CLINICAL INVESTIGATIONAL PROTOCOL

PROTOCOL NO: LAI2014-1

Version 3.1 15 September2016

A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin™ Nu-3 on Infected Diabetic Ulcers of Subjects With Type I or II Diabetes Mellitus

Lakewood Amedex Inc.
3030 University Pkwy
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CONFIDENTIALITY STATEMENT

TITLE PAGE

Title:	A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin™ Nu-3 on Infected Diabetic Ulcers of Subjects with Type I or II Diabetes Mellitus
Protocol No.:	LAI2014-1
Protocol Version:	Version 3.1
Version Date:	15 September 2016
Investigational Product:	Bisphosphocin™ Nu-3
Study Director:	[redacted]
Sponsor:	Lakewood Amedex Inc 3030 University Pkwy Sarasota, FL 34243 Tel.: +1 941 225 2515 Fax: +1 941 225 2511

Signature on behalf of the Sponsor:

Signature: _____ Date:_____

INVESTIGATOR PROTOCOL AGREEMENT PAGE

- I, the undersigned, am responsible for the conduct of the trial at this site and agree to the following:
- I understand and will conduct the trial according to the protocol, any approved protocol amendments, ICH GCP and all applicable regulatory authority requirements and national laws.
- I have sufficient time to properly conduct and complete the trial within the agreed trial period, and I have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.
- I will ensure that all staff at the clinical trial site, who are involved in the trial conduct are adequately trained regarding the protocol and their responsibilities.

Signed:

Principal Investigator	**Date**

1 SYNOPSIS

Name of Sponsor: Lakewood Amedex Inc

Protocol No.: LAI2014-1

Name of Test Product: Bisphosphocin™ Nu-3

Clinical Investigation Center(s): Approximately 2 to 4 sites in USA

Title of Clinical investigation: A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin™ Nu-3 on Infected Diabetic Ulcers of Subjects With Type I or II Diabetes Mellitus

Patient Population: Male and Female subjects 18-85 years of age suffering from diabetes mellitus and a chronic infected diabetic ulcer(s).

Sample Size: Maximum 50 subjects in total across five study cohorts

Examination Points: Day 1, 2, 9, 15

Study Design

Multi center, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study

Clinical investigation Objective(s) and Endpoint:

	Objective	Endpoint	Variable
Primary	To assess the safety and tolerability of escalating doses of topically applied bisphosphocin™ Nu-3	• Treatment emergent events related to clinical investigation product	• Vital signs, • CBC analysis, CMP analysis, • Physical examination,
Secondary	• To assess the clinical and microbiological response to bisphosphocin™ Nu-3 • To support determination of the appropriate dose range of Nu-3 to be employed in future clinical studies.	• Reduction of pathogenic bacteria • Visual evaluation of the ulcer	• Aerobic and anaerobic culture and sensitivity • DUSS Score

Clinical investigation Duration (Per subject)

Total Clinical investigation duration: (Recruitment period) + screening + treatment and evaluation + follow-up.

- Screening Period: - 7 days to -4 hours
- Treatment: 7 Days
- Follow-up: 24 hours (± 2 hours) after first treatment; 8 days + 1 day) after first treatment
- End of clinical study: 15 days ± 1 day after first treatment

Randomization

Randomization will be conducted in blocks of five to a ratio of 4:1 (active:placebo). Qualified subjects will be enrolled and randomized in: 8:8:8:8:8:10 ratio to either Nu-3 at one of five possible dose levels (0.1%, 1%, 2%, 5% and 10% Nu-3 solution in saline (w/v)), or to placebo, respectively.

Inclusion Criteria

1. Men and women between the ages of 18 and 85.
2. Voluntary written consent, given before performance of any clinical investigation-related procedure not part of standard medical care, 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
4. Diabetic foot ulcer(s) with a DUSS Score of 0 to 3
5. Ulcerated area(s) of not more than two (2) ulcers between 0.5 to 6 cm^2
6. Any female of child bearing age must consent to use medically acceptable birth control for the duration of the study
7. Female subjects must meet at least one of the following additional criteria:
    a. Surgically sterile with bilateral tubal ligation or hysterectomy.
    b. Post-menopausal for at least one year.
    c. If of child-bearing potential, practicing an acceptable method of birth control for the duration of the clinical investigation as judged by the Investigator, such as condoms, foams, jellies, diaphragm, intrauterine device or abstinence.
8. Subjects willing to undergo pre-and post-clinical investigation blood collection, physical exams and laboratory investigations.

Exclusion Criteria

1. A DUSS Score above 3.
2. DUSS Probing to Bone = "Yes"
3. An ulcer area(s) greater than 6 cm^2 or more than two (2) ulcers
4. Any subject that has received systemic or topical antibiotics within the last seven (7) days
5. Any subject on topical antimicrobial treatment for their infected diabetic foot ulcer whose ulcer is responding to treatment
6. Any subject that would be unable to follow the protocol procedures, safely monitor the infection status at home, and return for schedule visits
7. Positive pregnancy test at Screening or Visit 2
8. Active infection as demonstrated by temperature > 37.5 ^oC and clinical features of active infection.
9. Known immunosuppression or taking immunosuppressive agents including systemic steroids.
10. History of severe co-morbidity with expected patient survival ≤ 6 months.
11. Pregnancy or lactation
12. Intake of investigational drugs within 28 days prior to enrollment.
13. History of concurrent condition that, in the Investigator's opinion, would jeopardize the safety of the subject or compliance with the protocol.
14. Likely inability to comply with the protocol or cooperate fully with the investigator and site personnel.
15. Unwillingness or language barrier precluding adequate understanding of the trial procedure or cooperation with trial site personnel.
16. Known or suspected active abuse of alcohol, narcotics or non-prescription drugs.
17. Other planned surgical procedures within 30 days prior to or 30 days post-index procedure.
18. Prior enrolment in this clinical trial

Test Product

- 0.1% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v)
- 1% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v)
- 2% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v)
- 5% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v)
- 10% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v)
- 0.9% saline

Test Kit

Test Product kits will be labelled and contain a 0.5 oz dropper bottle containing 15 mL of Test Product packaged in a cardboard carton with a foam insert for protection. These kits will be pre-labelled with kit numbers. The contents of the kits will not be known to the pharmacist, designee or clinical staff. Upon randomization of the subject, the pharmacist or designee will receive a randomization number along with a kit number that is available at that site. The kit with the corresponding kit number will be used for dose administration.

Administration

Test Product will be administered to the infected diabetic foot ulcer and surrounding intact skin at a dose of 3 drops from a dropper bottle per 1 cm2 of ulcer area. This will be explained to test subjects so they know the exact number of square

After application of the Test Product is complete, the wound will be left open to air for 5 min and then will be dressed as per the following: Owens Gauze will be trimmed to cover the entire dimension of the ulcer and overlaid into the ulcer. Gauze dressings (2 x 2 inches) will be placed over the Owens Gauze to a depth of 1/4 inch followed by a circumferential wrap of 2 inch Kerlex. The dressing will be secured with paper tape applied to the circumferential dressing as to not come in contact with the underlying skin.

Clinical investigation Procedure

Subjects found eligible based on the inclusion/exclusion criteria 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. For subjects enrolled in the study, activities will consist of a Screening/Baseline Visit and three (4) scheduled visits. Screening can take place any time within 7 days to 4 hours prior to the start of the study. Screening will include:

Visit 1 (Day -7 to -4 hours) - Screening/Baseline: Following signed written informed consent and confirmation of eligibility, demographics and medical history, and limited physical examination per protocol, urine pregnancy test (if applicable), baseline laboratory tests (blood chemistries, hematology, coagulation profile, and urinalysis), and baseline subject disease assessments (as specified in the schedule of events table) will be performed at this visit.

- **Demography data, medical history, general physical examination of all organ systems, and vital signs (blood pressure, heart rate, and temperature)**: Perform a physical exam and assess vital signs per hospital protocol within the specified timeframe for screening.
- **Vital signs measurements**: Weight, temperature, resting blood pressure, heart rate, and respiratory rate
- **Hematology**: WBC, RBC, Hgb, Hct, MCV, MCH, HCHC, RDW, MPV, Platelets, Neutrophils Auto % (abs), Immature Granulocyte Auto % (abs), Lymphocytes Auto % (abs), Monocytes Auto % (abs), Eosinophils Auto % (abs), Basophils Auto % (abs)
- **Biochemistry:** Sodium, Potassium, Bicarbonate or CO2, Chloride, Total Bilirubin, Creatinine, BUN, Calcium, Alkaline Phosphatase, ALT, AST, Total Protein, Albumin, Anion Gap ,eGFR, Globulin, A/G ration
- **For females of child-bearing potential:** pregnancy test (urine) to be performed at baseline.
- **Baseline Subject Disease Assessment:** Visual examination of chronic ulcer, score target ulcer using DUSS and DFI Wound Scoring System, Swab wound to culture for microbiological examination

Visits 2 (Day 1) SAD Treatment: Subjects will return after the screening visit for Single Ascending Dose Treatment. Subject disease assessments will be performed as specified in the schedule of events table.

- Vital signs (blood pressure, heart rate, temperature) will be examined within 10 minutes prior to the start of treatment
- Baseline Subject Disease Assessment: Visual examination of chronic ulcer, score target ulcer using DUSS Scoring System, Swab wound to culture for microbiological examination – Not necessary if Visit 2 is the same day as Visit 1
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ System – Not necessary if Visit 2 is the same day as Visit 1

In SAD Treatment, eligible subjects will be treated with a single application of Test Product. Test Product will be administered to the infected diabetic foot ulcer and surrounding intact skin at a dose of 3 drops from a dropper bottle per 1 cm^2 of ulcer area, left open to air for 5 minutes and then dressed using Owens gauge, Kerlex and tape. The ulcer with a non-abrasive bandage following the initial observation period and the subject will be released with verbal instructions to leave the bandage on the wound and return for a follow up visit within 24 h ± 2 h.

Visits 3 (Day 2 [24 h ± 2 h.]) MAD Treatment: Subjects will return after the Single Ascending Dose Treatment. Subject disease assessments will be performed as specified in the schedule of events table.

- Vital signs (blood pressure, heart rate, temperature) will be examined within 10 minutes prior to the start of treatment
- Baseline Subject Disease Assessment: Visual examination of chronic ulcer, score target ulcer using DUSS Scoring System, Swab wound to culture for microbiological examination

The Principal Investigator will visually exam the chronic ulcer to determine if a subject is eligible to continue in the study. Eligible subjects will be provided with his/her designated test product kit and instructed to apply the product twice daily for one (1) week (7 days). Test product application will be demonstrated, and subject instruction sheet reviewed, using the assigned test product. The subjects will be observed applying the first dose in the clinic to ensure compliance (administering Test Product to the infected diabetic foot ulcer and surrounding intact skin at a dose of 3 drops from a dropper bottle per 1 cm^2 of ulcer area, left open to air for 5 minutes and then dressed using Owens gauge, Kerlex and tape.). The subject will be scheduled for the next follow-up visit, with a reminder to bring the test article kit to the visit.

Visit 4 (Day 9 + 1) Follow-Up to 7 Twice Daily Treatments:

The study subject will return for a follow-up evaluation to the clinical site on Day 9 ± 1day. 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
- Baseline laboratory tests (blood chemistries and hematology)
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using the DUSS and DFI Wound Scoring System
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ System
- Collection of a sample from the ulcer for microbiological assessment

Subjects will be given a 7 day supply of non-abrasive bandage and written instructions on the proper care and hygiene to include keeping the ulcer clean and bandage dry until the Day 15 follow up visit. In addition, subjects will be told to call if there is any worsening of the ulcer with regard to pain, infection, or swelling.

Visit 5 (Day 15 ± 1) End of Study (EOS) Visit

The subject shall attend final end-of-study visit 15 days ± 1 day after receiving Test Product. 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
- Physical examination
- Baseline laboratory tests (blood chemistries and hematology)
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using the DUSS and DFI Wound Scoring System
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ System
- Collection of a sample from the ulcer for microbiological assessment

At Every Visit: All adverse events (local and systemic), concurrent procedures, and changes in concomitant medications during the study will be recorded on the source documents and case report forms (CRFs). 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.

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 an adverse event, he/she will be followed until resolution/stabilization of the adverse event.

If a subject prematurely withdraws from the study they 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
- Physical examination
- Baseline laboratory tests (blood chemistries and hematology)
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using the DUSS System

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).

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 are summarized by dose group and time point of collection. Descriptive statistics are calculated for quantitative safety data, and frequency counts are compiled for classification of qualitative safety data. In addition, a mean change from baseline table is provided for vital signs and a shift table describing out of normal range shifts is provided for clinical laboratory results.
Changes in physical examinations are described in the text of the final report.

Justification of Sample Size
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 of safety and efficacy and will be considered the intent-to-treat (ITT) population. Last-observation-carried-forward (LOCF) will be used to impute missing values for efficacy variables.

A subject will be included in the per-protocol (PP) efficacy analysis population if all of the following criteria are met:

- Subject meets the inclusion/exclusion criteria;
- Subject has not taken or applied any interfering concomitant medications
- Subject has completed Visit 4/Day 15 (± 2 days) and has missed no more than one Follow-Up Visit;
- Subject has been compliant with the dosing regimen (i.e., subject must take at least 80% of the expected doses up to and including the final visit).

Interim Analysis
A safety review will be conducted after each cohort to confirm proceeding 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.

2 SCHEDULE OF EVENTS

	Screening/ Baseline	Treatment			EOS	Early Termination
VISIT/CONTACT	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	
DAY	**-7 Day to -4 hour**	**Day 1**	**Day 2**	**Day 9 + 1**	**Day 15 ± 1**	
Eligibility Screening, Inclusion, Exclusion	X					
Informed Consent	X					
Demographics & Medical History	X					
Physical Exam	X				X	X
Vital Signs	X	X	X	X	X	X
Urine Pregnancy Test[1] (WOCBP)	X				X	X
Laboratory Tests – Safety Labs	X			X	X	X
Visual Examination of Chronic Ulcer	X	X	X	X	X	X
Photo Documentation and Calculation of target ulcer area and depth – ARANZ Silhouette™ Medical System	**X**	**X**	**X**	**X**	**X**	**X**
Score Target Ulcer using DUSS System	X	X	X	X	X	X
Rating of the infected ulcer using DFI Wound Scoring system	**X**			**X**	**X**	
Swab wound to culture for Microbiological Evaluation	X	X	X	X	X	X
Investigation Product Single Treatment		X				
Subject Instruction Sheet Reviewed & Distributed		X				
Subject Diary Distributed		X				
Subject Diary Collected				X		X
Dispense Test Article			X			
Test Article Accountability				X		X
Concomitant Medication	<---------- >					X
Adverse Events	<---------- >					X

[1] 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.

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4 ABBREVIATIONS AND DEFINITIONS

Abbreviation	Meaning
° C	degrees Celsius
° F	degrees Fahrenheit
AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
API	Active Pharmaceutical Ingredient
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
CMP	Comprehensive Metabolic Panel
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events v4.0
DFI	Diabetic Foot Infection
DSMB	Data and Safety Monitoring Board
DUSS	Diabetic Ulcer Severity Score
ELSD	Evaporative Light Scatter Detection
FDA	Food and Drug Administration
GCP	Good Clinical Practice
hCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IB	Investigator's Brochure
ICH	International Committee on Harmonization
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IR	Infrared Spectroscopy
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LAI	Lakewood-Amedex, Inc
LCMS	Liquid Chromatography Mass Spectrometry
LLN	Lower Limit of Normal

MAD	Multiple Ascending Dose
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
MOA	Mechanism of Action
MRSA	Methicillin Resistant *Staphylococcus aureus*
MS	Mass Spectroscopy
NDM-1	New Delhi Metallo-Beta Lactamase-1
NMR	Nuclear Magnetic Resonance
PBS	Phosphate Buffered Saline
PI	Principal Investigator
RBCs	Red Blood Cells
SAD	Single Ascending Dose
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TS	Trypic Soy
TSBD	Trypic Soy Broth with 1% Dextrose
ULN	Upper Limit of Normal
US	United States
WBC	White Blood Cell

5 BACKGROUND

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 which 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[2] bacteria strains, 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 [redacted]. 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*.

Diabetic foot infections are one of the most common complications for people who suffer from diabetes and a frequent cause of hospitalization**Error! Reference source not found.**. ccording to the American Diabetes Association approximately 8.3% of the US population suffers from diabetes and in 2006 there were 65,700 non-traumatic amputations performed on diabetics. In addition, almost half of diabetic patients that have an amputation will develop an ulcer on the remaining limb within 18 months after surgery. The treatment of a diabetic foot infection is complicated as it requires both antimicrobial therapy to cure the infection and proper wound care management to heal the ulcer. Curing the infection is particularly challenging since 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 60-80% of chronic infections involve biofilm formation which makes the bacteria more resistant to traditional antibiotics**Error! Reference source not found.**. proper anagement 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.

6 RATIONALE

Nu-3 is a thymidine-based therapeutic compound indicated for the reduction of pathogenic bacteria associated with chronic diabetic foot ulcers. The animal data from both rodent and swine models of wound injury and its anti-fungal as well as anti-bacterial activity suggest that treatment with Nu-3 may reduce and potentially eliminate pathogenic bacteria in patients

[2] In recent years, the Infectious Diseases Society of America has highlighted a faction of antibiotic-resistant bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) – acronymically dubbed 'the ESKAPE pathogens' – capable of 'escaping' the biocidal action of antibiotics and mutually representing new paradigms in pathogenesis, transmission and resistance.

with chronic diabetic foot ulcers. The topical route of administration will be the appropriate method of delivery for humans. The low order of toxicity in rodents suggests that Nu-3 has a very low risk of toxicity to humans at the proposed doses. The doses selected for evaluation are within the FDA guidelines of the recommended safety margin of one tenth of the NOAEL.

Prior to determining the efficacy of Nu-3 in patients, safety of Nu-3 related to skin irritation was established in male and female healthy volunteers in a Human Repeat Insult Patch Test. Briefly, 20 microliters of positive control (0.1% sodium lauryl sulfate), test product (2% Nu-3 in 0.85% saline), or negative control (0.9% saline) was randomly applied using occlusive patches to either abraded or non-abraded (intact) skin for 20 consecutive days on at least 30 subjects. A total of 35 subjects completed the study and were scored on all six sites. There was a difference among products for both abraded and intact skin sites. The conclusion from the study with regards to irritation was as follows:

Abraded Skin: Positive Control > 2% Nu-3 > Negative Saline control

Intact Skin: Positive Control > 2% Nu-3 = Negative Saline Control

7 OBJECTIVE

The objective of this study is to evaluate Nu-3 for safety, tolerability, and microbiological response to bacteria in patients with chronic diabetic foot ulcer, but excluding any ulcer caused by an underlying DUSS Probing to Bone = “Yes”.

This study will specifically examine the ability of different doses of Nu-3 to eliminate pathogenic bacteria in patients with chronic diabetic foot ulcers. Dose and regimen information will be obtained for future clinical studies of Nu-3.

7.1 Primary Objective

The primary objective of this study is

- To assess the safety and tolerability of a twice daily, 7-day repeat topical doses of Nu-3 in adult male and female patients with chronically infected diabetic ulcer.

7.2 Secondary Objectives

The secondary objectives of the study are:

- To assess the clinical and microbiological response to bisphosphocin™ Nu-3.
- To support determination of the appropriate dose range of Nu-3 to be employed in future clinical studies.

8 STUDY DESIGN

8.1 Overall Study Design

This study is a Phase 1/2a prospective, multi-center, blinded, randomized (with respect to placebo), dose escalation, twice daily for 7 consecutive days study of Nu-3 solution administered topically in patients with chronic diabetic foot ulcer.

8.2 Number of Centers

This study will be conducted at two to four investigative site.

8.3 Cohorts

The dose of Nu-3 administered will be escalated in five planned sequential cohorts, with 10 subjects in each cohort, to be administered Placebo (0.9% saline) or Nu-3 doses of 0.1% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v) (Cohort 1), 1% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v) (Cohort 2), 2% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v) (Cohort 3), 5% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v) (Cohort 4), 10% solution of Bisphosphocin™ Nu-3 in 0.9% saline (w/v) (Cohort 5) at twice daily for 7 consecutive days.

Additional cohorts may be enrolled, if needed, as provided by protocol.

8.4 Placebo Control

In each Cohort, 8 subjects will be randomized to receive Nu-3, and 2 subjects will be randomized to receive Placebo.

8.5 Number of Subjects

Approximately 50 subjects will be enrolled, of which approximately 40 will be exposed to Nu-3. Numbers may vary due to replacement of subjects, addition of cohorts as permitted by protocol, or stopping of study.

8.6 Study Visits

Subjects will have 5 nominally scheduled study visits: Screening (Day -7 to -4 hours), Single Dose Visit (Day 1), Safety Review and Multiple Dose Visit (Day 2), Safety Review Visit (Day 9 ±1 day) and Final Visit (Day 15 ± 2 days). Additional visits for screening procedures and/or follow-up (e.g., in follow-up of Adverse Events) may be necessary.

8.7 Study Duration

From consent and screening through final visit, a subject's duration of participation may vary between approximately 2 and 3 weeks. No individual subject will be randomized more than once in this study.

8.8 Dose Selection and Rationale

While mechanisms of the demonstrated therapeutic effect of Nu-3 in the animal models, and of the proposed therapeutic effect in humans, are unknown, structural characteristics of Nu-3 have not suggested homology with any genetically encoded human biological molecule. There is no known suggestion of ligand-receptor specificity, and in particular no suggestion of immune system effect at the proposed doses. Preclinical studies have not suggested a species specificity for any pharmacodynamic effect.

Accordingly, Nu-3 is not regarded as a higher-risk agent, and a conventional method for determination of maximum safe starting dose is considered appropriate for this first-in-human study.

8.9 First Dose Plan

The dosing schedule for all cohorts is intended to minimize potential risk to subjects' safety. It is known that the types, severities, time courses and pharmacokinetic correlates of adverse effects observed in preclinical studies are not necessarily predictive of experience in human subjects, at any dose. This plan further recognizes that major acute toxicities of pharmaceuticals in human subjects may on rare occasions not be predicted by preclinical

experience, warranting additional caution in administering first doses to humans. As such caution has been advised in particular regarding biopharmaceuticals, it is noted that Nu-3 is not categorized as a biopharmaceutical. A traditional risk-reduction strategy has been to apply large safety factors (divisors of human equivalent doses corresponding to NOAELs in appropriate animal toxicology models) in determining the Maximum Safe Starting Dose for first-in-human study. A further risk-reduction strategy, adopted here, is to separate first and second exposures more widely in time than has been typical study procedures.

Preclinical experience suggests that possible adverse effects after a single dose topical exposure to NU-3 would be unlikely. Accordingly, the dose plan is as follows:

- Qualified patients for all Cohorts are screened from Day -7 to -4 hours. Patients understand (disclosed in informed consent) that their qualification for enrollment is not finalized until the time of enrollment. They understand that their order of enrollment is randomly determined.
- On Day -7 to -4 hours, baseline procedures are performed (demographics and medical history, and limited physical examination per protocol, urine pregnancy test (if applicable), baseline laboratory tests (blood chemistries, hematology, coagulation profile, and urinalysis), and baseline subject disease assessments).
- On Day 1, the patients is randomized and dosed to either Nu-3 or Placebo.
- On the next following day, Day 2, if the Investigator observes no dose-limiting toxicity (Section 8.10.6) in the patient dosed on Day 1, the Investigator will distribute the investigational product kit and instructed to apply the product twice daily for one (1) week (7 days).
- If the Investigator observes a suggestion of dose-limiting toxicity, the procedure specified in Section 8.10.3 is followed.

8.10 Safety Review

8.10.1 Safety Review Committee

Membership of the Safety Review Committee includes the Sponsor's designated Medical Monitor, the Medical Director of the CRO, Medical Director of Lakewood Amedex, and other member(s) as deemed appropriate by Sponsor.

8.10.2 Safety Review Procedure

Decisions regarding dose escalation and/or modification (including option of next cohort repeating at same dose, or doses intermediate to or reduced from those planned per protocol) are based upon review by the Safety Review Committee of safety data to the date of the Safety Review Visit (including data at least through the Day 8 Safety Review Visit) for the current cohort and safety data of the preceding cohorts to the date of the Safety Review Visit.

- Safety Review meeting may be conducted by teleconference.
- Decisions of the Safety Review Committee must be unanimous among the members participating in the review and are recorded in meeting minutes.
- Draft minutes of Safety Review Committee meetings are reviewed by all members who attended. Proposed changes are reviewed by all members who attended. Final draft of minutes is approved by consensus.
- Individual members' approvals of final draft minutes are documented to study regulatory file.
- Final draft of minutes is placed in study regulatory file and provided to IRB.

8.10.3 Cohort 1 Day 1a Experience

If a Subject dosed on Day 1 of Cohort 1 experiences any therapeutic adverse event around the wound area:

- Further dosing of Cohort 1 is suspended.
- The Safety Review Committee meets to consider available safety data.
- The Safety Review Committee decides and documents rationale for further course of action, which may include resumption of study conduct.

8.10.4 Unblinding for Safety Review

If the Safety Review Committee decides that unblinding of dose randomization is necessary for decision regarding continued dosing and/or dose escalation, the unblinding is done in a manner that minimizes the extent of unblinding and of its potential effects on assessments of adverse events. This action is documented in the minutes of the Safety Review Committee meeting.

8.10.5 Decision Criteria

8.10.5.1 Stopping of Dose Administration and/or Escalation

Further study drug dose administration or escalation will be stopped:

1. by Sponsor, for any reason at any time;
2. by Principal Investigator or Safety Review Committee, if subjects' safety is considered to be at unacceptable risk;
3. by Safety Review Committee, according to these criteria:
    - If 2 or more of the Nu-3-treated subjects in a cohort experience a DLT, dose escalation is stopped;
    - If 1 of the Nu-3-treated subjects experiences a DLT, the cohort is expanded to include 4 additional subjects (Nu-3:Placebo ratio of 3:1); and
    - If 2 or more of the Nu-3-treated subjects in the expanded cohort (of 8+4=12 subjects) experience a DLT, dose escalation is stopped;
    - If only 1 of the Nu-3-treated subjects in the expanded cohort experiences a DLT (i.e., no additional subjects experience a DLT following cohort expansion), stopping of dose escalation is not required.

8.10.5.2 Definition of Dose-Limiting Toxicity (DLT)

Dose-Limiting Toxicity in an individual subject is defined as any Grade 2 or higher Adverse Event (AE) toxicity according to National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0 (CTCAE – See Appendix 16.1) that is considered by the Investigator to be at least possibly related to study drug.

8.10.5.3 Definition of Maximum Tolerated Dose (MTD)

If dose escalation is stopped according to criteria of 8.10.4 and the Safety Review Committee does not decide to administer a dose lower than the level at which dose escalation was stopped and higher than the next lowest tested level, the next lowest tested level will be considered the maximum tolerated dose (MTD).

8.11 Subsequent Cohort Dose Plan

Anticipated Dose Plan – Upon completion of Safety Review of experience to date, if the Safety Review Committee determines that there is no reason to do otherwise, the dose plan for the subsequent cohort is:

- Randomization and dosing proceed no sooner than 7 days after the last Day 1 dosing of the preceding cohort.
- Dose of study drug administered to subjects in the next cohort is the next higher dose anticipated by protocol (e.g., 1% Nu-3 solution dose cohort after 0.1% Nu-3 solution dose cohort).
- Ten subjects are randomized such that eight dose with Nu-3 and two dose with Placebo.
- A single subject is dosed at a given scheduled time.
- Dosing of successive subjects are separated in time by an interval that may vary between cohorts but must be at least 20 minutes. Safety Review Committee may specify this interval.

9 SELECTION AND WITHDRAWAL OF SUBJECTS

Subjects with diabetic chronic foot ulcer and with a DUSS Score of 0 to 3, ulcerated area(s) of not more than two (2) ulcers between 0.5 to 6 cm^2, and no underlying DUSS Probing to Bone = "Yes" will be enrolled. This study will enrol 10 evaluable subjects per group in 5 groups for a total of 50 evaluable subjects. An evaluable subject is one who has received investigational product and has returned for the 15 ± 2 Day follow-up visit. Up to approximately 55 patients will be enrolled in order to have a population minimum of 50 evaluable subjects at the end of the trial.

9.1 Inclusion and Exclusion of Subjects

To be included in this study, subjects must meet the following inclusion/exclusion criteria:

9.1.1 Inclusion Criteria

1. Men and women between the ages of 18 and 85.
2. Voluntary written consent, given before performance of any clinical investigation-related procedure not part of standard medical care, 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
4. Diabetic foot ulcer(s) with a DUSS Score of 0 to 3
5. Ulcerated area(s) of not more than two (2) ulcers between 0.5 to 6 cm^2
6. Any female of child bearing age must consent to use medically acceptable birth control for the duration of the study
7. Female subjects must meet at least one of the following additional criteria:
    d. Surgically sterile with bilateral tubal ligation or hysterectomy.
    e. Post-menopausal for at least one year.
    f. If of child-bearing potential, practicing an acceptable method of birth control for the duration of the clinical investigation as judged by the Investigator, such as condoms, foams, jellies, diaphragm, intrauterine device or abstinence.
8. Subjects willing to undergo pre-and post-clinical investigation blood collection, physical exams and laboratory investigations..

9.1.2 Exclusion Criteria

1. A DUSS Score above 3.
2. DUSS Probing to Bone = "Yes"
3. An ulcer area(s) greater than 6 cm^2 or more than two (2) ulcers

4. Any subject that has received systemic or topical antibiotics within the last seven (7) days
5. Any subject on topical antimicrobial treatment for their infected diabetic foot ulcer whose ulcer is responding to treatment
6. Any subject that would be unable to follow the protocol procedures, safely monitor the infection status at home, and return for schedule visits
7. Positive pregnancy test at Screening or Visit 2
8. Active infection as demonstrated by temperature > 37.5 ^{o}C and clinical features of active infection.
9. Known immunosuppression or taking immunosuppressive agents including systemic steroids.
10. History of severe co-morbidity with expected patient survival ≤ 6 months.
11. Pregnancy or lactation
12. Intake of investigational drugs within 28 days prior to enrollment.
13. History of concurrent condition that, in the Investigator's opinion, would jeopardize the safety of the subject or compliance with the protocol.
14. Likely inability to comply with the protocol or cooperate fully with the investigator and site personnel.
15. Unwillingness or language barrier precluding adequate understanding of the trial procedure or cooperation with trial site personnel.
16. Known or suspected active abuse of alcohol, narcotics or non-prescription drugs.
17. Other planned surgical procedures within 30 days prior to or 30 days post-index procedure.
18. Prior enrolment in this clinical trial

9.2 **Withdrawal of Subjects**

A subject must be withdrawn from the study prior to completion for any of the following reasons:

- Whenever the subject decides that it is in his/her best interest to withdraw
- Whenever the investigator decides that it is in the subject's best interest to be withdrawn
- An adverse event or illness which may, in the investigator's opinion, significantly affect the subject or study
- Non-compliance
- Sponsor administrative reasons.

Withdrawal of consent for follow-up should be accompanied by documentation of the reason for withdrawal. Withdrawal of consent for treatment should be distinguished from withdrawal of consent for follow-up visits and from withdrawal of consent for non-patient contact follow-up, e.g., medical records checks. Subjects requesting withdrawal should be informed that withdrawal of consent for follow-up will jeopardize the public health value of the study.

Subjects who withdraw should be explicitly asked about the contribution of possible adverse events to their decision to withdraw consent, and any adverse event information elicited should be documented.

Preferably the subject should withdraw consent in writing and, if the subject or the subject's representative refuses or is physically unavailable, the site should document and sign the reason for the subject's failure to withdraw consent in writing. The informed consent for the study should note that although a subject is free to leave the study and stop taking study medication, the investigators hope the patient will remain for follow up status evaluations.

Data from subjects who withdraw from the study will still be used in the statistical analysis. A decision to replace a withdrawn subject will be made on a case-by-case basis after discussions between the investigator, Medical Monitor, and Sponsor. Generally speaking, a subject who does not meet the definition of "evaluable" (i.e., received investigational product and has returned for the Day 2 follow-up visit) may be replaced. However, all subjects, whether withdrawn or not, will be included in ITT population for safety evaluations of Nu-3.

10 Investigational Products

10.1 Study Drug and Placebo

	Nu-3	Placebo
Manufacturer	Xcelience LLC 5415 West Laurel Street Tampa, Florida 33607 USA	
Route	Topical	
Formulation	0.1% Nu-3 in 0.9% saline (w/v) 1% Nu-3 in 0.9% saline (w/v) 2% Nu-3 in 0.9% saline (w/v) 5% Nu-3 in 0.9% saline (w/v) 10% Nu-3 in 0.9% saline (w/v)	0.9% sterile saline for injection

All study drug is provided in a 0.5 oz. oval low density ethylene dropper bottle. The bottle opening is plugged with a 13 mm extended controlled dropper tip and sealed with a 15/415 extend tip closure. [redacted]

10.2 Dose Schedule

The anticipated dosing plan is that each subject will receive a single topical administration of Nu-3 or Placebo on Day 1 followed by twice daily for 7 day administration of Nu-3 or Placebo on Day 2 as outlined in the following table. Actual randomization assignments and conduct of study may vary from this, as provided in 8.10 Safety Review.

Cohort	Nu-3 in 0.9% saline (w/v)					Total Dosed Nu-3 + Placebo
	Dose 1 0.1%	Dose 2 1%	Dose 3 2%	Dose 4 5%	Dose 5 10%	
1	8	--	--	--	--	10
2	--	8	--	--	--	10
3	--	--	8	--	--	10
4	--	--	--	8	--	10
5	--	--	--		8	10
						50

10.3 Drug Shipping, Packaging and Labeling

Study drug is shipped to the investigative site from [redacted] in standard packing which consists of an outer shipping container (20 x 17 x 12.5), an inner container (12 x 12 x 6) and two foam inserts each holding a quantity of 25 vials. Ice is packed on the bottom and around all three sides of the foam containers. Contents of the label(s) will meet all applicable regulatory requirements.

Study drug is packaged in a 0.5 oz. oval low density ethylene dropper bottle plugged with a 13 mm extended controlled dropper tip and sealed with a 15/415 extend tip closure

The following draft label will be affixed to all containers of Nu-3 drug product, indicating the appropriate storage condition.

DRAFT LABEL

GC: X; LID: X; CARTON	
Protocol #:	LAI2014-1
Product:	Bisphosphocin Nu-3 topical antibiotic or placebo
Bottle Number:	####
Lot Number*:	14K###
Directions for Use:	As directed, apply twice daily to the affected area
Storage:	Room Temperature^
Caution:	New Drug-Limited by federal law to Investigational Use Keep out of reach of children
Manufacturer:	Lakewood Amedex Inc., Sarasota, FL (941)225-2515

*Lot number represents the group of kits being assembled for each cohort.

^Room temperature is defined according to the USP as the temperature that is prevailing in a work area.

10.4 Study Drug Handling and Storage

10.4.1 Handling and Storage

Upon arrival of study drug shipping container at the investigative site, it is immediately opened by pharmacy personnel in a secure location, and contents are verified to match the shipping documentation.

Study drug is stored at the investigative site in a secure location routinely accessible only to Pharmaceutical Services personnel, under these environmental conditions: at ambient temperature.

10.4.2 Study Drug Accountability

The investigative site maintains records of study drug accountability and reconciliation throughout the course of the study. Records show the amount(s) of study drug received from Sponsor, administered to subjects, wasted/lost (with description of circumstances), and returned to Sponsor and/or Sponsor's designee. Only subjects enrolled and randomized in this study may receive study drug.

10.5 **Randomization**

Subjects are assigned to dosing with Nu-3 or Placebo according to the randomization schedule prepared prior to study start and provided by Sponsor/CRO. In the event that an expanded or unscheduled cohort is added, Sponsor/CRO will provide the randomization schedule for that cohort prior to admission of the cohort to the investigative site. The randomization scheme was predetermined and ordered in blocks of five (5). Five study kits are provided to each investigational site utilized to maintain the randomization scheme.

10.6 **Blinding and Unblinding**

This is a double-blind study. The randomization schedule for each cohort is provided by Sponsor/CRO as a sequential list in a sealed envelope. At the investigative site, procedural precautions are taken to avoid unblinding due to visual inspection of Nu-3 and/or Placebo prepared for dosing.

Unblinding occurs only in the event of an Adverse Event, or pregnancy of a subject's partner, and only if knowledge of the administered study drug is judged likely to affect the medical treatment decisions related to the subject or pregnant partner, or if required for decision-making by the Safety Review Committee. In the event of unblinding, the Investigator notifies the Sponsor within 24 hours of breaking the blind and provides formal, written documentation to the Sponsor within 5 working days. This documentation includes the identity of the individual(s) who have become aware of randomization assignment(s), the date on which the blind was broken, and a description of the event motivating the unblinding. The Investigator also records in relevant source documents that blind was broken, reason for unblinding, and the date/time of that occurrence.

The Safety Review Committee may request unblinding to assist in Safety Review and decisions regarding further conduct of study. To the extent possible while meeting the need for appropriate Safety Review, the Investigator and other investigative site personnel are not unblinded. The existence of unresolved Adverse Events at the time of unblinding is given consideration. If an Investigator is unblinded, that Investigator is not subsequently involved in the management or assessment of AEs for Subjects whose dose assignments have been unblinded.

10.7 **Study Drug Preparation**

Study drug is kept under supervision of pharmacy personnel until delivered to the area designated for study drug administration.

10.8 **Study Drug Administration**

10.8.1 Study Drug Administration Procedure

Licensed Clinical Operations personnel at the investigative site administer each dose of Study Drug to study. Test Product will be administered to the infected diabetic foot ulcer and surrounding intact skin at a dose of 3 drops from a dropper bottle per 1 cm^2 of ulcer area.

After application of the Test Product is complete, the wound will be left open to air for 5 min and then will be dressed as per the following: Owens Gauze will be trimmed to cover the entire dimension of the ulcer and overlaid into the ulcer. Gauze dressings (2 x 2 inches) will be placed over the Owens Gauze to a depth of 1/4 inch followed by a circumferential wrap of

2 inch Kerlex. The dressing will be secured with paper tape applied to the circumferential dressing as to not come in contact with the underlying skin.

10.8.2 Disposition of Dose Administration Materials

Following study drug administration, materials possibly containing remaining visible study drug or Placebo solution are placed in a closed container and returned to the Pharmacy for storage at ambient temperature until shipped to the Sponsor.

11 Concomitant Medications and Exposures

11.1 Prior Medications

All medications and herbal supplements used by a patient within 14 days prior to screening are recorded in the medical history. Dose, frequency, and purpose of use are recorded, as best the volunteer can recall.

11.2 Concomitant Medications

The Investigator notifies the Medical Monitor regarding all medications taken by a subject during study participation, for treatment of Adverse Events or pre-existing conditions.

12 TREATMENT OF SUBJECTS AND CONDUCT OF STUDY

Subjects can be screened up to seven days to 4 hours before treatment. Subjects who have been found eligible for the study based on the inclusion and exclusion criteria will have the study explained in detail. If the subject decides to participate in the study, the subject will be given a study specific ICF to sign. Only after the subject undergoes the informed consent process and provides a written informed consent, the screening evaluations shall be performed. Subjects can be consented at any time once identified by the site study staff as potentially eligible.

If a female of child-bearing potential is found to be pregnant, or is planning to become pregnant within one week of administering the study medication, then she is not eligible for the study.

Male or female patients of at least 18 years of age are candidates for this study. If the patient may meet entry criteria, the patient will be informed about the study. The investigator should also consider the patient's risk of injury prior to screening. Even after the inclusion and exclusion criteria are reviewed, if the investigator considers the patient a risk for injury, the patient should not be enrolled in the study.

12.1 Informed Consent

All patients are to give informed consent in accordance with the origins of the Declaration of Helsinki and applicable regional regulatory requirements (e.g. 21 CFR Part 50).

The patient will sign the Informed Consent Form before she/he enters the study, i.e., before screening bloods, screening assessments or any other study-related activity. The patient will be given sufficient time to consider the study's implications before deciding whether to participate. The format and content of the ICF must be agreed upon by Lakewood Amedex and the Institutional Review Board.

Should there be any amendments to the final protocol; the patient must agree to sign the amended ICF indicating that they re-consent to participate in the trial.

12.2 Visit 1 (Day -7 to -4 hours) - Screening

After granting of informed consent the patient will undergo screening. Screening includes the following:

- **Demography data, medical history, general physical examination of all organ systems, and vital signs (blood pressure, heart rate, and temperature)**: Perform a physical exam and assess vital signs per hospital protocol within the specified timeframe for screening.
- **Vital signs measurements**: Weight, temperature, resting blood pressure, heart rate, and respiratory rate
- **Hematology**: WBC, RBC, Hgb, Hct, MCV, MCH, HCHC, RDW, MPV, Platelets, Neutrophils Auto % (abs), Immature Granulocyte Auto % (abs), Lymphocytes Auto % (abs), Monocytes Auto % (abs), Eosinophils Auto % (abs), Basophils Auto % (abs)
- **Biochemistry:** Sodium, Potassium, Bicarbonate or CO2, Chloride, Total Bilirubin, Creatinine, BUN, Calcium, Alkaline Phosphatase, ALT, AST, Total Protein, Albumin, Anion Gap ,eGFR, Globulin, A/G ration
- **For females of child-bearing potential:** pregnancy test (urine or serum β-HCG) to be performed at baseline.
- **Baseline Patient Disease Assessment:** Visual examination of chronic ulcer, score target ulcer using DUSS Scoring System, Swab wound to culture for microbiological examination

Diabetic Ulcer Wound Scoring System (DUSS)

Parameter	Score	
Palpable Pedal Pulses	Presence = 0	Absence = 1
Probing to Bone	No = 0	Yes = 1
Location of Ulcer	Toe = 0	Foot = 1
Number of Ulcerations	Single = 0	Multiple = 1
Score Range	0	4

Diabetic Foot Ulcer Wound Infection Score

Parameter	Wound Infection Score			
	0	1	2	3
Purulent discharge	Absent			Present
Nonpurulant drainage (serious, sanguinous)	Absent	Mild		
Erythema	None	Mild pink, barely perceptible	Moderate pale red, defined edges	Severe red to dark red
Induration	None	Mild	Moderate	Severe
Tenderness (sign)	None	Mild	Moderate	Severe
Pain (symptoms)	None	Mild	Moderate	Severe
Local Warmth (relative to uninfected contralateral foot)	Same	Mildly increased	Moderately increased	Severely increased

Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ as described in Appendix A

A past medical/surgical history will be recorded for any conditions or treatments that are, in the opinion of the investigator, relevant to the current diagnosis. The previous condition will be recorded as a diagnosis rather than a previous symptom. The time of the previous diagnosis relative to the current admission will be provided (i.e., "6 months ago") to the best of the patient's knowledge. If the diagnosis was more than two years ago, then it can be recorded as "more than two years ago".

Prior medications will be recorded. Prior medications include medications that the patient is currently taken or has taken within the past fourteen days.

Physical examinations are performed at screening by the investigator or designee. The screening examinations will include general appearance, height, weight, skin, neck, eyes, ears, nose, throat, heart, lungs, abdomen, cervical and axillary lymph nodes, extremities, and nervous system. Height is measured in stocking or bare feet, and recorded in centimeters (cm). Weight is recorded in kilograms (kg).

Results of these investigations/assessments and baseline measurements obtained from the local laboratory will be reviewed by the Investigator prior to the start of the treatment. Patients who do not meet study entry criteria will be promptly notified and excluded from further participation in the study. Patients who continue to meet entry criteria will be enrolled in the study and assigned a patient number by the database. The patient can be randomized at any time following the screening.

12.3 Visit 2 (Day 1) SAD Treatment

Patients will return after the screening visit for Single Ascending Dose Treatment. Patient disease assessments will be performed as specified in the schedule of events table.

- Vital signs (blood pressure, heart rate, temperature) will be examined within 10 minutes prior to the start of treatment
- Baseline Patient Disease Assessment: Visual examination of chronic ulcer, score target ulcer using DUSS Scoring System, Swab wound to culture for microbiological examination – Not applicable if Visit 2 is the same day as Screening Visit
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ system - Not applicable if Visit 2 is the same day as Screening Visit

In SAD Treatment, eligible patients will be treated with a single application of Test Product. Test Product will be administered to the infected diabetic foot ulcer and surrounding intact skin at a dose of 1 to 3 drops from a dropper bottle per 1 cm^2 of ulcer area, left open to air for 5 minutes and then dressed using Owens gauge, Kerlex and tape. The ulcer with a non-abrasive bandage following the initial observation period and the patient will be released with verbal instructions to leave the bandage on the wound and return for a follow up visit within 24 h ± 2 h.

12.4 Visit 3 (Day 2 [24 h ± 2 h.]) MAD Treatment

Patients will return after the Single Ascending Dose Treatment. Patient disease assessments will be performed as specified in the schedule of events table.

- Vital signs (blood pressure, heart rate, temperature) will be examined within 10 minutes prior to the start of treatment
- Baseline Patient Disease Assessment: Visual examination of chronic ulcer, score target ulcer using DUSS Scoring System, Swab wound to culture for microbiological examination
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ System

The Principal Investigator will visually exam the chronic ulcer to determine if a patient is eligible to continue in the study. Eligible patients will be provided with his/her designated test product kit and instructed to apply the product twice daily for one (1) week (7 days). Test product application will be demonstrated, and patient instruction sheet reviewed, using the assigned test product. The patients will be observed applying the first dose in the clinic to ensure compliance (administering Test Product to the infected diabetic foot ulcer and surrounding intact skin at a dose of 1 to 3 drops from a dropper bottle per 1 cm^2 of ulcer area, left open to air for 5 minutes and then dressed using Owens gauge, Kerlex and tape.). The patient will be scheduled for the next follow-up visit, with a reminder to bring the test article kit to the visit.

12.5 **Visit 4 (Day 9 + 1) Follow-Up to 7 Twice Daily Treatments**

The study patient will return for a follow-up evaluation to the clinical site on Day 9 + 1. At this follow-up visit, the patient will undergo the following procedures and evaluations.

- Vital signs measurements including weight, temperature, resting blood pressure, heart rate, and respiratory rate
- Baseline laboratory tests (blood chemistries and hematology)
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using the DUSS and DFI Wound n System
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ System
- Collection of a sample from the ulcer for microbiological assessment
- Collection of Subject Home Treatment Log
- Collection of Investigational Product (IP)

Patients will be given a 7 day supply of non-abrasive bandage and written instructions on the proper care and hygiene to include keeping the ulcer clean and bandage dry until the Day 15 follow up visit. In addition, patients will be told to call if there is any worsening of the ulcer with regard to pain, infection, or swelling.

12.6 **Visit 5 (Day 15 ± 1) End of Study (EOS) Visit**

The patient shall attend final end-of-study visit 15 ± 1 days after receiving Test Product. At this follow-up visit, the patient will undergo the following procedures and evaluations.

- Vital signs measurements including weight, temperature, resting blood pressure, heart rate, and respiratory rate
- Physcial examination
- Baseline laboratory tests (blood chemistries and hematology)
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using the DUSS and DFI Wound Scoring System
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ System

- Collection of a sample from the ulcer for microbiological assessment

12.7 At Every Visit

All adverse events (local and systemic), concurrent procedures, and changes in concomitant medications during the study will be recorded on the source documents and case report forms (CRFs). 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.

12.8 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 an adverse event, he/she will be followed until resolution/stabilization of the adverse event.

If a subject prematurely withdraws from the study they 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
- Physcial examination
- Baseline laboratory tests (blood chemistries and hematology)
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using the DUSS System

12.9 Adverse Event Monitoring

Subjects will be monitored for any adverse events throughout the screening and until the end-of-study visit on Day 15. Adverse events will be specifically assessed by observation at Day 1 following administration of the first dose of investigational product, at Day 2 following the second dose administration, at Day 9 visit and at Day 15 visit. Abnormal and clinically significant measurements obtained from the local lab will be used to note adverse events.

Vital signs (blood pressure and heart rate) can be monitored per hospital/site protocol following administration of the first dose of investigational product, and at Day 2 following the second dose administration. Deviations from normal will be recorded as adverse events.

13 ASSESSMENT OF SAFETY

13.1 Physical Examination

Physical examinations are performed at screening and at the Day 15 End of Study Visit. The examinations will include general appearance, skin, neck, eyes, ears, nose, throat, heart, lungs, abdomen, cervical and axillary lymph nodes, extremities, and nervous system. Height is measured in stocking or bare feet, and recorded in inches, at screening. Weight is recorded in pounds at screening and Day 15 visit.

13.2 Vital Signs

Vital Signs, including one or more of systolic and diastolic blood pressure, pulse, and temperature, are recorded at screening, at Day 1 visit, at Day 2 visit, at Day 9 visit, and at the Day 15 Final Visit. See Schedule of Events for detail.

Consistent BP measurement in one arm is preferred to facilitate comparisons of serial BP measurements. However, optimal performance of other procedures (e.g., blood sampling) may require changing BP measurement from one arm to the other.

The investigator or designee will perform a thorough physical examination at the start of the study and brief physical examination at 15 days post dose administration. Any changes in the overall health and well-being of the subject during the study will be reported as an adverse event. If the subject is unable to return for the 15 day visit, then the physical exam is not mandatory. In this case, the subject's well-being may be assessed by phone.

An investigator will be in the premises during the investigational product administration and on call until the end of the study.

13.3 **Clinical chemistry**

A CMP (biochemistry and hematology) will be performed by the local lab at screening, and then repeated at Day 9 and Day 15 after the first dosing. Changes from baseline will be assessed as a safety parameter. If a change in a clinical chemistry parameter is observed and considered to be clinically significant by the investigator, the observation will be written up as an adverse event.

13.4 **Adverse events**

Subjects will be monitored for any adverse events throughout the screening and until the end-of-study visit on day 15. Adverse events will be specifically assessed by observation at Day 1 following administration of the first dose of investigational product, at Day 2 following the second dose administration, at Day 9 visit and at Day 15 visit.

The recording of adverse events is an important aspect of study documentation. It is of utmost importance that all staff members at the site involved in the study are familiar with the content of this section in order to document all adverse events according to the detailed guidelines set out below. During the study where there is safety evaluation, the principal investigator or site staff will be responsible for detecting, documenting and reporting AEs and SAEs.

An **adverse event (AE)** is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Events that are expected as a result of a procedure (other than drug administration of investigational product) that the subject undergoes should not be listed as an AE unless the event is more severe than expected or deemed significant by the Principal Investigator.

Timely and complete reporting of all AEs assists the medical staff and sponsor in identifying any untoward medical occurrence, thereby allowing:

1. protection of the safety of study subjects;
2. a greater understanding of the overall safety profile of the test article;
3. recognition of dose-related test article toxicity;
4. appropriate modification of study protocols;
5. improvements in study design or procedures; and
6. adherence to worldwide regulatory requirements.

Test article is defined as a pharmaceutical form of an active ingredient (or "primary operational component" for devices) or vehicle/placebo being tested or used as a reference in

the study, whether blinded or unblinded. AEs may be either spontaneously reported or elicited during questioning and examination of a subject. All AEs must be completely recorded on the within the electronic source document system. If known, the Investigator should report the diagnosis of the underlying illness or disorder, rather than its individual symptoms. Subjects experiencing AEs that cause interruption or discontinuation of test article, or those experiencing AEs that are present at the end of their participation in the study will receive follow-up as appropriate. If possible, report the outcome of any AE that caused permanent discontinuation or that was present at the end of the study particularly if the AE was considered by the investigator to be definitely, probably, or possibly related to test article.

The investigator will instruct the subject to report any adverse events that may occur during the study. At each visit, the investigator will ask the subject, in non-directive fashion, about any change in the subject's overall condition since the previous visit.

The severity of each adverse event, as judged by the Investigator, will be graded according to Common Terminology Criteria for Adverse Events v4.02 (CTCAE). For AEs not listed in the CTCAE table, the severity of adverse changes in physical signs or symptoms will be classified as follows (consistent with CTCAE):

Grade 1 Mild - asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 Moderate - minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
Grade 3 Severe - medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
Grade 4 Life-threatening - urgent intervention indicated.
Grade 5 Death - Death related to AE.

The investigator must determine the relationship of the AE to the test article according to the following categories:

Definite - An event that follows a reasonable temporal sequence from administration of the test article; that follows a known or expected response pattern to the test article; and that is confirmed by improvement on stopping or reducing the dosage, and reappearance of the event on repeated exposure (re-challenge).
Probable - An event that follows a reasonable temporal sequence from administration of the test article; that follows a known or expected response pattern to the test article; and that is confirmed by improvement on stopping or reducing the dosage of the test article; and that is unlikely to have been caused by concurrent/underlying illness or other drugs, procedures, or other causes.
Possible - An event that follows a reasonable temporal sequence from administration of the test article; that follows a known or expected response pattern to the test article; but may have been caused by concurrent/underlying illness, other drug, procedure, or other causes.
Unlikely - An event that does not follow a reasonable temporal sequence from administration of the test article; that does not follow a known or expected response pattern to the test article, or most likely was caused by concurrent/underlying illness, other drug, procedure, or other causes, because of their known effects.

Not Related - An event almost certainly caused by concurrent/underlying illness, other drug, procedure, or other causes.

An **adverse reaction** is any AE caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. For the purposes of prescription drug labelling, the term adverse reaction means an undesirable effect, reasonably associated with the use of a drug that may occur as part of its pharmacological action or may be unpredictable in its occurrence.

A **suspected adverse reaction** is any adverse event for which there is a reasonable possibility that the drug caused the event.

For the purposes of safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An event that is a **serious adverse event (SAE)** must be recorded on the AE eCRF and requires expeditious handling to comply with regulatory requirements.

An adverse event or suspected adverse reaction is considered "serious" if, in the opinion of either the investigator or sponsor, it results in any of the following outcomes:

- Death.
- Life-threatening event.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly/birth defect.
- Is an important medical event - defined as a medical event(s) that may not result in death, be life-threatening, or require hospitalization but, based upon appropriate medical judgment, may jeopardize the patient/subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events NOT considered to be SAEs are:

- Hospitalizations for the treatment, which was elective or pre-planned, of a pre-existing condition that did not worsen. A hospitalization planned after the index admission, such as a staged PCI procedure, or a planned prolonged hospitalization, such as coronary artery bypass surgery related to the index admission, will not be considered an SAE.
- Treatment on an emergency, outpatient basis, for an event not fulfilling any of the definitions of "serious" given above and not resulting in hospital admission.

The Medical Monitor, Lead Principal Investigator, or Sponsor may be consulted with any questions regarding the definition of "planned". The Medical Monitor may be consulted regarding other questions about SAEs.

Adverse events classified as "serious" require expeditious handling and reporting to the Medical Monitor, Safety Officer or designee to comply with regulatory requirements. **All SAEs, whether related or unrelated to test article, must be immediately reported by telephone to the Medical Monitor, Safety Officer or, in the event that neither is available, to the Project Manager listed on the first page of the protocol.** All SAEs should be entered in the eCRF as soon as possible. These include those SAEs listed in the protocol or Investigator Brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

All SAE are required to be captured on the study supplied SAE report form. Please contact the Safety Officer at [redacted]

If only limited information is initially available, follow-up reports are required. In the event of death, if an autopsy is performed, a copy of the report should be sent to the Sponsor or designee, if available.

As required, the Sponsor or designee will notify participating investigators of all suspected adverse reactions that are serious and unexpected. This notification will be in the form of an IND safety report of potential serious risks as soon as possible but no later than 15 calendar days after the Sponsor determines that the information is "reportable" according to the criteria listed in Section 312.32(c)(1)(i) to (iv) of the CFRs. These are:

- Serious and unexpected suspected adverse reactions,
- Findings from other studies including epidemiological studies, pooled analyses or other clinical studies that suggest a significant risk in humans exposed to the test articles,
- Findings from animal or in vitro tests that suggest a significant risk to humans exposed to the test articles, or reports of significant organ toxicity at or near the expected human exposure, and
- Clinically important increases in the rate of occurrence of serious suspected adverse reactions.

Upon receiving such notices, the investigator must review and retain the notice with the Investigator Brochure and immediately submit a copy of this information to the responsible IRB according to local regulations. The investigator and IRB will determine if the ICF requires revision. The investigator should also comply with the IRB procedures for reporting any other safety information. Where required, submission of safety updates by the investigator to Health Authorities should be handled according to local regulations.

All AEs must be recorded on the AE CRF. AEs should be followed to resolution or stabilization (if possible), and reported as SAEs if they become serious. AEs will be collected starting when the subject signs the ICF and ending with the end-of-study visit on Day 15. For subjects who withdraw from the study, the collection of AEs will end 15 days after the first dose administration. If any adverse event is present when a subject completes the study or if a

subject is withdrawn from the study and the adverse event has still not resolved at this time, additional follow-up will be performed, as appropriate. The investigator will continue to follow-up all serious adverse events or other adverse events that are considered to be related to the investigational products, until it is resolved or assessed to be stable by the Investigator. This follow-up may be extended beyond the end of the study period. An adverse event can reach one of the following outcomes:

- Completely recovered from the AE
- Recovered with sequelae
- Ongoing
- Ongoing at the time of death
- Death
- Unknown

14 ASSESSMENT OF EFFICACY

14.1 Primary Efficacy Endpoint Assessment

The primary efficacy endpoint is to assess the microbiological response to bisphosphocin™ Nu-3 as determined by reduction of pathogenic bacteria following Nu-3 treatment. The microbiological assessments from the local lab will be used to measure aerobic and anaerobic culture and sensitivity at screening, after a single dose treatment (Day 1), after a second dose treatment (Day 2), after twice daily for 7 day treatment, at Day 15.

14.2 Secondary Efficacy Endpoint Assessment

The primary efficacy endpoint is to assess the clinical response to bisphosphocin™ Nu-3 as determined by visual evaluation of the ulcer following Nu-3 treatment. The measurement of this variable will be determined by the DUSS score at screening, after a single dose treatment (Day 1), after a second dose treatment (Day 2), after twice daily for 7 day treatment, at Day 15.

15 PHARMACOKINETIC ASSESSMENT

There are no pharmacokinetic assessments being conducted in this study.

16 STATISTICAL CONSIDERATIONS

This study is designed to assess the safety and efficacy of Nu-3. Safety will be assessed by monitoring clinical laboratory parameters and treatment emergent adverse events. Efficacy will be assessed using endpoints of the clinical and microbiological response to Nu-3

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

For all parameters, a comparison will be made of each active treatment group versus placebo using the appropriate statistical test. Unless otherwise indicated, all statistical tests will be

two-sided and differences will be considered statistically significant if the associated p-value is less than 0.05. All data will be presented in data listings.

A formal statistical analysis plan will be provided in a separate document. The Statistical Analysis Plan will contain additional details of the planned analysis along with tables, listings and figures that will become part of the Clinical Study Report. The Statistical Analysis Plan will be finalized prior to the database lock.

16.1 **Justification of Sample Size and Power Analysis**

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

16.2 **Analysis Populations**

All randomized subject will be accounted for in a listing for subject disposition. All randomized subjects will be included in the intent-to-treat (ITT) population. All subjects administered any amount of investigational product will be included in the Safety population. The per-protocol (PP) population will include all subjects who meet enrollment criteria and receive all doses of investigational product as required by the protocol and who have no major protocol violations. Major protocol violations are defined as follows:

1. Failure to properly obtain consent or subject not consented prior to dosing.
2. Subject not meeting the entry criteria and enrolled without a written waiver.
3. Subject randomized in the incorrect treatment arm.
4. Subject given incorrect dose (overdose/under-dose).
5. Data not available from the post-dose central laboratory assessment.
6. Failure to complete the study.
7. Performing study procedure(s) not described in the approved protocol.

All safety and efficacy analyses will be performed on all populations.

16.3 **Interim Analysis**

A safety review will be conducted after each cohort to confirm proceeding to the next dose level.

16.4 **Analysis of Baseline Data**

Demographic and baseline characteristics will be summarized using descriptive statistics. Continuous variables will be summarized by mean, median, standard deviation (SD), minimum and maximum. Categorical variables will be summarized by counts and percentages.

16.5 Analysis of Primary Outcome Measure

The microbiological response of Nu-3 to eliminate pathogenic bacteria will be determined for each group. Aerobic and anaerobic culture and sensitivity will be assessed at screening, after a single dose treatment (Day 1), after a second dose treatment (Day 2), after twice daily for 7 day treatment, at Day 15. An uncorrected chi-square analysis or a Fisher's Exact test will be used to compare each treatment group to the placebo group. An associated p-value less than 0.05 will be used to define statistical significance. An associated p-value less than 0.016 (0.05 ÷ 5; Bonferroni correction) will be considered highly conclusive.

The clinical response of Nu-3 via visual evaluation of the ulcer will be determined for each group. DUSS Score will be assessed at screening, after a single dose treatment (Day 1), after a second dose treatment (Day 2), after twice daily for 7 day treatment, and at Day 15. An uncorrected chi-square analysis or a Fisher's Exact test will be used to compare each treatment group to the placebo group. An associated p-value less than 0.05 will be used to define statistical significance. An associated p-value less than 0.016 (0.05 ÷ 5; Bonferroni correction) will be considered highly conclusive.

17 REGULATORY AND ADMINISTRATIVE ISSUES

17.1 Subject Information and Informed Consent

The risks and benefits of participating in the study, including their right to withdraw at any time, will be verbally explained to each potential subject prior to entering the study. Prior to any screening tests or procedures for the study, the subject must sign and date the IRB/IEC approved informed consent form (which also defines the risk and benefits of participating in the study).

The investigator shall seek consent only under circumstances that provide the subject sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject shall be in language understandable to the subject. No informed consent may include any exculpatory language through which the subject is made to waive or appear to waive any of their legal rights, or releases or appears to release the investigator, Lakewood Amedex, the institution, or its agents from liability for negligence. The subject, or legal guardian, must be able to comprehend the informed consent form and sign prior to subject enrollment.

The study sponsor must agree with the final IRB/IEC-approved consent form prior to initiation of the study. The original signed consent forms will be retained by the Investigator and a copy provided to each subject.

If any significant new information develops during the conduct of the study which may affect the subject's willingness to continue participation, written consent for continued participation in the study must be obtained from each subject.

17.2 Pre-study Documentation

The following documentation must be received by the Sponsor prior to the initiation of the trial:

- Qualified Investigator Study Undertaking form/Statement of Investigator (FDA Form 1572 or QIU (Canada))
- Certification of Financial Disclosure

- Curricula vitae and current medical licenses of the Investigator and all Sub-Investigators
- Completed “Investigator Approval”
- Written documentation of Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approval for the protocol and informed consent form, signed by the IRB/IEC chairperson or designee. The protocol and the informed consent form should be clearly identified by protocol number or title and date of approval.
- Copy of the informed consent form that was reviewed and approved by the IRB/IEC
- Current normal laboratory values for all tests required in the protocol and current laboratory certification

17.3 Independent/ Institutional Ethics Committee Approval

The study protocol, the Investigator's Brochure, the informed consent form, advertising and subject recruitment procedures must be approved by an appropriately comprised IRB/IEC according to the Declaration of Helsinki, ICH Guidelines and other applicable regulations before the study is initiated at the investigator site.

Any protocol modifications or revisions to the informed consent form require prior written approval by Lakewood Amedex. No deviations from or changes to protocol or informed consent form can be initiated without prior IRB/IEC approval of the appropriate amendment, except when necessary to eliminate immediate harm to the subjects or when the change(s) involves only logistical or administrative aspects of the study.

The Investigator is required to report the following to the IRB/IEC to the extent necessary under local requirements:

- Periodic reports on the progress of the study
- Deviations from, or changes to, the protocol to eliminate immediate harm to the study subjects
- Change that increases the risk to the subject or significantly affects the conduct of the study
- All serious adverse events
- New information that may adversely affect the safety of the subjects or the conduct of the study
- Final study report

In addition, updates to the Investigator’s Brochure during the study must be submitted to the IRB/IEC for approval or acknowledgement (as appropriate).

17.4 Ethical Conduct of the Study

The study will be conducted in accordance with the ethical principles with origin in the Declaration of Helsinki and are consistent with Good Clinical Practice, applicable regulatory requirements, protocol, in accordance with the sponsor and CRO’s standard operating procedures (SOPs). Any significant deviation from the protocol must be reported immediately to the Study Medical Monitor.

Subject identity will be kept confidential and to the extent permitted by applicable laws and regulations will not be made publicly available. If the results of the study are published, subject identity will remain confidential.

18 DATA RECORDING AND COLLECTION

18.1 Source Documents

Source documents are records maintained at the investigator site through the electronic source documentation SureSource™ Source documents include signed informed consent forms, written progress notes, laboratory reports, etc. All source documents and subject assessments must be maintained at the investigator site. The site staff will use caution to avoid any unblinding through normal reading lab reports, clinical notes, pharmacy reports, etc.

Any corrections should be made by a single line drawn through the entry, adding the correct information, initialing and dating by the person making the change, and preferably indicating why the change was required.

18.2 Case Report Forms (CRFs)

Case Report Forms will not be utilized for LAI2014-1. All study information will be captured directly in the electronic source documentation system SureSource™. The Principal Investigator is responsible for all information collected on subjects enrolled at their investigational site. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the investigator. A copy of each subjects enrolled at a specific investigational site will be provided to the Principal Investigator at the completion of the study.

19 MONITORING, AUDIT, RETENTION AND CONFIDENTIALITY OF STUDY RECORDS

The Investigator is required to prepare and maintain adequate individual eCRFs provided for each subject and accurate case histories (e.g., medical records) designed to record all observations and other data pertinent to the study for each study participant. This also includes accurate documentation of investigational product accountability.

All case report forms (eCRFs) will be kept up to date and the Principal Investigator will be responsible for reviewing each subject's completed eCRFs. These will be reviewed by the sponsor/CRO at regular intervals to ensure protocol compliance, compare eCRFs with original source documents for accuracy and completeness of data recording, review study logs to assess drug accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. Periodically, the facilities used in the study may be reviewed. The review of the subject's medical records will be performed in a manner that subject confidentiality is maintained.

In addition, a study center may be audited in depth for study quality assurance by Lakewood Amedex, a CRO or an external auditor on behalf of Lakewood Amedex, or inspected by a national regulatory authority (such as the FDA or its equivalent) with or without prior notification, at random or for cause. The audit may include review of all source documents,

drug records, original clinic notes, some or all of the facilities, etc. Direct access to original source data will be required for inspections/audits, which will be carried out giving due consideration to data protection and subject confidentiality.

The Investigator must arrange for retention of study documents according to specific country requirements/regulations, after the last approval of a marketing application and until there are no pending or contemplated marketing applications in the ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents may be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Lakewood Amedex. It is Lakewood Amedex's responsibility to inform Investigator/Institution as to when these documents no longer need to be retained. Regardless of the above time frame, destruction of study records at any time requires confirmation by Lakewood Amedex. Lakewood Amedex shall retain ownership of all CRFs, data analyses, and reports that result from this study.

Monitors, auditors and other authorized agents of Lakewood Amedex, the CRO, the IRB/IEC approving this research, the FDA, and others (as applicable) will be granted direct access to the study subjects' original medical records for verification of clinical study procedure and data, without violating the confidentiality of the subjects, to the extent permitted by the law and regulations. Any presentations of the results of the study at meetings or in publications will not contain subject names.

20 STUDY TERMINATION

The study may be terminated by the investigator or the Sponsor. If, in the opinion of the investigator, clinical observations made during the study suggest that it may be unwise to continue, he or she may stop the study. A study termination by the investigator will be reported to the sponsor.

In addition, a written statement fully documenting the reasons for this action will be submitted to the Sponsor by the Investigator within five working days.

In the event that the Sponsor chooses to discontinue or terminate the study, appropriate notification will be given to the Investigator.

21 CONFIDENTIALITY AND PUBLICATION POLICY

The investigator agrees, by signing the protocol, to keep all information provided by Lakewood Amedex in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC. Study documents provided by Lakewood Amedex (such as protocol, Investigator's Brochures, CRFs, etc.) will be stored appropriately to ensure confidentiality. The information provided to the investigator may not be disclosed to others without direct written authorization from Lakewood Amedex, except to the extent required by local regulations and necessary to obtain informed consent from subjects who wish to participate in the study.

Investigators may publish results generated from this study provided the publication is reviewed and approved by Lakewood Amedex and a copy of the proposed oral or written publication is submitted to Lakewood Amedex for review at least 60 days prior to submission

for publication or presentation. Lakewood Amedex will respond to the investigator within 30 days with any changes or deletions in technical or confidential information, and investigator agrees to make such changes or deletions prior to publication or presentation.

No patent application based on the results of the study may be made by the Investigator nor may assistance be given to any third party to make such an application without the written authorization of the Study Sponsor.

22 REFERENCES

ICH E6 Guidelines for Good Clinical practice

ICH E2A Clinical Safety Data Management: Definitions and Standards for Expedited Reporting

23 APPENDICES

Appendix A: Use of the Aranz Medical Silhouette™ System for Photo documentation of the DFU and Calculation of Area and Depth, and Volume.

Capturing a Wound Image

1. Log in to Silhouette Connect and Select the Patient to be imaged.
2. Review the patient details and select Capture Images Button. The laser lines should come on from the Silhouette Star Camera.
3. To capture the wound image position the Silhouette Star Camera directly above the wound and move the camera up or down so that the laser lines form a star. The center of the laser line star should be over the deepest part of the wound.
4. Capture an image of the wound by pressing the bottom on the camera.
5. Repeat the process to capture multiple images.
6. All images will be uploaded to the Silhouette Connect Database.

Calculating the Ulcer Area, Volume, and Depth

1. Select the Ulcer image to be used for assessment on the tablet.
2. Enlarge the image so the perimeter of the ulcer is easily visible
3. Outline the perimeter of the ulcer by either clicking around the perimeter so that the entire ulcer is surrounded by dots or tracing the perimeter of the ulcer with a pointer or finger.
4. Clicking on the circular target to initiate calculation of the area, volume, and depth of the ulcer by the SilhouetteConnect software.
5. Click on the Notes(?) button to enter comments on the ulcer margins, appearance, and surrounding skin
6. Click Generate pdf report and Save to Store it to the database.
7. Upload .pdf report to the SureSource™ Database.

Appendix B: Subject Home Treatment Log:

Subject ID No.:_____

Study Drug Number:_____

Issue Date:_____ **Return Date:**_____

Instructions: Subjects/caregivers should log both the date and approximate time of each treatment and the person administering the study drug should initial in the corresponding box. A space is also provided for the subject/caregiver to write any comments concerning the administration or observations of the ulcer.

Day	Treatment 1 Date/Time	Initials	Therapeutic Footwear Worn	Length of Time Footwear Worn	Treatment 2 Date/Time	Initials	Therapeutic Footwear Worn	Length of Time Footwear Worn	Comments
1			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
2			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
3			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
4			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
5			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
6			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
7			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	

CLINICAL STUDY PROTOCOL

Study Title:	A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin Nu-3 on Infected Diabetic Ulcers of Subjects With Type I or II Diabetes Mellitus
Sponsor:	Lakewood-Amedex, Inc. 3030 University Pkwy Sarasota, FL 34243
IND No.: **EudraCT Number**	[redacted] Not Applicable
Indication:	Diabetic Foot Infections
Protocol ID:	LAI2014-1
Protocol Coordinator:	[redacted]
Medical Monitor	[redacted]
Protocol Version/Date:	V2.0 24 September 2015

CONFIDENTIALITY STATEMENT

The information contained in this document, particularly unpublished data, is the property or under control of Lakewood-Amedex, and is provided to you in confidence as an investigator, potential investigator, or consultant, for review by you, your staff, and an applicable Institutional Review Board or Independent Ethics Committee. The information is only to be used by you in connection with authorized clinical studies of the investigational drug described in the protocol. You will not disclose any of the information to others without written authorization from Lakewood-Amedex, Inc., except to the extent necessary to obtain informed consent from those persons to whom the drug may be administered.

SIGNATURE PAGE

Lakewood-Amedex Bisphosphocin NU-3 Clinical Protocol

A PHASE I/IIa, RANDOMIZED DOUBLE BLIND, PLACEBO CONTROLLED, DOSE ESCALATING STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF TOPICALLY APPLIED BISPHOSPHOCIN™ NU-3 ON INFECTED DIABETIC ULCERS OF SUBJECTS WITH TYPE I OR II DIABETES MELLITUS

Version 2.0

24 September 2015

Approved by:

[redacted]

STUDY ACKNOWLEDGEMENT

A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin™ Nu-3 on Infected Diabetic Ulcers of Subjects With Type I or II Diabetes Mellitus

Version 2.0: 24 September 2015

INVESTIGATOR STATEMENT

I have read the protocol and Investigator's Brochure, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Lakewood-Amedex. I will discuss this material with them to ensure that they are fully informed about the drugs and the study.

Principal Investigator Name (Printed)	Signature

Date

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

° C	degrees Celsius
° F	degrees Fahrenheit
AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
API	Active Pharmaceutical Ingredient
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
CMP	Comprehensive Metabolic Panel
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events v4.0
DFI	Diabetic Foot Infection
DSMB	Data and Safety Monitoring Board
DUSS	Diabetic Ulcer Severity Score
ELSD	Evaporative Light Scatter Detection
FDA	Food and Drug Administration
GCP	Good Clinical Practice
hCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IB	Investigator's Brochure
ICH	International Committee on Harmonization
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IR	Infrared Spectroscopy
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LAI	Lakewood-Amedex, Inc
LCMS	Liquid Chromatography Mass Spectrometry

LLN	Lower Limit of Normal
MAD	Multiple Ascending Dose
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
MOA	Mechanism of Action
MRSA	Methicillin Resistant *Staphylococcus aureus*
MS	Mass Spectroscopy
NDM-1	New Delhi Metallo-Beta Lactamase-1
NMR	Nuclear Magnetic Resonance
PBS	Phosphate Buffered Saline
PI	Principal Investigator
RBCs	Red Blood Cells
SAD	Single Ascending Dose
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TS	Trypic Soy
TSBD	Trypic Soy Broth with 1% Dextrose
ULN	Upper Limit of Normal
US	United States
WBC	White Blood Cell

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PROTOCOL SUMMARY

Title:	A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin™ Nu-3 on Infected Diabetic Ulcers of Subjects With Type I or II Diabetes Mellitus
Phase:	I/IIa
Population:	Up to 30 subjects over 18 years of age suffering from diabetes mellitus and a chronic infected diabetic ulcer(s).
Number of Sites:	2
Study Duration:	6 months
Subject Participation Duration:	44 days (includes screening visit, study treatment and all follow-ups visits through Day 15 post treatment.
Description of Agent or Intervention:	Bisphosphocin™ Nu-3 to be applied as a topical solution to the ulcer and covered by a bandage. This study includes three dose ascending cohorts. Within each cohort there are two phases with both a single ascending dose and multiple ascending dose arms. The three dose levels (cohorts) are 0.1% (1mg/ml, anticipated low dose), 1% (10mg/ml, anticipated therapeutic dose, and 2% (20mg/ml, anticipated high dose).
Objectives:	**Primary:** To assess the safety and tolerability of escalating doses of topically applied bisphosphocin™ Nu-3, [redacted], to an open infected ulcer. Assessment of safety will be determined by visual evaluation of the ulcer, vital signs, CBC analysis, CMP analysis, and physical examination, as well as the incidence and severity of emergent adverse events that occur during the study participation. The study will be monitored by the Safety Monitoring Committee (SMC). **Secondary:** To assess the clinical and microbiological response to bisphosphocin™ Nu-3 administered topically to the ulcer, including the improvement of wound appearance and elimination of the pathogenic bacteria.
Estimated Time to Complete Enrollment:	24 Weeks

Study Design:	**Allocation:** Randomized, Placebo-Controlled **Endpoint:** Safety and Tolerability **Enrollment Model:** Sequential Assignment **Masking:** Double Blind (Subject, Caregiver, Investigator) Primary Purpose: Safety
Description of Study Design	Phase I/IIa, three cohort ascending dose with two dosing arms per cohort, study in Type I or II diabetes mellitus subjects with a chronic infected diabetic ulcer defined as having a DUSS score of 0 to 3 and DFI wound score of 1 to 9. The study proposes to use the same subject in SAD Arm 1 and MAD Arm 2 of each cohort The study is designed to run the cohorts in series with the completion of the first cohort before initiating the next dosing level. At all study visits the ulcer will be visually examined for any changes and photographed using the Aranz Medical Silhouette™ system that will calculate area and depth of the ulcer. In Arm 1, eligible subjects will be treated with a single application of Nu-3 or placebo in 4 to 1 ratio to judge the initial safety of Nu-3 over a brief one (1) hour interval and 24-hr interval post application. Bisphosphocin Nu-3 will be applied topically to the chronic infected ulcer, covered with a non-abrasive bandage following the initial observation period. The subject will be released with verbal instructions to leave the bandage on the wound and return for a follow up visit within 24h ± 2h. At the follow up visit, the bandage will be removed, the ulcer visually examined and the subject cleared for the MAD Arm 2 based on the recommendation of the PI and absence of any SAEs. In Arm 2, eligible subjects which are those who have been approved by the PI after the Visit 2 examination will be instructed in the proper application of bisphosphocin Nu-3. The subjects will be observed applying the first dose in the clinic to ensure compliance. Subjects will then be given a 7 day supply and sent home to continue treatment. Visit 4 or earlier in the case of any adverse events, subjects will return to the clinic for an examination, including visual examination of the ulcer, vital signs, adverse events, photo documentation, collection of a sample for microbiology and concomitant medication use. A final follow up visit will be scheduled +7 days after last dose of study medication (Day 15) for a complete examination as described above.

1 INTRODUCTION

1.1 Background Information

Infectious disease represents a growing threat to world health due to the emergence of new viruses and the increase in bacterial resistance to antibiotics[1,2]. It is now estimated that almost one-third of the world population or 2 billion people are infected with *Mycobacterium tuberculosis* with between 3-5% of these cases being multidrug or extremely drug resistant[3]. In addition, bacteria have repeatedly demonstrated the ability to become resistant to antibiotics much faster than new compounds or sub-classes can be identified. The NDM-1 resistance gene represents a perfect example of the ability of bacteria to not only acquire resistance but broad spectrum resistance on a single plasmid that provides resistance against most of the major or widely used classes of antibiotics. This highlights the shortcomings of current and past antibiotic research which has relied on derivatives of existing classes that all share a common or similar core structure making them susceptible to inactivation by a single enzyme. In addition, traditional antibiotics share a related mechanism of action in that they all function through a target protein or receptor molecule to inhibit a cellular process to kill the bacteria allowing the bacteria to rapidly become resistant by acquiring a beneficial mutation in the target molecule[4].

Over the past ten years, the amount of research and development invested by large pharmaceutical companies in the discovery of novel antibiotics has steadily declined while bacteria have become increasingly resistant to the currently available classes of antibiotics. According to the Centers for Disease Control and Prevention, approximately 2 million people each year are infected with bacteria resistant to at least one antibiotic which results in around 23,000 deaths[5]. Methicillin resistant *Staphylococcus aureus* (MRSA) has become a major public health issue resulting in the closing of several public schools for cleaning and demonstrating that this is a problem no longer restricted to hospitals. No other factor highlights the need for a greater effort into the research and development of novel anti-bacterial compounds than the ever increasing ability of bacteria to rapidly acquire resistance to current and new derivatives of existing antibiotics. The recent identification of a strain of *Streptococcus* resistant to more than 18 different antibiotics highlights this fact and the obvious need for novel anti-infective compounds like Bisphosphocins.

Lakewood Amedex has discovered a new class of broad spectrum antimicrobial, termed bisphosphocins™, formerly known as nubiotics, that have been proven effective *in vitro* at killing 70 different strains of bacteria, including all Category A pathogens and *in vivo* against the difficult to treat infections caused by *Francisella tularensis*, *Helicobacter pylori*, and *Pseudomonas aeruginosa*. Bisphosphocins are a new class of synthetic broad spectrum antimicrobial that are characterized by a core structure bridging two phosphate groups attached to even chain alkyl or alcohol groups. The compounds are activated through an acidification process which is reversible by exposing the compounds to a buffer or base. The compounds were found to be bactericidal and effective in killing slow growing or stationary bacteria. Preclinical studies have demonstrated the effectiveness of these compounds in the treatment of a number of serious bacterial infections[6,7,8]. In addition, the compounds were initially developed as a homeopathic treatment and were found to reduce the severity of several common infection

associated with acne, diabetic foot infection, and skin infections[9]. The compounds function through a unique mechanism of action, are amendable to multiple formulations, and routes of administrations enabling a clinical development plan distinct from most traditional antibiotics. The current clinical development plan entails development of a topical formulation of bisphosphocin™ Nu-3 for a number of topical infections such as diabetic foot infections, chronic urinary tract infections, and pulmonary infections using direct delivery of the compound to the site of the infection. This clinical approach will demonstrate initial safety in humans and keep delivery of the antimicrobial localized to the site of infection and thus decrease the chance of bacteria becoming resistant. This clinical study will also yield important data for planning follow on clinical trials, including those involving Nu-3 for systemic administration to treat serious gram negative infections such as complicated urinary tract infections.

Diabetic foot infections are one of the most common complications for people who suffer from diabetes and a frequent cause of hospitalization[10]. According to the American Diabetes Association approximately 8.3% of the US population suffers from diabetes and in 2006 there were 65,700 non-traumatic amputations performed on diabetics. In addition, almost half of diabetic patients that have an amputation will develop an ulcer on the remaining limb within 18 months after surgery. The treatment of a diabetic foot infection is complicated as it requires both antimicrobial therapy to cure the infection and proper wound care management to heal the ulcer. Curing the infection is particularly challenging since 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 60-80% of chronic infections involve biofilm formation which makes the bacteria more resistant to traditional antibiotics[11]. Therefore, 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.

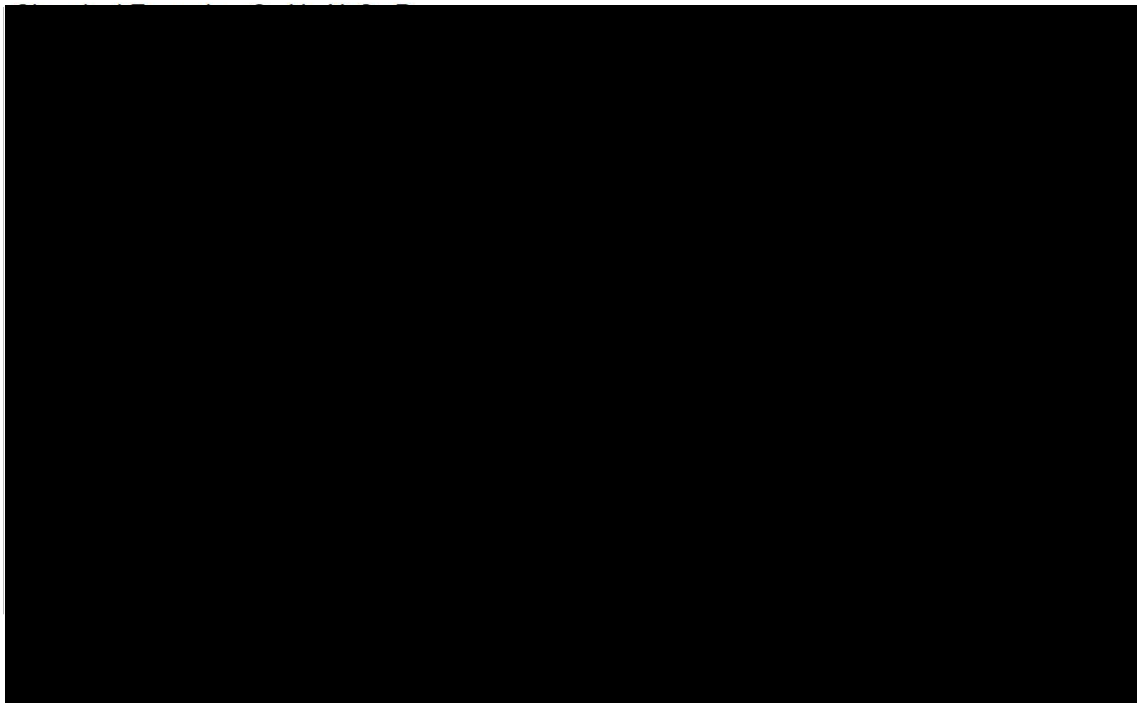
Bisphosphocins™, a new class of broad spectrum antimicrobials, possess a number of characteristics that make these compounds extremely well-suited to the management of diabetic foot infections. First, bisphosphocins™ are rapidly and directly bactericidal functioning through a unique mechanism of action providing the ability to quickly cure an infection. The compounds are fairly low in molecule weight, have an excellent safety profile, and have shown good penetration into wounds. In addition, there are no known bacterial mechanisms of resistance and no resistant bacteria have been identified to date eliminating a common cause of treatment failure[7]. Finally, bisphosphocins have been demonstrated highly effective at killing bacteria established in biofilms[12] which is increasingly become associated with many chronic infections. The preclinical studies conducted on the bisphosphocin™ class clearly support their evaluation in the proposed Phase I/IIa safety study in diabetic foot infections.

1.2 Bisphosphocin Nu-3

1.2.1 General Information

Bisphosphocins™ were originally discovered by [redacted] while conducting research in the field of antisense technology and attempting to kill bacteria using gene silencing. The initial compounds identified were protonated/acidified oligonucleotides that demonstrated bactericidal activity independent of nucleotide sequence. Further experimentation expanded the class to protonated/acidified nucleotides and derivative molecules that exhibit chemical stability, acid pH resistance, and nuclease resistance enabling formulations for oral, intravenous, pulmonary, and topical delivery. The lead compound, designated Nu-3, is a thymidine derivative that would be considered to be in the nucleotide analogue class of drugs (Figure 1). Experimental data has elucidated a non-receptor dependent mechanism of action involving disruption of the bacterial cell membrane causing depolarization and rapid cell death. Bisphosphocins™ have an extremely broad spectrum of activity *in vitro* testing against over 70 different bacterial strains carrying various antibiotic resistant markers. There is currently no known mechanism of resistance to the bisphosphocin class and because they were synthetically derived there is no natural pool of resistant bacteria. In addition, panelling the compounds against a number of strains with known antibiotic resistance or clinical isolates has failed to identify any cross-resistance or resistance mechanisms. The following preclinical pharmacological and toxicology data is provided as a brief summary of the activity of bisphosphocin Nu-3 to support its potential as a treatment for diabetic foot infections and this clinical protocol should be read in conjunction with the Investigator Brochure on the product.

Figure 1. Structure, Chemical Name and Chemical Formula of Bisphosphocin™ Nu-3



1.2.2 Pre-Clinical Pharmacology and Toxicology

Specific Pharmacology

Initial experiments revealed that bisphosphocins™ had an antimicrobial activity distinct from any currently existing antibiotic class. Extensive *in vitro* and i*n vivo* experiments have been conducted to further define their activity, identify their mechanism of action, and begin to establish a dose range to allow entry in human clinical trials. An important factor to consider in the review of the following experimental results is that it is now known bisphosphocins™ do not follow the simple inhibitory kinetic model of one compound binding to one target molecule of the current classes of antibiotics but a more complex kinetic model involving the binding of multiple molecules to a bacterium resulting in direct bacteria cell death. For this reason, the current hypothesis is bisphosphocin™ dosing *in vivo* will be based on total dose delivered and not on maintaining a circulating level above the minimum inhibitory concentration or at the site of infection to achieve an efficacy outcome because the molecules rapidly bind and kill bacteria as opposed to inhibiting growth. Therefore, the therapeutic effect of bisphosphocins™ can be manipulated by not only controlling the dose but frequency and duration of treatment not because of the pharmacokinetics of the compound but due to the fact that each dose will result in a reduction of bacterial load. The following experiments are presented to further define, distinguish and compare the activity of the bisphosphocin class from traditional antibiotics in order to develop a comprehensive clinical plan to enable evaluation in humans. The majority of the experimental data presented in the following sections was performed in the laboratory of outside collaborators or researchers who then published their results[6,7,8,12] or by independent Contract Research Organization following the current guidelines for antimicrobial testing. Due to the unique MOA of the bisphosphocin™ class some standard assays were modified to enable the evaluation which has been noted in the protocol. Table 1 and Table 2 summarizes the activity of Nu-3 in the standard MIC assay against bacterial pathogens commonly found in diabetic foot infections and against common fungal pathogens.

Table 1. Bisphosphocin Nu-3 Activity in a Minimum Inhibitory Assay according to CLSI Guidelines

Organism	Number of Strains	MIC mg/ml
Acinetobacter baumannii	N=15	5-10
Enterococcus faecalis, VRE	N=15	5
Enterococcus faecalis, VSE	N=15	5
Enterococcus faecium, VRE	N=15	5
Enterococcus faecium, VRE	N=15	5
Escherichia coli	N=15	10
Proteus mirabilis	N=15	5-10
Proteus vulgaris	N=15	5-10
Pseudomonas aeruginosa	N=5	2.5-5

Organism	Number of Strains	MIC mg/ml
Pseudomonas aeruginosa	N=15	5-10
Serratia marcescens	N=15	10
Staphylococcus aureus, MSSA	N=15	5-10
Staphylococcus aureus, MRSA	N=15	5-10
Staphylococcus epidermidis, MRSE	N=15	10
Staphylococcus epidermidis, MSSE	N=15	10
Streptococcus agalactiae	N=15	10

Table 2. Anti-fungal Activity of Bisphosphocin Nu-3

Yeast Strain	ATCC #	Drug Resistance	MIC mg/ml
			Nu3
Candida albicans	44373	5-Fluorocytosine	≤0.53
Candida albicans	44374		≤0.53
Saccharomyces pastorius	2366		≤0.07
Trichophytan mentagrophytes var. *interdigitale*	200099		5.0

Evaluation of Nu-3 Activity against Bacteria Established in Biofilm

The following studies were performed by [redacted] and were designed to evaluate the activity of Nu-3 against bacteria established in biofilms which is increasing becoming associated with chronic infections[12,unpublished]. Biofilm was formed in borosilicate glass tubes by standard methods and then treated with Nu-3 or kept as untreated controls. As shown in Table 3, Nu-3 was able to penetrate the biofilm and kill the bacteria as judged by the lack of growth in Nu-3 treated tubes.

Table 3. Bactericidal activity of Nu-3 against bacteria established in biofilm

Bacterial Strain	Designation	Assessment	Treatment			
					Nu-3 Exposure (10mg/ml)	
			Media	Control	10min	30min
Klebsiella pneumoniae Kp-S1	Clinical	Growth	N.G.	1.1×10^9 cfu/ml	N.G.	N.G.
		Biofilm				
Acinetobacter baumannii	BAA-1605	Growth	N.G.	2×10^9 cfu/ml	N.G.	N.G.
		Biofilm				
Staphylococcus epidermidis	35984	Growth	N.G.	2.7×10^7 cfu/ml	N.G.	N.G.
		Biofilm				
Staphylococcus epidermidis	PCI1200	Growth	N.G.	5.2×10^8 cfu/ml	N.G.	N.G.
		Biofilm				
Staphylococcus aureus	NRS684	Growth	N.G.	4.3×10^8 cfu/ml	5×10^8 cfu/ml	8.6×10^8 cfu/ml
		Biofilm				
Staphylococcus aureus	NRS732	Growth	N.G	7.6×10^8 cfu/ml	N.G	N.G
		Biofilm				

Assessment of the rate of kill of Nu-3 in 0.85% normal saline:

Bacteria in the log phase of growth were collected by centrifugation, re-suspended in sterile saline (0.85% NaCl) at 1 x 10^5 CFU/ml. Nu-3 in sterile saline was added to a final concentration of 0.2 to 10mg/ml to the bacteria and the bacteria/Nu-3 mixture incubated at room temperature. At time points ranging from 5 to 60 minutes, aliquots were removed, diluted and plated onto TS agar plates for quantitation of the number of colony forming units (CFU's). After 24h at 37°C, the number of CFU's were determined and the Nu-3 concentrations and exposure times resulting in 100% kill were determined (Table 4).

Table 4. Time and concentration dependent activity of Nu-3 against biofilm forming bacterial strains.

Bacterial Strain	Nu-3 Concentration			
	0.625 (mg/ml)	**1 (mg/ml)**	**5 (mg/ml)**	**10 (mg/ml)**
K. pneumoniae Sp1	15 min		5 min	
K. pneumoniae Sp5		15 min	5 min	
A. baumannii BAA-1605	30 min		10 min	5 min
S. aureus NRS684				60 min
S. aureus NRS732				60 min

Mouse Suture Wound Infection Model

Briefly, a 10cm length of suture was threaded onto a needle and soaked for 45 minutes in undiluted cultures of *S. aureus* cvcc2248 or *P. aeruginosa* cvcc 5668 at 35°C for 8 hours. The needle and sutures were removed, placed on filter paper, and dried at 4°C until implantation. A 1-cm long piece of suture was implanted under the skin in the shaved and washed mid-back of anesthetized animals using knots to secure it in place. An incision was made between the knots on top of the suture line with a scalpel. The animals were then divided into groups and treated as indicated at 4h and 8h post-surgery and then 3 times daily for 3 days with either placebo, Nu-3 or ciprofloxacin ointment. On Day 5 the animals were euthanized and a 1cm^2 area surrounding and including the wound was harvest for homogenization and CFU counts (Table 5). The initial inoculum (i.e. CFU of bacteria in 1 cm length of suture) was determined by vortexing 1-cm of suture in bacterial growth media and plating serial dilutions to obtain bacteria counts.

Table 5. Log reduction in bacterial cell counts in surgical wound model

Treatment	Mean Bacterial Count ± SD (Log10 CFU/Wound)	
	P. aeruginosa	***S. aureus***
Initial Inoculation	4.93 ± 0.33	4.79 ± 0.31
Untreated	7.52 ± 0.57	7.64 ± 0.58
Glycerin	7.34 ± 0.27	7.47 ± 0.34
5% Nu-3 in Saline	5.62 ± 0.61	5.23 ± 0.5
5% Nu-3 in Glycerin	5.07 ± 0.53	4.51 ± 0.55
Ciprofloxacin HCl Ointment	4.82 ± 0.58	5.63 ± 0.31

Mouse Superficial Skin Polymicrobial Infection Model

Briefly, mice are pre-treated with cyclophosphamide four day prior to initiating the infection to suppress the immune system. The mice are anesthetized and kept sedated while the fur on the back dorsal surface is shaved using electric clippers followed by 'wet shaving' with a disposable razor. The skin is then sterilized with a betadine wash followed by alcohol swab and abraded with a +50 grit emery board that results in a smooth, red, shiny appearance with no bleeding. A droplet of the bacterial inoculum is placed directly on the abraded skin that contains *Pseudomonas aeruginosa* UNT034-1 (ATCC 27853) 6.35 Log10 CFU/ mouse and *S. aureus* UNT006-4 (smith) 5.65 Log10 CFU/ mouse. The infected area is treated with 50 microliters of varying concentrations of bisphosphocin Nu-3, gentamycin, mupirocin, or normal saline twice daily for 3 days. The mice are then euthanized, the area of infected skin excised, and the CFU of both bacteria is determined by culture or serial diluted samples of homogenized tissue (Table 6). The data shows that the top 5% dose of bisphosphocin Nu-3 results in a 1 log reduction in the CFU count of both *S. aureus* and *P. aeruginosa*.

Table 6. Mouse Superficial Skin Polymicrobial Infection Bacterial Counts

Compound	Dose	*S. aureus*		*P. aeruginosa*	
		CFU	SD	CFU	SD
NU3	1.0 mg/mL	9.22	0.19	6.35	0.21
	10 mg/mL	8.78	0.24	6.81	0.23
	20 mg/mL	9.00	0.27	7.08	0.19
	50 mg/mL	7.61	1.35	5.65	1.16
Gentamicin	0.3%	4.40	0.85	5.17	0.87
Mupirocin	2.0%	6.32	1.01	6.95	0.51
Vehicle	saline	8.73	0.25	6.3	0.42
Infection Control	Day 4	8.72	0.52	6.68	0.69
	4 hr	5.40	0.45	4.33	0.83

Preclinical Toxicology

Summary of Preclinical Toxicology Data

Nu-3 is the first in a new class of broad spectrum antimicrobials, termed bisphosphocins, being developed to treat a wide range of serious bacterial infections. Chemically Nu-3 would be considered to be in the nucleotide analog class of drugs and the following set of preclinical studies was designed based on the preclinical and clinical data collected over the past 50 years on this class. The nucleotide analog class of drugs have a good safety profile even when administered long term at low doses and toxicities are generally associated with their interference of normal DNA/RNA synthesis.

Nu-3 or [redacted] is a thymidine analog with both the 3' and 5' sites blocked by butyl phosphate which prevents it from causing early chain termination by incorporation by the polymerase unlike most compounds in this class. Nu-3 is activated through an acidification step that results in an acidic or protonated compound that when dissolved in water has a pH between 2 and 2.5. The compound can be neutralized or unactivated by a strong base or buffer such as sodium bicarbonate. In addition, the molecule was designed to minimize the potential for toxic side effects by utilizing a thymidine core molecule, natural phosphate linkages, and even carbon butyl blocking groups, all of which are either found in the body and/or feed into natural recycling pathways.

A summary of the preclinical toxicology studies completed with Nu-3 is presented in Table 7. The data indicates that Nu-3 is safe and well-tolerated when administered systemically or

topically and no specific toxicities were identified that would be cause for concern for the proposed Phase I/IIa study in subjects with infected diabetic foot ulcers. In general, the data indicates that Nu-3 functions through a membrane depolarization mechanism of action that is highly specific for bacterial cell membranes.

Table 7. Summary of Nu-3 Preclinical Toxicology Data

Study	Test Article/Dose	Species	Lot #	Result
Genotoxicity				
Bacterial Reverse Mutation Assay (GLP)	Nu-3 ACT	*In vitro*	LWA-01R-14	Non-mutagenic
Bacterial Reverse Mutation Assay (GLP)	Nu-3 NEUT	*In vitro*	LWA-01R-14	Non-mutagenic
Systemic Toxicity Study				
7-Day Daily Repeat IV Toxicology (GLP)	0, 2, 10, 50mg/kg	Rats	CCS-2009/ST-03/00114	NOAEL at 50mg/kg No drug related findings
IV Max Tolerated Dose (GLP)	0, 250, 500, 1000mg/kg	Rats	LWA-01-14	NOEL and NOEAL at 250mg/kg $LD_{50} \geq$ 500mg/kg
IV Max Tolerated Dose Study (Non-GLP)	0, 250, 500, 750, 1000mg/kg	Mice	LWA-01-14	$LD_{50} \geq$ 1000mg/kg
Oral Max Tolerated Dose Study (Non-GLP)	1000, 1256, 1580, 1988, 2500mg/kg	Mice	PN 1127	LD_{50} 2001mg/kg according to the Karber method
Acute Single IV Dose (Non-GLP)	50, 200mg/kg	Rat	PN 1127	No observable effect on blood chemistry (CBC, metabolic panel)
Local Tolerance Studies				
Primary Dermal Irritation (GLP)	10% Nu-3	Rabbit	LWA-01R-14	Not a primary irritant
MapTek EpiOcular™ Toxicity (GLP)	1%, 2.5%, 5% Nu-3	*In vitro*	LWA-01-14	1% - Non-irritating, Minimal; 2.5%, 5% - Moderate irritants; No significant IL1α stimulation for all doses
In vitro Sensitization (GLP)	10%, 6.8%, 4.6%, 3.2%, 2.2%, 1.5%, 1%, 0.68%	In vitro	LWA-01-14	4.6% Sensitizer, All other concentrations are Non-sensitizers
3 Day Ophthalmic Irritation Study (Non-GLP)	1%	Rat	PN 1127	No sign of irritation
28 Day Repeat Dermal Toxicity (Non-GLP)	10%	Rat	PN1127	No pathological changes in skin, no clinical observations

1.2.3 Clinical Trials of Bisphosphocin Nu-3

Bisphosphocin™ Nu-3 is a new chemical entity that has not been evaluated under a formal IND application in human clinical studies. The compound was initial developed as a homeopathic treatment for a variety of topical infections as it was considered to fall under the HPUS DNA guidelines[9]. As a homeopathic treatment, Nu-3 was used topically to treat a variety of infections including acne, urethritis, ear infections, diabetic foot infections, and certain fungal infections. The information collected from these homeopathic treatments revealed that Nu-3 applied topically in solution for up to 14 days was able to improve the severity of an infection based visual observation.

1.3 Rationale for Current Study

The proposed clinical study is designed to demonstrate the safety and tolerability of increasing doses of topically applied bisphosphocin™ Nu-3 on a chronic diabetic foot infection. In addition, the study is designed to collect data on the efficacy of Nu-3 as it relates to eradication of the infection and wound healing to enable planning of future trials. The 3 dosing levels and length of treatment were selected based on *in vitro*, *in vivo*, and toxicology studies of Nu-3 and its unique mechanism of action. While the current trial is considered a first-in-human trial, the design also has taken into consideration data collected from the development of the compound initially as a homeopathic treatment where the use as a topical solution was able to reduce the severity of several common topical infections. Recent studies have shown that a high percentage of chronic wounds have biofilm associated which interferes with wound healing. This trial is intended to evaluate the efficacy of an antimicrobial with known activity against biofilm encased bacteria to not only eliminate the infection but potentially improve wound healing. For this reason, bisphosphocin Nu-3 is being formulated in a normal saline solution so that a clearer picture can be gained of its effect on the wound without the interference of an ointment base. Bisphosphocins have been shown to effectively reduce the severity of the infection in preclinical animal model testing, but these models have limits as they do not accurately mimic the complex environment of a chronic wound infection and in many cases rely on immunosuppression to maintain the infection. This first in human study is designed to collect both safety and efficacy data so that the parameters such as dose, duration and frequency of administration can be refined for future more intensive studies. Based on the data collected from preclinical studies, it is anticipated that a therapeutic effect may be observed in the middle (1% w/v, or 10 mg/ml) and high dose (2% w/v, or 20 mg/ml) cohorts that could justify extending the treatment course an additional seven (7) days. An extension of the treatment duration to fourteen (14) days would only be initiated after five subjects in Cohorts 2 and 3 completed the seven (7) day treatment and approval was given by the SMC. The decision to extend treatment of individual subjects showing a clinical response would then be made by the principal investigator.

Potential Risks

This clinical trial is a first in human trial so there is a potential risk of an adverse reaction from the study drug. Bisphosphocin Nu-3, however would be considered a nucleotide analog which is a class of drugs with a strong safety record so the likelihood of this particular risk is considered

minimal. The major distinction between bisphosphocin Nu-3 and the nucleotide analog class are that both 3' and 5' sites are blocked and a novel acid activation step that is reversed when the compounds are exposed to buffers or base. Therefore, it is anticipated that any reaction would be localized since the sodium bicarbonate in the blood would neutralize the compound which is supported by inhibitory effect of blood in MIC assays. In addition to a reaction from the study drug, there is also the possibility that the drug is ineffective and the infection worsens which could necessitate more aggressive therapy or treatment.

Potential Benefits

The major benefit of the proposed study will be an improvement in the wound due to the elimination of the pathogenic bacteria that is inhibiting healing. Bisphosphocin™ Nu-3 is a new class of broad spectrum antimicrobial so it is unlikely that the pathogenic bacteria will be outside its spectrum of activity or resistant, which is a major cause of treatment failure in this indication. The dose levels have been selected so that two cohorts are likely to be receiving efficacious dose levels of drug with a satisfactory safety margin so that some improvement in wound appearance is anticipated in some subjects in these cohorts. In addition to directly eliminating the pathogenic bacteria associated with the chronic ulcer, it is anticipated that a second potential benefit would be the elimination of any biofilm encased bacteria that would then make the infection more susceptible to traditional therapy as a follow up therapy. Any of these outcomes would benefit the subject as it would reduce the risk of limb loss due to a worsening infection and the pain.

2 OBJECTIVES

Objective/Purpose: The primary objective of this study is to assess the safety and tolerability of Nu-3 when applied topically to a chronically infected diabetic ulcer.

A secondary objective of this study is to obtain preliminary data on the microbiological activity of Nu-3 as measured by clinical wound assessments and presence of pathogenic bacteria. Based on preclinical data, it is anticipated that improvement in the clinical wound assessment may be observed in the middle and high dose (1% and 2%) cohorts.

Statement of Hypothesis: Nu-3 is a broad spectrum antimicrobial with a unique mechanism of action that involves cell membrane depolarization and rapid bacterial cell death. As an antimicrobial it is particularly well suited for development as a topical for Diabetic Foot Infections for the following reasons:

1. These infections tend to be polymicrobial in nature, involve biofilm, and require an antibiotic(s) with a broad spectrum of activity.
2. Many of the bacteria in these infected wounds are resistant to one or more classes of antibiotics making treatment difficult
3. The directly bactericidal mode of action of Nu-3 should translate into an immediate reduction in the severity of the infections and offers new options in dosing.
4. Research has shown that many chronic infections are due to the formation of biofilm which is inherently resistant to treatment by traditional antibiotics but susceptible to treatment by bisphosphocins such as Nu-3

The proposed clinical trial design is aimed at defining the initial parameters for safety and tolerability in an infected wound based on the currently available preclinical data on Nu-3. The trial is focused on DFIs of mild to moderate severity and an area of 0.5 to 6 cm^2 as a population of subjects that will provide the data across a range of pathogens and ulcers of varying duration. In addition, the middle and high dose (1% and 2% Nu-3) cohorts were selected so that some clinical improvement in wound assessment be observed which will enable better planning of following clinical trials.

Route of Administration: Study medication will be applied topically directly to the infected ulcer

Dosage: The study includes a sequential ascending dose protocol with Cohort 1 at 0.1% w/v or 1mg/ml, Cohort 2 at 1% w/v or 10mg/ml, and Cohort 3 at 2% w/v or 20mg/ml. In addition, two subjects in each cohort will be randomized to placebo. The proposed doses and treatment duration were based on data from *in vitro* antimicrobial assays, preclinical animal efficacy models, and toxicology studies and were selected to demonstrate safety at a high dose that provides a significant therapeutic margin lethal to all tested bacteria and fungi to date while proven safe in animal toxicology studies.

Dosing Regime: To establish the initial safety and tolerability of Nu-3, a twice daily application of the compound was selected for this clinical trial based on preclinical studies on efficacy and toxicology. Subjects will be required to keep a daily log of when treatments are applied and contacted daily by a member of the study team.

Ulcer Imaging and Area Calculations: Photographs of the ulcer(s) will be taken at study visits using the Aranz Medical Silhouette™ system which allows capture of a color image and computer calculation of ulcer(s) area, depth, and volume with more consistent and greater accuracy than traditional methods.

Randomization: Eligible subjects will be randomly assigned in a 4:1 ratio to Nu-3 (n=8) or matching placebo (n=2) in each cohort.

Treatment Period: Day 1 (in clinic): Day 2 (end of SAD/Beginning of MAD) through Day 8

Follow-Up Period: Day 15

Selection of Study Population: Type I or II diabetes mellitus subjects with a chronic infected lower extremity ulcer defined as having a DUSS score of 0 to 3 and a DFI wound score 1 to 9 as this is the most appropriate population to assess the safety of a new drug aimed at treating infected wounds. The proposed inclusion criteria have been selected to define a subject population that will have a diverse spectrum of pathogens and range of chronic ulcers to ensure a proper assessment of safety and tolerability as well as data to better define follow on clinical studies. The DFI wound score range is intended to select subjects with mild to moderate infection which have the best potential to respond to the treatment. A radiograph of the affected area will be performed during the screening period to rule out the possibility that the ulcer is due to an underlying osteomyelitis. Preclinical studies have demonstrated the safety of Nu-3 on mammalian cells, intact and damaged skin, and when injected intravenously, therefore exposure of healthy subject to Nu-3 would provide little if any additional safety information.

3 STUDY DESIGN

Phase I/IIa, three cohort double-blind ascending dose with two dosing phases per cohort, study in diabetes mellitus subjects with a chronic infected diabetic ulcer defined as having a DUSS score between 0 and 3 and a DFI wound score between 1 and 9. The study proposes to use the same subject(s) in SAD Phase 1 and MAD Phase 2 of each cohort.

The study is designed to conduct the cohorts sequentially with the completion of the first cohort before initiating the next dosing level.

In Phase 1, eligible subjects will be treated with a single application of Nu-3 or placebo in a 4 to 1 ratio to judge the initial safety of Nu-3 over a brief 15-30 minute interval and 24 hour interval. Bisphosphocin Nu-3 will be applied topically to the chronic infected ulcer (DUSS score: 1-3; DFI wound score: 1-9), and the area observed over a 15-30 minute time period and photographed. If no visible reaction is observed, the ulcer is covered with a bandage. The subject will be released with verbal instructions to leave the nonabrasive, hypoallergenic bandage on the wound, avoid getting the bandage wet, and return for a follow up visit within 24 ± 2h. At the follow up visit, the bandage will be removed, the ulcer visually examined, photographed using the Aranz Medical Silhouette™ system, and the subject cleared for the MAD Phase 2 based on the recommendation/decision of the PI and on the absence of any drug related SAEs.

In Phase 2, eligible subjects, which are those who have been approved by the PI after the Visit 2 examination, will be instructed in the proper application of bisphosphocin Nu-3 or placebo. In the clinic, the subjects will be observed applying the first dose to ensure compliance. The subject will then be given a 7-day supply of study drug, non-abrasive bandages, a home diary, written instructions for care and hygiene, and sent home to continue treatment. On Day 9 ± 2h or earlier in the case of any adverse events, subjects will return to the clinic for an examination, including visual evaluation of the ulcer, vital signs, adverse events, photo documentation using the Aranz Medical Silhouette™ system, collection of a sample for microbiological assessment, and concomitant medication use. A final follow up visit will be scheduled for Day 15 for a complete examination as described above. Based on the data collected from preclinical studies, it is anticipated that a therapeutic effect may be observed in the middle (1% w/v, or 10 mg/ml) and high-dose (2% w/v, or 20 mg/ml) cohorts.

SUBJECT POPULATION

3.1 Selection and Exclusion of Subjects

Subjects, greater than 18 years of age, will be recruited from the area surrounding the Phase I/IIa Clinical Trial Centers as well as existing subjects of the center. Strategies for recruiting subjects will include web based advertising and social media sites, or traditional advertising in local newspapers all using IRB-approved materials. It is anticipated that 50-60 subject will be screened to identify the 30 subject needed for the proposed study. Subjects will be allowed as

much time as necessary to review and give informed signed prior to any study related procedures being performed.

3.2 Inclusion Criteria

1. Signed Informed Consent Form must be obtained for the subject and if necessary the subject's caregiver prior to any study related procedures being performed
2. Non-hospitalized ambulatory subjects suffering from Diabetes mellitus, Type I or II
3. Male or Female older than 18 yrs
4. Diabetic foot ulcer(s) with a DUSS Score of 0 to 3
5. A radiograph, MRI, and/or CAT scan evaluation within the last seven (7) days to determine the ulcer is not caused by an osteomyelitic bone infection
6. Infection as defined by the IDSA as ≥ 2 classic findings of inflammation or purulence
7. Ulcerated area(s) of not more than two (2) ulcers between 0.5 to 6 cm^2 as calculated by the Aranz Medical Silhouette™ system
8. Infection must be localized to the area of the ulcer and defined as mild (superficial and limited in size and depth) with a DFI Wound Score between 1 and 6
9. Subject must agree to be treated as an outpatient, follow all protocol procedures, return for all schedule visits, and provide informed consent
10. Any female of child bearing age must consent to use medically acceptable birth control for the duration of the study

3.3 Exclusion Criteria

1. A DUSS Score above 3
2. Any ulcer caused by an underlying osteomyelitic bone infection
3. Assessed with a Moderate to Severe Infection, including abscesses, extensive gangrene, symptoms of systemic infection, or a limb threatening infection. DFI Wound Score above 6
4. Hemoglobin A1c (HbA1c) level ≥ 9.0%
5. An ulcer area(s) greater than 6 cm^2 or more than two (2) ulcers
6. Any subject that has received systemic or topical antibiotics within the last seven (7) days
7. Any subject on topical antimicrobial treatment for their infected diabetic foot ulcer whose ulcer is responding to treatment
8. Any subject that would be unable to follow the protocol procedures, safely monitor the infection status at home, and return for schedule visits
9. Positive pregnancy test at Screening or Visit 2
10. Positive drug or alcohol test at Screening Visit or Visit 2, unless determined by the Principal Investigator (PI) the positive result would not impair full participation in the clinical research study

4 STUDY DRUGS

4.1 Randomization, Blinding, and Drug Product/Placebo Packaging and Labeling

The drug product kits will be labeled as shown in Table 8 to ensure blinding of both the Principal Investigator and Subject. The kits will contain a 0.5 oz dropper bottle containing either 15 ml of Bisphosphocin™ Nu-3 or Placebo packaged in a cardboard a carton with a foam insert for protection. Both the carton and dropper bottle will be labelled.

The randomization will consist of two materials that have been allocated within 3 Cohorts.

Material 1: Bisphosphocin™ Nu-3 at 0.1%, 1%, or 2% w/v

Material 2: Matching Placebo

Within each Cohort, a material blocking ratio of 4:1 has been utilized with three (3) blocks per cohort. All Cohorts utilize a different bottle number range with ranges set at Cohort 1 from 0101 to 0115, Cohort 2 from 0201-0215, and Cohort 3 from 0301 to 0315. All cohorts will have a different starting Randomization Specification Seed value which will result in a different statistical outcome.

Table 8. Product Label Specifications

GC: X; LID: X; CARTON	
Protocol #:	LAI2014-1
Product:	Bisphosphocin Nu-3 topical antibiotic or placebo
Bottle Number:	####
Lot Number*:	14K###
Directions for Use:	As directed, apply twice daily to the affected area
Storage:	Room Temperature^
Caution:	New Drug-Limited by federal law to Investigational Use Keep out of reach of children
Manufacturer:	Lakewood Amedex Inc, Sarasota, FL (941)225-2515

*Lot number represents the group of kits being assembled for each cohort.

^Room temperature is defined according to the USP as the temperature that is prevailing in a work area.

4.2 Description and Handling of Bisphosphocin Nu-3

Nu-3 or [redacted] is a thymidine analog with both the 3' and 5' sites blocked by butyl phosphate which prevents it from causing early chain termination by incorporation by the polymerase unlike most compounds in this class. Nu-3 is

activated through an acidification step that results in an acidic or protonated compound that when dissolved in water has a pH between 2 and 2.5. The antibacterial activity can be neutralized by a strong base or buffer. Prior non-human investigations indicate that all doses being used in this study are likely to be safe, medical personnel and caregivers should use caution when handling the drug to avoid exposure of sub-MIC levels through inadvertent application to the skin and normal skin flora that could potentially lead to resistant strains.

4.3 Dosage and Administration of Bisphosphocin Nu-3 or Placebo

Bisphosphocin Nu-3 will be dosed at three different concentrations of 0.1% w/v (1mg/ml), 1% w/v (10mg/ml), and 2% w/v (20mg/ml) in 0.85% normal saline. Placebo will be 0.85% normal saline solution. The drug or placebo will be administered as a single dose and then twice daily for 7 days. The drug or placebo will be administered to the infected diabetic foot ulcer and surrounding intact skin at a dose of 1-3 drops from a dropper bottle per 1 cm^2 of ulcer area.

4.4 Prior and Concomitant Medications

Subjects are allowed to continue on any concomitant prescribed medication for medical conditions not related to the treatment of the infected diabetic ulcer. All systemic and topical antibiotics must be stopped seven (7) days prior to admission to the proposed study.

5 STUDY PROCEDURES

5.1 Subject Enrollment and Treatment Assignment

Screened subjects who sign the Informed Consent and meet all the inclusion criteria and none of the exclusion criteria will be considered eligible for study participation. Eligible subjects will be assigned to Cohort 1 until a total of 10 subjects are enrolled, followed by Cohort 2, and Cohort 3. There will be a minimum of 7 days between the last subject completing the Day 9 assessment from each cohort and the first subject enrolled into the next cohort. All subjects who are eligible for enrollment will be randomly assigned in a 4:1 ratio to NU-3 (n=8) or matching placebo (n=2) in each cohort. The dose escalation will proceed following the Safety Monitoring Committee recommendation/approval.

5.2 Pre-treatment Assessments

The schedule of evaluations and procedures that must be performed at specific time points is described in the following sections. The Time and Event Schedule (Appendix B) summarizes the frequency and timing of various safety evaluations and clinical microbiological sampling. As soon as a subject is considered to be a potential subject for this study and prior to any other study procedures, the nature of the study will be explained to him/her by the study investigator or designee and the potential subject will be asked to provide written informed consent. Informed consent must be obtained prior to any study procedures being conducted.

5.2.1 Screening Visits

Screening Visit/Visit 1

Subjects diagnosed with diabetes mellitus and suffering from a chronic infected diabetic foot ulcer will be evaluated for entry criteria during a screening period conducted within the 30 days prior to bisphosphocin Nu-3 or placebo treatment. After informed consent has been obtained, the following procedures will be completed for each subject prior to inclusion in the study.

- Medical History
- Physical Examine with vital signs
- Concomitant medications
- Urine Pregnancy tests (HCG), if applicable
- Drug and alcohol blood toxicity screen
- Draw blood for CBC and CMP
- Review of shoe gear worn
- Radiograph, MRI, and/or CAT scan evaluations of the infected ulcer within the last seven (7) days to confirm the absence of osteomyelitis
- Scoring of the diabetic ulcer using the DUSS Scoring system

- Rating of the infection using the DFI Wound Scoring system
- Examination of Chronic Ulcer(s)
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ as described in Appendix C.

Diabetic Ulcer Wound Scoring System (DUSS)

Parameter	Score	
Palpable Pedal Pulses	Presence = 0	Absence = 1
Probing to Bone	No = 0	Yes = 1
Location of Ulcer	Toe = 0	Foot = 1
Number of Ulcerations	Single = 0	Multiple = 1
Score Range	0	4

Diabetic Foot Ulcer Wound Infection Score

Parameter	Wound Infection Score			
	0	1	2	3
Purulent discharge	Absent			Present
Nonpurulant drainage (serious, sanguinous)	Absent	Mild		
Erythema	None	Mild pink, barely perceptible	Moderate pale red, defined edges	Severe red to dark red
Induration	None	Mild	Moderate	Severe
Tenderness (sign)	None	Mild	Moderate	Severe
Pain (symptoms)	None	Mild	Moderate	Severe
Local Warmth (relative to uninfected contralateral foot)	Same	Mildly increased	Moderately increased	Severely increased

5.3 Enrollment/Baseline in Single Ascending Dose Arm (SAD)

Treatment Visit/Visit 2 (Day 1)

- A urine pregnancy test must be performed prior to starting Nu-3 treatment in all women of child-bearing potential and a negative result must be documented.
- A urine drug and alcohol toxicology screen must be performed before starting Nu-3 treatment for each subject and a negative result must be documented.
- Draw blood for CBC and CMP

- Study subject will be assigned to the open cohort and treatment number per standard procedure. The treatment number will correspond with the dropper bottle number that will be used for treatment.
- Before the treatment begins, the following data/samples will be collected and procedures completed:
  - Concomitant medications
  - Vital signs
  - Debridement, if deemed necessary by the PI
  - Collection of sample from the ulcer for microbiological assessment
- Study Treatment for SAD Arm will be applied topically to the chronic ulcer and the area observed over a fifteen minute period for any acute reactions prior to being covered by a non-abrasive hypoallergenic bandage. The Nu-3 or placebo solution will be applied from a pre-packaged dropper bottle at a rate of 1-3 drops per 1 cm^2 of ulcer making sure to cover the entire ulcer as well as up to 0.5 cm of surrounding tissue.
- One hour after the treatment the following data will be collected:
  - Vital signs
  - Signs and symptoms of adverse events (e.g. redness of the ulcer, swelling, fever, chills)
- One hour post-treatment the study subject will be allowed to leave the clinic and instructed to call or return to the office if any of the following occur:
  - Increased pain or swelling of the infected ulcer or surrounding area.
  - Any observed increase in redness or swelling of the ulcer.

5.4 Follow-up Single Ascending Dose Arm

Post Treatment Visits/Visit 3 Day 2 (24+/-2 h from Visit 2)

Study subjects will return to the clinical site for a follow-up evaluation 24±2h post treatment prior to starting the multiple ascending dose arm (MAD). At this follow-up visit, the subject will undergo the following procedures and evaluations to determine if they can be cleared for enrollment in the multiple ascending dose arm (MAD).

- Vital sign measurements including, weight, temperature, resting blood pressure, heart rate, and respiratory rate.
- Assessment of adverse events since the previous study visit
- Visual examination of the ulcer
- Photo documentation and calculation of ulcer area and depth using the Aranz Medical Silhouette™ system

5.5 Enrollment/Baseline in Multiple Ascending Dose (MAD) Arm

Treatment Visit/Visit 3 (Day 2)

- Subjects will be instructed on the proper procedure for applying study treatment for the MAD Arm and observed performing the first application to the chronic ulcer and applying the non-abrasive hypoallergenic bandage. The Nu-3 or placebo solution will be applied from a pre-packaged dropper bottle at a rate of 1-3 drops per 1 cm^2 of ulcer making sure to cover the entire ulcer as well as up to 0.5cm of surrounding tissue. The subject will be provided the Subject Treatment Log in Appendix D.
- The study subject will be discharged from Visit 3 with a seven-day supply of study medication and supplies. The study subject will be instructed to continue treatment until the night before their next Visit.
- The study subject will be allowed to leave the clinic and instructed to call or return to the clinic if any of the following occur:
  - Increased pain or swelling of the infected ulcer or surrounding area.
  - Any observed increase in redness or swelling of the ulcer.

5.6 Subjects will be provided a Subject Home Treatment Log (Appendix B) and instructed in the proper method of documenting the timing of treatments and recording of any observations.

Follow-Up 7 Day Multiple Ascending Dose Arm Visit/Visit 4 (Day 9+1)

The study subject will return for a follow-up evaluation to the clinical site on Day 9 (or the next day) following the final MAD treatment. At this follow-up visit, the subject will undergo the following procedures and evaluations.

- Vital sign measurements including weight, temperature, resting blood pressure, heart rate, and respiratory rate.
- Radiograph, MRI, and/or CAT evaluations of the infected ulcer to rule out the development of osteomyelitis, if determined by the Principal Investigator to be necessary
- Concomitant medication use
- Draw blood for CBC and CMP.
- Assessment of adverse events and interim history since the previous study visit
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using both the DUSS and DFI Wound Scoring Systems
- Photo documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ system
- Collection of a sample from the ulcer for microbiological assessment
- Subjects will be given a 7 day supply of non-abrasive bandage and written instructions on the proper care and hygiene to include keeping the ulcer clean and bandage dry until the Day 15 follow up visit. In addition, subjects will be told to call if there is any worsening of the ulcer with regard to pain, infection, or swelling.

Subjects will be given a 7 day supply of non-abrasive bandage and given written instructions on proper care and hygiene which includes keeping the ulcer clean and bandaged dry until the Day 15 Final Study Visit. In addition, subjects will be told to call if there is any worsening of the ulcer with regard to pain, infection, or swelling.

5.7 Final Study Visit – Visit 5 (7-Day MAD)

Post Treatment Final Visit (Day 15)

All study subjects will have the following procedures performed at their final visit (Day 15)

- Vital sign measurements including weight, temperature, resting blood pressure, heart rate and respiratory rate
- Physical examinations
- Visual examination of the chronic ulcer
- Scoring of the Ulcer(s) using both the DUSS and DFI Wound Scoring Systems
- Photo documentation and calculation of ulcer area and depth using the Aranz Medical Silhouette™ System
- Assessment of adverse events and interim history since the previous study visit
- Collection of a sample from the ulcer for microbiological evaluation

5.8 Early Termination Visit

If a subject withdraws prior to completing the study, the reason for withdrawal will be documented on source documentation and in the CRF. If a subject withdraws early due to an adverse event, he/she will be followed until resolution/stabilization of the adverse event.

If a subject prematurely withdraws from the study they will be asked to complete the study procedures and evaluations performed in the final study visit at the time of withdrawal from the study (i.e. Study subject withdraws at the time of visit and consents to having procedures/evaluations done):

- Vital sign measurements including weight, temperature, resting blood pressure, heart rate and respiratory rate
- Physical examinations
- Visual examination of the chronic ulcer
- Photo documentation and calculation of ulcer area and depth using the Aranz Medical Silhouette™ system
- Concomitant medication use
- Assessment of adverse events and interim history since the previous study visit
- Collection of a sample from the ulcer for microbiological evaluation

Administrative withdrawals will be replaced to ensure that each cohort includes a total of 10 subjects who have completed the entire treatment. The study sponsor has designated the following person to be in charge of the shipping of study drug as they will be unblinded and able to match the proper replacement kit.

Subjects withdrawn from the study due to an AE/SAE related to the study drug will not be replaced.

5.9 Unscheduled Visit

Unscheduled visits will be documented using the CRFs used for the Day 1 follow-up visit determined to be required by the PI, except in the case of photo documentation which must be performed. If the unscheduled visit occurs as a result of an event, additional testing consistent with the event will be performed.

6 ADVERSE EVENTS AND TOXICITY MANAGEMENT

6.1 Specification of Safety Parameters

The primary endpoint safety variables will be the incidence of localized swelling surrounding the ulcer, development of a rash, joint pain, and any adverse changes in the appearance of the ulcer.

The Safety Monitoring Committee (SMC) will receive safety reports outlining the number of subjects enrolled in each cohort to date, summary of the safety data Day 0 – 15 for the most recent cohort, adverse events, and serious adverse events listed by grade as well as all laboratory tests data. These reports will present the data blinded and will be unblinded only if necessary to determine the cause of an AE or SAE at the request of the SMC. In addition, safety data from completed cohorts will be summarized in the same manner and included in the report. All information on adverse events will be provided in a table.

6.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

6.2.1 Adverse Events

Adverse Event: According to the ICH E6 guidelines, an AE is defined as any untoward medical occurrence in a subject/subjects in a clinical study regardless of its relationship to the administration of the study drug. Therefore, an AE is any unintended, unfavorable, or unexpected sign, laboratory test, symptom, reaction, or disease temporarily associated with a clinical subject/subjects during the duration of the study. The occurrence of an AE may come to the attention of study personnel at a study visit and during interviews of subjects seeking medical care, or upon reviews conducted by study monitors. During all visits and interviews after administration of the study drug, clinical staff will inquire about any anticipated adverse events or symptoms using standardized questions. The data will be recorded on the appropriate CRFs,

All AEs whether local or systemic reactions not meeting the criteria for “serious adverse events” should be documented on the appropriate CRF. The information to be recorded should include a description of the event, time of onset, clinician’s assessment of the severity, relationship to the study drug (assessment to be conducted only by those with the qualified training and authority to make a diagnosis and include the CMO, MD, PA, Nurse Practitioner, DO, DPM in podiatry and DDS), and time to resolution or stabilization. All AEs that occur during the study regardless of their association with the study drug must be documented appropriately and followed to adequate resolution.

Any AE (i.e. a new event or an exacerbation of a pre-existing condition) with an onset date after the screening visit up to the last day on study (including follow-up), should be recorded as an AE on the appropriate CRF page(s).

An AE does not include:

- Medical or surgical procedures) e.g. surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an adverse event.
- Pre-existing diseases or conditions or laboratory abnormalities present or detected prior to the screening visit, that do not worsen.
- Situations where an untoward medical occurrence has not occurred (e.g. hospitalization for elective surgery, social and/or convenience admissions).
- Overdose of either study drug or concomitant medication without any signs or symptoms unless the subject is hospitalized for observation.

Severity of Event: All AEs will be assessed by the investigator and recorded on the appropriate CRF page, including the date of onset and resolution, severity, relationship to study drug or study procedures, outcome and action taken with study medication. The clinician will assess all AEs using a protocol defined grading system as follows.

Scale	Definition
Grade 1	No intervention required because the event is mild or asymptomatic. A clinical or diagnostic observation.
Grade 2	Minimal to moderate intervention and/or localized event that requires non-invasive treatment
Grade 3	A debilitating or incapacitating condition, severe or medically significant that is not life threatening that requires hospitalization or prolonged treatment
Grade 4	Requiring urgent intervention because the event is life threatening
Grade 5	Death of a subject due to an adverse event.

Relationship to Study Products:

6.2.2 Reactogenicity

Adverse events associated with the study product may include vesicle (blistering) development, burning sensation at the ulcer site (may be masked by diabetic neuropathy), increase in local temperature, and expansion of necrotic tissue surrounding the ulcer.

6.2.3 Serious Adverse Events

A **serious adverse event** (SAE) is defined as follows:

- Any adverse drug experience occurring at any dose that results in any of the following outcomes:
- Death;
- Life-threatening situation (subject is at **immediate** risk of death);
- In-patient hospitalization or prolongation of existing hospitalization (excluding those for study therapy or placement of an indwelling catheter, unless associated with other serious events);
- Persistent or significant disability/incapacity;
- Congenital anomaly/birth defect in the offspring of a subject who received study drug;
- Other: medically significant events that may not result in death, be immediately life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, they jeopardize the Subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of such events are:

- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias or convulsions that do not result in hospitalization
- Development of drug dependency or drug abuse

Clarification of Serious Adverse Events

- Death is an outcome of an adverse event, and not an adverse event in itself. In reports of death due to "Disease Progression", where no other information is provided, the death will be assumed to have resulted from progression of the disease being treated with the study drug(s).
- All deaths, regardless of cause or relationship, must be reported for subjects on study and for deaths occurring within 30 days of last study drug dose or within 30 days of last study evaluation, whichever is longer.
- "Occurring at any dose" does not imply that the subject is receiving study drug at the time of the event. Dosing may have been given as treatment cycles or interrupted temporarily prior to the onset of the SAE, but may have contributed to the event.
- "Life-threatening" means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death, if it had occurred with greater severity.
- Complications that occur during hospitalizations are AEs. If a complication prolongs hospitalization, it is an SAE.

- "In-patient hospitalization" means that the subject has been formally admitted to a hospital for medical reasons, for any length of time. This may or may not be overnight. It does not include presentation and care within an emergency department.
- The investigator should attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. In such cases the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms.

A distinction should be drawn between serious and severe AEs. An AE that is assessed as Grade 4 (potentially life-threatening) should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 4. An event is defined as "serious" when it meets one of the pre-defined outcomes as described above in Section 6.2.3.

6.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Laboratory abnormalities are usually not recorded as adverse events or serious adverse events unless they are associated with clinical signs and/or symptoms. However, laboratory abnormalities (e.g. clinical chemistry, hematology, urinalysis, etc.) independent from the underlying medical condition, that require medical or surgical intervention, or lead to study drug interruption or discontinuation must be recorded as an AE, as well as an SAE, if applicable. In addition, laboratory or other abnormal assessments (e.g. electrocardiogram, X-rays, vital signs) that are associated with sign and/or symptoms must be recorded as an AE or SAE if they meet the definition of an adverse event (or serious adverse event) as described in the protocol. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis.

Severity should be recorded and graded according to Common Terminology Criteria for Adverse Events v4.0 (CTCAE). For adverse events associated with laboratory abnormalities, the event should be graded based on the clinical severity in the context of the underlying conditions, which may or may not be in agreement with the grading of the laboratory abnormality.

6.3 Serious Advent Event Reporting Procedures

The study sponsor is required to expedite to worldwide regulatory authorities reports of Serious Adverse Drug Reactions or Suspected Unexpected Serious Adverse Reactions (SUSARs); therefore, the study sponsor or its delegate must be notified immediately regarding the occurrence of any SAE that occurs after the subject consents to participate in the study, including SAEs resulting from protocol-associated procedures performed from screening onwards. The procedures for reporting all SAEs, regardless of causal relationship, are as follows:

- Record the SAE on the AE CRF and complete the "Serious Adverse Event Report" form.
- E-mail or fax the SAE form to the attention of the Drug Safety Manager within 24 hours of the investigator's knowledge of the event. Contact information is as follows:

Lakewood-Amedex Inc. Drug Safety Manager [redacted]

Medical Monitor: [redacted]

- For fatal or life-threatening events, also e-mail or fax copies of hospital case reports, autopsy reports, and other documents when requested and applicable. Transmission of such documents should occur with Personal Subject Details de-identified, without losing the traceability of a document to the Subject Identifiers.

- The study sponsor or its delegate may request additional information from the investigator to ensure the timely completion of accurate safety reports.

The investigator must take all therapeutic measures necessary for resolution of the SAE. Any medications necessary for treatment of the SAE must be recorded onto the concomitant medication section of the subject's CRF and the event description section of the SAE form.

Follow-up of adverse events will continue through the last day on study (including the follow-up off-study medication period of the study) and/or until the investigator and/or study sponsor determine that the subject's condition is stable. The study sponsor or its delegate may request that certain adverse events be followed until resolution.

6.3.1 Reporting Pregnancy

The risks of treatment with bisphosphocin during pregnancy has not been evaluated.

The subject must be instructed to discontinue all study drugs and inform the investigator **immediately** if she becomes pregnant during the study.

The investigator should report all pregnancies to Lakewood-Amedex within 24 hours of becoming aware of the pregnancy. The investigator should counsel the subject regarding the possible effects of prior study drug exposure on the fetus and the need to inform the study site of the outcome of the pregnancy.

Any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or an SAE.

A spontaneous abortion is always considered to be a SAE and will be reported as described in the adverse and Serious Adverse Events section. Furthermore, any SAE occurring as an adverse pregnancy outcome post-study must be reported to Lakewood-Amedex.

Additionally, all pregnancies that occur during the study should be reported using the Pregnancy Report CRF page. Monitoring of the subject should continue until the conclusion of the pregnancy. The outcome should be reported to Lakewood-Amedex using the Pregnancy Outcome CRF page(s). If the end of the pregnancy occurs after the study has been complete the outcome should be reported directly to Lakewood-Amedex. Pregnancies that occur after the subject has discontinued study drugs do not require monitoring.

6.4 Safety Oversight

6.4.1 Independent Safety Monitor Committee

Clinical Response Evaluations:

The clinical outcome will be based on the Principle Investigator's judgment as to the improvement of the wound status from Visit 1 to Visit 4 (7-Day MAD) and Visits 5 and 6. The PI will score the ulcer(s) using both the DUSS and DFI Wound Scoring Systems.

Diabetic Ulcer Wound Scoring System (DUSS)

Parameter	**Score**	
Palpable Pedal Pulses	Presence = 0	Absence = 1
Probing to Bone	No = 0	Yes = 1
Location of Ulcer	Toe = 0	Foot = 1
Number of Ulcerations	Single = 0	Multiple = 1
Score Range	0	4

Diabetic Foot Ulcer Wound Infection Score

Parameter	**Wound Infection Score**			
	0	**1**	**2**	**3**
Purulent discharge	Absent			Present
Nonpurulant drainage (serious, sanguinous)	Absent	Mild		
Erythema	None	Mild pink, barely perceptible	Moderate pale red, defined edges	Severe red to dark red
Induration	None	Mild	Moderate	Severe
Tenderness (sign)	None	Mild	Moderate	Severe
Pain (symptoms)	None	Mild	Moderate	Severe
Local Warmth (relative to uninfected contralateral foot)	Same	Mildly increased	Moderately increased	Severely increased

An improvement in the DFI Wound Score will be used to indicate a clinical improvement of the wound and a change in the DUSS Score wound be used to indicate the healing of a small ulceration.

Microbiological Response Evaluations:

This assessment will be conducted on samples collected by the investigator on Visit 2, Visit 4, Visit 5, and Visit 6.of the study which will be cultured to determine: microbial load and identity of the infected pathogens or wound flora. The microbiological response will be determined by a comparison of the results pre- and post-treatment.

SMC Safety Reports:

Safety reports will be written by the SMC three times during the course of the study. The first will follow the first interim analysis (once the first SAD/MAD Cohort 1 reaches Day 9), the second report will follow the second interim analysis (once the second SAD/MAD Cohort 2 reaches Day 9), and the third report will follow the third interim analysis (once the third SAD/MAD Cohort 3 reaches Day 9). The study reports will be presented to the Principal Investigator at each clinical site and Lakewood-Amedex Inc.

6.4.2 Study Termination

This study may be terminated at any time by Lakewood-Amedex Inc. The PI and SMC may provide Lakewood-Amedex with recommendations regarding termination of the trial, however, Lakewood-Amedex ultimately makes the final decision. Reasons for terminating the study include:

- If a serious or unexpected adverse event occurs and the event is judged to be probably or definitively related to exposure to Nu-3, the study will be immediately suspended by the Principal Investigator pending review of all appropriate safety data. The event will be reported to the SMC, IRB, and Sponsor Medical Monitor within 24 hours or notification of its occurrence. No additional subjects will receive Nu-3 until a joint decision is reached between the Sponsor, PI, and SMC as to whether further doses can be given or the trial is terminated. Subjects currently in a MAD Arm will be contacted to ascertain treatment status and if any AEs have occurred. Absent any findings, subjects in the ongoing MAD arms will be allowed to complete treatment.
- Safety Monitoring Committee terminates the study based on interim safety review
- In the event of a clinical hold being placed on the study following a report of an AE to the FDA
- At the discretion of Lakewood-Amedex.

6.4.3 Removal of Subjects from the Trial or Study Drug

Subjects will be free to withdraw at any time for any reason, or they may be withdrawn if necessary, to protect their health and safety or the integrity of the study data. A subject will be removed from participation in the study if any of the following occur:

- Any clinical adverse event (AE), laboratory abnormality, intercurrent illness, or other medical condition or situation arises such that in the judgment of the PI continued participation in the study would not be in the best interest of the subject
- A protocol violation/deviation occurs that might compromise the integrity of the data, compliance, or subject safety
- Informed consent is withdrawn
- Subject is lost to follow-up

6.4.4 Handling Withdrawals

If a subject is withdrawn prematurely from the study after receiving Nu-3, the reason for withdrawal is to be documented in the source document and in the CRF. If the withdrawal of a subject is due to an AE, the subject will be followed until resolution/stabilization of the adverse event.

In the case of a subject prematurely withdrawing from the study, the following procedures and/or evaluations will be performed if possible at the time of withdrawal or as soon thereafter as possible (i.e. study subject withdraws at time of visit and consents to the procedures or consents to come in for a visit and the procedures)

- Photo documentation of ulcer site using the Aranz Medical Silhouette™ system.
- Collection of a sample for microbiological assessment
- Assessment of adverse events

In the event a subject is lost to follow-up, the site staff must make reasonable attempts to contact the subject. A minimum of two documented phone calls followed by a certified mailed letter is considered reasonable. The measures taken to follow up must be documented.

Administrative withdrawals will be replaced in order to achieve the objective of 10 subjects per cohort who receive a complete treatment regime. Subjects withdrawn from the study due to an AE/SAE related to the study drug will not be replaced.

7 STATISTICAL CONSIDERATIONS

7.1 Study Hypothesis

Study Hypothesis – Bisphosphocin Nu-3 solution applied topically to a chronic infected diabetic ulcer will be safe and well tolerated in subjects suffering from diabetes mellitus. In addition, the Nu-3 MAD Arm subjects may show improvement in the ulcer appearance, reduction in size, and improvement in both the DUSS score and DFI Wound Score.

The study hypothesis will be judged by the following two study objectives:

- Primary objective: To assess the safety and tolerability of escalating single and multiple doses of Nu-3, a novel antimicrobial of the bisphosphocin class, topically to an open wound/diabetic ulcer
- Secondary objectives: To assess clinical response of subjects of the ascending multiple dose arm of Nu-3 applied topically to an open wound/diabetic ulcer to determine clinical and microbiological response.

7.2 Sample Size Considerations

The number of treated subjects is based on the desire to gain preliminary safety, tolerability and efficacy information to support future work, while exposing a minimal number of subjects to the study procedures and medication. Inferential statistical testing is not the primary intent of the study, therefore, no formal sample size calculation was considered. Ten subjects per Cohort (1, 2, and 3), for a total not to exceed 30, is considered sufficient to show clinical significance for safety and preliminary efficacy analysis. The sample sizes for this study were set based on the trial design for dose escalation and safety evaluation requirements and not for statistical analysis.

7.3 Planned Interim Analysis

There will be an interim analysis to review safety data on each cohort of subjects when all members of a cohort complete Visit 4 (7-Day MAD Follow Up). In addition, an interim analysis will be conducted after the first 5 subjects of Cohort 2 and 3 complete Visit 4 (7-Day MAD Follow Up). The interim analysis will be performed by the Safety Monitoring Committee (SMC) which will be composed of three physicians and may include one infectious disease specialist, one diabetologist, and one orthopedic surgeon. The SMC will communicate by email or teleconference on at least 3 occasions during the study and more frequently if necessary.

No formal statistical interim analyses are planned for this Phase I/IIa study since it is primarily focused on evaluating safety and tolerability. Interim safety data on each cohort will be provided to the SMC when all members of a cohort have reached Day 3. The SMC will decide if the study can proceed to the next dose cohort (majority vote). The SMC will also determine if the study may proceed after receiving a report about a study related Grade 3 or 4 adverse event

(majority vote). The Chairman of the SMC will share their findings and recommendations with Lakewood Amedex and Principal Investigators.

7.3.1 Safety Review

Subjects will be monitored closely for the occurrence of study related adverse events during their 24-hr admission in the SAD Arm and for up to 7 days following their last Nu-3 treatment in the study. If any grade 3 or 4 adverse events occur and the event is judged to be probably or definitely related to having received the Nu-3, the study will be immediately suspended by the Principal Investigator pending review of all safety data. Adverse events determined to be related to the study drug will be reported to the SMC, Lakewood-Amedex, and second PI within 24 hours of notification of its occurrence. No additional subjects will receive Nu-3 depending on the joint decision of the SMC, Principal Investigator, and Lakewood Amedex Project Coordinator and CMO as to whether further doses can be given or the entire study should be terminated. In addition, the SMC will review all interim safety data prior to the next cohort beginning treatment. At each review, the SMC will determine whether the study may proceed to the next dose cohort (majority vote).

7.4 Statistical Analysis Overview

This study is designed to evaluate the safety, tolerability, and preliminary efficacy of a minimum of three dose levels of Nu-3 relative to placebo. Data summaries will be presented in order to assess the safety of Nu-3 and each Nu-3 dose group will be compared with placebo to determine the presence or absence of a treatment effect. Evaluation across all dose levels of Nu-3 will be conducted to determine if there is evidence of a dose response to any treatment effect noted. All subjects treated with placebo from all cohorts will be combined for any analysis. Evaluations will also be conducted within each subject over time to assess the rate of change (if any) in study outcome measures over the full course of the study treatment and follow-up periods. Inferential statistical testing is not the primary interest of this study.

All subjects receiving any amount of study treatment will be evaluated for safety, tolerability, and efficacy. An “intent to treat” approach will be followed in all data summaries, using all available data in the summaries and analysis for an assessment of safety and tolerability while an “as treated” approach will be used for microbiological and clinical wound assessment data summaries and reports.

All descriptive summaries and analyses will be presented in tabular and/or graphical form from all subjects as appropriate. These summaries and analysis will be described in detail in the Statistical Analysis Plan (SAP) which will be produced prior to breaking the study blind. All statistical summaries and analysis will be conducted using SAS®.

8 RESPONSIBILITIES

8.1 Investigator Responsibilities

8.1.1 Good Clinical Practice

The investigator will ensure that this study is conducted in accordance with the principles of the "Declaration of Helsinki" (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), International Conference on Harmonization (ICH) guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study subject. For studies conducted under a United States IND, the investigator will ensure that the basic principles of "Good Clinical Practice," as outlined in 21 CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," 21 CFR, part 50, 1998, and 21 CFR, part 56, 1998, are adhered to.

Since this is a "covered" clinical trial, the investigator will ensure that 21 CFR, Part 54, 1998, is adhered to; a "covered" clinical trial is any "study of a drug or device in humans submitted in a marketing application or reclassification petition subject to this part that the applicant or FDA relies on to establish that the product is effective (including studies that show equivalence to an effective product) or that make a significant contribution to the demonstration of safety." This requires that investigators and all sub-investigators must provide documentation of their financial interest or arrangements with Lakewood-Amedex or proprietary interests in the drug being studied. This documentation must be provided before participation of the investigator and any sub-investigator. The investigator and sub-investigator agree to notify Lakewood-Amedex of any change reportable interests during the study and for one year following completion of the study. Study completion is defined as the date that the last subject has completed the protocol defined activities.

This study is also subject to and will be conducted in accordance with 21 CFR, part 320, 1993, "Retention of Bioavailability and Bioequivalence Testing Samples."

8.1.2 Institutional Review Board (IRB)/Independent Ethics Committee (IEC) Approval

This protocol and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) will be submitted by the investigator to an IRB in compliance with FDA regulations for IRBs (21CFR Part 56). Approval from the IRB must be obtained **before** starting the study and must be documented in a letter to the investigator specifying the protocol number, protocol version, protocol date, documents reviewed, and date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of IRB approval must also be submitted to the IRB for approval before implementation.

8.1.3 Informed Consent

The investigator is responsible for obtaining written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and before undertaking any study-related procedures. The investigator must utilize an IRB approved consent form for documenting written informed consent. Each informed consent will be appropriately signed and dated by the subject or the subject's legally authorized representative and the person obtaining consent. The information in the informed consent must comply with 21CFR Part 50, Subpart B. The elements of the informed consent must be consistent with the requirements of 21CFR Part 50.25.

8.1.4 Confidentiality

The investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, date of birth, and an identification code (i.e., not names) should be recorded on any form or biological sample submitted to the Sponsor, IRB, or laboratory. The investigator must keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the trial.

The investigator agrees that all information received from Lakewood-Amedex, including but not limited to the Investigator Brochure, this protocol, CRFs, the investigational new drug, and any other study information, remain the sole and exclusive property of Lakewood-Amedex during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from Lakewood-Amedex. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

8.1.5 Study Files and Retention of Records

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following two categories: (1) investigator's study file, and (2) subject clinical source documents.

The investigator's study file will contain the protocol/amendments, CRF and query forms, IRB and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

The required source data are listed in the Source Data verification Plan, and should include sequential notes containing at least the following information for each subject:

- subject identification (name, date of birth, gender);
- documentation that subject meets eligibility criteria, i.e., history, physical examination, and confirmation of diagnosis (to support inclusion and exclusion criteria);

- participation in trial (including trial number);
- trial discussed and date of informed consent;
- dates of all visits;
- documentation that protocol specific procedures were performed;
- results of efficacy parameters, as required by the protocol;
- start and end date (including dose regimen) of trial medication (preferably drug dispensing and return should be documented as well);
- record of all adverse events and other safety parameters (start and end date, and preferably including causality and intensity);
- concomitant medication (including start and end date, dose if relevant; dose changes should be motivated);
- date of trial completion and reason for early discontinuation, if applicable.

All clinical study documents must be retained by the investigator until at least 2 years after the last approval of a marketing application in an ICH region (i.e., United States, Europe, or Japan) and until there are no pending or contemplated marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if required by applicable regulatory requirements, by local regulations, or by an agreement with Lakewood-Amedex. The investigator must notify Lakewood-Amedex before destroying any clinical study records.

Should the investigator wish to assign the subject records to another party or move them to another location, Lakewood-Amedex should be notified in advance.

If the investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and Lakewood-Amedex to store these in sealed containers outside of the site so that they can be returned sealed to the investigator in case of a regulatory audit. When source documents are required for the continued care of the subject, appropriate copies should be made for storage outside of the site.

8.1.6 Case Report Forms

For each subject enrolled, a CRF must be completed and signed by the principal investigator or sub-investigator (as appropriate) within a reasonable time period after data collection. This also applies to records for those subjects who fail to complete the study (even during a pre-randomization screening period if a CRF was initiated). If a subject withdraws from the study, the reason must be noted on the CRF. If a subject is withdrawn from the study because of a treatment-limiting adverse event, thorough efforts should be made to clearly document the outcome.

8.1.7 Drug Accountability

The investigator or designee (i.e., pharmacist) is responsible for ensuring adequate accountability of all used and unused investigational medicinal product, placebos, and comparators. This includes acknowledgment of receipt of each shipment of study product (quantity and condition), subject dispensing records, and returned or destroyed study product. Dispensing records will document quantities received from the study sponsor and quantities dispensed to subjects, including kit number, date dispensed, subject identifier number, subject initials, and the initials of the person dispensing the medication.

At study initiation, the monitor will evaluate the site's standard operating procedure for investigational medicinal product disposal/destruction in order to ensure that it complies with Lakewood-Amedex requirements. Drug may be returned on an ongoing basis during the study, if appropriate. At the end of the study, following final drug inventory reconciliation by the monitor, the study site will ship any remaining investigational product (to include empty containers) to [redacted] for disposal.

All drug supplies and associated documentation will be periodically reviewed and verified by the study monitor over the course of the study.

8.1.8 Inspections

The investigator understands that source documents for this trial must be made available to appropriately qualified personnel from Lakewood-Amedex or its representatives, to IRBs or to regulatory authority or health authority inspectors.

8.1.9 Protocol Compliance

The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance maybe either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- Compliance with Protocol sections 4.5.1, 4.5.2, and 4.5.3
- Quality Assurance and Quality Control, section 5.1.1
- Noncompliance sections 5.20.1, and 5.20.2

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All significant deviations must be promptly reported to

Lakewood Amedex Inc. to the attention of [redacted] via fax. Reports can be sent to Lakewood -Amedex as specified below copying the CRA assigned to your site:

- Via the web-based PD forms submission
- FAX submission (1[redacted]): utilizing the Protocol Deviation form and the email/fax transmittal form, or
- E-mail submission ([redacted]) utilizing the Protocol Deviation form and the email/fax transmittal form.

All deviations from the protocol must be addressed in the study subject source documents. A completed copy of the LAI Protocol Deviation form must be maintained in the regulatory file, as well as in the subject's source document. Significant protocol deviations must be sent to the local IRB/EC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB/EC requirements.

8.2 Sponsor Responsibilities

8.2.1 Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by Lakewood-Amedex. All protocol modifications must be submitted to the IRB in accordance with local requirements. Approval must be obtained before changes can be implemented.

8.2.2 Study Report and Publications

A clinical study report will be prepared by Protocol Coordinator and provided to the regulatory agency(ies). Lakewood-Amedex will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). Note that an abbreviated report may be prepared in certain cases.

Lakewood-Amedex Inc. will have the sole decision on whether or not to publish the results of this research. The Company encourages its scientists and collaborators to published the results of any research sponsored by the Company but only after a review of the data by the Company to ensure that any discoveries or trade secrets are properly protected by issued, pending, or newly filed patents or patents applications.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials registration policy as a condition for publication. This policy requires that all clinical trials except Phase I studies be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. Lakewood-Amedex, Inc. will be responsible for registering the clinical trial with an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before subject enrollment.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subject to intervention or comparison groups to study the cause-and-effect relationship between medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g. Phase I trials) would be exempt from this policy.

8.3 Joint Investigator/Sponsor Responsibilities

8.3.1 Access to Information for Monitoring

In accordance with ICH Good Clinical Practice (ICH GCP) guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the CRFs for consistency.

The monitor is responsible for routine review of the CRFs at regular intervals throughout the study to verify adherence to the protocol and the completeness, consistency, and accuracy of the data being entered on them. The monitor should have access to any subject records needed to verify the entries on the CRFs. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

8.3.2 Access to Information for Auditing or Inspections

Representatives of regulatory authorities or of Lakewood-Amedex may conduct inspections or audits of the clinical study. If the investigator is notified of an inspection by a regulatory authority the investigator agrees to notify the Lakewood-Amedex medical monitor immediately. The investigator agrees to provide to representatives of a regulatory agency or Lakewood-Amedex access to records, facilities, and personnel for the effective conduct of any inspection or audit.

8.3.3 Study Discontinuation

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authority(ies), and IRB/IECs. In terminating the study, Lakewood-Amedex and the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

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10 APPENDICES

Appendix A. Project Timeline and Flow Chart

		Obtain Informed Consent		
		Screen Subject		
		Assign to Open Cohort		
SAD Arm 1: Cohort 1 – 0.1% or 1mg/ml (n=10) (2 Placebo; 8 Nu-3)				
Perform Informed Consent and Document Pregnancy and Drug Screen <24h Before Treatment Apply Treatment Follow up Visit 3				
PI Review at 24hr Examination No Safety Issues. Proceed to MAD Arm – Cohort 1		SAD Arm 1:Cohort 2 – 1% or 10mg/ml (n=10) (2 Placebo; 8 Nu-3)		
Instruct on Treatment Observe Subject Apply 1st Treatment Follow up Visit 5		Perform Informed Consent and Document Pregnancy and Drug Screen <24h Apply Treatment Follow up Visit 3		
SMC Review After All Subject Have Completed Cohort 1 No Safety Issues. Proceed to Next Cohort		PI Review at 24hr Examination No Safety Issues. Proceed to MAD Arm Cohort 2		SAD Arm 1: Cohort 3 – 2% or 20mg/ml (n=10) (2 Placebo; 8 Nu-3)
		Instruct on Treatment Observe Subject Apply 1st Treatment Follow up Visit 5		Perform Informed Consent and Document Pregnancy and Drug Screen <24h Before Treatment Review Informed Consent & Document t, Apply Treatment Follow up Day 2
		SMC Review After All Subject Have Completed Cohort 2 No Safety Issues. Proceed to Next Cohort		PI Review at 24hr Examination No Safety Issues. Proceed to MAD Arm 2 Cohort 3

				Instruct on Treatment Observe Subject Apply 1st Treatment Follow up Visit 5

Appendix B: Schedule of Events

Event	Visit 1 Screening Visit	Visit 2 SAD Treatment	Visit 3[a]	Visit 4[b]	Visit 5[c]	Early Termination (ET) Visit
Informed Consent Process	X					
Medical History	X					
Physical Exam	X				X	X
Vital Signs[1]	X	X[2]	X	X	X	X
CBC, CMP Blood Draw	X	X		X	X	X
Collect ulcer tissue sample for microbiological evaluation	X	X		X	X	X
Urine Pregnancy Test (HCG)[3]	X	X				
Drug and ETOH toxicity screen	X	X				
Visual Examination of Chronic Ulcer	X	X	X	X	X	X
Radiograph, MRI and/or CT evaluation of the infected ulcer[4]	X			X		
Photo Documentation and Calculation of target ulcer area and depth – ARANZ Silhouette™ Medical System	X	X	X	X	X	X
Score target ulcer using DUSS Scoring System	X	X		X	X	
Rating of the infected ulcer using DFI Wound Scoring system	X			X	X	
Investigational Product		X	X			
Concomitant Medication	X	X	X	X	X	X
Adverse Event	X	X[5]	X	X	X	X

Key

[a] Visit window 24 hours ± 2 hours post Visit 2

[b] Visit window 8 days + 1 day post Visit 2

[c] Visit window 14 days ± 1 day post Visit 2

[1] Height and weight (screening, Day 1, follow-up visit and ET), temperature, resting blood pressure (BP), heart and respiration rate

[2] Resting BP, heart and respiration rate to be obtained prior to and 1 hour post SAD Treatment

[3] Urine pregnancy test for all female subjects of childbearing potential

[4] Radiography, MRI and/or CT evaluation required within 2 weeks of Visit 1 (Screening Visit), at all other visits only necessary if determined by PI [5] Upon SMC review and safety approval of 1st five (5) subjects in Cohorts 2 and 3, Principal Investigator (PI) may elect to extend treatment to fourteen (14) days if in the opinion of the PI the subject is showing improvement at Visit 4.

[5] Signs and symptoms of adverse events to be obtained 1 hour post SAD Treatment

Appendix C: Use of the Aranz Medical Silhouette™ System for Photo documentation of the DFU and Calculation of Area and Depth, and Volume.

Capturing a Wound Image

1. Log in to Silhouette Connect and Select the Patient to be imaged.
2. Review the patient details and select Capture Images Button. The laser lines should come on from the Silhouette Star Camera.
3. To capture the wound image position the Silhouette Star Camera directly above the wound and move the camera up or down so that the laser lines form a star. The center of the laser line star should be over the deepest part of the wound.
4. Capture an image of the wound by pressing the bottom on the camera.
5. Repeat the process to capture multiple images.
6. All images will be uploaded to the SilhouetteConnect Database on the tablet.

Calculating the Ulcer Area, Volume, and Depth

1. Select the Ulcer image to be used for assessment on the tablet.
2. Enlarge the image so the perimeter of the ulcer is easily visible
3. Outline the perimeter of the ulcer by either clicking around the perimeter so that the entire ulcer is surrounded by dots or tracing the perimeter of the ulcer with a pointer or finger.
4. Clicking on the circular target to initiate calculation of the area, volume, and depth of the ulcer by the SilhouetteConnect software.
5. Click on the Notes(?) button to enter comments on the ulcer margins, appearance, and surrounding skin
6. Click Generate pdf report and Save to Store it to the database.
7. Click Send to export to the ClinicalInk Database.

Appendix D: Subject Home Treatment Log:

Subject ID No.:_____

Study Drug Number:_____

Issue Date:_____ **Return Date:**_____

Instructions: Subjects/caregivers should log both the date and approximate time of each treatment and the person administering the study drug should initial in the corresponding box. A space is also provided for the subject/caregiver to write any comments concerning the administration or observations of the ulcer.

Day	Treatment 1 Date/Time	Initials	Therapeutic Footwear Worn	Length of Time Footwear Worn	Treatment 2 Date/Time	Initials	Therapeutic Footwear Worn	Length of Time Footwear Worn	Comments
1			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
2			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
3			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
4			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
5			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
6			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	
7			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐ 5-8 hrs. ☐ 9-12 hrs.	

CLINICAL STUDY PROTOCOL

Study Title:	A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin Nu-3 on Infected Diabetic Ulcers of Subjects With Type I or II Diabetes Mellitus
Sponsor:	Lakewood-Amedex, Inc. 9015 Town Center, Pkwy, Unit 110 Bradenton, FL 34202
IND No.: **EudraCT Number**	Not Applicable
Indication:	Diabetic Foot Infections
Protocol ID:	LAI2014-1
Protocol Coordinator:	[redacted]
Medical Monitor	[redacted]
Protocol Version/Date:	v1.0 31 October 2014

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Lakewood-Amedex Bisphosphocin NU-3 Clinical Protocol

A PHASE I/IIa, RANDOMIZED DOUBLE BLIND, PLACEBO CONTROLLED, DOSE ESCALATING STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF TOPICALLY APPLIED BISPHOSPHOCIN™ NU-3 ON INFECTED DIABETIC ULCERS OF SUBJECTS WITH TYPE I OR II DIABETES MELLITUS

Version 1.0

31 October 2014

Approved by:

[redacted]

STUDY ACKNOWLEDGEMENT

A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin™ Nu-3 on Infected Diabetic Ulcers of Subjects With Type I or II Diabetes Mellitus

Version 1.0: 31 October 2014

INVESTIGATOR STATEMENT

I have read the protocol and Investigator's Brochure, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Lakewood -Amedex. I will discuss this material with them to ensure that they are fully informed about the drugs and the study.

_____ _____

Principal Investigator Name (Printed) Signature

_____

Date

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

° C	degrees Celsius
° F	degrees Fahrenheit
AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
API	Active Pharmaceutical Ingredient
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
CMP	Comprehensive Metabolic Panel
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events v4.0
DFI	Diabetic Foot Infection
DSMB	Data and Safety Monitoring Board
DUSS	Diabetic Ulcer Severity Score
ELSD	Evaporative Light Scatter Detection
FDA	Food and Drug Administration
GCP	Good Clinical Practice
hCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IB	Investigator's Brochure
ICH	International Committee on Harmonization
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IR	Infrared Spectroscopy
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LAI	Lakewood-Amedex, Inc
LCMS	Liquid Chromatography Mass Spectrometry

LLN	Lower Limit of Normal
MAD	Multiple Ascending Dose
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
MOA	Mechanism of Action
MRSA	Methicillin Resistant *Staphylococcus aureus*
MS	Mass Spectroscopy
NDM-1	New Delhi Metallo-Beta Lactamase-1
NMR	Nuclear Magnetic Resonance
PBS	Phosphate Buffered Saline
PI	Principal Investigator
RBCs	Red Blood Cells
SAD	Single Ascending Dose
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TS	Trypic Soy
TSBD	Trypic Soy Broth with 1% Dextrose
ULN	Upper Limit of Normal
US	United States
WBC	White Blood Cell

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PROTOCOL SUMMARY

Title:	A Phase I/IIa, Randomized Double Blind, Placebo-Controlled, Dose Escalating Study to Evaluate the Safety and Tolerability of Topically Applied Bisphosphocin™ Nu-3 on Infected Diabetic Ulcers of Subjects With Type I or II Diabetes Mellitus
Phase:	I/IIa
Population:	Up to 30 subjects over 18 years of age suffering from diabetes mellitus and a chronic infected diabetic ulcer(s).
Number of Sites:	2
Study Duration:	6 months
Subject Participation Duration:	44 days (includes screening visit, study treatment and all follow-ups visits through Day 15 or Day 22 post treatment.
Description of Agent or Intervention:	Bisphosphocin™ Nu-3 to be applied as a topical solution to the ulcer and covered by a bandage. This study includes three dose ascending cohorts. Within each cohort there are two phases with both a single ascending dose and multiple ascending dose arms. The three dose levels (cohorts) are 0.1% (1mg/ml, anticipated low dose), 1% (10mg/ml, anticipated therapeutic dose, and 2% (20mg/ml, anticipated high dose).
Objectives:	**Primary:** To assess the safety and tolerability of escalating doses of topically applied bisphosphocin™ Nu-3, [redacted] to an open infected ulcer. Assessment of safety will be determined by visual evaluation of the ulcer, vital signs, CBC analysis, CMP analysis, and physical examination, as well as the incidence and severity of emergent adverse events that occur during the study participation. The study will be monitored by the Safety Monitoring Committee (SMC). **Secondary:** To assess the clinical and microbiological response to bisphosphocin™ Nu-3 administered topically to the ulcer, including the improvement of wound appearance and elimination of the pathogenic bacteria.
Estimated Time to Complete Enrollment:	24 Weeks

Study Design:	**Allocation:** Randomized, Placebo-Controlled **Endpoint:** Safety and Tolerability **Enrollment Model:** Sequential Assignment **Masking:** Double Blind (Subject, Caregiver, Investigator) **Primary Purpose:** Safety
Description of Study Design	Phase I/IIa, three cohort ascending dose with two dosing arms per cohort, study in Type I or II diabetes mellitus subjects with a chronic infected diabetic ulcer defined as having a DUSS score of 0 to 3 and DFI wound score of 1 to 9. The study proposes to use the same subject in SAD Arm 1 and MAD Arm 2 of each cohort The study is designed to run the cohorts in series with the completion of the first cohort before initiating the next dosing level. Prior to initiating treatment of the infected diabetic ulcer, subject will be evaluated for skin sensitivity to Nu-3 by applying 1 drop to the forearm and monitoring the site for any acute reaction over a 10-15 minute interval for any sign of reddening, swelling, and/or irritation. In addition, at all study visits the ulcer will be visually examined for any changes and photographed using the Aranz Medical Silhouette™ system that will calculate area and depth of the ulcer. In Arm 1, eligible subjects will be treated with a single application of Nu-3 or placebo in 4 to 1 ratio to judge the initial safety of Nu-3 over a brief 15-30 minute interval and 24-hr interval. Bisphosphocin Nu-3 will be applied topically to the chronic infected ulcer, covered with a non-abrasive bandage following the initial observation period. The subject will be released with verbal instructions to leave the bandage on the wound and return for a follow up visit within 24h ± 2h. At the follow up visit, the bandage will be removed, the ulcer visually examined and the subject cleared for the MAD Arm 2 based on the recommendation of the PI and absence of any SAEs. In Arm 2, eligible subjects which are those who have been approved by the PI after the Visit 2 examination will be instructed in the proper application of bisphosphocin Nu-3. The subjects will be observed applying the first dose in the clinic to ensure compliance. Subjects will then be given a 7 day supply and sent home to continue treatment. Visit 4 or earlier in the case of any adverse events, subjects will return to the clinic for an examination, including visual examination of the ulcer, vital signs, adverse events, photo documentation, collection of a sample for microbiology and concomitant medication use. For Cohorts 2 and 3, an extension of the treatment duration to fourteen (14) days would only be initiated after five subjects in Cohorts 2 and 3 completed

	the seven (7) day treatment and approval was given by the SMC. The decision to extend treatment of individual subjects showing a clinical response would then be made by the Principal Investigator. A final follow up visit will be scheduled +7 days after last dose of study medication (Day 15 or Day 22) for a complete examination as described above.

1 INTRODUCTION

1.1 Background Information

Infectious disease represents a growing threat to world health due to the emergence of new viruses and the increase in bacterial resistance to antibiotics[1,2]. It is now estimated that almost one-third of the world population or 2 billion people are infected with *Mycobacterium tuberculosis* with between 3-5% of these cases being multidrug or extremely drug resistant[3]. In addition, bacteria have repeatedly demonstrated the ability to become resistant to antibiotics much faster than new compounds or sub-classes can be identified. The NDM-1 resistance gene represents a perfect example of the ability of bacteria to not only acquire resistance but broad spectrum resistance on a single plasmid that provides resistance against most of the major or widely used classes of antibiotics. This highlights the shortcomings of current and past antibiotic research which has relied on derivatives of existing classes that all share a common or similar core structure making them susceptible to inactivation by a single enzyme. In addition, traditional antibiotics share a related mechanism of action in that they all function through a target protein or receptor molecule to inhibit a cellular process to kill the bacteria allowing the bacteria to rapidly become resistant by acquiring a beneficial mutation in the target molecule[4].

Over the past ten years, the amount of research and development invested by large pharmaceutical companies in the discovery of novel antibiotics has steadily declined while bacteria have become increasingly resistant to the currently available classes of antibiotics. According to the Centers for Disease Control and Prevention, approximately 2 million people each year are infected with bacteria resistant to at least one antibiotic which results in around 23,000 deaths[5]. Methicillin resistant *Staphylococcus aureus* (MRSA) has become a major public health issue resulting in the closing of several public schools for cleaning and demonstrating that this is a problem no longer restricted to hospitals. No other factor highlights the need for a greater effort into the research and development of novel anti-bacterial compounds than the ever increasing ability of bacteria to rapidly acquire resistance to current and new derivatives of existing antibiotics. The recent identification of a strain of *Streptococcus* resistant to more than 18 different antibiotics highlights this fact and the obvious need for novel anti-infective compounds like Bisphosphocins.

Lakewood Amedex has discovered a new class of broad spectrum antimicrobial, termed bisphosphocins™, formerly known as nubiotics, that have been proven effective *in vitro* at killing 70 different strains of bacteria, including all Category A pathogens and *in vivo* against the difficult to treat infections caused by *Francisella tularensis*, *Helicobacter pylori*, and *Pseudomonas aeruginosa*. Bisphosphocins are a new class of synthetic broad spectrum antimicrobial that are characterized by a core structure bridging two phosphate groups attached to even chain alkyl or alcohol groups. The compounds are activated through an acidification process which is reversible by exposing the compounds to a buffer or base. The compounds were found to be bactericidal and effective in killing slow growing or stationary bacteria. Preclinical studies have demonstrated the effectiveness of these compounds in the treatment of a

number of serious bacterial infections[6,7,8]. In addition, the compounds were initially developed as a homeopathic treatment and were found to reduce the severity of several common infection associated with acne, diabetic foot infection, and skin infections[9]. The compounds function through a unique mechanism of action, are amendable to multiple formulations, and routes of administrations enabling a clinical development plan distinct from most traditional antibiotics. The current clinical development plan entails development of a topical formulation of bisphosphocin™ Nu-3 for a number of topical infections such as diabetic foot infections, chronic urinary tract infections, and pulmonary infections using direct delivery of the compound to the site of the infection. This clinical approach will demonstrate initial safety in humans and keep delivery of the antimicrobial localized to the site of infection and thus decrease the chance of bacteria becoming resistant. This clinical study will also yield important data for planning follow on clinical trials, including those involving Nu-3 for systemic administration to treat serious gram negative infections such as complicated urinary tract infections.

Diabetic foot infections are one of the most common complications for people who suffer from diabetes and a frequent cause of hospitalization[10]. According to the American Diabetes Association approximately 8.3% of the US population suffers from diabetes and in 2006 there were 65,700 non-traumatic amputations performed on diabetics. In addition, almost half of diabetic patients that have an amputation will develop an ulcer on the remaining limb within 18 months after surgery. The treatment of a diabetic foot infection is complicated as it requires both antimicrobial therapy to cure the infection and proper wound care management to heal the ulcer. Curing the infection is particularly challenging since 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 60-80% of chronic infections involve biofilm formation which makes the bacteria more resistant to traditional antibiotics[11]. Therefore, 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.

Bisphosphocins™, a new class of broad spectrum antimicrobials, possess a number of characteristics that make these compounds extremely well-suited to the management of diabetic foot infections. First, bisphosphocins™ are rapidly and directly bactericidal functioning through a unique mechanism of action providing the ability to quickly cure an infection. The compounds are fairly low in molecule weight, have an excellent safety profile, and have shown good penetration into wounds. In addition, there are no known bacterial mechanisms of resistance and no resistant bacteria have been identified to date eliminating a common cause of treatment failure[7]. Finally, bisphosphocins have been demonstrated highly effective at killing bacteria established in biofilms[12] which is increasingly become associated with many chronic infections. The preclinical studies conducted on the bisphosphocin™ class clearly support their evaluation in the proposed Phase I/IIa safety study in diabetic foot infections.

1.2 Bisphosphocin Nu-3

1.2.1 General Information

Bisphosphocins™ were originally discovered by [redacted] while conducting research in the field of antisense technology and attempting to kill bacteria using gene silencing. The initial compounds identified were protonated/acidified oligonucleotides that demonstrated bactericidal activity independent of nucleotide sequence. Further experimentation expanded the class to protonated/acidified nucleotides and derivative molecules that exhibit chemical stability, acid pH resistance, and nuclease resistance enabling formulations for oral, intravenous, pulmonary, and topical delivery. The lead compound, designated Nu-3, is a thymidine derivative that would be considered to be in the nucleotide analogue class of drugs (Figure 1). Experimental data has elucidated a non-receptor dependent mechanism of action involving disruption of the bacterial cell membrane causing depolarization and rapid cell death. Bisphosphocins™ have an extremely broad spectrum of activity *in vitro* testing against over 70 different bacterial strains carrying various antibiotic resistant markers. There is currently no known mechanism of resistance to the bisphosphocin class and because they were synthetically derived there is no natural pool of resistant bacteria. In addition, panelling the compounds against a number of strains with known antibiotic resistance or clinical isolates has failed to identify any cross-resistance or resistance mechanisms. The following preclinical pharmacological and toxicology data is provided as a brief summary of the activity of bisphosphocin Nu-3 to support its potential as a treatment for diabetic foot infections and this clinical protocol should be read in conjunction with the Investigator Brochure on the product.

Figure 1. Structure, Chemical Name and Chemical Formula of Bisphosphocin™ Nu-3

1.2.2 Pre-Clinical Pharmacology and Toxicology

Specific Pharmacology

Initial experiments revealed that bisphosphocins™ had an antimicrobial activity distinct from any currently existing antibiotic class. Extensive *in vitro* and i*n vivo* experiments have been conducted to further define their activity, identify their mechanism of action, and begin to establish a dose range to allow entry in human clinical trials. An important factor to consider in the review of the following experimental results is that it is now known bisphosphocins™ do not follow the simple inhibitory kinetic model of one compound binding to one target molecule of the current classes of antibiotics but a more complex kinetic model involving the binding of multiple molecules to a bacterium resulting in direct bacteria cell death. For this reason, the current hypothesis is bisphosphocin™ dosing *in vivo* will be based on total dose delivered and not on maintaining a circulating level above the minimum inhibitory concentration or at the site of infection to achieve an efficacy outcome because the molecules rapidly bind and kill bacteria as opposed to inhibiting growth. Therefore, the therapeutic effect of bisphosphocins™ can be manipulated by not only controlling the dose but frequency and duration of treatment not because of the pharmacokinetics of the compound but due to the fact that each dose will result in a reduction of bacterial load. The following experiments are presented to further define, distinguish and compare the activity of the bisphosphocin class from traditional antibiotics in order to develop a comprehensive clinical plan to enable evaluation in humans. The majority of the experimental data presented in the following sections was performed in the laboratory of outside collaborators or researchers who then published their results[6,7,8,12] or by independent Contract Research Organization following the current guidelines for antimicrobial testing. Due to the unique MOA of the bisphosphocin™ class some standard assays were modified to enable the evaluation which has been noted in the protocol. Table 1 and Table 2 summarizes the activity of Nu-3 in the standard MIC assay against bacterial pathogens commonly found in diabetic foot infections and against common fungal pathogens.

Table 1. Bisphosphocin Nu-3 Activity in a Minimum Inhibitory Assay according to CLSI Guidelines

Organism	Number of Strains	MIC mg/ml
Acinetobacter baumannii	N=15	5-10
Enterococcus faecalis, VRE	N=15	5
Enterococcus faecalis, VSE	N=15	5
Enterococcus faecium, VRE	N=15	5
Enterococcus faecium, VRE	N=15	5
Escherichia coli	N=15	10
Proteus mirabilis	N=15	5-10
Proteus vulgaris	N=15	5-10

Organism	Number of Strains	MIC mg/ml
Pseudomonas aeruginosa	N=5	2.5-5
Pseudomonas aeruginosa	N=15	5-10
Serratia marcescens	N=15	10
Staphylococcus aureus, MSSA	N=15	5-10
Staphylococcus aureus, MRSA	N=15	5-10
Staphylococcus epidermidis, MRSE	N=15	10
Staphylococcus epidermidis, MSSE	N=15	10
Streptococcus agalactiae	N=15	10

Table 2. Anti-fungal Activity of Bisphosphocin Nu-3

Yeast Strain	ATCC #	Drug Resistance	MIC mg/ml
			Nu3
Candida albicans	44373	5-Fluorocytosine	≤0.53
Candida albicans	44374		≤0.53
Saccharomyces pastorius	2366		≤0.07
Trichophytan mentagrophytes var. *interdigitale*	200099		5.0

Evaluation of Nu-3 Activity against Bacteria Established in Biofilm

The following studies were performed by [redacted] and were designed to evaluate the activity of Nu-3 against bacteria established in biofilms which is increasing becoming associated with chronic infections[12,unpublished]. Biofilm was formed in borosilicate glass tubes by standard methods and then treated with Nu-3 or kept as untreated controls. As shown in Table 3, Nu-3 was able to penetrate the biofilm and kill the bacteria as judged by the lack of growth in Nu-3 treated tubes.

Table 3. Bactericidal activity of Nu-3 against bacteria established in biofilm

Bacterial Strain	Designation	Assessment	Treatment			
					Nu-3 Exposure (10mg/ml)	
			Media	Control	10min	30min
Klebsiella pneumoniae Kp-S1	Clinical	Growth	N.G.	1.1×10^9 cfu/ml	N.G.	N.G.
		Biofilm				
Acinetobacter baumannii	BAA-1605	Growth	N.G.	2×10^9 cfu/ml	N.G.	N.G.
		Biofilm				
Staphylococcus epidermidis	35984	Growth	N.G.	2.7×10^7 cfu/ml	N.G.	N.G.
		Biofilm				
Staphylococcus epidermidis	PCI1200	Growth	N.G.	5.2×10^8 cfu/ml	N.G.	N.G.
		Biofilm				
Staphylococcus aureus	NRS684	Growth	N.G.	4.3×10^8 cfu/ml	5×10^8 cfu/ml	8.6×10^8 cfu/ml
		Biofilm				
Staphylococcus aureus	NRS732	Growth	N.G	7.6×10^8 cfu/ml	N.G	N.G
		Biofilm				

Assessment of the rate of kill of Nu-3 in 0.85% normal saline:

Bacteria in the log phase of growth were collected by centrifugation, re-suspended in sterile saline (0.85% NaCl) at 1 x 10^5 CFU/ml. Nu-3 in sterile saline was added to a final concentration of 0.2 to 10mg/ml to the bacteria and the bacteria/Nu-3 mixture incubated at room temperature. At time points ranging from 5 to 60 minutes, aliquots were removed, diluted and plated onto TS agar plates for quantitation of the number of colony forming units (CFU's). After 24h at 37°C, the number of CFU's were determined and the Nu-3 concentrations and exposure times resulting in 100% kill were determined (Table 4).

Table 4. Time and concentration dependent activity of Nu-3 against biofilm forming bacterial strains.

Bacterial Strain	Nu-3 Concentration			
	0.625 (mg/ml)	**1 (mg/ml)**	**5 (mg/ml)**	**10 (mg/ml)**
K. pneumoniae Sp1	15 min		5 min	
K. pneumoniae Sp5		15 min	5 min	
A. baumannii BAA-1605	30 min		10 min	5 min
S. aureus NRS684				60 min
S. aureus NRS732				60 min

Mouse Suture Wound Infection Model

Briefly, a 10cm length of suture was threaded onto a needle and soaked for 45 minutes in undiluted cultures of *S. aureus* cvcc2248 or *P. aeruginosa* cvcc 5668 at 35°C for 8 hours. The needle and sutures were removed, placed on filter paper, and dried at 4°C until implantation. A 1-cm long piece of suture was implanted under the skin in the shaved and washed mid-back of anesthetized animals using knots to secure it in place. An incision was made between the knots on top of the suture line with a scalpel. The animals were then divided into groups and treated as indicated at 4h and 8h post-surgery and then 3 times daily for 3 days with either placebo, Nu-3 or ciprofloxacin ointment. On Day 5 the animals were euthanized and a 1cm^2 area surrounding and including the wound was harvest for homogenization and CFU counts (Table 5). The initial inoculum (i.e. CFU of bacteria in 1 cm length of suture) was determined by vortexing 1-cm of suture in bacterial growth media and plating serial dilutions to obtain bacteria counts.

Table 5. Log reduction in bacterial cell counts in surgical wound model

Treatment	Mean Bacterial Count ± SD (Log10 CFU/Wound)	
	P. aeruginosa	***S. aureus***
Initial Inoculation	4.93 ± 0.33	4.79 ± 0.31
Untreated	7.52 ± 0.57	7.64 ± 0.58
Glycerin	7.34 ± 0.27	7.47 ± 0.34
5% Nu-3 in Saline	5.62 ± 0.61	5.23 ± 0.5
5% Nu-3 in Glycerin	5.07 ± 0.53	4.51 ± 0.55
Ciprofloxacin HCl Ointment	4.82 ± 0.58	5.63 ± 0.31

Mouse Superficial Skin Polymicrobial Infection Model

Briefly, mice are pre-treated with cyclophosphamide four day prior to initiating the infection to suppress the immune system. The mice are anesthetized and kept sedated while the fur on the back dorsal surface is shaved using electric clippers followed by 'wet shaving' with a disposable razor. The skin is then sterilized with a betadine wash followed by alcohol swab and abraded with a +50 grit emery board that results in a smooth, red, shiny appearance with no bleeding. A droplet of the bacterial inoculum is placed directly on the abraded skin that contains *Pseudomonas aeruginosa* UNT034-1 (ATCC 27853) 6.35 Log10 CFU/ mouse and *S. aureus* UNT006-4 (smith) 5.65 Log10 CFU/ mouse. The infected area is treated with 50 microliters of varying concentrations of bisphosphocin Nu-3, gentamycin, mupirocin, or normal saline twice daily for 3 days. The mice are then euthanized, the area of infected skin excised, and the CFU of both bacteria is determined by culture or serial diluted samples of homogenized tissue (Table 6). The data shows that the top 5% dose of bisphosphocin Nu-3 results in a 1 log reduction in the CFU count of both *S. aureus* and *P. aeruginosa*.

Table 6. Mouse Superficial Skin Polymicrobial Infection Bacterial Counts

Compound	Dose	*S. aureus*		*P. aeruginosa*	
		CFU	SD	CFU	SD
NU3	1.0 mg/mL	9.22	0.19	6.35	0.21
	10 mg/mL	8.78	0.24	6.81	0.23
	20 mg/mL	9.00	0.27	7.08	0.19
	50 mg/mL	7.61	1.35	5.65	1.16
Gentamicin	0.3%	4.40	0.85	5.17	0.87
Mupirocin	2.0%	6.32	1.01	6.95	0.51
Vehicle	saline	8.73	0.25	6.3	0.42
Infection Control	Day 4	8.72	0.52	6.68	0.69
	4 hr	5.40	0.45	4.33	0.83

Preclinical Toxicology

Summary of Preclinical Toxicology Data

Nu-3 is the first in a new class of broad spectrum antimicrobials, termed bisphosphocins, being developed to treat a wide range of serious bacterial infections. Chemically Nu-3 would be considered to be in the nucleotide analog class of drugs and the following set of preclinical studies was designed based on the preclinical and clinical data collected over the past 50 years on this class. The nucleotide analog class of drugs have a good safety profile even when administered long term at low doses and toxicities are generally associated with their interference of normal DNA/RNA synthesis.

Nu-3 or [redacted] is a thymidine analog with both the 3' and 5' sites blocked by butyl phosphate which prevents it from causing early chain termination by incorporation by the polymerase unlike most compounds in this class. Nu-3 is activated through an acidification step that results in an acidic or protonated compound that when dissolved in water has a pH between 2 and 2.5. The compound can be neutralized or unactivated by a strong base or buffer such as sodium bicarbonate. In addition, the molecule was designed to minimize the potential for toxic side effects by utilizing a thymidine core molecule, natural phosphate linkages, and even carbon butyl blocking groups, all of which are either found in the body and/or feed into natural recycling pathways.

A summary of the preclinical toxicology studies completed with Nu-3 is presented in Table 7. The data indicates that Nu-3 is safe and well-tolerated when administered systemically or

topically and no specific toxicities were identified that would be cause for concern for the proposed Phase I/IIa study in subjects with infected diabetic foot ulcers. In general, the data indicates that Nu-3 functions through a membrane depolarization mechanism of action that is highly specific for bacterial cell membranes.

Table 7. Summary of Nu-3 Preclinical Toxicology Data

Study	Test Article/Dose	Species	Lot #	Result
Genotoxicity				
Bacterial Reverse Mutation Assay (GLP)	Nu-3 ACT	*In vitro*	LWA-01R-14	Non-mutagenic
Bacterial Reverse Mutation Assay (GLP)	Nu-3 NEUT	*In vitro*	LWA-01R-14	Non-mutagenic
Systemic Toxicity Study				
IV Max Tolerated Dose (GLP)	0, 250, 500, 1000mg/kg	Rats	LWA-01-14	NOEL and NOEAL at 250mg/kg $LD_{50} \geq$ 500mg/kg
IV Max Tolerated Dose Study (Non-GLP)	0, 250, 500, 750, 1000mg/kg	Mice	LWA-01-14	$LD_{50} \geq$ 1000mg/kg
Oral Max Tolerated Dose Study (Non-GLP)	1000, 1256, 1580, 1988, 2500mg/kg	Mice	PN 1127	LD_{50} 2001mg/kg according to the Karber method
Acute Single IV Dose (Non-GLP)	50, 200mg/kg	Rat	PN 1127	No observable effect on blood chemistry (CBC, metabolic panel)
Local Tolerance Studies				
Primary Dermal Irritation (GLP)	10% Nu-3	Rabbit	LWA-01R-14	Not a primary irritant
MapTek EpiOcular™ Toxicity (GLP)	1%, 2.5%, 5% Nu-3	*In vitro*	LWA-01-14	1% - Non-irritating, Minimal; 2.5%, 5% - Moderate irritants; No significant IL1α stimulation for all doses
In vitro Sensitization (GLP)	10%, 6.8%, 4.6%, 3.2%, 2.2%, 1.5%, 1%, 0.68%	In vitro	LWA-01-14	4.6% Sensitizer, All other concentrations are Non-sensitizers
3 Day Ophthalmic Irritation Study (Non-GLP)	1%	Rat	PN 1127	No sign of irritation
28 Day Repeat Dermal Toxicity (Non-GLP)	10%	Rat	PN1127	No pathological changes in skin, no clinical observations

1.2.3 Clinical Trials of Bisphosphocin Nu-3

Bisphosphocin™ Nu-3 is a new chemical entity that has not been evaluated under a formal IND application in human clinical studies. The compound was initial developed as a homeopathic treatment for a variety of topical infections as it was considered to fall under the HPUS DNA guidelines[9]. As a homeopathic treatment, Nu-3 was used topically to treat a variety of infections including acne, urethritis, ear infections, diabetic foot infections, and certain fungal infections. The information collected from these homeopathic treatments revealed that Nu-3 applied topically in solution for up to 14 days was able to improve the severity of an infection based visual observation.

1.3 Rationale for Current Study

The proposed clinical study is designed to demonstrate the safety and tolerability of increasing doses of topically applied bisphosphocin™ Nu-3 on a chronic diabetic foot infection. In addition, the study is designed to collect data on the efficacy of Nu-3 as it relates to eradication of the infection and wound healing to enable planning of future trials. The 3 dosing levels and length of treatment were selected based on *in vitro*, *in vivo*, and toxicology studies of Nu-3 and its unique mechanism of action. While the current trial is considered a first-in-human trial, the design also has taken into consideration data collected from the development of the compound initially as a homeopathic treatment where the use as a topical solution was able to reduce the severity of several common topical infections. Recent studies have shown that a high percentage of chronic wounds have biofilm associated which interferes with wound healing. This trial is intended to evaluate the efficacy of an antimicrobial with known activity against biofilm encased bacteria to not only eliminate the infection but potentially improve wound healing. For this reason, bisphosphocin Nu-3 is being formulated in a normal saline solution so that a clearer picture can be gained of its effect on the wound without the interference of an ointment base. Bisphosphocins have been shown to effectively reduce the severity of the infection in preclinical animal model testing, but these models have limits as they do not accurately mimic the complex environment of a chronic wound infection and in many cases rely on immunosuppression to maintain the infection. This first in human study is designed to collect both safety and efficacy data so that the parameters such as dose, duration and frequency of administration can be refined for future more intensive studies. Based on the data collected from preclinical studies, it is anticipated that a therapeutic effect may be observed in the middle (1% w/v, or 10 mg/ml) and high dose (2% w/v, or 20 mg/ml) cohorts that could justify extending the treatment course an additional seven (7) days. An extension of the treatment duration to fourteen (14) days would only be initiated after five subjects in Cohorts 2 and 3 completed the seven (7) day treatment and approval was given by the SMC. The decision to extend treatment of individual subjects showing a clinical response would then be made by the principal investigator.

Potential Risks

This clinical trial is a first in human trial so there is a potential risk of an adverse reaction from the study drug. Bisphosphocin Nu-3, however would be considered a nucleotide analog which is a class of drugs with a strong safety record so the likelihood of this particular risk is considered

minimal. The major distinction between bisphosphocin Nu-3 and the nucleotide analog class are that both 3' and 5' sites are blocked and a novel acid activation step that is reversed when the compounds are exposed to buffers or base. Therefore, it is anticipated that any reaction would be localized since the sodium bicarbonate in the blood would neutralize the compound which is supported by inhibitory effect of blood in MIC assays. In addition to a reaction from the study drug, there is also the possibility that the drug is ineffective and the infection worsens which could necessitate more aggressive therapy or treatment.

Potential Benefits

The major benefit of the proposed study will be an improvement in the wound due to the elimination of the pathogenic bacteria that is inhibiting healing. Bisphosphocin™ Nu-3 is a new class of broad spectrum antimicrobial so it is unlikely that the pathogenic bacteria will be outside its spectrum of activity or resistant, which is a major cause of treatment failure in this indication. The dose levels have been selected so that two cohorts are likely to be receiving efficacious dose levels of drug with a satisfactory safety margin so that some improvement in wound appearance is anticipated in some subjects in these cohorts. In addition to directly eliminating the pathogenic bacteria associated with the chronic ulcer, it is anticipated that a second potential benefit would be the elimination of any biofilm encased bacteria that would then make the infection more susceptible to traditional therapy as a follow up therapy. Any of these outcomes would benefit the subject as it would reduce the risk of limb loss due to a worsening infection and the pain.

2 OBJECTIVES

Objective/Purpose: The primary objective of this study is to assess the safety and tolerability of Nu-3 when applied topically to a chronically infected diabetic ulcer.

A secondary objective of this study is to obtain preliminary data on the microbiological activity of Nu-3 as measured by clinical wound assessments and presence of pathogenic bacteria. Based on preclinical data, it is anticipated that improvement in the clinical wound assessment may be observed in the middle and high dose (1% and 2%) cohorts that may justify extending the treatment for an additional 7 days.

Statement of Hypothesis: Nu-3 is a broad spectrum antimicrobial with a unique mechanism of action that involves cell membrane depolarization and rapid bacterial cell death. As an antimicrobial it is particularly well suited for development as a topical for Diabetic Foot Infections for the following reasons:

1. These infections tend to be polymicrobial in nature, involve biofilm, and require an antibiotic(s) with a broad spectrum of activity.
2. Many of the bacteria in these infected wounds are resistant to one or more classes of antibiotics making treatment difficult
3. The directly bactericidal mode of action of Nu-3 should translate into an immediate reduction in the severity of the infections and offers new options in dosing.
4. Research has shown that many chronic infections are due to the formation of biofilm which is inherently resistant to treatment by traditional antibiotics but susceptible to treatment by bisphosphocins such as Nu-3

The proposed clinical trial design is aimed at defining the initial parameters for safety and tolerability in an infected wound based on the currently available preclinical data on Nu-3. The trial is focused on DFIs of mild to moderate severity and an area of 0.5 to 6 cm^2 as a population of subjects that will provide the data across a range of pathogens and ulcers of varying duration. In addition, the middle and high dose (1% and 2% Nu-3) cohorts were selected so that some clinical improvement in wound assessment be observed which will enable better planning of following clinical trials.

Route of Administration: Study medication will be applied topically directly to the infected ulcer

Dosage: The study includes a sequential ascending dose protocol with Cohort 1 at 0.1% w/v or 1mg/ml, Cohort 2 at 1% w/v or 10mg/ml, and Cohort 3 at 2% w/v or 20mg/ml. In addition, two subjects in each cohort will be randomized to placebo. The proposed doses and treatment duration were based on data from *in vitro* antimicrobial assays, preclinical animal efficacy models, and toxicology studies and were selected to demonstrate safety at a high dose that provides a significant therapeutic margin lethal to all tested bacteria and fungi to date while proven safe in animal toxicology studies.

Dosing Regime: To establish the initial safety and tolerability of Nu-3, a twice daily application of the compound was selected for this clinical trial based on preclinical studies on efficacy and toxicology. Subjects will be required to keep a daily log of when treatments are applied and contacted daily by a member of the study team.

Ulcer Imaging and Area Calculations: Photographs of the ulcer(s) will be taken at study visits using the Aranz Medical Silhouette™ system which allows capture of a color image and computer calculation of ulcer(s) area, depth, and volume with more consistent and greater accuracy than traditional methods.

Randomization: Eligible subjects will be randomly assigned in a 4:1 ratio to Nu-3 (n=8) or matching placebo (n=2) in each cohort.

Treatment Period: Day 1 (in clinic): Day 2 (end of SAD/Beginning of MAD) through Day 8 or Day 15 (Cohort 2 and 3)

Follow-Up Period: Day 15 or Day 22 (Cohort 2 and 3)

Selection of Study Population: Type I or II diabetes mellitus subjects with a chronic infected lower extremity ulcer defined as having a DUSS score of 0 to 3 and a DFI wound score 1 to 9 as this is the most appropriate population to assess the safety of a new drug aimed at treating infected wounds. The proposed inclusion criteria have been selected to define a subject population that will have a diverse spectrum of pathogens and range of chronic ulcers to ensure a proper assessment of safety and tolerability as well as data to better define follow on clinical studies. The DFI wound score range is intended to select subjects with mild to moderate infection which have the best potential to respond to the treatment. A radiograph of the affected area will be performed during the screening period to rule out the possibility that the ulcer is due to an underlying osteomyelitis. Preclinical studies have demonstrated the safety of Nu-3 on mammalian cells, intact and damaged skin, and when injected intravenously, therefore exposure of healthy subject to Nu-3 would provide little if any additional safety information.

3 STUDY DESIGN

Phase I/IIa, three cohort double-blind ascending dose with two dosing phases per cohort, study in diabetes mellitus subjects with a chronic infected diabetic ulcer defined as having a DUSS score between 0 and 3 and a DFI wound score between 1 and 9. The study proposes to use the same subject(s) in SAD Phase 1 and MAD Phase 2 of each cohort

The study is designed to conduct the cohorts sequentially with the completion of the first cohort before initiating the next dosing level. Prior to initiating treatment of the ulcer, subjects will be administered a skin sensitivity test by applying a single drop of Nu-3 or placebo to the forearm and visually monitoring the area for 10-15 minutes for any acute reaction such as reddening, swelling, or irritation. If the skin sensitivity test is negative the subject is cleared to begin Arm 1 of the treatment.

A subject who has cleared the skin sensitivity test will have a microbiological sample taken from the infected diabetic ulcer(s) prior to initiating treatment. In Phase 1, eligible subjects will be treated with a single application of Nu-3 or placebo in a 4 to 1 ratio to judge the initial safety of Nu-3 over a brief 15-30 minute interval and 24 hour interval. Bisphosphocin Nu-3 will be applied topically to the chronic infected ulcer (DUSS score: 1-3; DFI wound score: 1-9), and the area observed over a 15-30 minute time period and photographed. If no visible reaction is observed, the ulcer is covered with a bandage. The subject will be released with verbal instructions to leave the nonabrasive, hypoallergenic bandage on the wound, avoid getting the bandage wet, and return for a follow up visit within 24 ± 2h. At the follow up visit, the bandage will be removed, the ulcer visually examined, photographed using the Aranz Medical Silhouette™ system, and the subject cleared for the MAD Phase 2 based on the recommendation/decision of the PI and on the absence of any drug related SAEs.

In Phase 2, eligible subjects, which are those who have been approved by the PI after the Visit 2 examination, will be instructed in the proper application of bisphosphocin Nu-3 or placebo. In the clinic, the subjects will be observed applying the first dose to ensure compliance. The subject will then be given a 7-day supply of study drug, non-abrasive bandages, a home diary, written instructions for care and hygiene, and sent home to continue treatment. On Day 9 ± 2h or earlier in the case of any adverse events, subjects will return to the clinic for an examination, including visual evaluation of the ulcer, vital signs, adverse events, photo documentation using the Aranz Medical Silhouette™ system, collection of a sample for microbiological assessment, and concomitant medication use. A final follow up visit will be scheduled for Day 15 for a complete examination as described above. Based on the data collected from preclinical studies, it is anticipated that a therapeutic effect may be observed in the middle (1% w/v, or 10mg/ml) and high-dose (2% w/v, or 20mg/ml) cohorts that could justify extending the treatment course an additional seven (7) days. An extension of the treatment duration to fourteen (14) days would only be initiated after five subjects in Cohorts 2 and 3 completed the seven (7) day treatment and approval was given by the SMC. The decision to extend treatment of individual subjects showing a clinical response would then be made by the principal investigator.

SUBJECT POPULATION

3.1 Selection and Exclusion of Subjects

Subjects, greater than 18 years of age, will be recruited from the area surrounding the Phase I/IIa Clinical Trial Centers as well as existing subjects of the center. Strategies for recruiting subjects will include web based advertising and social media sites, or traditional advertising in local newspapers all using IRB-approved materials. It is anticipated that 50-60 subject will be screened to identify the 30 subject needed for the proposed study. Subjects will be allowed as much time as necessary to review and give informed signed prior to any study related procedures being performed.

3.2 Inclusion Criteria

1. Non-hospitalized ambulatory subjects suffering from Diabetes mellitus, Type I or II
2. Male or Female older than 18yrs
3. Diabetic foot ulcer(s) with a DUSS Score of 0 to 3
4. A radiograph, MRI, and/or CAT scan evaluation within the last seven (7) days to determine the ulcer is not caused by an osteomyelitic bone infection
5. Infection as defined by the IDSA as ≥2 classic findings of inflammation or purulence
6. Ulcerated area(s) of not more than two (2) ulcers between 0.5 to 6 cm^2 as calculated by the Aranz Medical Silhouette™ system
7. Infection must be localized to the area of the ulcer and defined as mild (superficial and limited in size and depth) with a DFI Wound Score between 1 and 9
8. Subject must agree to be treated as an outpatient, follow all protocol procedures, return for all schedule visits, and provide informed consent.
9. Any female of child bearing age must consent to use medically acceptable birth control for the duration of the study.

3.3 Exclusion Criteria

1. A DUSS Score above 3
2. Any ulcer caused by an underlying osteomyelitic bone infection
3. Assessed with a Moderate to Severe Infection, including abscesses, extensive gangrene, symptoms of systemic infection, or a limb threatening infection. DFI Wound Score above 9.
4. An ulcer area(s) greater than 6 cm^2 or more than two (2) ulcers.
5. Any subject that has received systemic or topical antibiotics within the last seven (7) days
6. Any subject on topical antimicrobial treatment for their infected diabetic foot ulcer whose ulcer is responding to treatment
7. Any subject that would be unable to follow the protocol procedures, safely monitor the infection status at home, and return for schedule visits
8. Positive pregnancy test at Screening or Visit 2

4 STUDY DRUGS

4.1 Randomization, Blinding, and Drug Product/Placebo Packaging and Labeling

The drug product kits will be labeled as shown in Table 8 to ensure blinding of both the Principal Investigator and Subject. The kits will contain a 0.5 oz dropper bottle containing either 15 ml of Bisphosphocin™ Nu-3 or Placebo packaged in a cardboard a carton with a foam insert for protection. Both the carton and dropper bottle will be labelled.

The randomization will consist of two materials that have been allocated within 3 Cohorts.

Material 1: Bisphosphocin™ Nu-3 at 0.1%, 1%, or 2% w/v

Material 2: Matching Placebo

Within each Cohort, a material blocking ratio of 4:1 has been utilized with three (3) blocks per cohort. All Cohorts utilize a different bottle number range with ranges set at Cohort 1 from 101 to 115, Cohort 2 from 201-215, and Cohort 3 from 301 to 315. All cohort will have a different starting Randomization Specification Seed value which will result in a different statistical outcome.

Table 8. Product Label Specifications

GC: X; LID: X; CARTON	
Protocol #:	LAI2014-1
Product:	Bisphosphocin Nu-3 topical antibiotic or placebo
Bottle Number:	####
Lot Number*:	14K###
Directions for Use:	As directed, apply twice daily to the affected area
Storage:	Room Temperature^
Caution:	New Drug-Limited by federal law to Investigational Use Keep out of reach of children
Manufacturer:	Lakewood Amedex Inc, Sarasota, FL (941)225-2515

*Lot number represents the group of kits being assembled for each cohort.

^Room temperature is defined according to the USP as the temperature that is prevailing in a work area.

4.2 Description and Handling of Bisphosphocin Nu-3

Nu-3 or [redacted] is a thymidine analog with both the 3' and 5' sites blocked by butyl phosphate which prevents it from causing early chain termination by incorporation by the polymerase unlike most compounds in this class. Nu-3 is activated through an acidification step that results in an acidic or protonated compound that when

dissolved in water has a pH between 2 and 2.5. The antibacterial activity can be neutralized by a strong base or buffer. Prior non-human investigations indicate that all doses being used in this study are likely to be safe, medical personnel and caregivers should use caution when handling the drug to avoid exposure of sub-MIC levels through inadvertent application to the skin and normal skin flora that could potentially lead to resistant strains.

4.3 Dosage and Administration of Bisphosphocin Nu-3 or Placebo

Bisphosphocin Nu-3 will be dosed at three different concentrations of 0.1% w/v (1mg/ml), 1% w/v (10mg/ml), and 2% w/v (20mg/ml) in 0.85% normal saline. Placebo will be 0.85% normal saline solution. The drug or placebo will be administered as a single dose and then twice daily for 7 days. The drug or placebo will be administered to the infected diabetic foot ulcer and surrounding intact skin at a dose of 1-3 drops from a dropper bottle per 1 cm^2 of ulcer area.

4.4 Prior and Concomitant Medications

Subjects are allowed to continue on any concomitant prescribed medication for medical conditions not related to the treatment of the infected diabetic ulcer. All systemic and topical antibiotics must be stopped seven (7) days prior to admission to the proposed study.

5 STUDY PROCEDURES

5.1 Subject Enrollment and Treatment Assignment

Screened subjects who sign the Informed Consent and meet all the inclusion criteria and none of the exclusion criteria will be considered eligible for study participation. Eligible subjects will be assigned to Cohort 1 until a total of 10 subjects are enrolled, followed by Cohort 2, and Cohort 3. There will be a minimum of 7 days between the last subject completing the Day 9 assessment from each cohort and the first subject enrolled into the next cohort. All subjects who are eligible for enrollment will be randomly assigned in a 4:1 ratio to NU-3 (n=8) or matching placebo (n=2) in each cohort. The dose escalation will proceed following the Safety Monitoring Committee recommendation/approval.

5.2 Pre-treatment Assessments

The schedule of evaluations and procedures that must be performed at specific time points is described in the following sections. The Time and Event Schedule (Appendix B) summarizes the frequency and timing of various safety evaluations and clinical microbiological sampling. As soon as a subject is considered to be a potential subject for this study and prior to any other study procedures, the nature of the study will be explained to him/her by the study investigator or designee and the potential subject will be asked to provide written informed consent. Informed consent must be obtained prior to any study procedures being conducted.

5.2.1 Screening Visits

Screening Visit/Visit 1

Subjects diagnosed with diabetes mellitus and suffering from a chronic infected diabetic foot ulcer will be evaluated for entry criteria during a screening period conducted within the 30 days prior to bisphosphocin Nu-3 or placebo treatment. After informed consent has been obtained, the following procedures will be completed for each subject prior to inclusion in the study.

- Medical History
- Physical Examine with vital signs
- Concomitant medications
- Urine Pregnancy tests (HCG), if applicable
- Drug and alcohol blood toxicity screen
- Draw blood for CBC and CMP
- Review of shoe gear worn
- Radiograph, MRI, and/or CAT scan evaluations of the infected ulcer within the last seven (7) days to confirm the absence of osteomyelitis
- Scoring of the diabetic ulcer using the DUSS Scoring system

- Rating of the infection using the DFI Wound Scoring system
- Examination of Chronic Ulcer(s)
- Photo Documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ as described in Appendix C.

Diabetic Ulcer Wound Scoring System (DUSS)

Parameter	Score	
Palpable Pedal Pulses	Presence = 0	Absence = 1
Probing to Bone	No = 0	Yes = 1
Location of Ulcer	Toe = 0	Foot = 1
Number of Ulcerations	Single = 0	Multiple = 1
Score Range	0	4

Diabetic Foot Ulcer Wound Infection Score

Parameter	Wound Infection Score			
	0	1	2	3
Purulent discharge	Absent			Present
Nonpurulant drainage (serious, sanguinous)	Absent	Mild		
Erythema	None	Mild pink, barely perceptible	Moderate pale red, defined edges	Severe red to dark red
Induration	None	Mild	Moderate	Severe
Tenderness (sign)	None	Mild	Moderate	Severe
Pain (symptoms)	None	Mild	Moderate	Severe
Local Warmth (relative to uninfected contralateral foot)	Same	Mildly increased	Moderately increased	Severely increased

5.3 Enrollment/Baseline in Single Ascending Dose Arm (SAD)

Treatment Visit/Visit 2

- A urine pregnancy test must be performed prior to starting Nu-3 treatment in all women of child-bearing potential and a negative result must be documented.
- A urine drug and alcohol toxicology screen must be performed before starting Nu-3 treatment for each subject and a negative result must be documented.
- Draw blood for CBC and CMP

- Study subject will be assigned to the open cohort and treatment number per standard procedure. The treatment number will correspond with the dropper bottle number that will be used for treatment.
- A skin sensitivity test will be performed prior to initiating dosing by applying a single drop of Bisphosphocin Nu-3/Placebo to the subject's forearm and observing the area over a 10-15 minute interval for any reddening, swelling, or irritation. A negative result must be documented before initiating treatment of the ulcerated area.
- Before the treatment begins, the following data/samples will be collected and procedures completed:
  - Concomitant medications
  - Vital signs
  - Debridement, if necessary
  - Collection of sample from the ulcer for microbiological assessment
- Study Treatment for SAD Arm will be applied topically to the chronic ulcer and the area observed over a fifteen minute period for any acute reactions prior to being covered by a non-abrasive hypoallergenic bandage. The Nu-3 or placebo solution will be applied from a pre-packaged dropper bottle at a rate of 1-3 drops per 1 cm^2 of ulcer making sure to cover the entire ulcer as well as up to 0.5 cm of surrounding tissue.
- One hour after the treatment the following data will be collected:
  - Vital signs
  - Signs and symptoms of adverse events (e.g. redness of the ulcer, swelling, fever, chills)
- One hour post-treatment the study subject will be allowed to leave the clinic and instructed to call or return to the office if any of the following occur:
  - Increased pain or swelling of the infected ulcer or surrounding area.
  - Any observed increase in redness or swelling of the ulcer.

5.4 Follow-up Single Ascending Dose Arm

Post Treatment Visits/Visit Day 3 (24+/-2 h from Visit 2)

Study subjects will return to the clinical site for a follow-up evaluation 24±2h post treatment prior to starting the multiple ascending dose arm (MAD). At this follow-up visit, the subject will undergo the following procedures and evaluations to determine if they can be cleared for enrollment in the multiple ascending dose arm (MAD).

- Vital sign measurements including, weight, temperature, resting blood pressure, heart rate, and respiratory rate.
- Assessment of adverse events since the previous study visit
- Visual examination of the ulcer

- Photo documentation and calculation of ulcer area and depth using the Aranz Medical Silhouette™ system

5.5 Enrollment/Baseline in Multiple Ascending Dose (MAD) Arm

Treatment Visit/Visit 3

- Subjects will be instructed on the proper procedure for applying study treatment for the MAD Arm and observed performing the first application to the chronic ulcer and applying the non-abrasive hypoallergenic bandage. The Nu-3 or placebo solution will be applied from a pre-packaged dropper bottle at a rate of 1-3 drops per 1 cm^2 of ulcer making sure to cover the entire ulcer as well as up to 0.5cm of surrounding tissue. The subject will be provided the Subject Treatment Log in Appendix D.
- The study subject will be discharged from Visit 3 with a seven-day supply of study medication and supplies. The study subject will be instructed to continue treatment until the night before their next Visit.
- The study subject will be allowed to leave the clinic and instructed to call or return to the clinic if any of the following occur:
  - Increased pain or swelling of the infected ulcer or surrounding area.
  - Any observed increase in redness or swelling of the ulcer.
- Subjects will be provided a Subject Home Treatment Log (Appendix B) and instructed in the proper method of documenting the timing of treatments and recording of any observations

.

Follow-Up 7 Day Multiple Ascending Dose Arm Visit/Visit 4 (Day 9+1)

The study subject will return for a follow-up evaluation to the clinical site on Day 8 (or the next day) following the final MAD treatment. At this follow-up visit, the subject will undergo the following procedures and evaluations.

- Vital sign measurements including weight, temperature, resting blood pressure, heart rate, and respiratory rate.
- Radiograph, MRI, and/or CAT evaluations of the infected ulcer to rule out the development of osteomyelitis, if determined by the Principal Investigator to be necessary
- Concomitant medication use
- Draw blood for CBC and CMP.
- Assessment of adverse events and interim history since the previous study visit
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using both the DUSS and DFI Wound Scoring Systems
- Photo documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ system
- Collection of a sample from the ulcer for microbiological assessment

- Subjects will be given a 7 day supply of non-abrasive bandage and written instructions on the proper care and hygiene to include keeping the ulcer clean and bandage dry until the Day 14 follow up visit. In addition, subjects will be told to call if there is any worsening of the ulcer with regard to pain, infection, or swelling.
- In Cohorts 2 and 3, if after review of the safety data of the first 5 subjects, the SMC finds no safety concerns with the treatment, approval may be given by the PI to extend treatment an additional seven (7) days for those subjects whom are showing a clinical benefit in their judgment. Subject selected for continued treatment will be scheduled to return for Visit 5 on Day 16+1 for assessment.
- Subjects not selected to continue treatment will be given a 7 day supply of non-abrasive bandage and given written instructions on proper care and hygiene which includes keeping the ulcer clean and bandaged dry until the Day 15 Final Study Visit. In addition, subjects will be told to call if there is any worsening of the ulcer with regard to pain, infection, or swelling.

Follow-Up 14 Day Multiple Ascending Dose Arm Visit/Visit 5 (Day 16+1)

Study subject will return for a follow-up evaluation to the clinical site on Day 15 or the next day following the final MAD treatment. At this follow-up visit, subject will undergo the following procedures and evaluations.

- Vital sign measurements including weight, temperature, resting blood pressure, heart rate, and respiratory rate.
- Radiograph, MRI, and/or CAT evaluations of the infected ulcer to rule out the development of osteomyelitis, if determined by the Principal Investigator to be necessary
- Concomitant medication use
- Draw blood for CBC and CMP.
- Assessment of adverse events and interim history since the previous study visit
- Visual examination of the ulcer
- Scoring of the Ulcer(s) using both the DUSS and DFI Wound Scoring Systems
- Photo documentation and Calculation of the Ulcer Area and Depth using the Aranz Medical Silhouette™ system
- Collection of a sample from the ulcer for microbiological assessment
- Subjects will be given a 7 day supply of non-abrasive bandage and given written instructions on proper care and hygiene which includes keeping the ulcer clean and bandaged dry until the Day 22 Final Study Visit. In addition, subjects will be told to call if there is any worsening of the ulcer with regard to pain, infection, or swelling.

5.6 Final Study Visit – Visit 5 (7-Day MAD) or Visit 6 (14-Day MAD)

Post Treatment Final Visit (Day 15 or 22 ± 1)

All study subjects will have the following procedures performed at their final visit (Day 15 or 22 ± 2)

- Vital sign measurements including weight, temperature, resting blood pressure, heart rate and respiratory rate
- Physical examinations
- Visual examination of the chronic ulcer
- Photo documentation and calculation of ulcer area and depth using the Aranz Medical Silhouette™ System
- Assessment of adverse events and interim history since the previous study visit
- Collection of a sample from the ulcer for microbiological evaluation

5.7 Early Termination Visit

If a subject withdraws prior to completing the study, the reason for withdrawal will be documented on source documentation and in the CRF. If a subject withdraws early due to an adverse event, he/she will be followed until resolution/stabilization of the adverse event.

If a subject prematurely withdraws from the study they will be asked to complete the study procedures and evaluations performed in the final study visit at the time of withdrawal from the study (i.e. Study subject withdraws at the time of visit and consents to having procedures/evaluations done):

- Vital sign measurements including weight, temperature, resting blood pressure, heart rate and respiratory rate
- Physical examinations
- Visual examination of the chronic ulcer
- Photo documentation and calculation of ulcer area and depth using the Aranz Medical Silhouette™ system
- Concomitant medication use
- Assessment of adverse events and interim history since the previous study visit
- Collection of a sample from the ulcer for microbiological evaluation

Administrative withdrawals will be replaced to ensure that each cohort includes a total of 10 subjects who have completed the entire treatment. The study sponsor has designated the following person to be in charge of the shipping of study drug as they will be unblinded and able to match the proper replacement kit.

[redacted]

Subjects withdrawn from the study due to an AE/SAE related to the study drug will not be replaced.

5.8 Unscheduled Visit

Unscheduled visits will be documented using the CRFs used for the Day 1 follow-up visit determined to be required by the PI, except in the case of photo documentation which must be performed. If the unscheduled visit occurs as a result of an event, additional testing consistent with the event will be performed.

6 ADVERSE EVENTS AND TOXICITY MANAGEMENT

6.1 Specification of Safety Parameters

The primary endpoint safety variables will be the incidence of localized swelling surrounding the ulcer, development of a rash, joint pain, and any adverse changes in the appearance of the ulcer.

The Safety Monitoring Committee (SMC) will receive safety reports outlining the number of subjects enrolled in each cohort to date, summary of the safety data Day 0 – 15 for the most recent cohort, adverse events, and serious adverse events listed by grade as well as all laboratory tests data. These reports will present the data blinded and will be unblinded only if necessary to determine the cause of an AE or SAE at the request of the SMC. In addition, safety data from completed cohorts will be summarized in the same manner and included in the report. All information on adverse events will be provided in a table.

6.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

6.2.1 Adverse Events

Adverse Event: According to the ICH E6 guidelines, an AE is defined as any untoward medical occurrence in a subject/subjects in a clinical study regardless of its relationship to the administration of the study drug. Therefore, an AE is any unintended, unfavorable, or unexpected sign, laboratory test, symptom, reaction, or disease temporarily associated with a clinical subject/subjects during the duration of the study. The occurrence of an AE may come to the attention of study personnel at a study visit and during interviews of subjects seeking medical care, or upon reviews conducted by study monitors. During all visits and interviews after administration of the study drug, clinical staff will inquire about any anticipated adverse events or symptoms using standardized questions. The data will be recorded on the appropriate CRFs,

All AEs whether local or systemic reactions not meeting the criteria for “serious adverse events” should be documented on the appropriate CRF. The information to be recorded should include a description of the event, time of onset, clinician’s assessment of the severity, relationship to the study drug (assessment to be conducted only by those with the qualified training and authority to make a diagnosis and include the CMO, MD, PA, Nurse Practitioner, DO, DPM in podiatry and DDS), and time to resolution or stabilization. All AEs that occur during the study regardless of their association with the study drug must be documented appropriately and followed to adequate resolution.

Any AE (i.e. a new event or an exacerbation of a pre-existing condition) with an onset date after the screening visit up to the last day on study (including follow-up), should be recorded as an AE on the appropriate CRF page(s).

An AE does not include:

- Medical or surgical procedures) e.g. surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an adverse event.
- Pre-existing diseases or conditions or laboratory abnormalities present or detected prior to the screening visit, that do not worsen.
- Situations where an untoward medical occurrence has not occurred (e.g. hospitalization for elective surgery, social and/or convenience admissions).
- Overdose of either study drug or concomitant medication without any signs or symptoms unless the subject is hospitalized for observation.
- Events related to the underlying condition under study should not be reported as adverse events unless they are considered to be study related.

Severity of Event: All AEs will be assessed by the investigator and recorded on the appropriate CRF page, including the date of onset and resolution, severity, relationship to study drug or study procedures, outcome and action taken with study medication. The clinician will assess all AEs using a protocol defined grading system as follows.

Scale	Definition
Grade 1	No intervention required because the event is mild or asymptomatic. A clinical or diagnostic observation.
Grade 2	Minimal to moderate intervention and/or localized event that requires non-invasive treatment
Grade 3	A debilitating or incapacitating condition, severe or medically significant that is not life threatening that requires hospitalization or prolonged treatment
Grade 4	Requiring urgent intervention because the event is life threatening
Grade 5	Death of a subject due to an adverse event.

Relationship to Study Products:

6.2.2 Reactogenicity

Adverse events associated with the study product may include vesicle (blistering) development, burning sensation at the ulcer site (may be masked by diabetic neuropathy), increase in local temperature, and expansion of necrotic tissue surrounding the ulcer.

6.2.3 Serious Adverse Events

A **serious adverse event** (SAE) is defined as follows:

- Any adverse drug experience occurring at any dose that results in any of the following outcomes:
- Death;
- Life-threatening situation (subject is at **immediate** risk of death);
- In-patient hospitalization or prolongation of existing hospitalization (excluding those for study therapy or placement of an indwelling catheter, unless associated with other serious events);
- Persistent or significant disability/incapacity;
- Congenital anomaly/birth defect in the offspring of a subject who received study drug;
- Other: medically significant events that may not result in death, be immediately life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, they jeopardize the Subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of such events are:

- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias or convulsions that do not result in hospitalization
- Development of drug dependency or drug abuse

Clarification of Serious Adverse Events

- Death is an outcome of an adverse event, and not an adverse event in itself. In reports of death due to "Disease Progression", where no other information is provided, the death will be assumed to have resulted from progression of the disease being treated with the study drug(s).
- All deaths, regardless of cause or relationship, must be reported for subjects on study and for deaths occurring within 30 days of last study drug dose or within 30 days of last study evaluation, whichever is longer.
- "Occurring at any dose" does not imply that the subject is receiving study drug at the time of the event. Dosing may have been given as treatment cycles or interrupted temporarily prior to the onset of the SAE, but may have contributed to the event.
- "Life-threatening" means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death, if it had occurred with greater severity.
- Complications that occur during hospitalizations are AEs. If a complication prolongs hospitalization, it is an SAE.

- "In-patient hospitalization" means that the subject has been formally admitted to a hospital for medical reasons, for any length of time. This may or may not be overnight. It does not include presentation and care within an emergency department.
- The investigator should attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. In such cases the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms.

A distinction should be drawn between serious and severe AEs. An AE that is assessed as Grade 4 (potentially life-threatening) should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 4. An event is defined as "serious" when it meets one of the pre-defined outcomes as described above in Section 6.2.3.

6.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Laboratory abnormalities are usually not recorded as adverse events or serious adverse events unless they are associated with clinical signs and/or symptoms. However, laboratory abnormalities (e.g. clinical chemistry, hematology, urinalysis, etc.) independent from the underlying medical condition, that require medical or surgical intervention, or lead to study drug interruption or discontinuation must be recorded as an AE, as well as an SAE, if applicable. In addition, laboratory or other abnormal assessments (e.g. electrocardiogram, X-rays, vital signs) that are associated with sign and/or symptoms must be recorded as an AE or SAE if they meet the definition of an adverse event (or serious adverse event) as described in the protocol. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis.

Severity should be recorded and graded according to Common Terminology Criteria for Adverse Events v4.0 (CTCAE). For adverse events associated with laboratory abnormalities, the event should be graded based on the clinical severity in the context of the underlying conditions, which may or may not be in agreement with the grading of the laboratory abnormality.

6.3 Serious Advent Event Reporting Procedures

The study sponsor is required to expedite to worldwide regulatory authorities reports of Serious Adverse Drug Reactions or Suspected Unexpected Serious Adverse Reactions (SUSARs); therefore, the study sponsor or its delegate must be notified immediately regarding the occurrence of any SAE that occurs after the subject consents to participate in the study, including SAEs resulting from protocol-associated procedures performed from screening onwards. The procedures for reporting all SAEs, regardless of causal relationship, are as follows:

- Record the SAE on the AE CRF and complete the "Serious Adverse Event Report" form.
- E-mail or fax the SAE form to the attention of the Drug Safety Manager within 24 hours of the investigator's knowledge of the event. Contact information is as follows:

Lakewood-Amedex Inc. Drug Safety Manager

Medical Monitor:

- For fatal or life-threatening events, also e-mail or fax copies of hospital case reports, autopsy reports, and other documents when requested and applicable. Transmission of such documents should occur with Personal Subject Details de-identified, without losing the traceability of a document to the Subject Identifiers.

- The study sponsor or its delegate may request additional information from the investigator to ensure the timely completion of accurate safety reports.

The investigator must take all therapeutic measures necessary for resolution of the SAE. Any medications necessary for treatment of the SAE must be recorded onto the concomitant medication section of the subject's CRF and the event description section of the SAE form.

Follow-up of adverse events will continue through the last day on study (including the follow-up off-study medication period of the study) and/or until the investigator and/or study sponsor determine that the subject's condition is stable. The study sponsor or its delegate may request that certain adverse events be followed until resolution.

6.3.1 Reporting Pregnancy

The risks of treatment with bisphosphocin during pregnancy has not been evaluated.

The subject must be instructed to discontinue all study drugs and inform the investigator **immediately** if she becomes pregnant during the study.

The investigator should report all pregnancies to Lakewood-Amedex within 24 hours of becoming aware of the pregnancy. The investigator should counsel the subject regarding the possible effects of prior study drug exposure on the fetus and the need to inform the study site of the outcome of the pregnancy.

Any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or an SAE.

A spontaneous abortion is always considered to be a SAE and will be reported as described in the adverse and Serious Adverse Events section. Furthermore, any SAE occurring as an adverse pregnancy outcome post-study must be reported to Lakewood-Amedex.

Additionally, all pregnancies that occur during the study should be reported using the Pregnancy Report CRF page. Monitoring of the subject should continue until the conclusion of the pregnancy. The outcome should be reported to Lakewood-Amedex using the Pregnancy Outcome CRF page(s). If the end of the pregnancy occurs after the study has been complete the outcome should be reported directly to Lakewood-Amedex. Pregnancies that occur after the subject has discontinued study drugs do not require monitoring.

6.4 Safety Oversight

6.4.1 Independent Safety Monitor Committee

Clinical Response Evaluations:

The clinical outcome will be based on the Principle Investigator's judgment as to the improvement of the wound status from Visit 1 to Visit 4 (7-Day MAD) and Visits 5 and 6. The PI will score the ulcer(s) using both the DUSS and DFI Wound Scoring Systems.

Diabetic Ulcer Wound Scoring System (DUSS)

Parameter	Score	
Palpable Pedal Pulses	Presence = 0	Absence = 1
Probing to Bone	No = 0	Yes = 1
Location of Ulcer	Toe = 0	Foot = 1
Number of Ulcerations	Single = 0	Multiple = 1
Score Range	0	4

Diabetic Foot Ulcer Wound Infection Score

Parameter	Wound Infection Score			
	0	1	2	3
Purulent discharge	Absent			Present
Nonpurulant drainage (serious, sanguinous)	Absent	Mild		
Erythema	None	Mild pink, barely perceptible	Moderate pale red, defined edges	Severe red to dark red
Induration	None	Mild	Moderate	Severe
Tenderness (sign)	None	Mild	Moderate	Severe
Pain (symptoms)	None	Mild	Moderate	Severe
Local Warmth (relative to uninfected contralateral foot)	Same	Mildly increased	Moderately increased	Severely increased

An improvement in the DFI Wound Score will be used to indicate a clinical improvement of the wound and a change in the DUSS Score wound be used to indicate the healing of a small ulceration.

Microbiological Response Evaluations:

This assessment will be conducted on samples collected by the investigator on Visit 2, Visit 4, Visit 5, and Visit 6.of the study which will be cultured to determine: microbial load and identity of the infected pathogens or wound flora. The microbiological response will be determined by a comparison of the results pre- and post-treatment.

SMC Safety Reports:

Safety reports will be written by the SMC three times during the course of the study. The first will follow the first interim analysis (once the first SAD/MAD cohort 1 reaches Day 9), the second report will follow the second interim analysis (once the second SAD/MAD cohort 2 reaches Day 9), and the third report will follow the third interim analysis (once the third SAD/MAD cohort 3 reaches Day 9). The study reports will be presented to the Principal Investigator at each clinical site and Lakewood-Amedex Inc.

6.4.2 Study Termination

This study may be terminated at any time by Lakewood-Amedex Inc. The PI and SMC may provide Lakewood-Amedex with recommendations regarding termination of the trial, however, Lakewood-Amedex ultimately makes the final decision. Reasons for terminating the study include:

- If a serious or unexpected adverse event occurs and the event is judged to be probably or definitively related to exposure to Nu-3, the study will be immediately suspended by the Principal Investigator pending review of all appropriate safety data. The event will be reported to the SMC, IRB, and Sponsor Medical Monitor within 24 hours or notification of its occurrence. No additional subjects will receive Nu-3 until a joint decision is reached between the Sponsor, PI, and SMC as to whether further doses can be given or the trial is terminated. Subjects currently in a MAD Arm will be contacted to ascertain treatment status and if any AEs have occurred. Absent any findings, subjects in the ongoing MAD arms will be allowed to complete treatment.
- Safety Monitoring Committee terminates the study based on interim safety review
- In the event of a clinical hold being placed on the study following a report of an AE to the FDA
- At the discretion of Lakewood-Amedex.

6.4.3 Removal of Subjects from the Trial or Study Drug

Subjects will be free to withdraw at any time for any reason, or they may be withdrawn if necessary, to protect their health and safety or the integrity of the study data. A subject will be removed from participation in the study if any of the following occur:

- Any clinical adverse event (AE), laboratory abnormality, intercurrent illness, or other medical condition or situation arises such that in the judgment of the PI continued participation in the study would not be in the best interest of the subject
- A protocol violation/deviation occurs that might compromise the integrity of the data, compliance, or subject safety
- Informed consent is withdrawn
- Subject is lost to follow-up

6.4.4 Handling Withdrawals

If a subject is withdrawn prematurely from the study after receiving Nu-3, the reason for withdrawal is to be documented in the source document and in the CRF. If the withdrawal of a subject is due to an AE, the subject will be followed until resolution/stabilization of the adverse event.

In the case of a subject prematurely withdrawing from the study, the following procedures and/or evaluations will be performed if possible at the time of withdrawal or as soon thereafter as possible (i.e. study subject withdraws at time of visit and consents to the procedures or consents to come in for a visit and the procedures)

- Photo documentation of ulcer site using the Aranz Medical Silhouette™ system.
- Collection of a sample for microbiological assessment

- Assessment of adverse events

In the event a subject is lost to follow-up, the site staff must make reasonable attempts to contact the subject. A minimum of two documented phone calls followed by a certified mailed letter is considered reasonable. The measures taken to follow up must be documented.

Administrative withdrawals will be replaced in order to achieve the objective of 10 subjects per cohort who receive a complete treatment regime. Subjects withdrawn from the study due to an AE/SAE related to the study drug will not be replaced.

7 STATISTICAL CONSIDERATIONS

7.1 Study Hypothesis

Study Hypothesis – Bisphosphocin Nu-3 solution applied topically to a chronic infected diabetic ulcer will be safe and well tolerated in subjects suffering from diabetes mellitus. In addition, the Nu-3 MAD Arm subjects may show improvement in the ulcer appearance, reduction in size, and improvement in both the DUSS score and DFI Wound Score.

The study hypothesis will be judged by the following two study objectives:

- Primary objective: To assess the safety and tolerability of escalating single and multiple doses of Nu-3, a novel antimicrobial of the bisphosphocin class, topically to an open wound/diabetic ulcer
- Secondary objectives: To assess clinical response of subjects of the ascending multiple dose arm of Nu-3 applied topically to an open wound/diabetic ulcer to determine clinical and microbiological response.

7.2 Sample Size Considerations

The number of treated subjects is based on the desire to gain preliminary safety, tolerability and efficacy information to support future work, while exposing a minimal number of subjects to the study procedures and medication. Inferential statistical testing is not the primary intent of the study, therefore, no formal sample size calculation was considered. Ten subjects per Cohort (1, 2, and 3), for a total not to exceed 30, is considered sufficient to show clinical significance for safety and preliminary efficacy analysis. The sample sizes for this study were set based on the trial design for dose escalation and safety evaluation requirements and not for statistical analysis.

7.3 Planned Interim Analysis

There will be an interim analysis to review safety data on each cohort of subjects when all members of a cohort complete Visit 4 (7-Day MAD Follow Up). In addition, an interim analysis will be conducted after the first 5 subjects of Cohort 2 and 3 complete Visit 4 (7-Day MAD Follow Up). The interim analysis will be performed by the Safety Monitoring Committee (SMC) which will be composed of three physicians and may include one infectious disease specialist, one diabetologist, and one orthopedic surgeon. The SMC will communicate by email or teleconference on at least 3 occasions during the study and more frequently if necessary.

No formal statistical interim analyses are planned for this Phase I/IIa study since it is primarily focused on evaluating safety and tolerability. Interim safety data on each cohort will be provided to the SMC when all members of a cohort have reached Day 3. The SMC will decide if the study can proceed to the next dose cohort (majority vote). The SMC will also determine if the study may proceed after receiving a report about a study related Grade 3 or 4 adverse event

(majority vote). The Chairman of the SMC will share their findings and recommendations with Lakewood Amedex and Principal Investigators.

7.3.1 Safety Review

Subjects will be monitored closely for the occurrence of study related adverse events during their 24-hr admission in the SAD Arm and for up to 7 days following their last Nu-3 treatment in the study. If any grade 3 or 4 adverse events occur and the event is judged to be probably or definitely related to having received the Nu-3, the study will be immediately suspended by the Principal Investigator pending review of all safety data. Adverse events determined to be related to the study drug will be reported to the SMC, Lakewood-Amedex, and second PI within 24 hours of notification of its occurrence. No additional subjects will receive Nu-3 depending on the joint decision of the SMC, Principal Investigator, and Lakewood Amedex Project Coordinator and CMO as to whether further doses can be given or the entire study should be terminated. In addition, the SMC will review all interim safety data prior to the next cohort beginning treatment. At each review, the SMC will determine whether the study may proceed to the next dose cohort (majority vote).

7.4 Statistical Analysis Overview

This study is designed to evaluate the safety, tolerability, and preliminary efficacy of a minimum of three dose levels of Nu-3 relative to placebo. Data summaries will be presented in order to assess the safety of Nu-3 and each Nu-3 dose group will be compared with placebo to determine the presence or absence of a treatment effect. Evaluation across all dose levels of Nu-3 will be conducted to determine if there is evidence of a dose response to any treatment effect noted. All subjects treated with placebo from all cohorts will be combined for any analysis. Evaluations will also be conducted within each subject over time to assess the rate of change (if any) in study outcome measures over the full course of the study treatment and follow-up periods. Inferential statistical testing is not the primary interest of this study.

All subjects receiving any amount of study treatment will be evaluated for safety, tolerability, and efficacy. An “intent to treat” approach will be followed in all data summaries, using all available data in the summaries and analysis for an assessment of safety and tolerability while an “as treated” approach will be used for microbiological and clinical wound assessment data summaries and reports.

All descriptive summaries and analyses will be presented in tabular and/or graphical form from all subjects as appropriate. These summaries and analysis will be described in detail in the Statistical Analysis Plan (SAP) which will be produced prior to breaking the study blind. All statistical summaries and analysis will be conducted using SAS®.

8 RESPONSIBILITIES

8.1 Investigator Responsibilities

8.1.1 Good Clinical Practice

The investigator will ensure that this study is conducted in accordance with the principles of the "Declaration of Helsinki" (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), International Conference on Harmonization (ICH) guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study subject. For studies conducted under a United States IND, the investigator will ensure that the basic principles of "Good Clinical Practice," as outlined in 21 CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," 21 CFR, part 50, 1998, and 21 CFR, part 56, 1998, are adhered to.

Since this is a "covered" clinical trial, the investigator will ensure that 21 CFR, Part 54, 1998, is adhered to; a "covered" clinical trial is any "study of a drug or device in humans submitted in a marketing application or reclassification petition subject to this part that the applicant or FDA relies on to establish that the product is effective (including studies that show equivalence to an effective product) or that make a significant contribution to the demonstration of safety." This requires that investigators and all sub-investigators must provide documentation of their financial interest or arrangements with Lakewood-Amedex or proprietary interests in the drug being studied. This documentation must be provided before participation of the investigator and any sub-investigator. The investigator and sub-investigator agree to notify Lakewood-Amedex of any change reportable interests during the study and for one year following completion of the study. Study completion is defined as the date that the last subject has completed the protocol defined activities.

This study is also subject to and will be conducted in accordance with 21 CFR, part 320, 1993, "Retention of Bioavailability and Bioequivalence Testing Samples."

8.1.2 Institutional Review Board (IRB)/Independent Ethics Committee (IEC) Approval

This protocol and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) will be submitted by the investigator to an IRB in compliance with FDA regulations for IRBs (21CFR Part 56). Approval from the IRB must be obtained **before** starting the study and must be documented in a letter to the investigator specifying the protocol number, protocol version, protocol date, documents reviewed, and date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of IRB approval must also be submitted to the IRB for approval before implementation.

8.1.3 Informed Consent

The investigator is responsible for obtaining written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and before undertaking any study-related procedures. The investigator must utilize an IRB approved consent form for documenting written informed consent. Each informed consent will be appropriately signed and dated by the subject or the subject's legally authorized representative and the person obtaining consent. The information in the informed consent must comply with 21CFR Part 50, Subpart B. The elements of the informed consent must be consistent with the requirements of 21CFR Part 50.25.

8.1.4 Confidentiality

The investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, date of birth, and an identification code (i.e., not names) should be recorded on any form or biological sample submitted to the Sponsor, IRB, or laboratory. The investigator must keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the trial.

The investigator agrees that all information received from Lakewood-Amedex, including but not limited to the Investigator Brochure, this protocol, CRFs, the investigational new drug, and any other study information, remain the sole and exclusive property of Lakewood-Amedex during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from Lakewood-Amedex. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

8.1.5 Study Files and Retention of Records

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following two categories: (1) investigator's study file, and (2) subject clinical source documents.

The investigator's study file will contain the protocol/amendments, CRF and query forms, IRB and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

The required source data are listed in the Source Data verification Plan, and should include sequential notes containing at least the following information for each subject:

- subject identification (name, date of birth, gender);
- documentation that subject meets eligibility criteria, i.e., history, physical examination, and confirmation of diagnosis (to support inclusion and exclusion criteria);

- participation in trial (including trial number);
- trial discussed and date of informed consent;
- dates of all visits;
- documentation that protocol specific procedures were performed;
- results of efficacy parameters, as required by the protocol;
- start and end date (including dose regimen) of trial medication (preferably drug dispensing and return should be documented as well);
- record of all adverse events and other safety parameters (start and end date, and preferably including causality and intensity);
- concomitant medication (including start and end date, dose if relevant; dose changes should be motivated);
- date of trial completion and reason for early discontinuation, if applicable.

All clinical study documents must be retained by the investigator until at least 2 years after the last approval of a marketing application in an ICH region (i.e., United States, Europe, or Japan) and until there are no pending or contemplated marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if required by applicable regulatory requirements, by local regulations, or by an agreement with Lakewood-Amedex. The investigator must notify Lakewood-Amedex before destroying any clinical study records.

Should the investigator wish to assign the subject records to another party or move them to another location, Lakewood-Amedex should be notified in advance.

If the investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and Lakewood-Amedex to store these in sealed containers outside of the site so that they can be returned sealed to the investigator in case of a regulatory audit. When source documents are required for the continued care of the subject, appropriate copies should be made for storage outside of the site.

8.1.6 Case Report Forms

For each subject enrolled, a CRF must be completed and signed by the principal investigator or sub-investigator (as appropriate) within a reasonable time period after data collection. This also applies to records for those subjects who fail to complete the study (even during a pre-randomization screening period if a CRF was initiated). If a subject withdraws from the study, the reason must be noted on the CRF. If a subject is withdrawn from the study because of a treatment-limiting adverse event, thorough efforts should be made to clearly document the outcome.

8.1.7 Drug Accountability

The investigator or designee (i.e., pharmacist) is responsible for ensuring adequate accountability of all used and unused investigational medicinal product, placebos, and comparators. This includes acknowledgment of receipt of each shipment of study product (quantity and condition), subject dispensing records, and returned or destroyed study product. Dispensing records will document quantities received from the study sponsor and quantities dispensed to subjects, including kit number, date dispensed, subject identifier number, subject initials, and the initials of the person dispensing the medication.

At study initiation, the monitor will evaluate the site's standard operating procedure for investigational medicinal product disposal/destruction in order to ensure that it complies with Lakewood-Amedex requirements. Drug may be returned on an ongoing basis during the study, if appropriate. At the end of the study, following final drug inventory reconciliation by the monitor, the study site will ship any remaining investigational product (to include empty containers) to Xcelience Inc. for disposal.

All drug supplies and associated documentation will be periodically reviewed and verified by the study monitor over the course of the study.

8.1.8 Inspections

The investigator understands that source documents for this trial must be made available to appropriately qualified personnel from Lakewood-Amedex or its representatives, to IRBs or to regulatory authority or health authority inspectors.

8.1.9 Protocol Compliance

The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance maybe either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- Compliance with Protocol sections 4.5.1, 4.5.2, and 4.5.3
- Quality Assurance and Quality Control, section 5.1.1
- Noncompliance sections 5.20.1, and 5.20.2

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All significant deviations must be promptly reported to

Lakewood Amedex Inc. to the attention of [redacted] via fax. Reports can be sent to Lakewood -Amedex as specified below copying the CRA assigned to your site:

- Via the web-based PD forms submission
- FAX submission (1[redacted]): utilizing the Protocol Deviation form and the email/fax transmittal form, or
- E-mail submission ([redacted]) utilizing the Protocol Deviation form and the email/fax transmittal form.

All deviations from the protocol must be addressed in the study subject source documents. A completed copy of the LAI Protocol Deviation form must be maintained in the regulatory file, as well as in the subject's source document. Significant protocol deviations must be sent to the local IRB/EC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB/EC requirements.

8.2 Sponsor Responsibilities

8.2.1 Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by Lakewood-Amedex. All protocol modifications must be submitted to the IRB in accordance with local requirements. Approval must be obtained before changes can be implemented.

8.2.2 Study Report and Publications

A clinical study report will be prepared by Protocol Coordinator and provided to the regulatory agency(ies). Lakewood-Amedex will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). Note that an abbreviated report may be prepared in certain cases.

Lakewood-Amedex Inc. will have the sole decision on whether or not to publish the results of this research. The Company encourages its scientists and collaborators to published the results of any research sponsored by the Company but only after a review of the data by the Company to ensure that any discoveries or trade secrets are properly protected by issued, pending, or newly filed patents or patents applications.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials registration policy as a condition for publication. This policy requires that all clinical trials except Phase I studies be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. Lakewood-Amedex, Inc. will be responsible for registering the clinical trial with an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before subject enrollment.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subject to intervention or comparison groups to study the cause-and-effect relationship between medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g. Phase I trials) would be exempt from this policy.

8.3 Joint Investigator/Sponsor Responsibilities

8.3.1 Access to Information for Monitoring

In accordance with ICH Good Clinical Practice (ICH GCP) guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the CRFs for consistency.

The monitor is responsible for routine review of the CRFs at regular intervals throughout the study to verify adherence to the protocol and the completeness, consistency, and accuracy of the data being entered on them. The monitor should have access to any subject records needed to verify the entries on the CRFs. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

8.3.2 Access to Information for Auditing or Inspections

Representatives of regulatory authorities or of Lakewood-Amedex may conduct inspections or audits of the clinical study. If the investigator is notified of an inspection by a regulatory authority the investigator agrees to notify the Lakewood-Amedex medical monitor immediately. The investigator agrees to provide to representatives of a regulatory agency or Lakewood-Amedex access to records, facilities, and personnel for the effective conduct of any inspection or audit.

8.3.3 Study Discontinuation

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authority(ies), and IRB/IECs. In terminating the study, Lakewood-Amedex and the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

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10 APPENDICES

Appendix A. Project Timeline and Flow Chart

		Obtain Informed Consent		
		Screen Subject		
		Assign to Open Cohort		
SAD Arm 1: Cohort 1 – 0.1% or 1mg/ml (n=10) (2 Placebo; 8 Nu-3)				
Perform Informed Consent and Document Pregnancy and Drug Screen <24h Before Treatment Perform Skin Sensitization Test, Apply Treatment Follow up Visit 3				
PI Review at 24hr Examination No Safety Issues. Proceed to MAD Arm – Cohort 1		SAD Arm 1:Cohort 2 – 1% or 10mg/ml (n=10) (2 Placebo; 8 Nu-3)		
Instruct on Treatment Observe Subject Apply 1st Treatment Follow up Visit 5		Perform Informed Consent and Document Pregnancy and Drug Screen <24h Before Treatment Sensitization Test, Apply Treatment Follow up Visit 3		
SMC Review After All Subject Have Completed Cohort 1 No Safety Issues. Proceed to Next Cohort		PI Review at 24hr Examination No Safety Issues. Proceed to MAD Arm Cohort 2		SAD Arm 1: Cohort 3 – 2% or 20mg/ml (n=10) (2 Placebo; 8 Nu-3)
		Instruct on Treatment Observe Subject Apply 1st Treatment Follow up Visit 5 SMC Review after first 5 subjects completed If approval given by SMC extension of treatment to fourteen (14) days MAD at PI discretion Follow-up Visit 6		Perform Informed Consent and Document Pregnancy and Drug Screen <24h Before Treatment Review Informed Consent & Document Perform Skin Sensitization Test, Apply Treatment Follow up Day 2
		SMC Review After All Subject Have Completed Cohort 2		PI Review at 24hr Examination No Safety Issues. Proceed to MAD

		No Safety Issues. Proceed to Next Cohort		Arm 2 Cohort 3
				Instruct on Treatment Observe Subject Apply 1st Treatment Follow up Visit 5 SMC Review after first 5 subjects completed If approval given by SMC extension of treatment to fourteen (14) days MAD at PI discretion Follow-up Visit6

Appendix B: Schedule of Events

Event	Visit 1 Screening Visit	Visit 2 SAD Treatment	Visit 3[a]	Visit 4[b]	Visit 5[c]	Visit 6[d]	Early Termination (ET) Visit
Informed Consent Process	X						
Medical History	X						
Physical Exam	X				X	X	X
Vital Signs[1]	X	X[2]	X	X	X	X	X
CBC, CMP Blood Draw	X	X		X	X	X	X
Collect ulcer tissue sample for microbiological evaluation	X	X		X	X	X	X
Urine Pregnancy Test (HCG)[3]	X	X					
Drug and ETOH toxicity screen	X	X					
Visual Examination of Chronic Ulcer	X	X	X	X	X	X	X
Radiograph, MRI and/or CT evaluation of the infected ulcer[4]	X			X			
Photo Documentation and Calculation of target ulcer area and depth – ARANZ Silhouette™ Medical System	X	X	X	X	X	X	X
Score target ulcer using DUSS Scoring System	X	X		X	X	X	
Rating of the infected ulcer using DFI Wound Scoring system	X			X	X	X	
Skin Sensitivity Test		X					
Investigational Product		X	X	X[5]			
Concomitant Medication	X	X	X	X	X	X	X
Adverse Event	X	X[6]	X	X	X	X	X

Key

[a] Visit window 24 hours ± 2 hours post Visit 2

[b] Visit window 8 days + 1 day post Visit 2

[c] Visit window 14 days ± 1 day post Visit 2 for 7-Day MAD subjects or 15 days +1 day post Visit 2 for 14-Day MAD subjects

[d] Visit window 22 days ± 1 day post Visit 2 (Visit 6 only necessary for 14-day MAD subjects)

[1] Height and weight (screening, Day 1, follow-up visit and ET), temperature, resting blood pressure (BP), heart and respiration rate

[2] Resting BP, heart and respiration rate to be obtained prior to and 1 hour post SAD Treatment

[3] Urine pregnancy test for all female subjects of childbearing potential

[4] Radiography, MRI and/or CT evaluation required within 2 weeks of Visit 1 (Screening Visit), at all other visits only necessary if determined by PI

[5] Upon SMC review and safety approval of 1st five (5) subjects in Cohorts 2 and 3, Principal Investigator (PI) may elect to extend treatment to fourteen (14) days if in the opinion of the PI the subject is showing improvement at Visit 4.

[6] Signs and symptoms of adverse events to be obtained 1 hour post SAD Treatment

Appendix C: Use of the Aranz Medical Silhouette™ System for Photo documentation of the DFU and Calculation of Area and Depth, and Volume.

Capturing a Wound Image

1. Log in to Silhouette Connect and Select the Patient to be imaged.
2. Review the patient details and select Capture Images Button. The laser lines should come on from the Silhouette Star Camera.
3. To capture the wound image position the Silhouette Star Camera directly above the wound and move the camera up or down so that the laser lines form a star. The center of the laser line star should be over the deepest part of the wound.
4. Capture an image of the wound by pressing the bottom on the camera.
5. Repeat the process to capture multiple images.
6. All images will be uploaded to the SilhouetteConnect Database on the tablet.

Calculating the Ulcer Area, Volume, and Depth

1. Select the Ulcer image to be used for assessment on the tablet.
2. Enlarge the image so the perimeter of the ulcer is easily visible
3. Outline the perimeter of the ulcer by either clicking around the perimeter so that the entire ulcer is surrounded by dots or tracing the perimeter of the ulcer with a pointer or finger.
4. Clicking on the circular target to initiate calculation of the area, volume, and depth of the ulcer by the SilhouetteConnect software.
5. Click on the Notes(?) button to enter comments on the ulcer margins, appearance, and surrounding skin
6. Click Generate pdf report and Save to Store it to the database.
7. Click Send to export to the ClinicalInk Database.

Appendix D: Subject Home Treatment Log:

Subject ID No.:_____

Study Drug Number:_____

Issue Date:_____ **Return Date:**_____

Instructions: Subjects/caregivers should log both the date and approximate time of each treatment and the person administering the study drug should initial in the corresponding box. A space is also provided for the subject/caregiver to write any comments concerning the administration or observations of the ulcer.

Day	Treatment 1 Date/Time	Initials	Therapeutic Footwear Worn	Length of Time Footwear Worn	Treatment 2 Date/Time	Initials	Therapeutic Footwear Worn	Length of Time Footwear Worn	Comments
1			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.	
2			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.	
3			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.	
4			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.	
5			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.	
6			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.	
7			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.			☐ Yes ☐ No	☐1-4 hrs. ☐5-8 hrs. ☐9-12 hrs.	