was interrupted in 1 participant.

No Cox regression model could be developed with any significant covariates in either population. A Kaplan-Meier analysis of the ITT population demonstrated a mean time to healing of 59 days (95% CI, 48.3–69.3) and a median time to healing of 56 days (95% CI, 33–79). The corresponding mean and median times to healing for the PP population were 56 days (95% CI, 45.1–66.9) and 56 days (95% CI, 45.5–70.5), respectively. **Figures 8 and 9** show the Kaplan-Meier plots of time to heal for both study populations.

DISCUSSION

For more than 20 years, wound care has targeted regenerative medicine and the use of biologic scaffolds for their potential benefit on recalcitrant and difficult-to- heal DFUs.[10] Yet, the evidence remains limited, and there are concerns over safety and accessibility of these products.[14,16]

The blood clot product was safe and efficacious in treating a sample of patients with UT grade 1A and 2A neuropathic DFUs, a substantial proportion (n = 9; 45%) of which had a duration of at least 6 months and 25% (n = 5) had a duration of >1 year, and many had been previously treated with advanced therapies without success. Furthermore, there was a mean number of 8.8 comorbidities per participant, and participants were taking a mean of 9.9 medications, indicating their poor health status, which could have delayed wound healing. Nevertheless, nearly two-thirds of DFUs in the total study sample healed after about 8 weeks of treatment with the blood clot product. The mean AE rate was 1.6 for the ITT population and 1.7 for the PP population. Among the 32 AEs that occurred, the majority (n = 21; 65.6%) were mild, and 93.8% (n = 30) were unrelated to the blood clot product. Only 4 participants had the blood clot product treatment stopped as a result of their AEs. In addition, there were no complications involving venous access reported during this study, further supporting the safety of the blood clot product and its related procedures.

This was the first clinical investigation of the blood clot product in DFUs, and there is no other similar product currently available to compare results. Natural regenerative tissues, such as amniotic membranes, are increasingly investigated for their effect on chronic DFUs. Recent systematic reviews with meta-analyses of 6 and 7 randomized controlled trials (RCTs) have found that amniotic membranes applied to DFUs resulted in healing 2.32 times more frequently and 32 days faster than standard of care,[23] with a net difference in healing rates at 12 weeks.[24] Studies have reported 62% to 85.2% of DFUs healed with an amnion membrane treatment at 12 weeks,[25,26] and the treatment appeared to be safe with an AE rate of 44% (22/50), although 18% of subjects (9/50) had an infection of the

study ulcer.[25] A small RCT demonstrated the healing rate of DFUs treated with amnion/chorion membranes was 85% at 12 weeks, with an AE rate of 5% due to 1 AE that was not product related.[27] Randomized clinical trials evaluating the efficacy and safety of amnion/chorion allograft applied to DFUs reported higher healing rates of 92.5% and 97% among 20 and 32 patients, respectively.[28,29] This product also appears to be safe, with an AE rate of 15% reported and no product-related AEs.[28] Overall, limited safety data and weak generalizability due to small sample sizes and the risk of bias limit any comparative analysis with the blood clot product.

A previous pilot study[17] evaluated the effect of the blood clot product on chronic wounds of different etiologies and had a healing rate of 78% of wounds closed, comparable to the 72.2% of wounds healed in the PP population of the current study. No AEs were reported.[17] Participants in that pilot study[17] required considerably less applications of the blood clot product compared with participants in the current study (3.9 mean applications vs. 7.6). However, only 7 patients with 9 wounds were enrolled in that initial study,[17] and the current study enrolled only patients with diabetes and DFUs, which, combined with the multiple serious comorbidities experienced by these patients, pose a greater challenge to the wound healing process.

A chronic wound can be caused by an inhibited blood supply to the wound site.[9,17] The blood clot plays an essential role in the entire wound healing process.[17,30-36] The application of the blood clot product to the wound promotes the healing process by controlling inflammation, facilitating angiogenesis, and having the necessary wound repair and remodeling factors.[17] The additional safety benefit of the blood clot product is that this autologous wound dressing is made of the patient's own coagulated blood to prevent immunorejection.[17] The citrate, calcium, and kaolin used during the preparation procedure control the coagulation process and enable the blood clot product to be safely created at patient bedside in a practical manner.

LIMITATIONS

In addition to the limitations inherent to the case series design, this study was limited by its sample size and the fact that patients with less severe chronic DFUs were enrolled. However, 25% of wounds had a duration of longer than 1 year after multiple treatments failed to heal their DFUs. Furthermore, the patients' poor health status demonstrated by their multiple serious comorbidities reflected the real-world challenges of the wound care setting. There were 4 recurring ulcers reported during this study, 2 of which occurred in unhealed wounds. A future trial could evaluate a larger sample of DFUs and include a follow-up period after the treatment phase to confirm the long-term safety and efficacy of the blood clot product.

CONCLUSIONS

This study demonstrated that the application of the blood clot product, which is prepared at point of care, on chronic neuropathic DFUs is safe and efficacious to use on patients with multiple serious comorbidities. Only 2 of 32 (6.3%) AEs were possibly related to the device, and treatment with the blood clot product continued once they were resolved. There were no problems with venous access reported. These findings further support the beneficial therapeutic use of the blood clot product on chronic wounds previously reported on complicated chronic wounds of various etiologies.[17] A larger cohort study of the elderly population in specialized nursing facilities and a RCT comparing the blood clot product to standard of care are to be performed to confirm the long-term safety of this product in DFUs.

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Affiliations: [1]Barry University School of Podiatric Medicine, Miami Shores, FL; [2]Martin Foot and Ankle, York, PA; [3]Armstrong County Memorial Hospital, Kittanning, PA; [4]Strategic Solutions, Inc, Cody, WY; [5]RedDress Ltd, Pardes-Hanna, Israel; and [6]SerenaGroup, Inc, Cambridge, MA

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Disclosure: RedDress Ltd. (Pardes-Hanna Israel) sponsored this study, provided the study supplies and materials, assisted with the protocol development, and trained staff of each study site on the blood clot product application and procedure, wound debridement, cleansing, dressing, and offloading. Palmetto Clinical Consulting LLC (Anderson, SC) and the Atlantic Research Group (Charlottesville, VA) monitored the study sites. Marissa J. Carter was a paid consultant to RedDress Ltd during this study; Igal Kushnir and Alon Kushnir are shareholders in RedDress Ltd. There was no financial interest for any of the investigators or participants.

REFERENCES

1. Margolis DJ, Malay DS, Hoffstad OJ, et al. *Incidence of diabetic foot ulcer and lower extremity amputation among Medicare beneficiaries, 2006 to 2008. Data Points #2*. Rockville, MD: Agency for Healthcare Research and Quality; 2011. AHRQ Publication No. 10(11)-EHC009-1-EF.
2. Lavery LA, Armstrong DG, Harkless LB. Classification of diabetic foot wounds. *J Foot Ankle Surg*. 1996;35(6):528–531.
3. Rice JB, Desai U, Cummings AK, Birnbaum HG, Skornicki M, Parsons NB. Burden of diabetic foot ulcers for Medicare and private insurers [published online ahead of print November 1, 2013. Published correction appears in *Diabetes Care*. 2014;37(9):2660]. *Diabetes Care*. 2014;37(3):651–658.
4. Game FL, Apelqvist J, Attinger C, et al; International Working Group on the Diabetic Foot. Effectiveness of interventions to enhance healing of chronic ulcers of the foot in diabetes: a systematic review. *Diabetes Metab Res Rev*. 2016;32(Suppl 1):154–168.
5. Polanco TO, Xylas J, Lantis JC 2nd. Extracellular matrices (ECM) for tissue repair. *Surg Technol Int*. 2016;28:43–57.
6. Tracy LE, Minasian RA, Caterson EJ. Extracellular matrix and dermal fibroblast function in the healing wound. *Adv Wound Care* (New Rochelle). 2016;5(3):119–136.
7. Pereira M, Rybarczyk BJ, Odrljin TM, Hocking DC, Sottile J, Simpson-Haidaris PJ. The incorporation of fibrinogen into extracellular matrix is dependent on active assembly of a fibronectin matrix. *J Cell Sci*. 2002;115(Pt 3):609–617.
8. Tuan TL, Song A, Chang S, Younai S, Nimni ME. In vitro fibroplasia: matrix contraction,

cell growth, and collagen production of fibroblasts cultured in fibrin gels. *Exp Cell Res*. 1996;223(1):127-134.

9. Demidova-Rice TN, Durham JT, Herman IM. Wound healing angiogenesis: innovations and challenges in acute and chronic wound healing. *Adv Wound Care* (New Rochelle). 2012;1(1):17–22.
10. Turner NJ, Badylak SF. The use of biologic scaffolds in the treatment of chronic nonhealing wounds. *Adv Wound Care* (New Rochelle). 2015;4(8):490-500.
11. Supp DM, Boyce ST. Engineered skin substitutes: practices and potentials. *Clin Dermatol*. 2005;23(4):403–412.
12. Assi R, Foster TR, He H, et al. Delivery of mesenchymal stem cells in biomimetic engineered scaffolds promotes healing of diabetic ulcers [published online ahead of print March 17, 2016]. *Regen Med*. 2016;11(3):245–260.
13. Markeson D, Pleat JM, Sharpe JR, Harris AL, Seifalian AM, Watt SM. Scarring, stem cells, scaffolds and skin repair [published online ahead of print October 29, 2013]. *J Tissue Eng Regen Med*. 2015;9(6):649–668.
14. Mulder G, Tenenhaus M, D'Souza GF. Reduction of diabetic foot ulcer healing times through use of advanced treatment modalities. *Int J Low Extrem Wounds*. 2014;13(4):335–346.
15. Pushpoth S, Tambe K, Sandramouli S. The use of AlloDerm in the reconstruction of full-thickness eyelid defects. *Orbit*. 2008;27(5):337–340.
16. Naves CC. The diabetic foot: a historical overview and gaps in current treatment. *Adv Wound Care* (New Rochelle). 2016;5(5):191–197.
17. Kushnir I, Kushnir A, Serena TE, Garfinkel D. Efficacy and safety of a novel autologous wound matrix in the management of complicated, chronic wounds: a pilot study. *Wounds*. 2016;28(9):317–327.
18. Oyibo SO, Jude EB, Tarawneh I, Nguyen HC, Harkless LB, Boulton AJ. A comparison of two diabetic foot ulcer classification systems: the Wagner and the University of Texas wound classification systems. *Diabetes Care*. 2001;24(1):84–88.
19. Semmes J, Weinstein S, Ghent L, Teuber HL. *Somatosensory Changes after Penetrating Brain Wounds in Man*. Cambridge, MA: Harvard University Press. 1960.
20. Birke JA, Sims DS. Plantar sensory threshold in the ulcerated foot. *Lepr Rev*. 1986;57(3):261–267.
21. Woo KY, Sibbald RG. A cross-sectional validation study of using NERDS and STONEES to assess bacterial burden. *Ostomy Wound Manage*. 2009;55(8):40–48.
22. National Cancer Institute. Common Terminology Criteria for Adverse Events Version 4.0. Updated June 14, 2010. https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf.
23. Paggiaro AO, Menezes AG, Ferrassi AD, De Carvalho VF, Gemperli R. Biological effects of amniotic membrane on diabetic foot wounds: a systematic review. *J Wound Care*. 2018;27(Suppl 2):S19–S25.
24. Laurent I, Astère M, Wang KR, Cheng QF, Li QF. Efficacy and time sensitivity of amniotic membrane treatment in patients with diabetic foot ulcers: a systematic review and meta-analysis [published online ahed of print September 11, 2017]. *Diabetes Ther*. 2017;8(5):967–979.
25. Lavery LA, Fulmer J, Shebetka KA, et al; Grafix Diabetic Foot Ulcer Study Group. The efficacy and safety of Grafix(®) for the treatment of chronic diabetic foot ulcers: results of a multi-centre, controlled, randomised, blinded, clinical trial [published online ahead of print July 21, 2014]. *Int Wound J*. 2014;11(5):554–560.
26. Regulski M, Jacobstein DA, Petranto RD, Migliori VJ, Nair G, Pfeiffer D. A retrospective analysis of a human cellular repair matrix for the treatment of chronic wounds. *Ostomy Wound Manage*. 2013;59(12):38–43.
27. DiDomenico LA, Orgill DP, Galiano RD, et al. Aseptically processed placental membrane improves healing of diabetic foot ulcerations: prospective, randomized clinical trial. *Plast Reconstr Surg Glob Open*. 2016;4(10):e1095.
28. Zelen CM, Serena TE, Snyder RJ. A prospective, randomised comparative study of weekly versus biweekly application of dehydrated human amnion/chorion membrane allograft in the management of diabetic foot ulcers. *Int Wound J*. 2014;11(2):122–128.
29. Zelen CM, Serena TE, Gould L, et al. Treatment of chronic diabetic lower extremity ulcers with advanced therapies: a prospective, randomised, controlled, multi-centre comparative study examining clinical efficacy and cost [published online ahead of print December 23, 2015]. *Int Wound J*. 2016;13(2):272–282.
30. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med*. 1999;341(10):738–746.
31. Razzell W, Wood W, Martin P. Swatting flies: modelling wound healing and inflammation in Drosophila [published online ahead of print August 2, 2011]. *Dis Model Mech*. 2011;4(5):569–574.
32. Clark RA. Fibrin and wound healing. *Ann N Y Acad Sci*. 2001;936:355–367.
33. Miloro M, Ghali GE, Larsen P, Waite P. *Peterson's Principles of Oral and Maxillofacial Surgery*. 2nd ed. Raleigh, NC: PMPH-USA; 2004.
34. Laurens N, Koolwijk P, de Maat MP. Fibrin structure and wound healing. *J Thromb Haemost*. 2006;4(5):932–939.
35. Tonnesen MG, Feng X, Clark RA. Angiogenesis in wound healing. *J Investig Dermatol Symp Proc*. 2000;5(1):40–46.
36. Clark RAF, ed. *The Molecular and Cellular Biology of Wound Repair*. 2nd ed. New York, NY: Plenum Press;1996.

eTable 1. Patient inclusion and exclusion criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
Age ≥18 years with type 1 or 2 diabetes	Ulcer not of neuropathic diabetic foot pathophysiology
Texas grade 1A or 2A ulcer located distal to the malleolus, excluding ulcers between the toes; with no exposed capsule, tendon, or bone and no tunneling, undermining, or sinus tracts; a depth of ≤5mm; a postdebridement size of 1cm² to 12cm²	Participated in another clinical trial involving a device or a systematically administered investigational study drug or treatment within 30 days of screening visit
Ulcer duration of ≥30 days	Proven sepsis established by a blood culture from the previous 2 weeks, or confirmed active infection likely to interfere with the trial, such as a urinary tract infection
If multiple ulcers present, a study ulcer separated from other wounds by ≥2cm	History of alcohol or substance abuse within the previous 2 months
Previous thorough, independent wound assessment	Underlying osteomyelitis present
No clinical signs of infection for ulcer or affected limb	Treatment with hyperbaric oxygen within 5 days of screening or was scheduled to receive it during the study
Ulcer free of necrotic tissue postdebridement	History of or any of the following intercurrent illnesses or conditions: end stage renal disease, immunosuppression, severe malnutrition, liver disease, scleroderma, HIV/AIDS, connective tissue disorder, or exacerbation of sickle cell anemia
Adequate vascular perfusion of the affected limb, as defined by at least 1 of the following: ankle-brachial index ≥0.65 and ≤1.2, toe pressure (plethysmography) >50mmHg, transcutaneous partial pressure of oxygen >40mmHg, or skin perfusion pressure >30mmHg	Hemoglobin anemia (<10g/dL)
No anemia (hemoglobin ≤10%)	Life expectancy of <12 months
Demonstrated adequate offloading regimen	Cognitively impaired and either had a health care proxy or clearly did not understand the informed consent form
Willing to comply with study protocol, including having blood drawn to create blood clot product	History of coagulation problems, abnormal thrombocytes levels, or was receiving heparin intravenously; patients taking warfarin, aspirin, or clopidogrel were not excluded
If female and capable of conceiving or male and capable of insemination, then agreed to use an acceptable form of contraception during the study	Unable to have the required amount of blood drawn (up to 10mL/week)
	For female patients, pregnant or currently breastfeeding
	Received within the previous 30 days or was scheduled to receive a medication or treatment known to interfere with, or affect the rate and quality of, wound healing (eg, systemic steroids, immunosuppressive therapy, autoimmune disease therapy, cytostatic therapy within the past 12 months, dialysis, radiation therapy to the foot, vascular surgery, angioplasty, or thrombolysis)

eTable 2. Patient characteristics (n=20)

CHARACTERISTIC	N (%)
Age (y)	58.6 (10.5)[a]
Age ≥65 y	7 (35)
Gender	
Male	16 (80)
Female	4 (20)
Race/ethnicity	
White	17 (85)
African American	2 (10)
Hispanic	1 (5)
Smoking	
Never smoked	9 (45.0)
Last 12 months	5 (25.0)
Pack years (n=9)	28.3 (12.8)[a]
BMI (n=19)	34.4 (6)[a]
Underweight	0 (0)
Normal weight	0 (0)
Overweight	5 (25)
Obese	10 (50)
Morbidly obese	4 (20)
Ambulation	
Full	16 (80)
Limited	2 (10)
Wheelchair	2 (10)
ABI (n=19)	1.07 (0.14)[a]
Median HbA_{1c} [b]	9.0 (3.1)[c]
Osteomyelitis (x-ray)	0 (0.0)
CBCs abnormal [d]	12 (60.0)
PT abnormal	2 (10.0)
PTT abnormal	1 (5.0)

BMI: body mass index; ABI: ankle-brachial index; CBC: complete blood count; PT: prothrombin time; PTT: partial thromboplastin time

[a] Mean and standard deviation provided.
[b] Data missing for 8 subjects.
[c] Interquartile range provided in parenthesis.
[d] There were 12 subjects who had at least 1 CBC parameter below or above the normal value.

eTable 3. Selected patient comorbidities

COMORBIDITY	N (%)
Allergy count	
0	9 (45)
1	8 (40)
2	1 (5)
3	1 (5)
5	1 (5)
Allergy, antiseptics, irritation	1 (5)
Allergy, dressings, rash	3 (15)
Allergy, drugs, itching	2 (10)
Allergy, drugs, nausea	1 (5)
Allergies, drugs, other	5 (25)
Allergy, drugs, sleep disorder	1 (5)
Anemia (iron)	2 (10)
Back surgery	1 (5)
Coronary artery disease	2 (10)
Cataract surgery	1 (5)
Charcot arthropathy	2 (10)
Chronic heart failure	1 (5)
Depression	6 (30)
Diabetes type	
1	1 (5)
2	19 (95)
Diabetic neuropathy	20 (100)
Eye disease	1 (5)
Gastroesophageal reflux disease	5 (25)
Gastrointestinal ulcer	3 (15)
Hip surgery	1 (5)
Hyperlipidemia/cholesterolemia	12 (60)
Hypertension	13 (65)
Nutrition problem	0 (0)
Osteoarthritis	3 (15)
Osteomyelitis (history)	2 (10)
Paralysis	2 (10)
Previous chronic wound	6 (30)
Prior amputation (major)	1 (5)
Prior amputation (minor)	2 (10)
Peripheral vascular disease	2 (10)
Mean other comorbidity (standard deviation)	3.7 (2.2)
Mean total comorbidity count per patient (standard deviation)	8.8 (3.7)

eTable 4. Wound characteristics

CHARACTERISTIC	N (%)
University of Texas grade	
1	15 (75)
2	5 (25)
Semmes-Weinstein score (n=19)	8.7 (2.7)[a]
Wound location	
Toe	5 (25)
Midfoot	12 (60)
Heel	3 (15)
Wound age (wk)	36.4 (38.1)[a] Median: 23.4 (42)
Wound age ≥26 wk	9 (45)
Wound age ≥52 wk	5 (25)
Initial area (cm²)	2.5 (1.5)[a] Median: 1.9 (2.6)
Initial depth (mm)	2.4 (1.3)[a]
Ulcer new or recurring [b]	
New	14 (70)
Recurring	6 (20)
Infected at baseline	0 (0)
Wound exudate type	
Serous	6 (30)
Serosanguinous	14 (70)
Wound exudate amount (n=19)	
Light	13 (65)
Moderate	6 (35)
Ulcer status prior to treatment	
No change	9 (45)
Improving	7 (35)
Was healed, now open	3 (15)
Prior treatments (all)	0.9 (1.1)[a]
Antimicrobial dressings	5 (25)
Collagen dressings	4 (20)
Graft	1 (5)
Cellular- and/or tissue-based product	1 (5)
Hyperbaric oxygen therapy	3 (15)
Negative pressure wound therapy	4 (20)
Offloading	
None	2 (10)
Shoe	2 (10)
Boot	15 (75)
Total contact cast	1 (5)

[a] Mean and standard deviation provided.

[b] Recurring means the study ulcer includes all or a portion of a prior ulcer area that had previously healed.

eTable 5. Description of AEs by category (ITT population, n=20)

AE CATEGORY	N	SAE (n)	DRAE (n)	SEVERITY
Embolism	2	1	0	Severe=2
Gastrointestinal problem	2	0	0	Mild=1 Moderate=1
Infection, other	2	1	0	Mild=2
Infection, other wound	2	0	0	Moderate=2
Infection, study DFU	4	0	1	Mild=3 Moderate=1
New DFU	6	0	0	Mild=5 Moderate=1
Gout	1	0	0	Mild
Pain at study ulcer	1	0	1	Mild
Friction blister at study ulcer	1	0	0	Mild
Friction blister, other wound	1	0	0	Moderate
Prophylactic use antibiotics	1	0	0	Mild
Psychiatric issue	1	1	0	Moderate
Rash	1	0	0	Mild
Restless leg syndrome	1	0	0	Mild
Sudden increase area study ulcer	2	0	0	Mild=2 Moderate=1
Traumatic injury (not study wound)	2	0	0	Mild=1 Moderate=1
Traumatic injury (study wound)	1	0	0	Moderate=1
Urinary tract infection	1	0	0	Mild
Total	**32**	**3**	**2**	**Mild=21** **Moderate=9** **Severe=2**

AE: adverse event; ITT: intent-to-treat; SAE: serious adverse event; DRAE: device-related adverse event; DFU: diabetic foot ulcer

eTable 6. Proportion of patients experiencing AEs, SAEs, and DRAEs, and venous access issues for the ITT and PP populations

METRIC	PROPORTION OF ITT POPULATION (95% CI)	PROPORTION OF PP POPULATION (95% CI)
AEs (proportions)		
Clopper-Pearson	0.80 (0.563–0.943)	0.83 (0.586–0.964)
Wilson (no continuity correction)	0.80 (0.584–0.919)	0.83 (0.608–0.942)
SAEs (proportions)		
Clopper-Pearson	0.10 (0.012–0.317)	0.11 (0.014–0.347)
Wilson (no continuity correction)	0.10 (0.28–0.301)	0.11 (0.031–0.328)
DRAEs (proportions)		
Clopper-Pearson	0.05 (0.001–0.249)	0.06 (0.001–0.273)
Wilson (no continuity correction)	0.05 (0.009–0.236)	0.06 (0.010–0.258)
Lack of venous access (BCP)		
Clopper-Pearson	0 (0–0.168)	0 (0–0.185)
Wilson (no continuity correction)	0 (0–0.161)	0 (0–0.176)
Lack of venous access (other reason)		
Clopper-Pearson	0 (0–0.169)	0 (0–0.185)
Wilson (no continuity correction)	0 (0–0.161)	0 (0–0.176)

AE: adverse event; SAE: serious adverse event; DRAE: device-related adverse event; ITT: intent-to-treat; PP: per-protocol; CI: confidence interval; BCP: blood clot product