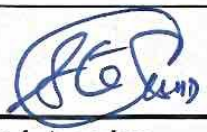
Lakewood-Amedex Inc.

LLA-Nu-3-CLIN002

A Phase 2a, Multi-Center, Randomized, Double-Blind, Placebo-Controlled Dose Escalating Study To Evaluate The Safety And Tolerability Of Topically Applied Bisphosphocin® Nu-3 Gel To Clinically Noninfected Chronic Diabetic Foot Ulcers (cDFU)

Sponsor:	**Lakewood-Amedex Inc. 3030 University Pkwy. Sarasota, FL 34243 USA Telephone: +1 941-225-2515 Fax: +1 941-225-2511**
IND No.:	**119428**
Date of Protocol:	**02-Dec-2021**
Contract Research Organization	**PERI**
Medical Monitor	**Sumita Paul 3030 University Pkwy. Sarasota, FL 34243 USA Telephone: +1 941-225-2516**
Version:	**1.0**

SPONSOR SIGNATURE OF PROTOCOL & PROTOCOL AMENDMENT(S)

Protocol Number:	LLA-Nu-3-CLIN002
Protocol Title:	A PHASE 2a, MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED DOSE ESCALATING STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF TOPICALLY APPLIED BISPHOSPHOCIN® NU-3 GEL TO CLINICALLY NONINFECTED CHRONIC DIABETIC FOOT ULCERS (cDFU)
Protocol Version and Date:	Version 1.0 02-Dec-2021
Amendment Number:	N/A; Original
Signatures:	 02Dec2021 Lakewood-Amedex Date *[Sumita Paul, MD, MPH* *Chief Medical Officer and SVP of Research & Development]*

INVESTIGATOR'S AGREEMENT

Protocol Number:	LLA-Nu-3-CLIN002
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Protocol Version and Date:	Version 1.0, 02-Dec-2021
Amendment Number:	N/A; Original

By signing this cover page, I attest that I have read and understand the contents of the Clinical Protocol LLA-Nu-3-CLIN002, Version 1.0, dated 02-Dec-2021. I agree to adhere to the design, conduct and reporting requirements of the study as stated in the clinical protocol and to my obligations to The Sponsor as described in the protocol and executed contracts between myself, my Institution and The Sponsor.

Investigator's Signature: _____

Investigator's Name: _____

Institution: _____

Date: (DD/MM/YYYY format) _____

1. SYNOPSIS

Name of Sponsor/Company:
Lakewood-Amedex

Name of Investigational Medicinal Product:
Bisphosphocin Nu-3 Gel

Protocol Number:	**Phase:**	**Country:**
LLA-Nu-3-CLIN002	2a	United States

Title of Study:
A PHASE 2a, MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED DOSE ESCALATING STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF TOPICALLY APPLIED BISPHOSPHOCIN® NU-3 GEL TO CLINICALLY NONINFECTED CHRONIC DIABETIC FOOT ULCERS (cDFU)

Study center(s): up to 5 sites

Subject Population: Male and female subjects age 18 years of age or older and up to and including 80 years of age suffering from diabetes mellitus and cDFU

Sample Size: Maximum of 32 subjects, 24 active and 8 placebo

Examination Points: Screening Day - 14, Standard-of-Care (SOC) Run-in Day - 7, Baseline Day 0, Day 7, Day 14, Day 21, Day 28 (End of Treatment/EOT) and Safety Follow-up Day 42 from first treatment. All visits are to be completed within a visit window of ± 2 days.

Study Design: Multi-Center, Randomized, Double-Blind, Placebo-Controlled, Dose Escalation Study

Clinical Investigation Objectives and Endpoints:

	Objective	**Endpoint [at 28 days]**	**Variable**
Primary	• To assess the safety and tolerability of escalating doses of topically applied Bisphosphocin Nu-3 Gel	• Treatment emergent events related to clinical investigational product	• Vital signs • Safety laboratory analysis • Local wound/skin assessments • Physical examination • Adverse events
Secondary	• To assess the clinical effects of Nu-3 on cDFU • To assess antimicrobiological effects of Nu-3 on wound microflora. • To support determination of the appropriate dose range of Nu-3 to be employed in	• Advanced digital imaging wound assessment post debridement • Pre and Post debridement digital pictures taken by clinical staff. • Changes in wound microbial population pre debridement	• Ulcer surface area • Ulcer depth • Culture and microflora microbiological analysis

	future clinical studies.	• Recommended Phase 2 Dose (RP2D)	

Clinical Investigation Duration (Per subject):
Screening + Standard-of-Care (SOC) Run-in + Treatment + Safety Follow-up.

- Screening & Run-in SOC: (14 days (± 2 days)).
- Treatment: 28 Days total
  - Safety and Efficacy evaluations: 7 days (±2 days); 14 days (±2 days); 21 days (±2 days); 28 days/EOT (±2 days).
- Safety Follow-up: 42 days (± 2 days) after first treatment

Randomization:
Randomization will be conducted to a ratio of 3:1 (active: placebo). All qualified subjects will be enrolled and randomized in two sequential Cohorts of 16 each, Cohort 1 to either Nu-3 gel at 5% or to placebo gel, and Cohort 2 to either 10% Nu-3 gel or to placebo gel. The two cohorts will be enrolled sequentially, each with 12 active and 4 placebo subjects, and safety will be evaluated by the independent Data Monitoring Committee (DMC) before proceeding to the next Cohort. At the completion of Cohort 2, the DMC will review all data and make a recommendation as to the appropriate dose for the next study.

Inclusion criteria

1. Men and women at least 18 years of age and up to 80 years of age, inclusive.
2. Voluntary written consent, given before performance of any clinical investigation-related procedure, and with the understanding that consent may be withdrawn at any time without prejudice to future medical care
3. Non-hospitalized ambulatory subjects suffering from diabetes mellitus, Type I or II per ADA criteria
4. The target ulcer is classified as a grade 1 ulcer according to a Wagner Scale. These ulcers are superficial, full-thickness ulcers limited to the dermis, not extending to the subcutis.
5. Target ulcer is between 2 and 10 cm^2 post debridement at screening and baseline.
6. The target ulcer must be no higher than the ankle (on or below the malleolus (ankle bone) with ≥50% below the malleolus.
7. Presence of a persistent cDFU for at least 4 weeks and not more than 1 year that has failed to respond to standard of care
8. Adequate vascular perfusion as evidenced by one of the following
   a. Dorsal transcutaneous oxygen measurement (TCOM) or a skin perfusion pressure (SPP) measurement of ≥ 40 mmHg
   b. Ankle Branchial Index (ABI) between 0.7 and 1.3 within 3 months of Screening using the extremity with the target ulcer
   c. Arterial Doppler ultrasound evaluating for biphasic or triphasic dorsalis pedis and posterior tibial vessels at the level of the ankle or a TBI (Toe Brachial Index) of > 0.6 is acceptable
9. Subject has a caregiver who is able to attend V1, and the Baseline visit (V3) and apply wound treatment along with study dressings for the study duration
10. Subject completed protocol-defined standardized wound care during the Screening and Run-in periods leading up to Day 0.
11. Must meet one of the following criteria:
    a. Female subjects of Non-Child-Bearing Potential defined as:

i. Postmenopausal for at least 1 year, or surgically sterilized (i.e., hysterectomy or bilateral oophorectomy more than 3 months prior to Screening), or
ii. Bilateral tubal ligation more than 6 months prior to Screening
iii. Must have a negative serum β-hCG pregnancy test at screening and not be breastfeeding prior to being administered with the study drug.

b. Male subjects of Non-Childbearing Potential defined as those vasectomized subjects whose vasectomy was performed 6 months prior to Screening or those diagnosed as sterile by a physician.
c. Females and Males of Childbearing Potential who practice an acceptable method of contraception defined as the use of any form of hormonal contraceptive, a barrier method with spermicide, condoms, intrauterine device, or abstinence from sexual intercourse starting at least 60 days prior to Screening and continuing at least 30 days following the last treatment.

12. Subjects must be willing to undergo all clinical investigation-related procedures and attend all required visits.
13. Subject must be willing to wear offloading RCW, if necessary throughout the duration of the clinical treatment

Exclusion criteria

1. Ulceration with exposed tendon, capsule, or bone
2. Suspicion of bone or joint infection by clinical or other criteria
3. Unable or unwilling to utilize the standardized offloading RCW as required per protocol
4. Target ulcer has decreased in area by ≥30% between the Screening (V1) and Baseline (V3) visits.
5. Any subject that is currently on/requires oral, systemic or topical antibiotics for the index limb, or is anticipated to require use during the course of the trial
6. Any subject that has vascular compromise requiring surgical intervention or has undergone vascular reconstruction or angioplasty less than 1 month prior to randomization. Any planned surgical procedures during the study participation
7. Serum Creatinine level >3.0 mg/dL
8. Hemoglobin A1c (HbA1c) >12%
9. Aspartate Aminotransferase (AST, GOT) and/or Alanine Aminotransferase (ALT, GPT) >3x the upper limit of normal
10. Acute active Charcot foot
11. The target ulcer is within 3 cm of any other ulcer.
12. Any subject that would be unable to safely monitor the infection status at home, and return for scheduled visits
13. History of immunosuppression or taking immunosuppressive agents including systemic corticosteroids, except stable daily doses of 5 mg/day or less for chronic conditions
14. Any subject with a life expectancy ≤ 6 months
15. Pregnancy, including a positive pregnancy test at Screening or Baseline, or lactation
16. Use of investigational drugs within 28 days prior to screening
17. History of concurrent condition that, in the Investigator's opinion, would jeopardize the safety of the subject or compliance with the protocol
18. Likely inability to comply with the protocol or cooperate fully with the investigator and site personnel
19. Known or suspected active abuse of alcohol, narcotics, or non-prescription drugs
20. Prior randomization in this clinical trial, or a previous Bisphosphocin study

Test Product

- 5% Bisphosphocin Nu-3 in gel
- 10% Bisphosphocin Nu-3 in gel
- Placebo gel at same pH as Bisphosphocin Nu-3 gel

Test Kit

Test Product kits will contain eighteen (18) 4 gm applicators of Test Product packaged individually in foil pouches. The kits and individual 4g applicators will be pre-labelled with kit numbers. The contents of the kits will not be known to any site personnel. Upon randomization of the subject through a centralized randomization service, the site personnel will receive a randomization number along with a kit number that is available at that site. The kit will be used for dose administration twice daily for one week. A new Test Product Kit will be dispensed to the subject at each of the subsequent visits on Days 7, 14, and 21.

Administration

Gel (Commercially acquired Hydrogel from V1 to V3 and after V7 through V8. Test Product gel between V3 and V7) will be applied topically to the target ulcer twice daily, roughly every 12 hours at the same times of day for four weeks during the entire study. Optimum evening treatment would occur at bedtime, once the subject is in bed for the evening. After application of each dose of the Test Product is complete, the wound will be dressed as per the following: Mepitel or equivalent will be trimmed to cover the entire dimension of the ulcer and overlaid into the ulcer, followed by Tegaderm dressing covering the entire dimension of the ulcer. Padding and Coban Lite 2- layer dressing or equivalent will be placed over the Mepitel covered with Tegaderm. Application of Test Product or any gel should not occur on the day of V7, or on the day of an early term visit. During the treatment phase, no gel other than Test Product can be administered.

Standardization of various processes are essential including subject/caregiver instruction; debridement; offloading; wound measurement/ digital imaging, digital photography and microbiological sampling and analysis as described in various appendices.

Statistical Analysis

Summary tables (descriptive statistics and/or frequency tables) will be provided for all baseline variables, efficacy variables, and safety variables. Continuous variables will be described by descriptive statistics (n, mean, standard deviation, minimum, maximum, median, 95% confidence limits for the mean) as detailed in the statistical analysis plan.

Frequency tables will be used to report adverse event frequency by system organ class (SOC), preferred term (PT), severity, and relationship to test article. All subjects receiving the test article will be included in the safety analyses.

Safety data including laboratory evaluations and vital signs assessments will be summarized by dose group and time point of collection. Descriptive statistics will be calculated for quantitative safety data, and frequency counts will be compiled for classification of qualitative safety data. In addition, a mean change from baseline table will be provided for vital signs and a shift table describing out of normal range shifts will be provided for clinical laboratory results.

Changes in physical examinations will be described in the text of the final report.

Efficacy data including clinical and microbiological response will be summarized by dose group and will include analysis per the statistical analysis plan.
Justification of Sample Size for Ascending Dose Study This is a pilot dose-ranging study. No sample size calculations were conducted. **Analysis Populations** All statistical processing will be performed using SAS® unless otherwise stated. All subjects enrolled in the study that were dispensed and applied test article at least once will be included in the analysis and will be considered in the intent-to-treat (ITT) population. **Interim Analysis** A safety review by an independent DMC will be conducted after each cohort to confirm it is acceptable to proceed to the next dose level.
Safety Assessments Adverse events: Treatment-emergent adverse events will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent adverse events will be summarized by body system and preferred term. **Parameters Requiring Pause in Treatment** Any one of the following would require a pause in treatment for the subject: wound gangrene; osteomyelitis; G3-4 AE; SAE related to study drug Occurrence of ≥3 of the above criteria in any given cohort would require a pause in treatment for the cohort, with subsequent actions determined based on the DMC charter.

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2. LIST OF ABBREVIATIONS

AE	adverse event
ADR	adverse drug reaction
AESI	adverse events of special interest
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BED	biologically effective dose
cDFU	chronic diabetic foot ulcer
CRO	contract research organization
DMC	Data Monitoring Committee
ECG	electrocardiogram
eCRF	electronic case report form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
hCG	human chorionic gonadotropin
ICF	informed consent form
ICH	International Council for Harmonization
ID	Identification
IMP	investigational medicinal product
INR	international normalized ratio
IRB	institutional review board
IRT	interactive response technology system
MedDRA	Medical Dictionary for Regulatory Activities
MOA	mechanism of action
NOAEL	no-observed adverse effect level
PK	Pharmacokinetics
PO	orally administered
RCW	Removable cast walker
SAE	Serious adverse event
SOC	Standard of Care (chronic diabetic foot ulcer)
TEAE	Treatment-Emergent Adverse Event
TGS	Toxicity Grading Scale (for Healthy Adult and Adolescent Volunteers)

ULN	Upper limit of normal

3. INTRODUCTION/BACKGROUND

3.1. Background

The treatment of a diabetic foot ulcer is complicated as it requires both antimicrobial treatment to cure any underlying infection (with or without clinical signs of infection) and proper wound care management to heal the ulcer. Curing any infection is particularly challenging because the infection tends to be polymicrobial in nature, requiring a broad-spectrum antibiotic, the ulcer is prone to re-infection, and the pathogenic bacteria are increasingly becoming resistant to most front-line therapies. In addition, recent studies have estimated that approximately 60% to 80% of chronic infections involve biofilm formation, which makes the bacteria more resistant to traditional antibiotics. Proper management of an infected diabetic foot ulcer requires an antimicrobial therapy to cure or clear the infection to allow proper wound management or therapy to heal the ulcer and prevent relapse.

Lakewood-Amedex Inc has developed a novel class of synthetic broad-spectrum antimicrobials, termed Bisphosphocins. Bisphosphocins function through a novel directly bactericidal mechanism of action that is not mediated through a single receptor/target protein. The Bisphosphocin mechanism of action appears specific for microbial membranes with minimal effect on mammalian cell membranes. In addition to possessing activity against all the ESKAPE bacteria strains (a group of antibiotic-resistant bacteria [Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter] recently identified by the Infectious Diseases Society of America that are capable of escaping the biocidal action of antibiotics), including those resistant to antibiotics, Bisphosphocins are effective against stationary, slow-growing, and bacteria encased in biofilm. Bisphosphocins represent a new class of antimicrobials and a potentially important new class of therapeutics needed to treat the increasing number of antibiotic resistant bacterial and fungal infections.

Nu-3 is a thymidine analogue with butylphosphate blocking groups at both the 3’ and 5’ positions (chemical name (2R,3S)-2-((butoxy(hydroxy)phosphoryloxy)methyl)-5-(5-methyl- 2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl butyl phosphate or thymidine-3’,5’-bis-(n-butylphosphate)). Nu-3 exhibits effective in vitro spectrum of activity at killing 70 different strains of bacteria, including all Category A pathogens and in vivo infections caused by Francisella tularensis, Helicobacter pylori, and Pseudomonas aeruginosa.

3.2. Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of LA-Bisphosphocin-Nu-3 gel may be found in the Investigator’s Brochure.

4. OJECTIVE AND ENDPOINTS

	Objective	Endpoint [at 28 days]	Variable
Primary	• To assess the safety and tolerability of escalating doses of topically applied Bisphosphocin Nu-3 Gel	• Treatment emergent events related to clinical investigational product	• Vital signs • Safety laboratory analysis • Local wound/skin assessments • Physical examination • Adverse events
Secondary	• To assess the clinical effects of Nu-3 on cDFU • To assess antimicrobial effects of Nu-3 on wound microflora. • To support determination of the appropriate dose range of Nu-3 to be employed in future clinical studies.	• Advanced digital imaging wound assessment post debridement • Pre and Post debridement digital pictures taken by clinical staff. • Changes in wound microbial population pre debridement • Recommended Phase 2 Dose (RP2D)	• Ulcer surface area • Ulcer depth • Culture and microflora microbiological analysis

5. OVERALL STUDY DESIGN

5.1. Design

This is a Phase 2a, multi-center, randomized, double-blind, placebo-controlled, dose escalation study to evaluate the safety and tolerability of twice daily (BID) topically administered concentrations of Nu-3 gel in subjects with Type 1 and 2 diabetic non-healing target chronic diabetic foot ulcer (cDFU). The study will be conducted in 4 phases: Screening, SOC Run-In, Treatment, and a Safety Follow-up.

Subjects will have the study explained to them and will be provided the study specific informed consent form (ICF). For eligible subjects, the screening evaluations will be performed after the subject provides a written informed consent. For subjects enrolled in the study, activities will consist of two visits prior to randomization: screening visit, and a run-in visit; this will be followed by six scheduled visits (total = 8 visits). Screening will commence 14 days (± 2 days) prior to baseline visit.

During the screening period, a target cDFU will be established and characterized using information obtained from the subject's medical history. At the end of the screening and the beginning of the run-in SOC periods, subjects will have their selected target lesion assessed again. Those subjects whose target ulcers are shown to have ≥ 30% decrease in area will be considered as responders and will be discontinued from the study.

Non-responder subjects will enter the active treatment period and will be randomized in a 3:1 ratio into one of these dose cohorts in a dose escalating manner:

- Cohort 1: 5% Nu-3 gel + SOC (n = 12) or placebo gel + SOC (n = 4)
- Cohort 2: 10% Nu-3 gel + SOC (n = 12) or placebo gel + SOC (n = 4)

Study drug will be applied BID to the target cDFU for 28 days (± 2 days).

The decision to dose-escalate to the next higher concentration will be based on the recommendation of the Data Monitoring Committee (DMC) that will review all of the safety data from the previous subject cohort concurrent with the safety follow-up period. This decision will be based on pre-defined Dose Limiting Toxicity criteria (DLT), which will be detailed in the DMC charter. The maximum tolerated dose (MTD) will be considered as the dose level below that for which a DLT was observed. If the criteria for a DLT occurs in the initial dosing cohort then the study will be halted.

All randomized subjects will continue to receive SOC throughout the study per Appendix 4.

After completing the active treatment period, subjects will return after 2 weeks for a follow-up safety evaluation.

5.2. Selection of the Target Ulcer

The target cDFU will be established and characterized using information obtained from the subject's medical history and physical examination as well as data from the following tests and measurements: Doppler Sonography, Transcutaneous oxygen tension ($TcPO_2$), and AB index. The target ulcer should not be within 3 cm of any other ulcer. In addition, qualitative and semiquantitative measurement of ulcer

depth and measurement of maximal width/length, depth and shape, respectively will be obtained. Final classification will employ the Wagner Scale.

5.3. Wagner Grade/Description of Ulcer

The Wagner system assess ulcer depth and presence of osteomyelitis or gangrene by using the grades listed below. The Wagner Scale outlined in this section will be used for grading ulcers for eligibility.

0. Pre- or post-ulcerative lesion completely epithelialized
1. Superficial, full-thickness ulcer limited to the dermis, not extending to the subcutis
2. Ulcer of the skin extending through the subcutis with exposed tendon or bone and without osteomyelitis or abscess formation
3. Deep ulcers with osteomyelitis or abscess formation
4. Localized gangrene of the toes or the forefoot
5. Foot with extensive gangrene

5.4. Study Procedures

Two dose escalating cohorts are planned using the following dose escalation procedure.

- Each dose escalation cohort will consist of 16 entry and responder criteria qualified subjects randomized in a 3:1 (12 active and 4 placebo) ratio following completion of the 14-day Screening/Run-in period. The initial dosing cohort will receive Nu-3, 5% gel or matching vehicle gel applied BID to the target cDFU.
- The decision whether to escalate the dose to the next higher concentration, e.g., 10% Nu-3 gel will be based on the recommendation of the DMC review of all safety data from the previous subject cohort. This decision will be based on pre-defined Dose Limiting Toxicity criteria (DLT), which will be detailed in the DMC Charter. The MTD will be considered as the dose level below that for which a DLT was observed. If such occurs in the initial dosing cohort then the study will be halted.

Subjects will have the study explained to them and will be provided the study specific informed consent form (ICF). The screening evaluations will be performed after the subject provides a written informed consent.

Subjects enrolled in the study will participate in four periods: Screening, SOC Run-In, Treatment and Safety Follow-up.

Randomized subjects will apply Test Product twice daily, approximately 12 hours apart and at approximately the same times each day. Optimum evening treatment would occur at bedtime, once the subject is in bed for the evening. Test Product will be applied topically to the diabetic foot ulcer from one-use single dose 4 gm tube. The cDFU will be dressed using Mepitel or equivalent, Tegaderm, padding, as needed and Coban Lite 2-Layer Compression Bandage System or equivalent. The subject will be

instructed to leave the bandage on the wound until applying the next dose and thereafter morning and evening for 28 days±2 days. Subjects and caregivers will be given written instructions on the proper care and hygiene to include keeping the ulcer clean and the bandage dry.

Standardized offloading will continue over the course of the study for those subjects with study ulcers located on the plantar or lateral surfaces of the foot or any location experiencing weight-bearing or shear forces as determined by the Investigator. These subjects will wear the RCW up to their exit from the study. The RCW will be applied to the subject's foot after the ulcer dressings are applied as directed by the Investigator.

For all visits during the treatment period, if subjects are seen at the clinic before mid-day, they will not replace the dressing applied the evening before prior to the clinic visit, until after the visit procedures are complete. The site staff or caregiver will then apply the morning dressing.

If subjects are seen at the clinic after mid-day, they will replace the gel dressing in the morning. After all the visit procedures are complete the site staff or caregiver will apply the evening dressing and this will be left in place until the following morning.

Test Product will not be applied on the morning of the final EOT visit or the ET visit.
Sharp Excisional Debridement is required at V1, permitted at V2 and prohibited thereafter.
Light Debridement is permitted at V3 – V6
No debridement is allowed at V7 or ET
Sharp excisional debridement or light sharp scalpel debridement is permitted at V8

Adherence to the study design requirements, including those specified in Schedule of Events, is essential and required for study conduct.

Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study treatment.

All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the subject's routine clinical management (e.g., blood count) and obtained before signing of the ICF may NOT be utilized for screening or baseline purposes. Study procedures and their timing are summarized in Schedule of Events. **Protocol waivers or exemptions are not allowed.**

5.5. Follow-up Safety Period

A 14-day follow-up safety and cDFU evaluation visit will be scheduled for all study subjects and will include assessments as per the Schedule of Events and as detailed above for V8.

5.6. End of Study Definitions

The end of the study is defined as the date of the last visit of the last subject in the study or last scheduled procedure shown in the Schedule of Events for the last subject in the trial. Sponsor retains the right to terminate the study at any time in accordance with all guidance and state and federal laws.

5.7. Scientific Rational for Study Design

One of the most important goals of this study is to identify the Recommended Phase 2b Dose (RP2D)/MTD with acceptable dose-limiting toxicity of the new drug. Among all dose-finding methods used in clinical studies, this design is the most standard approach used.

5.8. Justification for Starting Dose

The rationale for initial dose concentration selection is based on non-clinical data obtained from the 28-day toxicology results in Rats.

5.9. Study Oversight Committee

The decision whether to dose escalate-or-not to the next higher concentration, e.g., 10% Nu-3 gel will be based on the recommendation of the DMC review of all safety data from the previous subject cohort. This decision will be based on pre-defined Dose Limiting Toxicity criteria (DLT), which will be detailed in the DMC Charter. Details regarding the DMC's responsibilities will be outlined in a separate charter.

6. STUDY POPULATION

Inclusion criteria

1. Men and women at least 18 years of age and up to 80 years of age, inclusive.
2. Voluntary written consent, given before performance of any clinical investigation-related procedure, and with the understanding that consent may be withdrawn at any time without prejudice to future medical care
3. Non-hospitalized ambulatory subjects suffering from diabetes mellitus, Type I or II per ADA criteria
4. The target ulcer is classified as grade 1 ulcer according to a Wagner Scale. These ulcers are superficial, full-thickness ulcers limited to the dermis, not extending to the subcutis.
5. Target ulcer is between 2 and 10 cm2 post debridement at screening and baseline.
6. The target ulcer must be no higher than the ankle (on or below the malleolus (ankle bone) with ≥50% below the malleolus.
7. Presence of a persistent cDFU for at least 4 weeks and not more than 1 year that has failed to respond to standard of care
8. Adequate vascular perfusion as evidenced by one of the following
    a. Dorsal transcutaneous oxygen measurement (TCOM) or a skin perfusion pressure (SPP) measurement of ≥ 40 mmHg
    b. Ankle Branchial Index (ABI) between 0.7 and 1.3 within 3 months of Screening using the extremity with the target ulcer
    c. Arterial Doppler ultrasound evaluating for biphasic or triphasic dorsalis pedis and posterior tibial vessels at the level of the ankle or a TBI (Toe Brachial Index) of > 0.6 is acceptable
9. Subject has a caregiver who is able to attend baseline visit and apply wound treatment and study dressings for the study duration
10. Subject completed protocol-defined standardized wound care during the Screening and Run-in periods leading up to Day 0.
11. Must meet one of the following criteria:
    a. Female subjects of Non-Child-Bearing Potential defined as:
        i. Postmenopausal for at least 1 year, or surgically sterilized (i.e., hysterectomy or bilateral oophorectomy more than 3 months prior to Screening), or
        ii. Bilateral tubal ligation more than 6 months prior to Screening
        iii. Must have a negative serum β-hCG pregnancy test at screening and not be breastfeeding prior to being administered with the study drug.
    b. Male subjects of Non-Childbearing Potential defined as those vasectomized subjects whose vasectomy was performed 6 months prior to Screening or those diagnosed as sterile by a physician.
    c. Females and Males of Childbearing Potential who practice an acceptable method of contraception defined as the use of any form of hormonal contraceptive, a barrier method with spermicide, condoms, intrauterine device, or abstinence from sexual intercourse starting at least 60 days prior to Screening and continuing at least 30 days following the last treatment.
12. Subjects must be willing to undergo all clinical investigation-related procedures and attend all required visits.

Exclusion criteria

1. Ulceration with exposed tendon, capsule, or bone
2. Suspicion of bone or joint infection by clinical or other criteria

3. Unable or unwilling to utilize the standardized offloading RCW as required per protocol.
4. Target ulcer has decreased in area by ≥30% between Screening (V1) and Baseline (V3) visits
5. Any subject that is currently on/requires oral, systemic or topical antibiotics, or is anticipated to require use during the course of the trial
6. Any subject that has vascular compromise requiring surgical intervention or has undergone vascular reconstruction or angioplasty less than 1 month prior to randomization. Any planned surgical procedures during the study participation
7. Serum Creatinine level >3.0 mg/dL
8. Hemoglobin A1c (HbA1c) >12%
9. Aspartate Aminotransferase (AST, GOT) and/or Alanine Aminotransferase (ALT, GPT) >3x the upper limit of normal
10. Acute active Charcot foot
11. The target ulcer is within 3 cm of any other ulcer.
12. Any subject that would be unable to safely monitor the infection status at home, and return for scheduled visits
13. History of immunosuppression or taking immunosuppressive agents including systemic corticosteroids, except stable daily doses of 5 mg/day or less for chronic conditions
14. Any subject with a life expectancy ≤ 6 months
15. Pregnancy, including a positive pregnancy test at Screening or Baseline, or lactation
16. Use of investigational drugs within 28 days prior to screening
17. History of concurrent condition that, in the Investigator's opinion, would jeopardize the safety of the subject or compliance with the protocol
18. Likely inability to comply with the protocol or cooperate fully with the investigator and site personnel
19. Known or suspected active abuse of alcohol, narcotics, or non-prescription drugs
20. Prior randomization in this clinical trial, or a previous Bisphosphocin study

6.1. Pregnancy and contraception guidance

Pregnancy and contraception guidance is provided in Appendix 1.

6.2. Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (COSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study may be rescreened. Rescreened subjects should be assigned a different screening number from their initial screening and can be re-screened up to two times.

7. TREATMENTS

Study treatment is defined as any investigational treatment(s) or placebo intended to be administered to a study subject according to the study protocol.

7.1. Treatments Administered

Study Treatment Name:	Bisphosphocin®	Placebo
Dosage formulation:	Gel	Gel
Unit dose strength(s)/Dosage level(s):	5% or 10% concentration	Not applicable
Route of Administration	Topical administration	Topical administration
Dosing instructions:	Gel to be applied twice daily (BID) for 28 days	Gel to be applied twice daily (BID) for 28 days
Packaging and Labeling	Test Product kits will be labelled and contain 18 applicators individually packaged in a foil pouch. These kits will be pre-labelled with Batch/Expiration Dating/study and kit ID numbers. used for dose administration.	Matching placebo kits will be labelled and contain 18 applicators individually packaged in a foil pouch. These kits will be pre-labelled with Batch/Expiration Dating/study and kit ID numbers. used for dose administration.

7.2. Standard of Care

Currently accepted cDFU SOC involves four principles:

1. Pressure relief or off-loading
2. Proper ulcer cleaning and debridement procedures with post procedure irrigation with normal saline
3. Infection management as defined by Lipsky et al, 2012 Infection Guidelines
4. Revascularization when indicated.

During the SOC Run-in phase, a commercially available hydrogel will be used to keep the wound moist, whereas either Nu-3 gel, or placebo gel will be used during the treatment arm. Mepitel or equivalent will be trimmed to cover the entire dimension of the ulcer and overlaid into the ulcer. This will then be covered by Tegaderm. Padding and Coban Lite 2- layer dressing or equivalent will be placed over the Mepitel or equivalent.

7.3. Dose Escalation

The decision to proceed to the next dose level of Nu-3 Gel will be made by the DMC based on safety and tolerability data obtained in each cohort. Review of the data will occur concurrent with the 14-day safety follow-up period.

This decision will be based on pre-defined DLT, which will be detailed in the DMC Charter. The MTD will be considered as the dose level below that for which a DLT was observed. If the criteria for a DLT occurs in the initial dosing cohort then the study will be halted.

Details regarding responsibilities of the DMC are outlined in a separate charter.

7.4. Method of Treatment Assignment

All subjects will be centrally assigned to randomized study treatment using an automated system. Before the study is initiated, directions for use will be provided to each site.

Returned study treatment should not be re-dispensed to the subjects.

7.5. Blinding

The contents of the study treatment kits will not be known to the pharmacist, designee, clinical staff, the CRO or Sponsor. Upon randomization of the subject, the pharmacist or designee will receive a randomization number and an associated Test Product Kit number of a kit at the site. The matching kit will then be dispensed to the subject for dose administration on the first week of the study. On subsequent visits the pharmacist or designee will receive additional Test Product Kit numbers that will match the Test Product Kits that have been delivered to the site.

The IVRS/IWRS will be programmed with blind-breaking instructions. The blind may be broken if, in the opinion of the investigator, it is in the subject's best interest for the investigator to know the study treatment assignment. The sponsor must be notified before the blind is broken unless identification of the study treatment is required for a medical emergency in which the knowledge of the specific blinded study treatment will affect the immediate management of the subject's condition. In this case, the sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and CRF, as applicable.

7.6. Preparation/Handling/Storage/Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

Only subjects enrolled in the study and their caregivers may receive and apply the study treatment and only authorized site staff may supply the study treatment. While at the clinical site, all study treatments should be stored in a secure, environmentally controlled, and monitored (manual or automated) area in

accordance with the labeled storage conditions (2-8 °C) with access limited to the investigator and authorized site staff. Subject and caregivers will be instructed to keep the product refrigerated while stored at their home.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e.., receipt, reconciliation, and final disposition of records).

Study treatment will be dispensed as Product Test Kits at the study visits as summarized in the Schedule of Events.

Subjects will be instructed to bring empty and unused drug applicators back to the site at V4, V5, V6 and V7. Returned study drug should be handled according to the drug accountability guidelines provided **and under no circumstances can returned Product Test Kits or their contents be re-dispensed to the subjects.**

Treatment compliance will be assessed by the dispensing and return records in the subject's case report form (CRF).

7.7. Concomitant Therapy

Any medication or vaccine (including over the counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving for 30 days prior to screening for the trial or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose, route and frequency

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

7.7.1. Prohibited Medications

Immunosuppressive agents including systemic corticosteroids, are prohibited. Inhaled or topical steroids are allowed.

Wound care products such as Regranex® (rhPDGF), Apligraft®, Dermagraft®, Oasis®, as well as any other skin equivalents or topical growth factors, are excluded for use during this trial. Wound cleansers containing antiseptics are prohibited for use during this study. Hyperbaric oxygen (HBO) treatment is also excluded. The use of silver nitrate sticks, styptic pencils or electrocautery is prohibited.

8. CLINICAL PROCEDURES

Subjects will have the study explained to them and will be provided the study specific informed consent form (ICF). The screening evaluations will be performed after the subject has been given the opportunity to ask questions and has signed a written informed consent. For subjects enrolled in the study, activities will consist of two screening/ SOC Run-in visits and six scheduled visits (total = 8 visits). Screening will begin at Day -14, 7 days (± 2 days) prior to the beginning of the Run-In visit, Day -7, 7 days before baseline.

General Information Visits 3 – 7:
Eligible subject's caregivers will apply Test Product twice daily, approximately 12 hours apart and at approximately the same times each day. Test Product will be applied topically to the target ulcer from a single dose applicator. The target ulcer will be dressed with Mepitel or equivalent which will be trimmed to cover the entire dimension of the ulcer and overlaid into the ulcer, followed by Tegaderm dressing covering the entire dimension of the ulcer. Padding and Coban Lite 2- layer dressing or equivalent will be placed over the Mepitel, or equivalent, covered with Tegaderm.

The subject and caregiver will be instructed to leave the bandage on the wound until applying the next dose and thereafter morning and evening for 28 days. Subjects and caregivers will be given written instructions on the proper care and hygiene to include keeping the ulcer clean and bandage dry at V3. The subject WILL NOT apply gel or change dressing on the day of their final visit.

Standardized offloading will continue over the course of the study. For all visits during the treatment period, if subjects are seen at the clinic before mid-day, the caregiver will not replace the dressing applied the evening before prior to the clinic visit, until after the visit procedures are complete. The caregiver will then apply the morning dressing.

If subjects are seen at the clinic after mid-day, they will replace the gel dressing in the morning. After all the visit procedures are complete the caregiver will apply in the clinic the evening dressing and leave in place until the following morning.

Visit 1 (Day -14 prior to baseline (+/- 2 days)) Screening:

- Informed consent and confirmation of eligibility
- Baseline subject disease assessments (as specified in the Schedule of Events table)
- Collect Demographic Data
- Twelve-month medical history
- Targeted physical examination: Perform a physical exam.
- Vital signs measurements: Weight, temperature, resting blood pressure, heart rate, and respiratory rate. Vital signs are to be obtained prior to collecting laboratory samples.
- Safety Laboratory Tests including tests noted in section 11.5.4
- For females of child-bearing potential: the pregnancy test (serum) performed at screening
- Selection of the target ulcer
- Screening Ulcer Assessment: Visual examination of selected target ulcer before and after debridement.
- The target ulcer is classified according to the Wagner Scale.

- Two wound swabs, one for culture and one for microflora examination will be taken BEFORE debridement or any cleansing. Samples collected in accordance with the Microbiological Assessment Appendix 5
- Sharp excisional debridement in accordance with debridement section of the Standard of Care Appendix 4
- Digital photos taken before and after debridement by site staff. Two photos are to be taken from a focal distance of 18 inches, and one photo taken close-up for a total of three digital images
- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions following debridement
- Measure Transcutaneous oxygen pressures ($TcPO_2$), obtain Ankle brachial pressure index or biphasic/triphasic doppler ultrasound
- Subject and care giver trained in application of dressing in accordance with the Appendix 4
- Hydrogel and SOC dressing applied in accordance with the Standard of Care Appendix 4
- Review and dispense subject instruction sheet
- Record all adverse events (local and systemic), concurrent procedures, and changes in concomitant medications on the source documents and case report forms (eCRFs)
- Dispense offloading RCW accordance with Appendix 6 and review expectations
- Begin offloading in accordance with Appendix 6

Visit 2 (Day -7 (+/- 2 days)) Run-In: All subjects will return to the clinic to be seen for wound care and ulcer examination. The following assessments will be completed at this visit.

- Vital signs measurements: Weight, temperature, resting blood pressure, heart rate, and respiratory rate
- Run-in Ulcer Assessment: Visual examination of chronic ulcer before and after debridement
- Sharp excisional debridement is allowed if necessary at Investigator's discretion
- Pre and post debridement digital photos taken by site personnel, if debridement is required. Two photos are to be taken from a focal distance of 18 inches, and one photo taken close-up for a total of three digital images
- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions following debridement
- Evaluate wound healing and discontinue any subject with a ≥30% or greater reduction in area as shown by the advanced digital imaging measurements
- Continued offloading applied in accordance with Appendix 6
- Hydrogel and SOC dressing applied in accordance with the Appendix 4

Visit 3 (Day 0) Baseline: All subjects will return after the screening period for assessment of study participation criteria and if these are satisfied, the subjects will be randomly assigned to Nu-3 gel or placebo gel treatment. Subject disease assessments will be performed as specified in the schedule of events table.

- Vital signs measurements: Weight, temperature, resting blood pressure, heart rate, and respiratory rate. Vital signs are to be obtained prior to collecting laboratory samples
- Urine pregnancy test for WOCBP prior to randomization.
- Serum pregnancy test will also be conducted for WOCBP

- 12 Lead ECG
- Safety Laboratory tests
- Baseline Ulcer Assessment: Visual examination of chronic ulcer before and after debridement (if required, only LIGHT debridement is allowed)
- Two wound swabs, one for culture and one for microflora examination are taken BEFORE debridement
- Light debridement, only if required
- Digital photos should be taken pre and post debridement, if debridement is required. Two photos are to be taken from a focal distance of 18 inches, and one photo taken close-up for a total of three digital images
- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions following debridement
- Subject randomized within electronic RTSM, obtaining kit number
- Subjects will be provided with his/her first designated test product kit and instructed to apply the product twice daily for 1 weeks (7±2 days). Test product application will be demonstrated, and subject instruction sheet reviewed, using the assigned test product
- The caregiver will be observed applying the first dose in the clinic to ensure compliance administering Test Product topically to the diabetic foot ulcer and surrounding intact skin. The target ulcer will be dressed with Mepitel, or equivalent, which will be trimmed to cover the entire dimension of the ulcer and overlaid into the ulcer, followed by Tegaderm dressing covering the entire dimension of the ulcer. Padding and Coban Lite 2- layer dressing or equivalent will be placed over the Mepitel, or equivalent, covered with Tegaderm
- The subject will be scheduled for the next study visit, with a reminder to bring the test article kit, including all tubes, whether they are empty, partially empty and/or unused tubes to the visit
- Record all adverse events (local and systemic), concurrent procedures, and changes in concomitant medications during the study on the source documents and case report forms (eCRFs)
- Continue offloading applied in accordance with Appendix 6

Visit 4 (Day 7±2 days.): Subjects will return after applying test product for 7 (+/- 2) days every 12 hours. Subject disease assessments will be performed as specified in the schedule of events table.

- Vital signs measurements: Weight, temperature, resting blood pressure, heart rate, and respiratory rate
- Ulcer Assessment: Visual examination of chronic ulcer before and after light debridement (if necessary)
- Light debridement, only if required
- Digital images should be taken pre and post debridement with the sponsor provided digital camera, if debridement is required. Two photos are to be taken from a focal distance of 18 inches, and one photo taken close-up for a total of three digital images
- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions following debridement (if debridement is required)
- Collect Test Product kit dispensed on Day 0, determine accountability
- Subjects will be provided with his/her second designated test product kit and instructed to apply the product twice daily for 1 week (7±2 days)

- Two wound swabs, one for culture and one for microflora examination are taken BEFORE debridement, if the wound is still open
- The subject will be scheduled for the next study visit, with a reminder to bring the test article kit and tubes, including empty, partially used, and unused tubes to the visit
- Record all adverse events (local and systemic), concurrent procedures, and changes in concomitant medications during the study on the source documents and case report forms (eCRFs)
- Continue offloading applied in accordance with Appendix 6

Visit 5 (Day 14±2 days): Subjects will return after applying test product for another 7 (+/- 2) days every 12 hours. Subject disease assessments will be performed as specified in the schedule of events table.

- Vital signs measurements: Weight, temperature, resting blood pressure, heart rate, and respiratory rate. Vital signs are to be obtained prior to collecting laboratory samples
- Safety Laboratory tests
- Serum pregnancy
- Ulcer Assessment: Visual examination of target ulcer before and after light debridement (only if required by Investigator.)
- Light debridement, only if required
- Take digital photos with sponsor provided camera both pre and post debridement, if debridement is required. Two photos are to be taken from a focal distance of 18 inches, and one photo taken close-up for a total of three digital images
- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions following debridement, if performed
- Two wound swabs, one for culture and one for microflora examination are taken BEFORE debridement, if the wound is still open
- Collect kit dispensed on Day 7, determine accountability
- Subjects will be provided with his/her third designated test product kit and instructed to apply the product twice daily for 1 week (7±2 days)
- The subject will be scheduled for the next study visit, with a reminder to bring the test article kit and tubes including empty, partially used and unused tubes to the visit
- Record all adverse events (local and systemic), concurrent procedures, and changes in concomitant medications during the study on the source documents and case report forms (eCRFs)
- Continue offloading applied in accordance with Appendix 6

Visit 6 (Day 21±2 days.): Subjects will return after applying test product for 7 (+/- 2) days every 12 hours. Subject disease assessments will be performed as specified in the schedule of events table.

- Vital signs measurements: Weight, temperature, resting blood pressure, heart rate, and respiratory rate
- Ulcer Assessment: Visual examination of chronic ulcer before and after debridement (only if required by Investigator).
- Light debridement, only if required
- Take digital images with sponsor provided camera both pre and post debridement, if debridement is required. Two photos are to be taken from a focal distance of 18 inches, and one photo taken

close-up for a total of three digital images

- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions following debridement (if debridement is performed, only light debridement may be performed).
- Two wound swabs, one for culture and one for microflora examination are taken BEFORE debridement, if the wound is still open
- Collect kit dispensed on Day 14, determine accountability
- Subjects will be provided with his/her fourth and final designated test product kit and instructed to apply the product twice daily for 1 week (7±2 days).
- The subject will be scheduled for the next study visit, with a reminder to bring the test article kit and all tubes including empty, partially used and unused tubes to the visit.
- Record all adverse events (local and systemic), concurrent procedures, and changes in concomitant medications during the study on the source documents and case report forms (eCRFs).
- Continue offloading applied in accordance with Appendix 6

Visit 7 (Day 28±2 days): The study subject will return for final efficacy evaluation to the clinical site on Day 28±2 days. At that visit, the subject will undergo the following procedures and evaluations.

- Vital signs measurements: Weight, temperature, resting blood pressure, heart rate, and respiratory rate. Vital signs are to be obtained prior to collecting laboratory samples
- 12 Lead ECG
- Safety Laboratory Tests
- Serum pregnancy
- Ulcer Assessment: Visual examination of chronic ulcer.
- **No debridement to be performed at this visit**
- Two wound swabs will be taken, one for microbiological culture and one for microflora examination (unless ulcer has completely re-epithelialized).
- Take digital photos with the digital camera provided by sponsor. Two photos are to be taken from a focal distance of 18 inches, and one photo taken close-up for a total of three digital images
- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions.
- Collect kit dispensed on Day 21, perform drug accountability
- Record all adverse events (local and systemic), concurrent procedures, and changes in concomitant medications during the study on the source documents and case report forms (eCRFs).
- Continue offloading applied in accordance with Appendix 6

If the ulcer remains open at visit 7, subjects will be given a supply of non-abrasive bandages and written instructions on weekly ulcer care until the Day 42 follow up visit (Visit 8). In addition, subjects will be told to call if there is any worsening of the ulcer with regard to pain, signs of infection, or swelling.

Standardized offloading will continue until the final study visit (Day 42±2 days) regardless of wound healing status.

Visit 8 (Day 42±2) End of Study (EOS) Visit: The subject shall attend final end-of-study 42±2 days post

Randomization. At this follow-up visit, the subject will undergo the following procedures and evaluations.

- Vital signs measurements including weight, temperature, resting blood pressure, heart rate, and respiratory rate. Vital signs are to be obtained prior to collecting laboratory samples
- 12 Lead ECG
- Physical examination
- Safety Laboratory Tests
- For females of child-bearing potential: pregnancy test (serum) to be performed
- Ulcer Assessment: Visual examination of chronic ulcer.
- Take digital photos with the digital camera provided by sponsor. Two photos are to be taken from a focal distance of 18 inches, and one photo taken close-up for a total of three digital images
- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions
- Record all adverse events (local and systemic), concurrent procedures, and changes in concomitant medications during the study on the source documents and case report forms (eCRFs)
- Physician may perform or not perform debridement according to his practice standards
- Offloading ends

Early Termination Visit: If a subject withdraws prior to completing the study, the reason for withdrawal will be documented. If a subject withdraws early due to a Serious Adverse Event, he/she will be followed until resolution/stabilization of the SAE.

If a subject prematurely withdraws from the study, subject will be asked to complete the study procedures and evaluations performed in the final study visit at the time of withdrawal from the study:

- Vital signs measurements including weight, temperature, resting blood pressure, heart rate, and respiratory rate. Vital signs are to be obtained prior to collecting laboratory samples
- 12 Lead ECG
- Physical examination
- Safety Laboratory Tests
- For females of child-bearing potential: serum pregnancy test to be performed.
- Ulcer Assessment: Visual examination of chronic ulcer
- **No debridement to be performed at this visit**
- Two wound swabs will be taken, one for microbiological culture and one for microflora examination (unless ulcer has completely re-epithelialized)
- Take digital photos with the digital camera provided by sponsor. Two photos are to be taken from a focal distance of 18 inches, and one photo taken close-up for a total of three digital images
- Employ an advanced digital imaging system to accurately measure the target foot ulcer dimensions.
- Offloading ends
- If early termination occurs before Day 28 the following should be completed:
    - Two wound swabs, one for culture and one for microflora examination, are collected, if the wound is still open
    - Collect kit dispensed on prior study visit, if applicable and determine accountability

9. SCHEDULE OF EVENTS

	Screening		Treatment					EOS	Early Termination
	Visit 1 Screening	Visit 2	Visit 3 Baseline	Visit 4	Visit 5	Visit 6	Visit 7 Final	Visit 8 Follow-up	ET
Event	-14 Day (±2 days)	-7 Day (±2 days)	Day 0 (±2 days)	Day 7 (±2 days)	Day 14 (±2 days)	Day 21 (±2 days)	Day 28 (±2 days)	Day 42 (±2 days)	
Eligibility Screening, Inclusion, Exclusion Criteria	X		X						
Informed Consent	X								
Demographics & Medical History	X								
Physical Exam	X							X	X
Vital Signs	X	X	X	X	X	X	X	X	X
Transcutaneous oxygen pressures, ABI's, doppler ultrasound and Ischemic Index	X								
12 Lead ECG			X				X	X	X
Urine Pregnancy Test ([2]WOCBP)			X						
Serum Pregnancy Test ([2]WOCBP)	X		X		X		X	X	X
Laboratory Tests – Safety Labs	X[5]		X		X		X	X	X
Concomitant Medication and Adverse Event Review	<---------- X ---------->								
Visual Examination of Chronic Ulcer	X	X	X	X	X	X	X	X	X
Wound Culture Swab Pre-Debridement	X		X	X[6]	X[6]	X[6]	X[6]		X[4]
Microflora Swab Pre - Debridement	X		X	X[6]	X[6]	X[6]	X[6]		X[4]
Advanced digital imaging system Post -Debridement	X	X	X	X	X	X	X[3]	X	X[3]
Digital images before and after debridement (sponsor provided camera)	X	X	X	X	X	X	X[3]	X	X[3]
Offloading	X	X	X	X	X	X	X	Study Off-Loading Ends	Study Off-Loading Ends
Subject Instruction Sheet Reviewed & Distributed	X		X						
Randomization within RTSM			X						
Dispense Test Article			X	X	X	X			
Assess Subject Compliance Test Article Accountability				X	X	X	X		X[4]

Debridement [1]	SD-R	SD-P	LSD	LSD	LSD	LSD	None	SD-P	None

[1]Sharp excisional debridement is required at V1 (SD-R), permissible (SD-P) at V2 and prohibited at subsequent visits. Only light debridement (LSD) is allowed at subsequent visits at the Investigator's discretion per appendix.

[2]Women of childbearing potential (WOCBP) include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal [defined as amenorrhea >12 consecutive months]. Even women who are using oral, implanted, or injectable contraceptive hormones, an intrauterine device (IUD), barrier methods (diaphragm, condoms, spermicidal) to prevent pregnancy, practicing abstinence, or where partner is sterile (e.g., vasectomy performed at least six months prior to the subject's initiation of treatment) should be considered to be of childbearing potential.

[3]At Visit 7 no debridement occurs but all imaging will still be captured

[4]Only performed if early termination occurs prior to Visit 7

[5]HbA1c collected

[6]Swabs will not be performed if the wound is closed

10. DISCONTINUATION/WITHDRAWAL CRITERIA

10.1. Discontinuation of Study Treatment

Participation in this study and receiving the study treatment is voluntary. A subject can withdraw from the study treatment at any time. The Principal Investigator/treating physician based on his/her clinical judgement and evaluation may at his/her discretion discontinue study treatment for safety, behavioral, compliance, or administrative reasons.

If the subject withdraws consent for study treatment after beginning, study visits and all study procedures should still be completed. The sponsor may retain and continue to use any data collected during the study for a subject who has a withdrawal of consent for study treatment.

10.2. Withdrawal from the Study

A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons.

If the subject withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a subject withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

See Schedule of Events for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed

10.3. Lost to Follow-Up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

11. ADVERSE EVENTS

The definitions of an AE or SAE is provided in Appendix 2.

AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative).

The investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study (see Appendix 2).

11.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from the signing of the ICF until the safety follow-up visit at Day 42 at the time points specified in the Schedule of Events.

All SAEs will be recorded and reported to the sponsor or designee within 24 hours, as indicated in Appendix 2. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Appendix 2.

11.2. Methods of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

11.3. Follow-up AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 10.3), or the subject is withdrawn from the study due to clinical deterioration precluding the subject’s safe participation in the study (as defined in Section 10.2). Further information on follow-up procedures is given in Appendix 2.

11.3.1. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study treatment under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor, will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

11.4. Pregnancy

Details of all pregnancies in female subjects and female partners of male subjects, will be collected after the start of study treatment and until 14 days (+/- 2 days) after last dose of study drug (during the safety follow up visit).

If a pregnancy is reported, the investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 1.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

11.5. Safety Assessments

Planned time points for all safety assessments are provided in Schedule of Events.

11.5.1. Physical Examinations.

Physical exams are conducted per institutional protocol. At minimum, the following system are to be reviewed:

- Ear, nose and throat (EENT)
- Cardiovascular
- Respiratory
- Gastrointestinal (GI)

- Musculoskeletal/Connective Tissue
- Neurological
- Endocrine/Metabolic
- Hematopoietic/Lymphatic

11.5.2. Vital Signs

Oral temperature, pulse rate, respiratory rate, and blood pressure will be assessed

Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (e.g., television, cell phones).

Vital signs (to be taken before blood collection for laboratory tests) will consist of 1 pulse and 3 blood pressure measurements (3 consecutive blood pressure readings will be recorded at intervals of at least 1 minute). The 3 blood pressure readings will be recorded on the CRF.

11.5.3. Electrocardiograms

Triplicate 12-lead ECGs will be obtained as outlined in Schedule of Events, using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT.

At each time point at which triplicate ECG are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart.

11.5.4. Clinical Safety Laboratory Assessments

The following clinical laboratory tests will be performed at the timepoints designated in the schedule of events.

- Hematology: WBC and differential count, RBC, Hgb, Hct, MCV, MCH, HCHC, RDW, MPV, Platelets
- Biochemistry: Sodium, Potassium, Bicarbonate or CO2, Chloride, Total Bilirubin, Creatinine, BUN, Calcium, Alkaline Phosphatase, ALT, AST, Total Protein, Albumin, eGFR, Globulin
- Coagulation as per Study Reference Manual
- Urinalysis as per Study Reference Manual
- Hemoglobin A1c (HbA1c) to be collected at Visit 1 only

All laboratory tests required above will be performed by the central lab. Local laboratory results are only permitted in the case of a medical emergency or if the central laboratory results are not available in time

for either study treatment administration and/or response evaluation. Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations. If a local sample is required in the case of an emergency, it is important that the sample for central analysis is obtained at the same time. Additionally, if the local laboratory results are used to make either a study treatment decision or response evaluation, the results must be entered into the CRF

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

Study subjects who have laboratory tests with values considered clinically significantly abnormal and designated as an AE during the study, should have repeat labs drawn after the last dose of study treatment and should continue repeat labs until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.

12. STATISTICAL CONSIDERATIONS

12.1. Sample Size Determination

This is a pilot dose-ranging study. No sample size calculations were conducted.

12.2. Population for Analysis

All statistical processing will be performed using SAS® unless otherwise stated. All subjects enrolled in the study that were dispensed and applied test article at least once will be included in the analysis and will be considered in the intent-to-treat (ITT) population.

12.3. Statistical Analysis

The statistical analysis plan will be developed and finalized before database lock and will describe the subject populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

12.3.1. Efficacy Analysis

Efficacy data including clinical and microbiological response will be summarized by dose group and will include analysis further defined in the statistical analysis plan.

12.3.2. Safety Analysis

Adverse Events: Treatment-emergent adverse events will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent adverse events will be summarized by body system and preferred term

12.3.3. Interim Analysis

A safety review by an independent DMC will be conducted after each cohort to confirm it is acceptable to proceed to the next dose level.

12.3.4. Parameters Requiring Pause in Treatment

Individual: wound gangrene; osteomyelitis; G3-4 AE; SAE related to study drug.

Cohort: ≥3 of the above individual criteria in any given cohort

13. STUDY GOVERNANCE CONSIDERATIONS

Study Governance considerations are provided in Appendix 3, including:

- Regulatory and ethical considerations
- Financial disclosure
- Informed consent process
- Committee structure

14. REFERENCES

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APPENDIX 1. GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

Definitions

Women of Childbearing Potential (WOCBP)

A women is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP

1. Premenarchal: premenopausal female with 1 of the following:
    - Document hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

    Note: Documentation can come from the site personnel's: review of the subject's medical records, medical examination, or medical history interview.

2. Postmenopausal female:
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance

Male Subjects

Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent.

Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration during trial participation and for one month following trial completion.

Refrain from donating sperm for the duration of the study and for one month after study completion or the last dose of study treatment.

Female Subjects

Highly Effective Contraceptive Methods That Are User Dependent(a)

Failure rate <1% per year when used consistently and correctly.

Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation.)

- Oral
- Intravaginal
- Transdermal

Progestogen only hormonal contraception associated with inhibition of ovulation

- Oral
- Injectable

Highly Effective Methods That Are User Independent

Implantable progestogen only hormonal contraception associated with inhibition of ovulation

- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion

Vasectomized Partner

A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.

Sexual Abstinence

Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment.

(a): Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects participating in clinical studies.

(b): Hormonal contraception may be susceptible to interaction with the study treatment, which may reduce the efficacy of the contraceptive method. In this case, two highly effective methods of contraception should be utilized during the treatment period and for at least 30 days after the last dose of study treatment.

Pregnancy Testing

- Serum and urine pregnancy testing will occur per the Schedule of Events.
- WOCBP should only be included after a confirmed menstrual period and a negative highly sensitive serum pregnancy test.
- A final serum pregnancy test will occur at the EOS/ET visit and at the safety follow-up safety visit at week 42 which occurs 14 days (+ or – 2 days) after last dose of study treatment.

Male Subjects with Partners who Become Pregnant

- The investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study.

- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Subjects who Become Pregnant

- The investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the sponsor within 24 hours of learning of a subject's pregnancy. The subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-study pregnancy related SAE considered reasonably related to the study treatment by the investigator will be reported to the sponsor as described in Appendix 1. While the investigator is not obligated to actively seek this information in former study subjects, he or she may learn of an SAE through spontaneous reporting.
- Any female subject who becomes pregnant while participating in the study will discontinue study treatment and be withdrawn from the study but will be followed for 8 weeks post-delivery.

APPENDIX 2: ADVERSE EVENTS DEFINITIONS AND PROCEDURES FOR RECORDING, EVALUATING, FOLLOW-UP, AND REPORTING

Definition of AE

- An AE is any untoward medical occurrence in a subject or clinical study subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment.

Note: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment. Local reactions to test product administration include but are not limited to pain, edema, rash, cellulitis, localized infectious processes, and any systemic reaction including fever, allergic reaction, and anaphylaxis, and would be considered to be AEs.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
- "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which in the opinion of the Investigator are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.

- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:

- Results in Death
    - The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
- Requires in subject hospitalization or prolongation of existing hospitalization
    - In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or ambulatory care setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in persistent disability/incapacity
    - The term disability means a substantial disruption of a person's ability to conduct normal life functions.
    - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect
- Other situations:
    - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording an AE and/or SAE

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is not acceptable for the investigator to send photocopies of the subject's medical records to the safety team in lieu of completion of the safety team/AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by the safety team. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to the safety team.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- An event is defined as 'serious' when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- The investigator will use clinical judgment to determine the relationship.
    - Unlikely - A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations. For the purpose of this protocol, the term unlikely will be considered not related to study medication and an "Adverse Event"
    - Possible - A clinical event, including laboratory test abnormality, with a reasonable time sequence to administrations of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear. For the purpose of this protocol an event that has possible relationship to study medication will be defined as a "Suspected Adverse Drug Reaction".
    - Probable - A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal. For the purpose of this protocol an event that has probable relationship to study medication will be defined as an "Adverse Drug Reaction".

  - Definite - This category applies to those AEs which are considered "definitely related" to the test drug. The relationship of an AE to study drug may be considered definite, if it meets the criteria for "probable" with a definite certainty as to the relationship to the test drug.
  - Unrelated - This category is applicable to those AEs that are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.) and do not meet the criteria for drug relationship listed under Unlikely, Possible, Probable, or Definite.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to the safety team. However, **it is very important that the investigator make an assessment of causality for every event before the initial transmission of the SAE data to the safety team.**
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by safety team to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide the safety team with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

SAE Reporting to the Safety Team via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to the safety team will be the electronic data collection tool
- If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next section).
- The site will enter the SAE data into the electronic system as soon as it becomes available.

- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form located within the Investigator Site File.
- Contacts for SAE reporting can be found in the project team list within the Investigator Site File.

SAE Reporting to the Safety Team via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the safety monitor.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in the study reference manual.

APPENDIX 3: STUDY GOVERNANCE CONSIDERATIONS

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - Applicable ICH Good Clinical Practice (GCP) Guidelines
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator or CRO and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.
- The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
    - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for one year after completion of the study.

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

- The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.
- Any remaining study samples cannot be used for optional exploratory research.
- Subjects who are rescreened are required to sign a new ICF and will be given a new screening number.

Data Protection

- Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Data Quality Assurance

- All subject data relating to the study will be recorded on electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF and dating it.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator in accordance with all state, federal and local laws and FDA guidance

after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Source Documentation

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- A source document can be almost anything. It is the original place or "source" where information is recorded.

Study and Site Closure

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study treatment development

APPENDIX 4: STANDARD OF CARE

Standard of care (SOC) Treatment consists of:

- Moist wound dressings,
- debridement of necrotic/non-viable tissue
- infection surveillance and management and
- offloading of weight bearing from vicinity of the ulcer.

The site will document that a weekly Test Product kit was used and that they provided materials and training to the subject and care giver, as applicable. Beginning at the screening visit, all subjects must have their study ulcer managed using the SOC procedures noted below.

Cleaning the Study Ulcer

Microbiology and microflora swabs will be obtained before cleansing the wound and/or debridement. The leg should be elevated for as much time as possible during this process. The ulcer and peri ulcer area will be cleansed to minimize bioburden and maceration. The investigator or designee will irrigate the ulcer with sterile normal saline solution. Sterile gauze will be used to pat the ulcer dry.

Maintenance of a Clean, Moist Ulcer Environment

One of the key elements to optimal wound healing is maintaining a moisture balance. Too much moisture and the wound become macerated; too little moisture and the essential healing processes cannot function. During Screening and SOC Run-In Periods: A commercially available hydrogel will be applied topically to the cDFU twice daily instead of the Test Product. Hydrogel will be applied during V1 and V2 and Test Product gel during V3 onward. The gel will be applied topically to the target cDFU twice daily (roughly every 12 hours at the same times of day) for four weeks (treatment period). Optimum evening treatment would occur at bedtime, once the subject is in bed for the evening. After application of each dose of the Test Product is complete, the wound will be dressed as per the following: Mepitel or equivalent will be trimmed to cover the entire dimension of the ulcer and overlaid into the ulcer, followed by Tegaderm dressing covering the entire dimension of the ulcer. Padding and Coban Lite 2- layer dressing will be placed over the Mepitel, or equivalent, covered with Tegaderm. Application of Test Product or any gel should not occur on the day of the final visit or on the day of an early term visit.

The dressing should be kept dry during showering.

Debridement of the Target Ulcer

Ulcer debridement is an essential technique and standard of care in the treatment of diabetic foot ulcerations. It is important to remove all non-viable and necrotic material from the target ulcer during V1 and V2 if needed. Sharp excisional debridement is allowed at V1 and V2, only light sharp scalpel debridement is allowed at V3, V4, V5 and V6. Light sharp scalpel debridement consists of the following:

- The index ulcer and surround skin are prepped with water or Saline
- Anesthesia, topical or injected is applied to the ulcer as necessary to reduce subject discomfort
- Remove any non-viable and necrotic tissue or slough using a scalpel or scissors
- If there is a noticeable “edge effect” on the periphery of the index ulcer, debride back to the adherent epidermis

- Excessive bleeding is controlled by using direct pressure only

No debridement is allowed at V7. During V8 either sharp excisional debridement or light sharp scalpel debridement is allowed, based on Investigator discretion. Particular care should be taken to protect healthy tissue, which has a red or deep pink (granulation tissue) appearance, this tissue should not be disrupted during debridement.

If any anesthetic medications are used for debridement, they should be added to the Concomitant Medication page of the eCRF. If extensive surgical debridement is necessary during the run-in period (i.e., general anesthesia is required), the subject is not a candidate for this trial. Likewise, if other forms of debridement (e.g., enzymatic) are required during run-in based on the opinion of the treating clinician, the subject should be screen failed.

The use of silver nitrate sticks, styptic pencils or electro-cautery is prohibited as these coagulation methods induce further tissue damage.

Wound Debridement Procedures V1 and V2:

Assess pain level and determine appropriate method of pain control.

- Cleanse wound with sterile saline.
- Utilize sterile tissue scissors, forceps, surgical blade and/or other appropriate surgical instruments to remove devitalized tissue, as clinically indicated.
- Following debridement, cleanse wound with sterile saline.
- Achieve hemostasis
- Apply appropriate wound dressing.

The test article will not be applied to the ulcer bed until complete hemostasis has been attained. Further debridement may be performed as required throughout the treatment period, as determined by the investigator or appropriately trained and qualified designee. Debridement may be performed by either the investigator or appropriately trained and qualified designee but should be performed by the same individual throughout the duration of the clinical study.

V1: Sharp excisional debridement is required (unless contraindicated in the opinion of the investigator).

V2: Sharp excisional debridement is permitted.

Wound Debridement Procedures V3 through V8:

V3, V4, V5, V6: Only light sharp scalpel debridement is allowed.

V7 and ET: No debridement allowed.

V8: Sharp excisional debridement or Light sharp scalpel debridement allowed.

During the Treatment Phase of the trial, other forms of debridement are NOT permitted. If this should happen, the type of debridement should be noted in the CRF; using other forms of debridement besides light debridement more than once during the treatment phase will result in the subject being withdrawn from the trial.

APPENDIX 5: MICROBIOLOGICAL ASSESSMENTS

Two wound swabs of ulcer will be sent for aerobic and anaerobic cultures of contaminating microbes, which will be performed by a central microbiology lab, and for molecular evaluation of microflora at V1, V3, V4, V5, V6 and V7 (and/or at early termination). Suitable culture samples must be obtained via wound swab. Biopsy, curettage, and needle aspirations are not allowed. If suitable culture material is not available due to healing of the ulcer, this will be documented by the investigator. All wound swabs will be obtained BEFORE debridement.

The eradication/persistence of each baseline pathogenic species will be determined based on culture results reported by the laboratory. If a subject's culture result is missing for V7, the baseline pathogen will be presumed eradicated if a) ulcer closure is achieved, b) because of ulcer healing there is a lack of suitable tissue sample/culturable material (as documented by the investigator), or c) the culture result is otherwise missing, and pathogen eradication was achieved in the last available culture result. In all other circumstances, baseline pathogen persistence (i.e., not eradication) will be presumed.

Process and Procedure for Microbiological and Microflora Swabs

The process for obtaining, storing and shipping swabs can be found in the study reference manual.

APPENDIX 6: OFFLOADING

If the ulcer is on the bottom or side of the foot, the subject will be fitted with a standardized off-loading device provided by the sponsor. Off-loading is defined as avoidance of all mechanical stress on the injured extremity and is essential for healing. Avoidance of weight bearing is essential. Trauma causes most plantar ulcers and ongoing trauma prevents healing. Because off-loading (not bearing ANY weight) is so critical to the healing process, subjects will be instructed to wear the RCW at all times except when bathing and to use an RCW at all times when walking or standing is required. Strategies for off-loading will be standardized for all subjects as follows:

All subjects with an ulcer on the bottom of the foot will be fitted with an RCW or other off-loading device during the Screening Visit, according to the following procedure:

- The size of the walker will be determined based on the subject's correct shoe size.
- The appropriate size of the insole will be inserted into the RCW. Once the target ulcer has been debrided, assessed, cleansed, dressed and secured, the RCW will be applied according to the manufacturer's instructions for use.
- The investigator or designee will ensure that the subject and/or family members are able to unfasten, remove and reapply the RCW properly according to the manufacturer's instructions for use.
- Subjects will be instructed to wear the RCW at all times except when bathing or sleeping. When bathing, the subject will be instructed to wear a slipper with a plastic bag worn over the slipper and tied at the ankle or higher.

Subjects will be educated on the importance of using the device to offload their cDFU and instructed on keeping dressings dry. In addition, subjects should be educated on wound infection and if they observe infection to call or visit the study site.

The off-loading RCW/protective device and SOC dressings to be used in this study will be provided by the Sponsor. The off-loading RCW/protective device will only be used by subjects who require offloading throughout the Screening and Treatment (i.e., those subjects with study ulcers located on the plantar or lateral surfaces of the foot or any location experiencing weight-bearing or shear forces as determined by the Investigator). These subjects will wear the RCW up to their exit from the study. The RCW will be applied to the subject's foot after the outer dressings are applied.

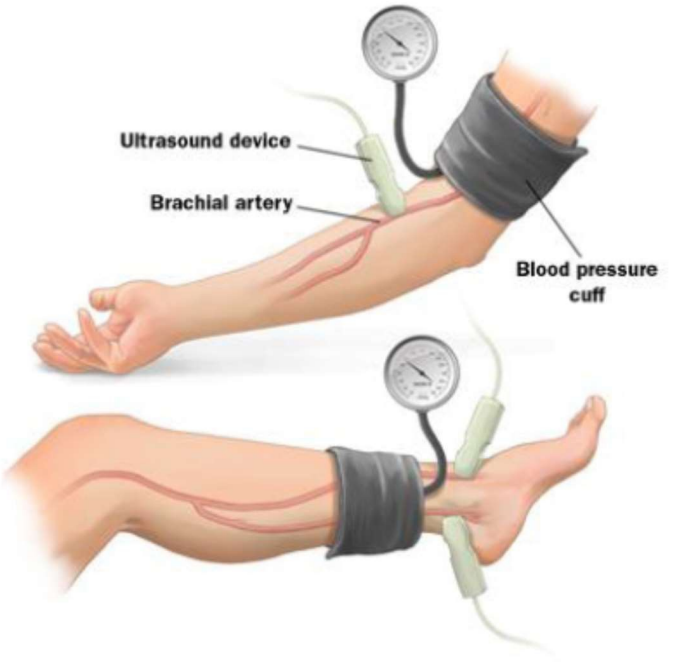
APPENDIX 7: VASCULAR PERFUSION ASSESSMENT

Vascular testing is performed by one of the following methods:

Screening: Perform one of the following: ABI (Preferred Assessment) The ABI is the ratio of the brachial pressure (i.e., blood pressure in the arm) to the blood pressure in the ankle. Briefly, the blood pressure is obtained in the arm (brachial) and then obtained in a similar manner in the ankle. To obtain the ratio (i.e., index), divide the systolic pressure from the ankle by that obtained in the arm. ABI is the Systolic Pressure Ankle /Systolic Pressure Arm.

Ankle-Brachial Index Technique

Place the subject in the supine position, with the arms and legs at the same level as the heart, for a minimum of 10 minutes before measurement. Select an appropriately sized blood pressure cuff for both the ankle and the arms (figure 1); the cuff width should be, at a minimum, 20% greater than the diameter of the extremity. The ankle cuff should be placed on the leg between the malleolus and the calf. Enough room should be left below both cuffs to permit placement of the ultrasound gel, so that the Doppler device can adequately detect the brachial, dorsalis pedis, and posterior tibial arteries. Obtain the brachial systolic pressures of both arms. Use the higher of the arm pressures in the ABI calculation. Obtain the pressure in the dorsalis pedis and posterior tibial arteries for the extremity with the target ulcer. Use the highest pressure for the ABI calculation. Ankle-Brachial Index = Highest ankle pressure/ Highest brachial pressure. Care should be taken to cover the ulcer during the ABI measurement. In addition, subjects should be informed that they may experience discomfort during the test secondary to the pressure exerted by the cuff in the area of skin breakdown.



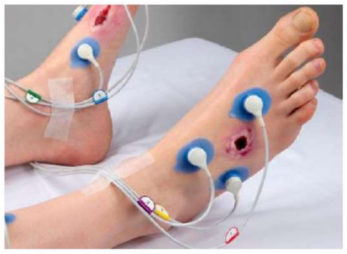
Arterial Doppler Ultrasound Technique

Place the subject in the supine position, with the arms and legs at the same level as the heart. Ultrasound gel will be applied to the ankle level to the anatomic position of the dorsalis pedis and posterior tibial vessels. The Doppler will then be applied to each vessel until a waveform is established, a printer will be used to document the Doppler waveform and at least three waveforms will be printed for each vessel and filed with source. The clinician will verify biphasic or triphasic waveforms.

Transcutaneous Oxygen Level ($TcPO_2$)

$TcPO_2$ is a measure of oxygen delivery to a local area. A special machine must be used to assess $TcPO_2$. Briefly, electrodes for the sensor will be placed onto the intact skin close to the study ulcer. The oxygen sensor then can read the oxygen content of the tissue and reports this as mmHg pressure. Each site should refer to the equipment's manual for specific and correct instructions for use. For this study, potential study candidates must have a $TcPO_2$ greater than 40 mmHg.

Technique: Place the subject in the supine position, with the arms and legs at the same level as the heart. Electrodes must be in contact with the tissue through the contact liquid. If there is air between the tissue and an electrode, TCOM values will be questionable. Erroneous readings may also occur if electrodes are placed directly over a bone or there is severe edema around the wound. For best results, tests should be conducted at ambient temperature (21-23º C), and the subjects should not have smoked nor had caffeine for several hours prior.



1. Calibrate the TCOM electrode—this takes about 15-20 minutes.
2. Clean the selected measuring site with alcohol or other skin-preparation solution.
3. Dry the site well with a gauze pad.
4. Take a standard fixation ring.
5. Remove the fixation ring from the protective film.
6. Apply the fixation ring to the measuring site as follows: • Press the center of the fixation ring onto the measuring site with a finger. • Run a finger around the rim circumference. • Press firmly to prevent leaks.
7. Fill the hole in the fixation ring with 3-5 drops of the contact liquid.
8. Affix the electrode into the fixation ring as follows: • Align the arrow on the electrode with one of the marks on the fixation ring. • Turn the electrode 90° clockwise to fasten it in the fixation ring.
9. Repeat steps 1 to 8 if more electrodes are to be applied; note: several electrodes can be calibrated at the same time.

It is sometimes advantageous to simultaneously use several electrodes placed strategically around the wound and calculate mean values from individual readings. The normal sequence of events for TCOM is measurement in air, the leg elevation test (optional), and the oxygen challenge. The optimal times for these events in terms of measurement time are, 20, 5, and 10 minutes, respective