

Protocol

PREPUP ONE: A Multicenter Randomized Trial Protocol Evaluating Early Wound-Area Response to Topical Wound-Bed Management Versus Clostridial Collagenase in Diabetic Foot Ulcers

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Background: Diabetic foot ulcers (DFUs) are associated with impaired healing, infection, hospitalization, amputation, mortality, and substantial health care costs. Building on prior randomized and observational evidence with ON101, PREPUP ONE will evaluate early wound-bed optimization with ON101 versus clostridial collagenase ointment using Week 4 percent area reduction (PAR).

Methods: PREPUP ONE is a prospective, multicenter, active-comparator, parallel-group randomized trial embedded in an adaptive hybrid master protocol. Approximately 100 adults with DFUs will be randomized 1:1 at up to 15 SerenaGroup research or affiliated centers to NWBOT + standard of care (SOC) or clostridial collagenase ointment (CCO) + SOC. Study products will be applied at treatment visit 1 (TV-1) through to TV-4. In addition, CCO will be applied once daily by participants at home, whereas NWBOT will be provider-applied at a frequency consistent with the applicable Instructions for Use and the healthcare provider's instructions during the 4-week treatment period. SOC includes wound cleansing, sharp debridement, moisture-balancing dressings, and offloading. Although treatment allocation cannot be masked, wound-area assessments will use protocol-specified digital imaging and physical examination and will be reviewed by an independent blinded adjudication panel using de-identified digital images.

The primary endpoint is Week 4 PAR measured by protocol-specified digital planimetry. The definitive frequentist analysis will use analysis of covariance adjusted for log baseline wound area and site or pooled site, with a proposed 10-PAR-point non-inferiority margin. A complementary Bayesian, estimation-only hurdle-gamma analysis of weekly wound area will derive Week 4 PAR from posterior model estimates. Participants will be followed for harms and selected patient-reported outcomes through Week 16. Supportive, nonrandomized comparisons with synthetic SOC, synthetic topical collagen, and matched topical hydrogel cohorts are planned.

Results: Results will be reported in prespecified interim and final analyses after the applicable data cuts, quality-control procedures, and source-document reconciliation are complete.

Conclusions: PREPUP ONE establishes a multicenter, randomized active-comparator framework to evaluate early wound response within a standardized US DFU care pathway. By integrating objective Week 4 PAR assessment with subsequent clinical and patient-reported outcomes, the trial is designed to generate clinically interpretable evidence on early wound improvement and its association with the subsequent clinical course and selected downstream clinical outcomes of DFU management.

Keywords: collagenase; diabetic foot ulcer; ON101; percent area reduction; randomized trial; wound bed preparation

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INTRODUCTION

More than 500 million people worldwide live with diabetes, most with type 2 diabetes.¹ Diabetic foot ulcers (DFUs) are a common complication associated with pressure, friction, gait abnormalities, neuropathy, peripheral arterial disease, and injury.^{2–4} An estimated 19% to 34% of people with diabetes develop a DFU, and affected patients face substantial five-year mortality.³ DFU care also imposes substantial costs and socioeconomic disparities.^{5–8}

Meaningful DFU outcomes extend beyond complete closure to include wound bed quality. A moist wound environment supports keratinocyte migration, angiogenesis, extracellular-matrix formation, and tissue repair.⁹ Percent area reduction (PAR) during the first several weeks of treatment provides an objective measure of early DFU response and has repeatedly been associated with subsequent wound closure. In a prospective multicenter study, Sheehan et al.¹⁰ identified a wound-area reduction of approximately 53% at Week 4 as the midpoint separating patients whose DFUs subsequently healed from those whose DFUs did not heal by Week 12. Subsequent studies similarly demonstrated that DFUs achieving approximately 50% or greater area reduction by Week 4 were substantially more likely to achieve later complete closure than wounds with a smaller early response.¹¹ More recent analyses have continued to support Week 4 PAR as a clinically informative prognostic measure.¹² Accordingly, early PAR can provide information regarding whether a wound is progressing along a favorable healing trajectory before complete closure can be assessed.

Bonvadis® Topical Matrix (ON101, ONENESS Biotech Co., Ltd., Taiwan) is a semi-viscous, semi-solid topical matrix formulation designed to conform to irregular wound geometry, provide consistent wound-surface coverage, and support effective wound-bed management. ON101 has been evaluated across randomized and observational clinical settings in patients with DFUs. In a multicenter randomized trial of 236 patients, complete wound closure during the 16-week treatment period occurred in 60.7% of patients treated with ON101 compared with 35.1% treated with an absorbent dressing.¹³ Subsequent analyses demonstrated directionally consistent healing effects across several subgroups with characteristics associated with hard-to-heal DFUs, while later real-world evidence also reported a higher likelihood of complete closure among patients treated with ON101.^{14,15} Economic analyses conducted in Taiwan and Singapore have further suggested that improvements in wound closure may have health-economic implications.^{16,17} Preliminary clinical experience in the US has provided additional feasibility and wound-response observations¹⁸; however, contemporary randomized evidence generated specifically within US wound-care practice remains limited. Together with the established prognostic relationship between early wound-area reduction and subsequent healing, these findings provided the rationale for a prospective US randomized comparison focused on early wound-bed optimization against an active wound-bed preparation comparator.

Clostridial collagenase ointment (CCO; Collagenase Santyl® Ointment, Smith+Nephew, US) is an enzymatic debridement treatment that selectively removes devitalized collagen and supports wound bed preparation. Available comparative evidence has evaluated topical therapies, including collagenase, for DFUs.¹⁹

PREPUP ONE is a prospective trial conducted across multiple centers. It uses a parallel-group active-comparator design with a hybrid master protocol. The protocol combines a randomized comparison of Bonvadis® Topical Matrix, referred to as novel wound-bed optimization therapy (NWBOT), + SOC with CCO + SOC and supportive external-control comparisons. The randomized comparison remains the sole basis for the confirmatory analysis; external comparisons are supportive. PREPUP ONE therefore focuses on the early treatment period, when differences in wound-bed progression may first become clinically apparent. The primary randomized comparison tests whether NWBOT produces at least comparable—and potentially

greater—Week 4 wound-bed improvement relative to CCO, while responder thresholds, granulation, and subsequent clinical follow-up are intended to characterize whether this early response is consistent with a favorable healing trajectory. With follow-up through Week 16 for safety, pain, wound-related quality of life, hospitalization, amputation, and escalation of wound care, the design of this study is intended to connect an early, quantitatively measured wound response with clinically relevant downstream observations while avoiding assumptions regarding benefits that have not yet been demonstrated.

OPEN SCIENCE

This trial was registered on ClinicalTrials.gov on 19 AUG 2026 with the protocol number of Pro04051910 and the trial identifier of NCT07779239. The record can be accessed on <https://clinicaltrials.gov/study/NCT07779239>.

This manuscript was prepared from protocol version 2, primary frequentist statistical analysis plan (SAP) version 1.0, and complementary Bayesian SAP version 1.0.

METHODS

There was no patient or public involvement in the development of the protocol, SAPs, or informed consent form.

Participants will be recruited from investigators' clinical practices at up to 15 SerenaGroup research or affiliated wound-care centers in the US and from the general public. Institutional review board (IRB)-approved social media advertising may be used to call for participants. The study population will consist of adults with diabetes receiving care for DFUs or minor-amputation wound sites. The protocol anticipates recruitment of at least 1.5 participants per site per month and overall study completion in approximately six months.

Investigational sites will be selected based on demonstrated wound-care experience, access to the relevant patient population, adequate facilities and resources, and a history of adherence with Good Clinical Practice (GCP). Investigators and sites will be required to attest that they have not been disqualified from clinical research by a government agency. New sites will undergo an in-person or virtual site-qualification visit conducted by the contract research organization (CRO) medical director or a senior designee. Qualification includes review of the facility and site standard operating procedures and interviews with the principal investigator, subinvestigators, and research staff. Established CRO sites that have participated satisfactorily in a CRO trial within the preceding 3 years may be exempt from a new qualification visit at the medical director's discretion. Site acceptance will require satisfactory qualification and confirmation of the regulatory approvals, personnel, facilities, and operational resources needed to conduct the trial.

Before activation, investigator licenses and current GCP training for all participating personnel will be documented in the electronic trial master file. Site personnel will complete protocol training covering eligibility criteria, study procedures, and endpoints, as well as study-specific instruction in electronic data capture, safety reporting, investigational-product handling, applicable devices, and clinical procedures such as debridement. The principal investigator will confirm that the site and delegated personnel are prepared to begin enrollment, after which a formal activation letter will authorize trial activities. Intervention-related procedures will be performed by appropriately licensed investigators, subinvestigators, or delegated clinical personnel working under principal-investigator oversight and trained in the applicable protocol and procedures. The site policies will not prescribe a numerical minimum for prior trials or procedures beyond

the requirements for relevant wound-care experience, applicable licensure, documented training, satisfactory qualification, and demonstrated readiness.

INTERVENTION AND COMPARATOR

Intervention

Bonvadis® Topical Matrix (NWBOT) is a nonsterile, water-based, preserved, semi viscous, semi-solid topical matrix formulation with proprietary extracts of Mexican mint and *Centella asiatica*, indicated for the management of partial- and full-thickness wounds, postsurgical wounds, first- and superficial second-degree burns, diabetic foot ulcers, venous stasis ulcers, and pressure ulcers.²⁰ NWBOT will be applied directly to the wound bed by physicians or other healthcare professionals to cover the entire irregular wound surface, support wound-bed management, and prepare the wound for subsequent interventions when clinically indicated.²⁰

Under the protocol-defined trial regimen, NWBOT is provider-applied at a frequency consistent with the applicable Instructions for Use and the healthcare provider's instructions.

Comparators

CCO (Collagenase Santyl® Ointment, Smith+Nephew, US) is an enzymatic debridement treatment indicated for removal of necrotic tissue from chronic dermal ulcers. Derived from *Clostridium histolyticum*, collagenase selectively digests collagen within devitalized tissue, facilitating wound bed preparation while preserving healthy tissue and supporting granulation tissue formation and re-epithelialization.

Three additional comparator cohorts will be evaluated in exploratory, supportive analyses:

sSOC: a synthetic standard of care comparator cohort derived from 10 US DFU randomized controlled trials, including prespecified comparator cohorts treated with foam dressings and/or calcium alginate dressings

sCOLL: SOC plus Synthetic topical collagen comparator cohort derived from a historical DFU clinical trial database

mHYDRO: a propensity score-matched external comparator cohort treated with topical hydrogel

STANDARD OF CARE

All participants in the prospectively randomized treatment groups will receive a protocol-defined standard of care (SOC) beginning at the initial treatment visit and continuing throughout the 4-week treatment phase. The common SOC procedures are intended to minimize between-site variability and include standardized wound cleansing, sharp selective debridement, moisture-balancing dressings, offloading when indicated, participant education, and assessment and documentation of treatment adherence. At treatment visit 1 (TV-1) through TV-4, the target-ulcer and periwound area will be assessed, photographed, and measured before and after debridement according to the protocol. At TV-5, open ulcers will undergo protocol-defined SOC, including wound assessment, cleansing, discretionary sharp selective debridement, digital imaging and measurement, dressing application, and offloading. Hyperbaric oxygen therapy (HBOT) and cellular, acellular, or matrix-like products (CAMPs) are prohibited during the 4-week randomized treatment phase.

At each treatment visit, the existing dressing will be removed, and the amount and type of exudate, odor, dressing integrity, target-ulcer appearance, and condition of the periwound skin will be assessed. The target-

ulcer and surrounding skin will be cleansed using normal sterile saline (NSS) as the default solution. Mild soap and water may be used only when explicitly permitted by the protocol. Antiseptic cleansing agents are not permitted during the treatment phase except under a protocol-defined exemption or after consultation with the medical monitor. Cleansing will include the plantar and dorsal surfaces, interdigital spaces, heel, and malleolar areas; interdigital areas will be dried thoroughly. Cleansing will be performed using sufficient volume of the NSS and gentle mechanical force to remove debris while minimizing trauma to viable tissue. The solution used, anatomical extent of cleansing, participant tolerance and pain, deviations from the protocol, and any adverse events will be documented.

Sharp selective debridement is the protocol-permitted debridement modality and will be performed by qualified clinicians trained and experienced in the procedure. After cleansing and assessment, devitalized tissue, including eschar, slough, visible biofilm, and other nonviable material, will be excised using sterile technique while preserving viable structures. Topical or local anesthesia may be administered as clinically indicated. Bleeding will be controlled by direct pressure whenever possible, with cautery used if necessary. The frequency and extent of debridement will be determined by the treating investigator based on wound condition, participant tolerance, and the balance between adequate removal of devitalized tissue and avoidance of excessive tissue trauma.

Enzymatic, autolytic, ultrasonic, hydrosurgical, and other non-sharp selective debridement modalities are prohibited during the treatment phase, except enzymatic debridement resulting from CCO is permitted for participants randomized to the CCO + SOC group only. Participants requiring extensive surgical debridement under general anesthesia are not eligible. Any nonpermitted debridement must be recorded; repeated use on more than one occasion will result in study withdrawal. Each debridement event will be documented in the medical record and case report form, including the date and visit, tissue type and extent removed, post-debridement wound-bed appearance, bleeding and method of hemostasis, participant pain and tolerance, and any protocol deviation.

Adequate sharp selective debridement is defined as removal of necrotic tissue, slough, and visible biofilm while preserving viable tissue and avoiding greater aggressiveness than clinically indicated. Each procedure will document tissue type and extent removed, post-debridement wound-bed appearance, bleeding and hemostasis, pain, and tolerance. In the comparator arm, CCO provides enzymatic debridement between weekly visits; consistent with the comparator IFU, CCO use will terminate when debridement of necrotic tissue is complete and granulation tissue is well established. Technique will be standardized through protocol training and required procedural documentation. Clinical research associates will monitor debridement records, and independent blinded assessors will review serial deidentified images to identify potential arm- or site-level differences in debridement.

After cleansing, debridement, imaging, and measurement, the assigned study product will be applied according to randomization group and covered with a protocol-approved calcium alginate or foam dressing supplied by CRO. Dressing absorbency will be selected according to the amount and type of exudate, wound characteristics, anatomical location, periwound condition, and investigator judgment. Foam may be selected for wounds with low exudate when clinically appropriate and consistent with protocol-defined dressing criteria. The protocol identifies alginate or hydrofiber, with a foam secondary dressing when needed, for moderate-to-heavy exudate; any option other than the protocol-approved calcium alginate or foam dressings requires medical-monitor authorization. Additional absorptive material may be added for highly exudative wounds with medical-monitor approval. Wet-to-dry dressings are prohibited.

Dressings will be applied to conform to the wound bed without overpacking, unnecessary bulk, or extension onto intact skin. Particular care will be taken to prevent dressing-related shear or pressure beneath offloading devices. At every visit, study personnel will document exudate amount and type, periwound condition, dressing type, dressing integrity, and any early dressing change and its rationale. Dressing selection or absorbency will be adjusted if strike-through, leakage, maceration, displacement, increased drainage or odor, or repeated early changes occur.

Protocol-directed offloading is required for plantar DFUs and other ulcers exposed to external pressure. Adequate perfusion and potential contraindications, including severe peripheral arterial disease, untreated infection, clinically important edema fluctuation, and inability to ambulate safely, will be evaluated before a device is selected. The preferred modalities are a removable fixed-ankle Walker (Foot Defender®, Defender Ops, US) or a total contact cast (TCC). A removable cast walker or fixed ankle walker may be used when a nonremovable option is unsuitable, and the alternative is approved by the medical monitor or required for participant safety. Participants must agree to use the prescribed offloading method for the duration of the study.

Device fit, integrity, pressure redistribution, participant comfort, skin condition, and adherence will be assessed at each visit. For a removable fixed-ankle Walker, staff will confirm proper sizing and fit, modify padding or inserts as clinically indicated, and instruct the participant to use the device continuously during ambulation unless otherwise directed. When a TCC is used, bony prominences will be protected, the foot and ankle will be positioned neutrally, and pressure will be distributed evenly. The initial cast change will generally occur 3–4 days after application and subsequent changes approximately weekly or sooner when clinically indicated. At each change, the skin and wound will be examined for pressure injury, maceration, new lesions, or other complications before the cast is reapplied or an alternative modality is selected. The modality, fit, modifications, time since the last change, participant-reported use, periods of nonuse, intolerance, and adverse effects will be recorded.

Dressings must be kept dry and intact, and participants must contact the study site if a dressing becomes soiled, displaced, or unexpectedly removed. However, the exception is made for the protocol-directed product application and dressing change. At TV-1 through TV-5, staff will record planned and actual product applications, dressing changes, missed applications, and participant-reported offloading use in the electronic data-capture system.

OBJECTIVES AND ENDPOINTS

The primary objective is to determine the between-arm difference in Week 4 PAR for NWBOT + SOC versus CCO + SOC as a quantitative measure of early wound response during wound-bed management. PAR is calculated by:

$$PAR_i = 100 \times \frac{A_{i0} - A_{i4}}{A_{i0}},$$

where A_{i0} is post-debridement target-ulcer area at TV-1/Day 0 and A_{i4} is area at TV-5/Day 28, both measured by protocol-specified digital planimetry. When multiple measurements fall within the Week 4 window, the measurement closest to Day 28 will be used; ties use the later measurement. Documented closure before Week 4 is assigned PAR of 100%. Other PAR values will not be truncated and may be negative.

The primary endpoint is the between-arm difference in Week 4 PAR derived from protocol-specified digital photographic planimetry at TV-1 and TV-5 for NWBOT + SOC versus CCO + SOC. Digital planimetry is the authoritative measurement; physical examination provides a supportive clinical assessment. The remainder of the study objectives and endpoints are shown in **Table 1**, in addition to the participant-level analysis metric.

Table 1. Study objectives, prespecified outcomes, and analysis features

Objective	Endpoint	Timepoint	Metric and Aggregation
Primary			
To determine the between-arm difference in PAR at Week 4 for NWBOT + SOC vs CCO + SOC	Between arm difference in PAR measured with digital photographic planimetry, using an imaging device, and physical examination for NWBOT + SOC vs CCO + SOC.	TV-1, TV-5	Adjusted between-group mean difference in PAR points
Secondary			
To determine the between-arm difference in proportion of subjects reaching PAR $\geq$ 40% at Week 4 for NWBOT + SOC versus CCO + SOC	The between-arm difference in the proportion of subjects reaching PAR $\geq$ 40% at 4 weeks for NWBOT + SOC versus CCO + SOC.	TV-1, TV-5	Risk and risk difference
To determine the difference in PAR at 4 weeks for NWBOT + SOC vs sSOC.	PAR measured with digital photographic planimetry, using an imaging device, and physical examination for NWBOT + SOC vs sSOC.	TV-1, TV-5	Adjusted mean difference using prespecified external-control methods
To determine the difference in PAR at 4 weeks for NWBOT + SOC vs sCOLL.	PAR measured with digital photographic planimetry, using an imaging device, and physical examination for NWBOT + SOC vs sCOLL.	TV-1, TV-5	Adjusted mean difference using prespecified external-control methods
To determine the difference in PAR at 4 weeks for NWBOT + SOC vs mHYDRO.	PAR measured with digital photographic planimetry, using an imaging device, and physical examination for NWBOT + SOC vs mHYDRO.	TV-1, TV-5	Adjusted mean difference using prespecified external-control methods

To determine the between-arm difference in the frequency and nature of adverse events in subjects receiving NWBOT + SOC vs CCO + SOC at 16 weeks.	The number of product- or procedure-related adverse events in subjects receiving NWBOT + SOC vs CCO + SOC.	Weekly, TV-1 through TV-5, FV	Participant incidence, event counts, and risk difference
To determine the between-arm difference in the frequency and nature of adverse events in subjects receiving NWBOT + SOC vs sSOC vs sCOLL vs mHYDRO.	The number of product- or procedure-related adverse events in subjects receiving NWBOT + SOC vs sSOC vs sCOLL vs mHYDRO.	Weekly, TV-1, TV-5, FV	Participant incidence, event counts, and risk difference
To determine the between-arm difference in the frequency and nature of serious adverse events in subjects receiving NWBOT + SOC vs CCO + SOC at 16 weeks.	The number of serious product- or procedure-related adverse events in subjects receiving NWBOT + SOC vs CCO + SOC at 16 weeks.	Weekly, TV-1, TV-5, FV	Risk and risk difference
To determine the between-arm difference in quality of life for subjects receiving NWBOT + SOC vs CCO + SOC using the Forgotten Wound Score (FWS) questionnaire.	Change in quality-of-Life based on the FWS in subjects receiving NWBOT + SOC vs CCO + SOC.	TV-1, TV-5, FV	Adjusted between-group mean change
To determine the between-arm difference in pain follow-up for subjects receiving NWBOT + SOC vs CCO + SOC.	Change in pain in the target-ulcer assessed using the Numeric scale. in subjects receiving NWBOT + SOC vs CCO + SOC.	Weekly, TV-1, TV-5	Adjusted between-group mean change
To determine the between-arm difference in pain follow-up for subjects receiving NWBOT + SOC vs CCO + SOC.	Change in pain in the target-ulcer assessed using the Numeric scale. in subjects receiving NWBOT + SOC vs CCO + SOC.	Weekly, TV-1, TV-5	Adjusted between-group mean change
To determine the between-arm difference in the rate of severe	The number of severe adverse wound-related outcomes—including	Weekly, TV-1, TV-5, FV	Risk and risk difference

adverse wound-related outcomes including lower extremity amputation, unplanned hospitalization, and wound deterioration requiring escalation of care, through 16 weeks of follow-up for subjects receiving NWBOT + SOC vs CCO + SOC.	lower extremity amputation, unplanned hospitalization, and wound deterioration requiring escalation of care in subjects receiving NWBOT + SOC vs CCO + SOC.		
Exploratory			
To determine the between-arm difference in the time to complete granulation for non-healing DFUs with the NWBOT + SOC vs CCO + SOC over 4 weeks (Non-Inferiority).	Time to complete granulation tissue for the target ulcer in subjects receiving NWBOT + SOC vs CCO + SOC.	Weekly, TV-1, TV-5	Time-to-event distribution and hazard ratio, if events permit
To determine the between-arm difference in proportion of subjects reaching PAR $\geq$ 50% at Week 4 for NWBOT + SOC versus CCO + SOC	The between-arm difference in the proportion of subjects reaching PAR $\geq$ 50% at 4 weeks for NWBOT + SOC versus CCO + SOC.	TV-1, TV-5	Risk and risk difference

CCO–Collagenase Santyl; mHYDRO–external comparator topical hydrogel; NWBOT–Novel Wound Bed Optimization Therapy; PAR–percent area reduction; SOC–standard of care; sSOC–synthetic standard of care; sCOLL–synthetic topical collagen comparator + standard of care; TV–treatment visit

Complete target-ulcer closure at Week 16 and closure confirmed through blinded review of final-visit images will be summarized as exploratory outcomes. The FWS is administered at TV-1, TV-5, and FV. Target-ulcer pain is assessed on a 0–10 numerical rating scale. Fitzpatrick skin phototype (I–VI) is recorded at baseline to characterize the study population.

PARTICIPANTS

Inclusion and Exclusion Criteria

The study population consists of adults with diabetes presenting with non-healing DFUs or eligible diabetes-related minor-amputation wound sites who are receiving care at participating wound care centers. Eligible minor-amputation wound sites must otherwise satisfy the target-wound criteria and will be analyzed within the randomized arms. Patients are recruited from clinic populations and undergo screening to determine eligibility in accordance with protocol-defined criteria.

To be eligible for the PREPUP ONE study, participants must:

be at least 18 years old; have type 1 or type 2 diabetes; have a target DFU measuring 1.0–15.0 cm² after debridement; have less than 50% granulation tissue as estimated by the principal investigator on physical examination; have a target-ulcer present after at least four weeks of SOC; have a target-ulcer on the foot with at least 50% below the malleolus and not on the dorsal toes; have an ulcer extending through dermis or subcutaneous tissue, possibly involving muscle, without exposed tendon or bone; have adequate perfusion documented within three months by ankle-brachial index 0.7–1.3, toe-brachial index ≥ 0.6 , transcutaneous oxygen measurement ≥ 40 mmHg, or biphasic pulse-volume recording; when multiple ulcers are present, have ulcers separated by at least 2 cm, with the largest eligible ulcer designated as the target; agree to the prescribed offloading method and weekly visits; and be willing and able to provide informed consent.

Participants will be excluded for: life expectancy under six months; an ulcer not secondary to diabetes; target-ulcer infection, surrounding cellulitis, exposed tendon or bone, or osteomyelitis; immunosuppressive therapy, systemic corticosteroids above 10 mg prednisone daily or equivalent, cytotoxic chemotherapy, or other medication judged likely to interfere with healing; topical steroid use on the ulcer within one month; HbA1c $\geq 12\%$ within three months; a partial-amputation deformity or Charcot foot that prevents adequate offloading; a target wound demonstrating $>30\%$ reduction in wound area over approximately 2 weeks before randomization, based on available historical medical-record measurements. (This threshold is used only as a historical screening rule-out and is distinct from the prospectively measured Week 4 responder endpoints); pregnancy or plans to become pregnant within six months; end-stage renal disease requiring dialysis; participation in an investigational-treatment trial within 30 days; a medical or psychological condition judged likely to interfere with assessments; use of HBOT or a cellular, acellular, or matrix-like product within 30 days; malnutrition, defined as a Mini Nutritional Assessment–Short Form score of 0–7; or known or suspected hypersensitivity to collagenase or a NWBOT ingredient.

VULNERABLE POPULATIONS

While vulnerable participants will not be specifically recruited, they may be present in the potential participant pool. The IRB-approved informed-consent form must be signed by the participant or a legally acceptable surrogate and by the investigator-designated research professional obtaining consent. Enrollment is limited to adults able to participate in the informed-consent process. A urine pregnancy test will be performed at screening/TV-1 for participants of childbearing potential. Pregnant individuals and those planning pregnancy within six months are excluded. Participants who become pregnant during the trial will discontinue study treatment, be withdrawn, and receive follow-up under SOC procedures.

SUBJECT WITHDRAWAL

Participation is voluntary, and participants may withdraw consent at any time without penalty or loss of benefits or medical care. The investigator or sponsor may end participation if continued involvement appears medically harmful, pregnancy occurs, study directions are not followed, eligibility changes, or another protocol-defined reason applies. Participants who withdraw early may be asked to complete TV-5 procedures but may refuse them. They will receive compensation only for completed scheduled visits and will return to wound care prescribed by their treating clinician.

SCREENING PHASE

Pre-screening will determine whether the investigator believes a potential participant may meet study qualifications. Screening, consent, eligibility confirmation, randomization, and treatment initiation normally occur at TV-1 (Day 0). Screening may occur up to seven days before TV-1. Screening includes medical history, physical examination, wound assessment, vascular testing, HbA1c testing, pregnancy testing when applicable, nutrition assessment, skin-type assessment, and ulcer-severity assessment. Individuals who initially fail screening may be re-screened up to two additional times; each re-screening requires re-consent and a new screening number.

STUDY PROCEDURES

At TV-1 through TV-4, the target-ulcer will be cleansed with normal sterile saline, photographed and measured before debridement, sharply debrided to remove nonviable tissue and slough, photographed and measured again, and covered with a protocol-approved calcium alginate or foam dressing. Plantar ulcers and ulcers exposed to pressure will receive protocol-defined offloading. Hyperbaric oxygen therapy and cellular, acellular, or matrix-like products are prohibited. At TV-5, open ulcers will undergo wound assessment, digital imaging and measurement, protocol-defined SOC, dressing application, and offloading without further randomized product application.

Participants receiving CCO must apply the assigned product once daily, whereas participants receiving NWBOT will receive provider-applied treatment at a frequency consistent with the applicable Instructions for Use and the healthcare provider's instructions. At TV-1 and each subsequent treatment visit at which continued product application is required, the site will dispense sufficient CCO to participants receiving CCO for use until the next scheduled clinic visit and provide CCO application and dressing-change instructions. At each weekly visit, staff will record planned and actual product applications, dressing changes, missed applications, and participant-reported offloading use in the electronic data-capture system; reinforce treatment-specific instructions; and dispense supplies as needed. Repeated non-adherence will be reviewed by the principal investigator and medical monitor and may lead to withdrawal. Study product will be held during target-ulcer infection and resumed when the investigator determines that the infection has resolved.

Participants will have five scheduled treatment-phase visits from Day 0 through Day 28 and a follow-up-only visit on Day 112 (± 3 days); no interim study visits are planned. Only the protocol-specified procedures listed for the follow-up visit will be performed. After Week 4, participants may receive any clinically indicated therapy; this care is outside the randomized treatment period and is not recorded as study data. Participants who withdraw early will be asked to complete TV-5 procedures; if permission is granted, survival status will be collected through the planned follow-up period. A participant is considered lost to follow-up after five telephone calls and three letters, including at least one certified letter.

Digital planimetry will use a MolecuLightDX device (MolecuLight Inc., Canada). Devices are calibrated during manufacture. Before acquiring study images, site staff will complete manufacturer e-training in device use, measurement, and interpretation. Clinical research associates will review image quality, query deficiencies, and escalate unresolved interpretation issues for medical review. Published validation of MolecuLightDX area measurement reported mean percentage error below 10% and intra- and interuser coefficients of variation below 10% across diverse wound types and skin tones.²¹ Baseline and Week 4 images used to derive the primary PAR endpoint, as well as presumed-closure and final-visit images, will be reviewed by two independent assessors blinded to treatment allocation and study site. Digital planimetry is the

authoritative source for primary PAR; physical examination is supportive. If the assessors disagree or an image is uninterpretable, a third blinded assessor will make the final determination, and any replacement measurement will follow the protocol-defined reacquisition procedures.

Table 2. Schedule of enrollment, intervention, and assessment

Procedure	TV-1 (Day 0)	TV-2 (Day 7)	TV-3 (Day 14)	TV-4 (Day 21)	TV-5 (Day 28)	FV-1 (Day 112)
Record Medical History and Demographic Information	X					
Assessment of Eligibility	X					
Sign Informed Consent Form	X					
Vascular Screening Test	X					
Physical Exam	X					
Pregnancy Test	X					
HbA1c	X					
Wagner Grade	X					
Mini Nutritional Assessment (MNA)	X					
Fitzpatrick Scale	X					
Randomization	X					
Assessment for AE and SAE	X	X	X	X	X	X
Review medication for changes		X	X	X	X	X
Vital Signs	X	X	X	X	X	
Wound Assessment	X	X	X	X	X	X
Pain Assessment	X	X	X	X	X	X
Forgotten Wound Score (FWA)	X				X	X
Study Ulcer Cleaning, Debridement (if applicable)	X	X	X	X	X	
Study Ulcer Area with Digital Images	X	X	X	X	X	X
Treatment Based On Randomization	X	X	X	X		
Apply Dressing	X	X	X	X	X	
Offloading	X	X	X	X	X	

DOCUMENTATION AND RECORDS

Clinical data will be entered directly into Greenlight Guru Clinical. Supplemental source records may include laboratory reports, images, medication records, and other clinical documents. Electronic case report forms will record all required data; missing fields must be explained. Investigators will review and electronically sign completed records. Study records will be retained for up to five years after study completion, and investigators must notify the sponsor at least 30 days before scheduled destruction.

The source protocol requires an informed consent form enrollment tracker documenting participant identifier, consent and verification status, consent date and version, re-consent, issues, and eligibility waivers. Signed consent forms will be uploaded when feasible; otherwise at minimum the first and signature pages will be retained with confirmation of Good Clinical Practice compliance.

STATISTICAL METHODS*Randomization and Stratification*

Individual participants will be randomized electronically in Greenlight Guru Clinical in a 1:1 ratio to NWBOT + SOC or CCO + SOC. The SAPs specify a non-inferiority framework for the primary randomized comparison. The protocol does not specify stratification factors; in particular, eligible minor-amputation wound sites will not be separately stratified. The master protocol additionally includes three non-randomized external-control cohorts: sSOC, sCOLL, and mHYDRO. External cohorts do not contribute to the primary randomized test. The randomization schedule will be generated and implemented within Greenlight Guru Clinical. Authorized site personnel will enroll eligible participants and initiate randomization within the system. Allocation will remain concealed until eligibility has been confirmed and the participant has been randomized; investigators and site personnel will not have access to the underlying allocation sequence or future treatment assignments. Study treatment will be assigned and dispensed according to the allocation generated by Greenlight Guru Clinical.

SAMPLE SIZE DETERMINATION

Approximately 100 participants, 50 per arm, are planned for the randomized comparison. The study separately anticipates at least 100 sSOC records and at least 50 mHYDRO records; sCOLL records will be included according to availability. These external-control records do not increase the randomized sample. The statistical assumptions used in this protocol, including the evidence informing the noninferiority margin and sample-size planning, were informed by aggregate data from ongoing and completed clinical trials in comparable DFU populations. These data represent the most relevant evidence currently available and support the planning assumptions for PREPUP ONE.

ANALYSIS POPULATIONS

Table 3 shows the population definitions that will be used.

Table 3. Analysis populations

Population	Definition	Use
Full Analysis Set/intent-to-treat (ITT)	All randomized participants with baseline target-ulcer area; analyzed as randomized; missing Week 4 PAR retained under the missing-data plan	Primary efficacy
Per-Protocol Set (PP)	Full Analysis Set participants without major deviations expected to materially affect Week 4 PAR; deviations classified before unblinding when possible	Non-inferiority consistency analysis
Safety Set	All randomized participants receiving at least one study-product application; analyzed according to treatment received	Safety
External-Control Sets	Participants from sSOC, sCOLL, or mHYDRO meeting harmonized criteria defined before unblinding	Supportive external comparisons

INTERIM ANALYSIS PLAN

The primary Frequentist and supplemental Bayesian SAPs specify an interim analysis for an external evidence package. The protocol publication plan also permits a preliminary clinical data report at approximately 25% of planned enrollment or another prespecified proportion. The interim data cut is descriptive and does not alter the prespecified randomized design, primary endpoint, statistical hypothesis, sample size, or analysis framework.

It attaches no efficacy, futility, stopping, sample-size-re-estimation, or design-change rule and no alpha spending. Safety oversight follows the protocol and contract research organization procedures. The analysis will be conducted by the designated statistical team using a quality-controlled data extract, with access restricted to authorized sponsor and contract research organization personnel.

OUTCOME ANALYSIS PLAN

The primary analysis will use analysis of covariance with Week 4 PAR as the dependent variable and treatment group, log baseline wound area, and site or pooled site as covariates. Site will be included as a fixed effect if cell size and stability permit. Sparse sites may be pooled; if pooling is not feasible, the fallback model will include treatment and log baseline wound area only. The adjusted mean difference, NWBOT minus CCO,

will be reported with a two-sided 95% confidence interval. Non-inferiority will be concluded if the lower confidence limit exceeds -10 PAR points.

Secondary continuous endpoints will use analysis of covariance on change from baseline or the endpoint value, adjusted for baseline when applicable. Binary endpoints will be summarized with risks, risk differences, and 95% confidence intervals; logistic regression may be used when stable. Time-to-event endpoints will use Kaplan–Meier summaries and Cox regression if event counts permit. Safety analyses will report participant incidence, event counts, severity, seriousness, relatedness, expectedness, and risk differences with 95% confidence intervals. Secondary, exploratory, safety, and external-control analyses are supportive or descriptive; no multiplicity adjustment is planned, and any p values will be interpreted as nominal and descriptive. Planned and actual product applications, dressing changes, missed applications, and participant-reported offloading use recorded in the electronic data-capture system will be summarized by arm; prespecified adherence deviations will inform the per-protocol set and sensitivity analyses.

The Bayesian SAP specifies an estimation-only analysis that complements rather than replaces the primary Frequentist analysis. Weekly post-baseline wound area will be modeled jointly across groups using a hurdle-gamma generalized linear mixed model with a log link, subject and site random intercepts, categorical visit, group, group-by-visit interaction, and log baseline wound area. If no complete closure occurs in any group by Week 4, a prespecified plain-gamma model will replace the hurdle-gamma model. Week 4 PAR and between-group PAR differences will be derived on each posterior draw from model-based mean areas.

Weakly informative priors will be used. Posterior mean and median differences, 95% equal-tailed and highest-posterior-density credible intervals, and descriptive posterior probabilities for differences above 0, 5, and 10 PAR points will be reported without a Bayesian success threshold. Four Markov chain Monte Carlo chains with at least 1,000 warm-up and 2,000 retained draws per chain are planned. Prespecified diagnostics include rank-normalized $R^2 < 1.01$, bulk and tail effective sample sizes above 400, divergence and tree-depth checks,

Missing Week 4 PAR due to missed assessment, early withdrawal, or loss to follow-up will be handled by multiple imputation under a missing-at-random assumption. Fully conditional specification with predictive mean matching will include treatment, log baseline area, site or pooled site, and available post-baseline area or PAR through Week 3. Documented closure before Week 4 will be assigned PAR of 100%. Death or major target-limb amputation before Week 4 will be assigned PAR of 0% and summarized as a safety event. Infection, product hold, discontinuation, and additional wound care will not remove a participant from the Full Analysis Set. Sensitivity analyses include Per-Protocol and complete-case analyses and delta-adjusted imputation subtracting 10 PAR points from imputed NWBOT values.

All external-control comparisons are exploratory and supportive and do not contribute to confirmatory inference. The primary Frequentist SAP states that their final method must be documented before unblinding. The later Bayesian SAP specifies no dynamic borrowing or discounting: external cohorts enter as direct comparator groups at full weight under weakly informative priors. The harmonized sSOC pool of at least 100 records will be covariate-adjusted with a source-trial random effect; mHYDRO will be propensity-score matched 1:1 to NWBOT; sCOLL will be included according to availability. External adjustment variables include baseline area, ulcer duration, Wagner grade, HbA1c, and age. Balance targets include absolute standardized mean differences below 0.10; comparisons with residual imbalance above 0.25 on a prognostic variable will be downgraded to exploratory. Sensitivity analyses include adjusted versus unadjusted estimates, overlap restriction, propensity-score alternatives, and leave-one-source-out analyses.

Analyses will be performed in Python using pandas, NumPy, SciPy, statsmodels, matplotlib, PyMC, Bambi, ArviZ, and other archived packages as needed. Final software versions, the operating environment, datasets, programs, seeds, outputs, and Bayesian inference objects will be retained.

BLINDING

As the intervention product is visibly distinguishable during treatment, blinding of participants and treating investigators is not feasible. Accordingly, PREPUP ONE is designed as an open-label study for both subjects and site personnel responsible for treatment administration. To reduce detection bias, an independent assessor will remain blinded to treatment allocation and study site. Panel members will evaluate deidentified baseline and Week 4 images used to derive primary PAR, as well as images obtained at the presumed wound-closure and final study visits, with information identifying treatment assignment removed. To further limit performance bias, standardized protocols for wound dressings and offloading will be implemented across both treatment groups, ensuring consistent application of the core components of SOC regardless of randomized assignment.

ETHICAL APPROVAL AND PATIENT CONSENT

The protocol, SAPs, and ICF were submitted to Advarra Institutional Review Board (IRB) and approved on 1st September 2026. Written approval will be provided to the investigator and sponsor before initiation. Any future amendments required will also be submitted to Advarra IRB for approval before implementation. Written informed consent must be obtained before any study procedure by an investigator-designated research professional.

The informed consent form describes randomization, study procedures, weekly treatment, risks, potential benefits, alternatives, privacy, injury, costs, compensation, and voluntary withdrawal. Study-related visits, procedures, assessments, supplies, and products will be sponsor-funded. After study exit, participants with unhealed ulcers will return to care prescribed by their treating clinician.

SAFETY CONSIDERATIONS

Adverse Events

Adverse events (AEs), serious adverse events (SAEs), adverse product effects, serious adverse product effects, product deficiencies, serious health threats, and unanticipated adverse product effects will be collected. At each scheduled and unscheduled visit, staff will review potential AEs and medication changes. Events will be documented by term, clinical detail, onset and resolution, relationship to product and procedure, expectedness, seriousness, severity, actions or treatments, and outcome. Hospitalizations, amputations, and infections will be ascertained and source-verified under the study safety SOPs. Severity will be classified as mild, moderate, or severe, and the medical monitor will review serious events.

SAEs and adverse product effects must be reported to the sponsor within three business days of investigator awareness. Other adverse events and product deficiencies must be reported within five business days. These timelines are superseded by any more stringent IRB/IEC or national reporting requirement verified before study initiation.

Target-ulcer infection and resolution will be determined clinically by the investigator based on physical examination and source documentation rather than molecular criteria. An infection occurring after randomization will be recorded as an adverse event and treated at the investigator's discretion with topical antimicrobial therapy and/or systemic antibiotics. The assigned study product will be withheld until the infection resolves, TCC offloading will be suspended during the active infection, and dressings will be changed at least every 72 hours. The participant may remain in the study unless management requires a procedure or treatment that violates the protocol. Study-product application may resume after the investigator determines that the infection has resolved.

RISKS AND BENEFITS

Protocol and consent materials identify risks from DFU progression; sharp selective debridement; infection or delayed healing; product irritation, sensitivity, or allergic reaction; offloading-related discomfort, skin injury, reduced mobility, imbalance, or falls; testing discomfort; wound photography; and loss of privacy or confidentiality. Unknown risks are possible. Pregnancy effects are unknown, and pregnancy results in treatment discontinuation and withdrawal.

Participants may benefit if assigned treatment and SOC improve wound bed preparation or healing, and the study may increase knowledge about DFU treatment; no individual benefit is promised. Alternatives include standard DFU care and other treatments recommended by the treating clinician.

DATA SHARING

Participants will receive a study identifier. Linking information will be kept separately, and electronic records will be password protected. Authorized representatives of Sponsor, CRO, the reviewing IRB, participating clinics, and regulatory agencies may inspect study records. Wound photographs will be framed to limit identifying features.

Electronic case report forms will be maintained in Greenlight Guru Clinical and analysis datasets, the operating environment, package versions, and output-generation programs will be archived. The ICF permits de-identified or limited datasets to be used by the study team or a qualified third party for future wound-care research.

Following publication of the primary study results, de-identified participant-level data supporting the published findings, the corresponding data dictionary, and statistical programming code may be made available upon reasonable request to the Sponsor. Requests must include a scientifically sound research proposal and statistical analysis plan and will be reviewed by the Sponsor in consultation with the study investigators. Approved requests will require execution of an appropriate Data Use Agreement (DUA) prior to data release. Access will be limited to de-identified data and supporting documentation necessary to reproduce the published analyses. Study protocol, statistical analysis plan, and other study materials may also be provided upon reasonable request, subject to Sponsor approval and applicable confidentiality, intellectual property, and regulatory requirements. Data containing information that could compromise participant confidentiality, proprietary Sponsor information, or contractual obligations will not be shared.

PUBLICATION PLAN

Clinical results are intended for publication or presentation at conferences. Conduct and results will be documented in clinical study reports. A preliminary clinical data report may be prepared from a prespecified interim data cut for regulatory, scientific, or evidence-development purposes. The interim data cut is descriptive and does not alter the prespecified randomized design, primary endpoint, statistical hypothesis, sample size, or analysis framework.

The ICF states that study results, including DFU photographs, may be published or presented without identifying participants and that individual research results are not planned to be provided to participants.

DISCUSSION

PREPUP ONE incorporates several design features intended to generate clinically interpretable comparative evidence in a modern US wound-care setting. Randomization against an established active wound-bed preparation therapy reduces the limitations inherent in uncontrolled or historical comparisons, while protocol-defined cleansing, sharp selective debridement, moisture-balancing dressings, and offloading are intended to reduce variability in background care across participating centers. Exclusion of rapidly improving ulcers before randomization further limits enrollment of wounds already demonstrating a favorable spontaneous trajectory. Serial digital wound measurement provides a standardized quantitative assessment of early response, and the prespecified continuous Week 4 PAR endpoint is complemented by clinically interpretable responder thresholds and longer-term safety and patient-reported assessments. Supportive external-control analyses may provide additional context but remain distinct from the randomized comparison because residual confounding cannot be eliminated through statistical adjustment alone. The randomized arms may differ in treatment administration and exposure because CCO is applied by the participant daily, whereas NWBOT will be provider-applied; electronic data-capture records of exposure and adherence will therefore be summarized by arm and examined in per-protocol and sensitivity analyses. Protocol-mandated sharp selective debridement may also reduce the necrotic substrate available to CCO, although training, documentation, monitoring, and blinded image review are intended to limit differential debridement.

Early wound-bed improvement is clinically relevant because DFU response may become apparent before complete closure, and published evidence associates early wound-area reduction with subsequent healing. PREPUP ONE prospectively tests whether Week 4 PAR with NWBOT is noninferior to that with an established enzymatic wound-bed preparation strategy. Favorable downstream findings accompanying greater early wound-area reduction would support the clinical relevance of the response but would not establish improved closure, limb preservation, infection, or treatment-burden outcomes. The trial is not powered for Week 16 closure or severe clinical outcomes; these downstream outcomes are exploratory and supportive.

From a health-system perspective, the clinical significance of an early wound-area response may extend beyond the measurement itself if differences persist during subsequent follow-up. PREPUP ONE therefore prospectively captures selected downstream outcomes, including wound-related hospitalization, escalation of wound care, lower-extremity amputation, pain, and patient-reported outcomes. These measures are intended to characterize whether the randomized treatment comparison is accompanied by differences in subsequent clinical course and treatment burden. Due to comprehensive healthcare use, direct medical costs, and preference-based health-utility measures are not collected as dedicated economic endpoints, any observed differences should be interpreted as clinical observations rather than as evidence of cost savings. They may,

however, provide empirical inputs for subsequent US-specific health-economic and resource-utilization analyses.

LIMITATIONS

A limitation of the current protocol is that use of comprehensive healthcare utilization, direct medical costs, and preference-based health-utility measures are not collected as dedicated economic endpoints. This limitation can be addressed in subsequent health-economic analyses by integrating the randomized treatment effects, healing trajectories, patient-reported outcomes, and observed clinical-event rates generated in PREPUP ONE with US-specific resource-utilization, cost, and health-utility inputs established U.S. data sources. Such analyses could extend the trial's clinical findings by evaluating whether earlier improvement in wound trajectory is associated with reduced longer-term treatment burden and improved value for patients and the U.S. health care system.

CONCLUSIONS

PREPUP ONE establishes a prospective, multicenter, randomized active-comparator framework to evaluate NWBOT + SOC against CCO + SOC within a standardized US DFU care pathway. By integrating objective Week 4 PAR assessment with protocol-defined wound care, longer-term clinical follow-up, and exploratory external-control analyses, the study is designed to evaluate early wound-area response and to describe its relationship to the subsequent course of DFU management. Any interpretation of the clinical role of NWBOT will depend on the direction and consistency of the primary result and supportive downstream findings; those downstream outcomes are not designed to establish reduced treatment burden or greater efficiency.

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Institutional Review Board Statement: The study will be conducted in accordance with the Declaration of Helsinki and was approved by the Advarra Institutional Review Board (Pro04051910).

Informed Consent Statement: Informed consent will be obtained from all subjects involved in the study prior to participation. All participants will provide written informed consent in accordance with Advarra IRB approved procedures and applicable regulatory requirements.

Data Availability Statement: De-identified participant-level data underlying the findings of this study may be made available by the corresponding author upon reasonable request, subject to sponsor approval, ongoing trial conduct considerations, and applicable data protection requirements.

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