Statistical Analysis Plan (SAP): LLA-Nu-3-CLIN002

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

Sponsor: Lakewood-Amadex, Inc.

CONFIDENTIAL

April 5, 2022

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Table 1. Version History

Version Number	Release Date	Change(s)	Reason for Change
1.0	April 5, 2022	N/A	Initial Release

Brief Trial Description

This study is a phase 2a, multi-center, double-blind, placebo-controlled, dose-escalating, parallel group, randomized controlled trial designed to evaluate the safety and tolerability of topically applied bisphosphocin NU-3 gel to clinically non-infected Wagner grade 1 diabetic foot ulcers (DFUs).

Two weeks after screening, subjects will be randomized 3:1 to bisphosphocin Nu-3 gel and standard of care (SOC) or placebo gel and SOC to treat Wagner 1 DFUs. Major inclusion criteria will be DFUs between 1.0 and 10 cm^2 initial area and having no wound ischemia.

Subjects will be seen weekly until 6 weeks after randomization unless the subject's wound healed, or the subject is withdrawn or lost to follow-up (i.e., early termination). The bisphosphocin Nu-3 or placebo gel will be applied starting on the day of randomization for 4 weeks twice daily. The study will be executed in two phases: cohort 1 and cohort 2, in which the doses for the intervention group will be 5% or 10% Nu-3 gel.

SOC in this study will be offloading of the DFU using a removable cast walker provided to the subject, appropriate sharp or surgical debridement (note: there will be several rules pertaining to prohibition on certain visits and degree of debridement), and infection management post-randomization as deemed appropriate by the investigator. Primary dressings will be Mepitel (or equivalent), Tegaderm, and padding/Coban Lite 2- layer dressing.

General

Descriptive statistical methods will be used to summarize the data from this study. These include number of subjects (n), mean and standard deviation (SD) for continuous variable data (medians and IQR [interquartile range] values will be given when the distribution of variable values is non-normal), and frequencies and percentages for categorical variable data. There will be no hypothesis testing performed. All data collected during the study will be reported and analyzed.

Unless specified otherwise, all statistical testing will be two-sided and performed using a significance (alpha) level of 0.05.

All statistical analyses will be conducted using PASW 27 (SPSS; IBM).

Sample Size Calculations

Since this is a pilot study focusing primarily on safety, no sample size calculations will be done. Based on general experience with phase 2a trials, it is planned to enroll 32 subjects (24 active and 8 placebo).

Randomization

Subjects eligible for the study after screening and run-in will be randomized 3:1 to bisphosphocin Nu-3 gel and SOC or placebo gel SOC.

Interim Analysis

A safety review will be conducted by an independent Data Monitoring Committee (DMC) after the first cohort to confirm it is acceptable to proceed to the next dose level.

Trial Stopping Rules

Subjects will be permitted to withdraw at any time and investigators can withdraw a patient based on the occurrence of an AE/SAE interfering with treatment or placing the subject's health in jeopardy, or pause treatment for any one these scenarios: (a) wound gangrene; osteomyelitis; G3-4 AE; SAE related to study drug. Occurrence of ≥3 of the above criteria in any given cohort will also require a pause in treatment for the cohort, with subsequent actions determined based on the DMC charter.

Patient Populations

The populations defined for the final analysis include intent-to-treat (ITT), modified-intent-to-treat (MITT), per protocol (PP), and safety populations are defined as shown in Table 2. All the defined populations will be analyzed unless the populations are the same; for example, if no subjects were withdrawn, lost to follow-up, or died, and all subjects received assigned doses, then the ITT and MITT groups would be identical and MITT analysis would be dropped.

Variable	Intent-to-treat (ITT)	Modified-intent-to-treat (MITT)	Per Protocol (PP)	Safety (SP)
All randomized subjects	✓			
Analysis per assigned treatment group	✓	✓		✓
Major protocol violation	✓	✓		✓
Withdrawn, died, or lost to follow-up	✓			✓
Received at least one treatment	✓	✓		✓
Received at least 80% of doses			✓	
Received no treatment	✓			

Definitions

Baseline

For all parameters, baseline will be defined as the last available data point just prior to the first treatment, which occurs at visit 3.

Visit window data including study endpoints

For each weekly visit a window of ± 2 days will be adopted for data.

Healing of Index Ulcer

Healing is defined as complete epithelialization of the ulcer without drainage observed in the index ulcer.

Date of healing
The date of healing is defined as the date of the first assessment of index ulcer healing.

Days to Index Ulcer healing
For subjects who achieve healing of index ulcer during the Treatment Phase, days to healing of Index Ulcer will be calculated. Time to first assessment of ulcer healing will be used for this time-to-event analysis.*

For the right censored subjects (i.e., subjects who do not have a wound healing event) the time to heal in days will be:

- 42 days if the subject completes the study
- Time of the early termination – date of randomization for those subjects who withdraw or terminate early from the study (i.e. the censored time)

*For calculation purposes, days to healing = date ulcer is first healed – date of randomization.

Percentage area reduction from baseline
The percent change in the surface area of the Index Ulcer (PAR) will be calculated using the following formula:

((A1–A2)/A1)*100

Where A1 is the baseline area (at randomization), and A2 is the area at the specified time point.

Statistical methodology

Missing data

Missing data may be the result of missed visits, subjects lost to follow-up or who have died, and may be endpoint data or covariate data. Missing continuous outcome data will be imputed in this study using the LOCF (last observation carried forward). If wound healing outcomes are missing these will be imputed as follows: (a) If an event removes the index ulcer, such as an amputation, the outcome will be scored as not healed; (b) If a subject dies, the outcome will be scored as not healed; (c) If a subject is withdrawn from the study, the outcome will be scored as not healed; and (d) for all remaining non-healed index ulcers in the drug groups, the outcomes will be based on non-right-censored outcomes in the SOC group to weight outcomes.

Data QC

1. Inspect for accuracy of input:
    a. Plausible values
    b. Outliers or out-of-range values
2. Evaluate degree and distribution of missing data; for non-endpoint data query PERI
3. Identify non-normal continuous variables and outliers; data for non-normal variables will include median/IQR.

Statistical Analysis

Analysis will be started once database lock for the trial is initiated and the final version of the SAP is released in Lakewood-Amedex' system.

Research hypotheses

Because this trial is focused on safety, no primary research hypotheses (hypotheses tested using statistical tests) will be tested. However, two secondary endpoints will be tested statistically, one of which has to meet a priori conditions to decide validity of testing.

Analysis

The following analyses will be conducted:

- Flow chart (subjects) with disposition according to CONSORT criteria. (see shell Figure 1).
- Overall trial statistics (enrollment dates, site numbers, numbers of subjects, group assignments)
- Withdrawals (including death), and loss to follow-up (total and by group assignment) with week of discontinuation and reason.
- Screen failure rate and screen failure reasons according to exclusion criteria

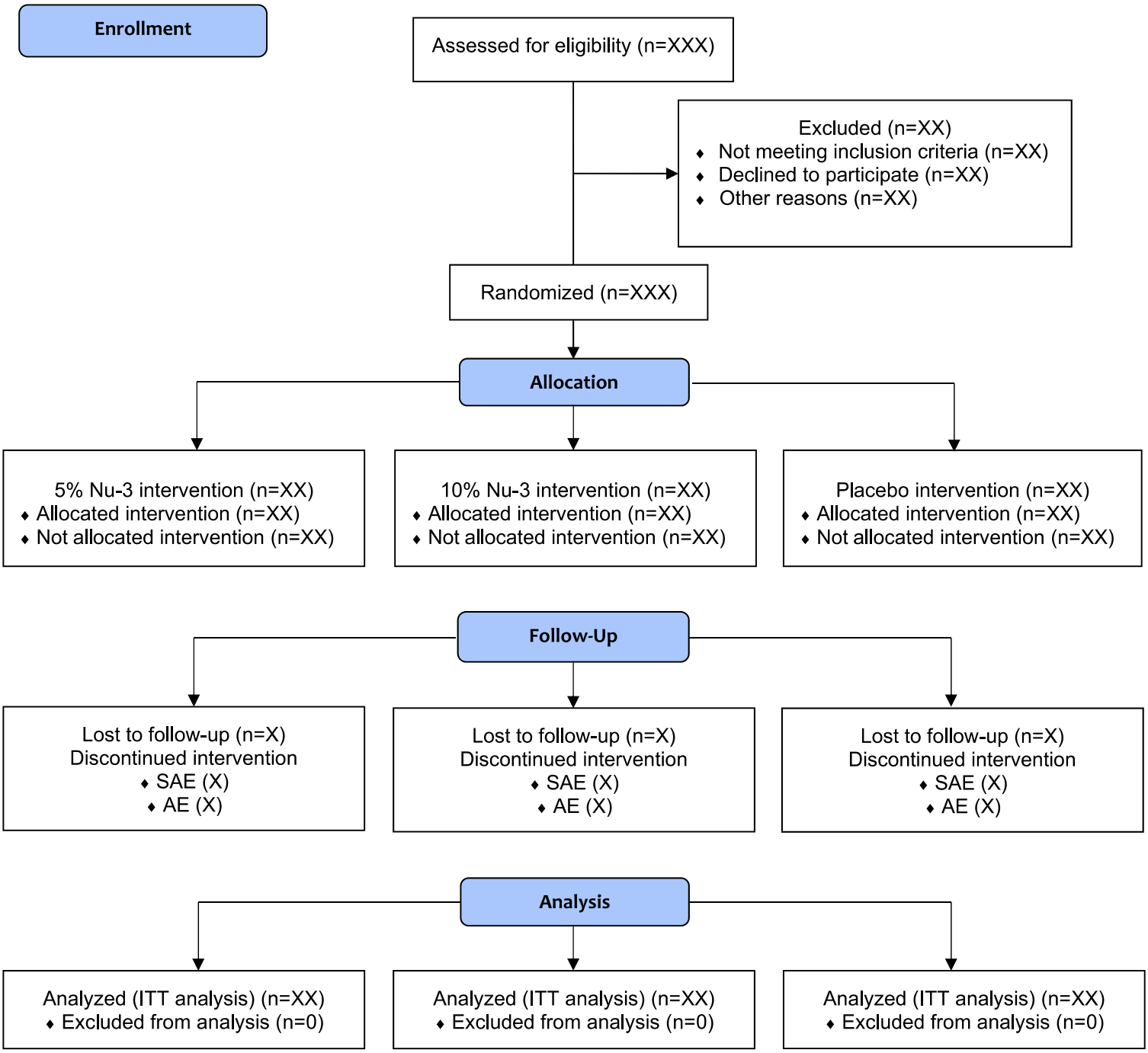
- Demographics between groups at baseline (subject-related and wound-related variables): patient age, smoking/alcohol status, BMI, HbA1c, creatinine levels; diabetes duration); number of concurrent DFUs, prior DFUs and amputations, history of DFU recurrence, history of and significant foot deformities (if available); DFU location, ulcer duration, initial area; baseline variables between groups will be tested using t tests, Mann-Whitney, chi square, and Fisher exact tests (as appropriate) without adjustment for multiplicity testing (see shell Tables 1 and 7).
- Subject comorbidities will be listed using first-level medDRA organ classes by treatment group (see shell Table 8); for each subject the total number of comorbidities will be calculated and then the means between groups will be tested in the same way as for demographics and added to shell Table 1
- Prescription medications will be listed using WHODrug classes by treatment group (see shell Table 9); for each subject the total number of prescription drugs will be calculated and then the means between groups will be tested in the same way as for demographics and added to shell Table 1
- The primary endpoint analysis will be a summary of the incidence of treatment-emergent adverse events (TEAEs), including subject and overall AE rates by treatment group:
    - Summary statistics for each treatment group (count of TEAEs per group/per patient; summary rates per group; notes of unexpected occurrences during the study; relevant decisions made) [text in report]
    - SAE (brief description; resolution; impact on study (e.g., withdrawal, etc.) [text in report]
    - **All** TEAEs by system organ class (SOC), preferred term (PT), severity, and relationship to test article (see shell Table 10).
- Other safety data will be analyzed as follows:
    - Laboratory evaluations and vital signs assessments will be summarized by dose group (including placebo group) and time point of collection (i.e., visit number starting with randomization visit) (see shell Table 2).
        - Tables will also be constructed for each subject per timeline (see Shell Table 3)

- Table describing change in value and percentage change from baseline for each visit will be provided for vital signs for each subject (see Shell Table 4)
- Table describing out-of-normal range shifts will be provided for clinical laboratory results for each subject, grouped according to dose group (See Shell Table 5)
- Table describing changes noted in physical examinations (at each visit after randomization) will be constructed for each subject, grouped according to dose group (see Shell Table 6)

- Efficacy date will be analyzed as follows:
  - Summary statistics of DFUs that healed by treatment group by 6 weeks (numbers and percentage healed); graph showing weekly percentage of wounds healed by treatment group
  - PAR weekly plot by treatment group; summary table of PAR values by week and treatment group
  - Time to heal within 6 weeks (summary time to heal statistics by treatment group) using Kaplan-Meier method; probability of wound healing (1-survival ≡ 1-wounds not healed) plot by treatment group. If the percentage healed figures for the 5% and 10% Nu-3 groups at 6 weeks is within 10% **and** a statistical power calculation shows that the proportions of wounds healed for the combined versus placebo groups is at least 80%, the times to heal will be tested using the logrank test.
  - Bacterial counts (CFU/g) for weeks 0-4 will be analyzed by (a) reporting the means and standard deviations for each group at each time point and (b) using GLMM (general linear modeling with repeated measures [weeks 1-4] and counts at week 0 as a factor)
  - Microbiological data will be summarized for each of the 3 treatment groups for each visit (visits 1, 3, 4, 5, 6, 7; unscheduled visit; early termination) as follows (a) list of species detected in each microbiological sample (Shell Table 11); and (b) susceptibility testing to the following drugs for each microbiological sample: penicillin, vancomycin, and linezolid (Shell Table 12)

    *A reason will be given in each instance in which susceptibility testing was *not* done (e.g., no growth).

Shell Figure 1. Note: Nu-3 = bisphosphocin Nu-3



Shell Table 1. Patient-related variables by treatment group. Figures in parentheses will be percentage for categorical variables and standard deviation (SD) of the mean for continuous variables; for key variables, such as initial wound area, medians and interquartile ranges (IQR) will be added. p values will have 2 significant figures

Variable	Nu-3 (5%)	Nu-3 (10%)	Placebo	p
Age (years)	xx.x (x.xx)	xx.x (x.xx)	xx.x (x.xx)	0.xx
Race				
Caucasian	x (x)	x (x)	x (x)	
African American	x (x)	x (x)	x (x)	0.xx
Hispanic	x (x)	x (x)	x (x)	
Gender				
Male	x (x)	x (x)	x (x)	0.xx
Female	x (x)	x (x)	x (x)	
Diabetes duration (years)	xx (x.xx)	xx (x.xx)	xx (x.xx)	0.xx
BMI	xx.x (x.xx)	xx.x (x.xx)	xx.x (x.xx)	0.xx
HbA1c	xx.x (x.xx)	xx.x (x.xx)	xx.x (x.xx)	0.xx
Creatinine	xx.x (x.xx)	xx.x (x.xx)	xx.x (x.xx)	0.xx
Comorbidity count	xx.x (x.xx)	xx.x (x.xx)	xx.x (x.xx)	0.xx
Prescription medication count	xx.x (x.xx)	xx.x (x.xx)	xx.x (x.xx)	0.xx

Nu-3: bisphosphocin Nu-3 gel

Shell Table 2. Laboratory evaluations and vital signs assessments by treatment group and visit number; randomization visit=1.

Analyte or Vital Signs **Visit** **Statistic**	**Nu-3 (5%)**	**Nu-3 (10%)**	**Placebo**
<Analyte (unit)> Visit 1 n Mean (SD) Median (IQR)	 xx xx.x (xx.xx) xx.x (xx.xx)	 xx xx.x (xx.xx) xx.x (xx.xx)	 xx xx.x (xx.xx) xx.x (xx.xx)
Visit 2 n Mean (SD) Median (IQR)	 xx xx.x (xx.xx) xx.x (xx.xx)	 xx xx.x (xx.xx) xx.x (xx.xx)	 xx xx.x (xx.xx) xx.x (xx.xx)
{Continue for all scheduled post-randomization visits and all analytes. Start a new page for every additional analyte}			
<Vital sign (unit)> Visit 1 n Mean (SD) Median (IQR)	 xx xx.x (xx.xx) xx.x (xx.xx)	 xx xx.x (xx.xx) xx.x (xx.xx)	 xx xx.x (xx.xx) xx.x (xx.xx)
Continue for all scheduled post-randomization visits and all vital signs. Start a new page for every additional vital sign}			

Nu-3: bisphosphocin Nu-3 gel

Shell Table 3. Laboratory evaluations and vital signs assessments for each subject by treatment group and visit number; randomization visit=1.

Analyte or Vital Signs **Visit** **Statistic**	**Nu-3 (5%)**	**Nu-3 (10%)**	**Placebo**
Subject ID <Analyte (unit)> Visit 1 Value	XX-XXX xx.x	XX-XXX xx.x	XX-XXX xx.x
Subject ID <Analyte (unit)> Visit 2 Value	XX-XXX xx.x	XX-XXX xx.x	XX-XXX xx.x
{Continue for all scheduled post-baseline visits and all analytes. Start a new page for every additional analyte}			
Subject ID <Vital sign* (unit)> Visit 1 Value	XX-XXX xx.x	XX-XXX xx.x	XX-XXX xx.x
Subject ID <Vital sign (unit)> Visit 2 Value	XX-XXX xx.x	XX-XXX xx.x	XX-XXX xx.x
{Continue for all scheduled post-baseline visits and all vital signs. Start a new page for every additional vital sign}			

Nu-3: bisphosphocin Nu-3 gel; *some vital signs will be paired—for example, blood pressure

Shell Table 4. Changes in vital signs assessments for each subject by treatment group and visit number compared to randomization visit [1].

Vital Signs **Visit** **Statistic**	**Nu-3 (5%)**	**Nu-3 (10%)**	**Placebo**
Subject ID <Vital sign* (unit)> <Visit 1, value> <Visit 2, change in vital sign> <Visit 2, percentage change in vital sign> {Continue for all scheduled post-baseline visits and all vital sign changes.}	XX-XXX xx.x (-) xx.x (-) xx.x%	XX-XXX xx.x (-) xx.x (-) xx.x%	XX-XXX xx.x (-) xx.x (-) xx.x%
{Continue for all scheduled post-baseline visits and all vital signs. Start a new page for every additional vital sign}			

Nu-3: bisphosphocin Nu-3 gel; *some vital signs will be paired—for example, blood pressure

Shell Table 5. Out of normal range shifts for laboratory evaluations for each subject by treatment group and visit number; randomization visit=1.

Analyte **Visit** **Statistic**	**Nu-3 (5%)**	**Nu-3 (10%)**	**Placebo**
Subject ID	XX-XXX	XX-XXX	XX-XXX
<Analyte (unit>			
Visit 1			
Missing	Y/N	Y/N	Y/N
Low	Y/N	Y/N	Y/N
Normal	Y/N	Y/N	Y/N
High	Y/N	Y/N	Y/N
Subject ID	XX-XXX	XX-XXX	XX-XXX
<Analyte (unit>			
Visit 2			
Missing	Y/N	Y/N	Y/N
Low	Y/N	Y/N	Y/N
Normal	Y/N	Y/N	Y/N
High	Y/N	Y/N	Y/N
{Continue for all scheduled post-baseline visits and all analytes. Start a new page for every additional analyte}			

Nu-3: bisphosphocin Nu-3 gel

Shell Table 6. Changes noted in physical examinations for each subject by treatment group and visit number; randomization visit=1. If there is no change, entry will be blank.

Physical Exam Change **Visit** **Description**	**Nu-3 (5%)**	**Nu-3 (10%)**	**Placebo**
Subject ID Visit 1* System organ class Preferred term(s)	XX-XXX Text	XX-XXX Text	XX-XXX Text
Subject ID Visit 2 System organ class Preferred term(s)	XX-XXX Text	XX-XXX Text	XX-XXX Text
{Continue for all scheduled post-baseline visits and all analytes. Start a new page for every additional subject}			

Nu-3: bisphosphocin Nu-3 gel; *Change from last screening visit

Shell Table 7 (wound-related variables). Figures in parentheses will be percentage for categorical variables and standard deviation (SD) of the mean for continuous variables; for variables that have a non-normal distribution of values, such as initial wound area, medians and interquartile ranges (IQR) will be added.

Variable	Nu-3 (5%)	Nu-3 (10%)	Placebo
Wound area (cm^2)	xx.x (x.xx) xx.x (x.xx)	xx.x (x.xx) xx.x (x.xx)	xx.x (x.xx) xx.x (x.xx)
Wound age (weeks)	xx.x (x.xx) xx.x (x.xx)	xx.x (x.xx) xx.x (x.xx)	xx.x (x.xx) xx.x (x.xx)
Number of prior DFUs 0 1 2 ≥3	 xx (xx) xx (xx) xx (xx) xx (xx)	 xx (xx) xx (xx) xx (xx) xx (xx)	 xx (xx) xx (xx) xx (xx) xx (xx)
Number of concurrent DFUs 0 1 2 ≥3	 xx (xx) xx (xx) xx (xx) xx (xx)	 xx (xx) xx (xx) xx (xx) xx (xx)	 xx (xx) xx (xx) xx (xx) xx (xx)
History of ulcer recurrence	xx (xx)	xx (xx)	xx (xx)
Prior amputation history (extremity, amputation type)	xx (xx)	xx (xx)	xx (xx)
Significant foot deformity history	xx (xx)	xx (xx)	xx (xx)
DFU location Plantar Dorsal	 xx (xx) xx (xx)	 xx (xx) xx (xx)	 xx (xx) xx (xx)
DFU location Toe Forefoot Midfoot Heel Ankle	 xx (xx) xx (xx) xx (xx) xx (xx) xx (xx)	 xx (xx) xx (xx) xx (xx) xx (xx) xx (xx)	 xx (xx) xx (xx) xx (xx) xx (xx) xx (xx)
Number of debridements	x (x)	x (x)	x (x)

Nu-3: bisphosphocin Nu-3 gel

Shell Table 8. Comorbidities noted at first physical examination for each subject by treatment group.

Physical Exam Change Visit Description	**Nu-3 (5%)**	**Nu-3 (10%)**	**Placebo**
Subject ID System organ class Preferred term(s) Comorbidity count	XX-XXX Text xx	XX-XXX Text xx	XX-XXX Text xx
{Continue for all subjects. Start a new page for every additional subject}			

Nu-3: bisphosphocin Nu-3 gel; *Change from last screening visit

Shell Table 9. Prescription medications noted at first examination for each subject by treatment group.

Physical Exam Change Visit Description	**Nu-3 (5%)**	**Nu-3 (10%)**	**Placebo**
Subject ID Drug class Preferred term(s) Prescription count	XX-XXX Text xx	XX-XXX Text xx	XX-XXX Text xx
{Continue for all subjects. Start a new page for every additional subject}			

Nu-3: bisphosphocin Nu-3 gel; *Change from last screening visit

Shell Table 10. Categorization of AEs by medDRA terms, severity, and relatedness.

TEAE Category	Nu-3 (5%)	Nu-3 (10%)	Placebo
System organ class List of classes Preferred term(s)	xx (xx)	xx (xx)	xx (xx)
{Continue for all system organ classes. Start a new page for each new system class as needed}			
Severity			
1	xx (xx)	xx (xx)	xx (xx)
2	xx (xx)	xx (xx)	xx (xx)
3	xx (xx)	xx (xx)	xx (xx)
4	xx (xx)	xx (xx)	xx (xx)
5	xx (xx)	xx (xx)	xx (xx)
Relatedness			
Not related	xx (xx)	xx (xx)	xx (xx)
Unlikely to be related	xx (xx)	xx (xx)	xx (xx)
Possibly related	xx (xx)	xx (xx)	xx (xx)
Probably related	xx (xx)	xx (xx)	xx (xx)
Definitely related	xx (xx)	xx (xx)	xx (xx)
TOTAL	xx	xx	xx

Nu-3: bisphosphocin Nu-3 gel

Shell Table 11. Microbiological species identification from wound swabs for each subject by treatment group and visit number; randomization visit=1.

Microbiological Identity Statistic	Nu-3 (5%)	Nu-3 (10%)	Placebo
Subject ID Visit 1 Species 1 {Continue listing for all species identified}	XX-XXX text	XX-XXX text	XX-XXX text
Subject ID Visit 3 Species 1 {Continue listing for all species identified}	XX-XXX text	XX-XXX text	XX-XXX text
{Continue for all visits for which data are available. Start a new page for every subject ID}			

Nu-3: bisphosphocin Nu-3 gel

Shell Table 12. Microbiological susceptibility testing to penicillin, vancomycin, and linezolid from wound swabs for each subject by treatment group and visit number; randomization visit=1.

Susceptibility Testing Statistic	Nu-3 (5%)	Nu-3 (10%)	Placebo
Subject ID	XX-XXX	XX-XXX	XX-XXX
Visit 1			
<Penicillin>			
Value*	text	text	text
Interpretation**	text	text	text
<Vancomycin>			
Value	text	text	text
Interpretation	text	text	text
<Linezolid>			
Value	text	text	text
Interpretation	text	text	text
Subject ID	XX-XXX	XX-XXX	XX-XXX
Visit 3			
<Penicillin>			
Value	text	text	text
interpretation	text	text	text
<Vancomycin>			
Value	text	text	text
Interpretation	text	text	text
<Linezolid>			
Value	text	text	text
Interpretation	text	text	text
{Continue for all visits for which data are available. Start a new page for every subject ID}			

Nu-3: bisphosphocin Nu-3 gel

*Values can have a variety of numerical formats; for example, 0.08, <=0.01, >2 (If susceptibility was not done a reason will be substituted instead of a value); **interpretation can only have one of 4 values: susceptible (S), intermediate (I), resistant (R), or not applicable (N).