Open-Label, Non-randomized, Non-controlled Protocol for the Treatment of Patients With Mildly Infected Diabetic Foot Ulcer Using PluroGel PN

CLINICAL PROTOCOL PGN-1300X

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Open-Label, Non-randomized, Non-controlled Protocol for the Treatment of Patients With Mildly Infected Diabetic Foot Ulcer Using PluroGel PN

PROTOCOL #   PGN-1300X

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I have read Protocol PGN1300X and I agree that it contains all of the necessary information in sufficient detail to conduct the clinical study as described. Furthermore, I agree to conduct the study as described within this protocol; no changes to the protocol will be made without prior approvals by the Sponsor and a duly-constituted Institutional Review Board (IRB) except when necessary to protect the safety, rights, or welfare of subjects.

My signature below confirms that my staff and I have carefully read and understand this study protocol, that I will personally conduct and supervise this study, and that I will ensure that all who participate in the conduct of the study are fully informed regarding the study and the study agent.

Termination of this study and agreement may be initiated at the Sponsor's option with written 30-day notice. If this trial is terminated prior to completion, I will cooperate with the Sponsor through the process of study closure. Commitments of both parties will continue as stated in the protocol and agreement except that payments made will be prorated in a way consistent with the proportion of work completed.

Principal Investigator Printed Name

Principal Investigator Signature     Date

Sponsor Designee  Printed Name

Sponsor Designee     Date

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ABSTRACT

Open-Label, Non-randomized, Non-controlled Protocol for the Treatment of Patients With Mildly Infected Diabetic Foot Ulcer Using PluroGel PN

This is an open-label, non-randomized, non-controlled, multi-site follow-up study to evaluate the safety and efficacy of topical PluroGel PN for up to 14 days in subjects with mildly infected diabetic foot ulcers who failed treatment, had a recurrence, or had a partial response (Day 14) in the companion double-blinded (evaluator-blind), randomized, vehicle-controlled study (PGN-1300) preceding it. The primary efficacy outcome will be complete clinical response at the Two-Week Follow-up Visit. Safety outcomes will be subject disposition, Adverse Events (AEs), development of drug resistance, clinical laboratory parameters, physical examination findings, and vital signs.

After initiation of treatment at the Enrollment Visit (Day 0), subjects will return to the investigational site for their first Treatment Visit on Day 3 (±1) and may return for subsequent Treatment Visits on Day 10 (±1) and Day 14 (±1); depending on their clinical response to treatment. The Day 14 Visit or the visit at which the subject discontinues early will be the End of Treatment Visit. Subjects will return for the Two Week Follow-up Visit approximately two weeks after the Day 14 Visit for final efficacy and safety assessments (unless starting an alternative [non-study] treatment or in circumstances requiring immediate withdrawal).

The investigator will immediately discontinue the subject and consider the initiation of an appropriate, alternative (non-study) therapy in the following instances:

- Clinical Treatment Failure at any visit - one or more signs or symptoms of the infection are worsening or the overall signs and symptoms of infection have not changed since the previous visit, or
- Recurrence at any visit - previously cured or improved infection shows worsening of signs or symptoms of infection, or
- Partial Response (at the Day 14 Visit) - most, but not all, signs and symptoms of infection have improved or resolved.

The treatment period is 14 days unless one of the above treatment failures occurs prior to the Day 14 Visit.

Inclusion Criteria

- Subjects who have one of the following outcomes on protocol PGN-1300:
  - Clinical Treatment Failure at any visit, or
  - Recurrence at any visit, or
  - Partial Response at the Day 14 Visit.

Exclusion Criteria

- Subjects with IDSA-defined moderate infection as per Appendix C, including cellulitis extending > 2 cm; lymphangitis; spread beneath the fascia; deep tissue abscess; osteomyelitis; gangrene; muscle, joint, or bone involvement.
Subjects with IDSA-defined severe infection as per Appendix C, including systemic toxicity or metabolic instability (e.g., fever, chills, tachycardia, hypotension, confusion, vomiting, leukocytosis, acidosis, hyperglycemia, or azotemia).

Subjects with systemic inflammatory response signs, as manifested by ≥2 of the following:
- Temperature >38°C or <36°C
- Heart rate >90 beats/min
- Respiratory rate >20 breaths/min or PaCO2 <32 mm Hg
- White blood cell count >12 000 or <4000 cells/μL or ≥10% immature (band) forms

Subjects with local wound complications (e.g., prosthetic materials).

Subjects requiring concurrent systemic antimicrobials during the study period for any infection, including diabetic foot ulcer.

Subjects who are expected to be unable to care for their ulcer because of hospitalization, vacation, disability, etc. during the study period, or are unable to safely monitor the infection status at home.

Subjects who have had an unexplained fever or chills during the week prior to enrollment.

Subjects with other conditions considered by the investigator to be reasons for disqualification that may jeopardize subject safety or interfere with the objectives of the trial (e.g., acute illness or exacerbation of chronic illness, lack of motivation, history of poor compliance).

Women who are breast feeding, pregnant, or not using contraception unless sterile.

Efficacy Endpoints

The primary efficacy endpoint will be:

Complete clinical response at the Two-Week Follow-up Visit according to the clinical response guidelines below:

- Complete Response (all signs and symptoms of infection resolved);
- Partial Response (most, but not all, signs and symptoms of infection improved or resolved);
- Treatment Failure (one or more signs or symptoms of infection are worsening or overall signs and symptoms of infection have not changed);
- Unevaluable (less than 3 days of study treatment or subject lost to follow-up); or
- Recurrence (a previously cured or improved infection showing worsening of signs or symptoms of infection).

The secondary efficacy endpoints for this study will be:

Complete or Partial Clinical Response at all post baseline visits other than the Two-Week Follow-up Visit.

Change in the Total Diabetic Foot Infection Score at all post-baseline visits.
Change in Microbiological Response at all post-baseline visits, according to the following guidelines:

- Complete Response (all organisms eradicated or no available material to culture);
- Microbiologic Persistence (Persistence of one or more of the original organisms present at Day 0);
- Colonization (Appearance of new organisms);
- Unevaluable (less than 3 days of study treatment or subject lost to follow-up).

Corynebacterium, Propionibacterium, or Bacillus species will not be considered as infecting organisms, unless one of these 3 species is isolated in pure culture.

Rate of Recurrence at the Two Week Follow-up Visit

Safety Endpoints

The safety endpoints will be:

- Incidence and Severity of Adverse Events
- Development of Drug Resistance
1 INTRODUCTION

Diabetic ulcers occur in over 600,000 people (3 to 8% of diabetics) in the United States and lead to over 60,000 amputations annually. Each foot ulcer is associated with a direct cost of approximately $5,000. The American Diabetes Association estimates that more than one-half of all amputations can be prevented by using proper patient therapy. Diabetic neuropathy leads to functional impairment of the microcirculation and can result in foot tissue hypoxia and reduced healing capacity, even in the presence of adequate blood flow. The standard treatment recommended by the American Diabetes Association consists of extensive debridement of necrotic tissue, treatment of infection, non-weight-bearing or offloading techniques that decrease the pressure applied on the affected extremity, and if indicated, arterial revascularization. Topical treatment of diabetic foot ulcers include enzymatic debridement, total contact casting, hyperbaric oxygen, antibiotics, growth factors, and dressings that keep the wound moist while protecting granulating tissue from mechanical injury. Foot infections are the most common problems in persons with diabetes. These individuals are predisposed to foot infections because of a compromised vascular supply secondary to diabetes. Local trauma and/or pressure (often in association with lack of sensation because of neuropathy), in addition to microvascular disease, may result in various diabetic foot infections that run the spectrum from simple, superficial cellulitis to chronic osteomyelitis.

Infections in patients with diabetes are difficult to treat, because these individuals have impaired microvascular circulation, which limits the access of phagocytic cells to the infected area and results in a poor concentration of antibiotics in the infected tissues. Treating a diabetic foot infection (DFI) requires proper wound care and appropriate antibiotic therapy. The antibiotic(s) selected must cover the often-polymicrobial organisms that cause these infections. A chosen antibiotic regimen must balance effectiveness against potential adverse effects and the likelihood of emergence of antibiotic resistance.

According to the internationally accepted and verified IDSA classification, wound depth and extent of erythema are important determinants of severity. A mild wound infection, for example, could be amenable to treatment with a topically administered anti-infective agent. Topical treatment has the advantages of avoiding systemic adverse effects, providing increased target site concentration, and allowing the use of agents not available for systemic therapy. An acceptable topical anti-infective agent would need to demonstrate activity against the spectrum of bacteria that are known to cause foot infections, and would need to avoid serious adverse effects, interference with wound healing, or induction of drug resistance.

This trial evaluates the safety and effectiveness of PluroGel PN, a topical combination product containing agents known to have significant activity against gram positive and gram negative organisms, which are those most commonly associated with DFI. PluroGel PN’s polymicrobial coverage and safety profile should make it a preferred agent for the treatment of mildly infected diabetic foot infections.

2 OBJECTIVES

The primary objective of this clinical study is to determine the efficacy of topical PluroGel PN in the treatment of mild infected diabetic foot ulcer infections.
The secondary objective of this clinical study is to determine the safety of topical PluroGel PN in the treatment of mild infected diabetic foot ulcer infections.

3 STUDY DESIGN

3.1 STUDY DESIGN OVERVIEW

This is an open-label, non-randomized, non-controlled, multi-site follow-up study to evaluate the safety and efficacy of topical PluroGel PN for up to 14 days in subjects with a mild diabetic foot ulcer (IDSA Criteria) who failed treatment, had a recurrence, or had a partial response (Day 14) in the companion double-blinded (evaluator-blind), randomized, vehicle-controlled study (PGN-1300) preceding it. The primary efficacy outcome will be complete clinical response at the Two-Week Follow-up Visit. Safety outcomes will be subject disposition, adverse events, development of drug resistance, clinical laboratory parameters, physical examination findings, and vital signs.

3.1.1 Efficacy Endpoints

The primary efficacy endpoint will be:

Complete clinical response at the Two-Week Follow-up Visit, according to the clinical response

- Complete Response (all signs and symptoms of infection resolved);
- Partial Response (most, but not all, signs and symptoms of infection improved or resolved);
- Treatment Failure (one or more signs or symptoms of infection are worsening or overall signs or symptoms have not changed);
- Unevaluable (less than 3 days of study treatment or subject lost to follow-up); or
- Recurrence (a previously cured or improved infection showing worsening of signs or symptoms of infection).

The secondary efficacy endpoints for this study will be:

Complete or Partial Clinical Response at all post-baseline visits other than the Two-Week Follow-up Visit.

Change in the Total Diabetic Foot Infection Score at all post-baseline visits.

Change in Microbiological Response at the Two-Week Follow-up Visit, according to the following guidelines:

- Complete Response (pathogens not detectable or no available material to culture);
- Microbiologic Failure (Detection of one or more of the original pathogens present at Day 0);
- Colonization (Detection of new pathogens not detected at Day 0);
- Unevaluable (less than 3 days of study treatment or subject lost to follow-up).
Corynebacterium, Propionibacterium, or Bacillus species will not be considered as infecting pathogens, unless one of these 3 species is isolated in pure culture.

Rate of Recurrence at the Two Week Follow-up Visit

3.1.2 Safety Endpoints

The safety endpoints will be:

- Incidence and Severity of Adverse Events
- Development of Drug Resistance

3.2 PATIENTSUBJECT ENROLLMENT

After the Investigator has determined that a subject meets the eligibility criteria and the enrollment is complete, a copy of the completed Subject Eligibility case report form must be faxed to the Arkios (859.317.5990) on the day of enrollment. Arkios will acknowledge receipt of the fax via fax or email response. The Unique Subject Identification number assigned during the subject’s participation in protocol PGN-1300 will continue to be used for this trial. No additional subject numbers will be assigned.

3.3 SUBJECT DISCONTINUATION

Subjects will be discontinued from the study for the following reasons:

- If a subject suffers an adverse event that, in the judgment of the Investigator, Sponsor, or Medical Monitor, presents an unacceptable risk to the subject;
- If a subject develops an intercurrent illness or complication that is not consistent with the protocol requirements or justifies withdrawal from the study;
- If the subject’s ulcer is considered a Clinical Treatment Failure, Recurrence, or Partial Response (Day 14) according to the protocol definition;
- If the subject withdraws consent;
- If the subject develops sensitivity to PluroGel PN components;
- If the subject develops an infection (other than that under study and requiring systemic antibiotic treatment).

4 STUDY PRODUCT AND DOSAGES

4.1 IDENTIFICATION AND DESCRIPTION OF STUDY PRODUCT

PluroGel PN is composed of the PluroGel gel carrier with two active pharmaceutical ingredients (APIs), polymyxin B (10,000 U/g) and nitrofurantoin (0.3%) (Table 1). The unique combination of polymyxin B and nitrofurantoin provides PluroGel PN with extensive antimicrobial activity against commonly seen bacterial and newer resistant topical infections.

The gel carrier, PluroGel is water-soluble. This makes the removal of the previously applied PluroGel PN easier, gentler, and faster, which could significantly reduce the pain associated with dressing changes of infected wounds.

The investigational product will be shipped in jars with a PluroGel PN label affixed.
Table 1. Active Pharmaceutical Ingredients in PluroGen's PluroGel PN

<table>
<thead>
<tr>
<th>API Name</th>
<th>Inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyxin B</td>
<td>10,000 U/g</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

4.2 STUDY PRODUCT STORAGE AND HANDLING

The Sponsor will arrange for study product to be shipped to the Investigator. To request additional supplies of study product, contact the Project Director (contact information is found on the cover of this protocol). **REFRIGERATE** study product at 34° to 46°F in a secured area with controlled access. The site should review the temperature tracking device included in the shipment and confirm that the study product remained within 34° to 46°F during the entire shipping period. The site will notify the Project Director immediately to report any out of range temperature excursions and wait for further direction before dispensing. Site personnel should advise the subject to store the product in the refrigerator once dispensed. **DO NOT FREEZE.**

The Investigator is responsible for maintaining inventory and accounting for all study product received from the Sponsor, dispensed from the clinic, and returned from subjects on a study product accountability log. When dispensing study product to the subject, site personnel should record the subject initials on the product label along with the date dispensed.

At the end of the study, all study product containers received by the Investigator (used and unused) will be returned to the sponsor after reconciliation is complete.

5 EXPERIMENTAL PROCEDURES

5.1 ELIGIBILITY CRITERIA

Male and female subjects aged 18 and older with a clinical diagnosis of a mildly infected diabetic foot ulcer according to the IDSA definition (see Appendix C) that meet the study inclusion/exclusion criteria are eligible to participate in this trial.

**Inclusion Criteria**

- Subjects who have one of the following outcomes on protocol PGN1300:
  - Clinical Treatment Failure at any visit, or
  - Recurrence at any visit, or
  - Partial Response at the Day 14 Visit.

**Exclusion Criteria**

- Subjects with IDSA-defined moderate infection as per Appendix C, including cellulitis extending > 2 cm; lymphangitis; spread beneath the fascia; deep tissue abscess; osteomyelitis; gangrene; muscle, joint, or bone involvement.
• Subjects with IDSA-defined severe infection as per Appendix C, including systemic toxicity or metabolic instability (e.g., fever, chills, tachycardia, hypotension, confusion, vomiting, leukocytosis, acidosis, hyperglycemia, or azotemia).

• Subjects with systemic inflammatory response signs, as manifested by ≥2 of the following:
  - Temperature >38°C or <36°C
  - Heart rate >90 beats/min
  - Respiratory rate >20 breaths/min or PaCO₂ <32 mm Hg
  - White blood cell count >12 000 or <4000 cells/μL or ≥10% immature (band) forms

• Subjects with local wound complications (e.g., prosthetic materials).

• Subjects requiring concurrent systemic antimicrobials during the study period for any infection, including diabetic foot ulcer.

• Subjects who are expected to be unable to care for their ulcer because of hospitalization, vacation, disability, etc. during the study period, or are unable to safely monitor the infection status at home.

• Subjects who have had an unexplained fever or chills during the week prior to enrollment.

• Subjects with other conditions considered by the investigator to be reasons for disqualification that may jeopardize subject safety or interfere with the objectives of the trial (e.g., acute illness or exacerbation of chronic illness, lack of motivation, history of poor compliance).

• Women who are breast feeding, pregnant, or not using contraception unless sterile.

5.2 EVALUATIONS

5.2.1 Enrollment Procedures

All subjects eligible for entry into the study will have the potential risks and benefits of all treatments and the overall study protocol explained to them. Each subject who wishes to participate in this study will provide written informed consent prior to any study-related procedures being performed.

If the inclusion and exclusion criteria are met and informed consent is granted by the subject, the subject will receive PluroGel PN. The following information will be collected for each enrolled subject and recorded in the Case Report Forms (CRFs):

• Unique Subject Identification number (assigned in protocol PGN-1300)
• Date of evaluation
• Subject date of birth, sex, height, and weight
• Subject race and ethnicity
• Pertinent medical history
• Previous treatments to ulcer
Additionally, clinical laboratory samples for assessment of safety, and microbiology culture will be obtained according to the procedures in Appendix D.

5.2.2 Photography and Measurements of the Wound

The target ulcer will be photographed after cleansing and debridement at the initial visit and at every subsequent visit, as needed. Instructions for photography are detailed in Appendix B. Subjects are not required to have their wounds photographed and photography is optional on the part of the site.

Measurement of the ulcer should be performed after debridement in the following fashion: length is the longest edge-to-edge measurement of the ulcer, width is taken from a perpendicular axis to the length, and depth is the deepest vertical measurement using a sterile swab. Subsequent measurements are obtained using the same orientation.

The following information regarding the target ulcer should be recorded in the source documents and will be entered on the case report form:

- Diabetic ulcer onset date
- Diabetic ulcer location
- Dimensions of ulcer (L x W x D)

Additional material that illustrates the proper photographic technique can be found in Appendix B.

5.2.3 Clinical Outcome

At each visit, the investigator will grade the clinical response as:

- Complete Response (all signs and symptoms of infection resolved);
- Partial Response (most, but not all, signs and symptoms of infection improved or resolved);
- Treatment Failure (one or more signs or symptoms of infection are worsening or the overall signs or symptoms have not changed);
- Unevaluable (less than 3 days of study treatment or subject lost to follow-up); or
- Recurrence (a previously cured or improved infection showing worsening of signs or symptoms of infection).

The investigator will immediately discontinue the subject and consider the initiation of an appropriate, alternative (non-study) therapy in the following instances:

- Clinical Treatment Failure at any visit, or
- Recurrence at any visit, or
- Partial Response at the Day 14 Visit.

5.2.4 Wound Assessment (DFI Score)

The target ulcer will be evaluated on Day 0 and at each subsequent visit. A “Total Diabetic Foot Infection Score” will be calculated combining the DFI-General Parameters Score with the DFI-Wound Measurement Scores according to the following criteria:
Table 2. Diabetic Foot Infection – General Parameters Score

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purulent Drainage</strong></td>
<td>Absent</td>
<td>-</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Nonpurulent drainage</strong></td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>(serous, sanguinous)</td>
<td>None</td>
<td>Mild: pink, barely perceptible</td>
<td>Moderate: pale red, defined edges</td>
<td>Severe: red to dark red</td>
</tr>
<tr>
<td><strong>Erythema</strong></td>
<td>None</td>
<td>Mild: pink, barely perceptible</td>
<td>Moderate: pale red, defined edges</td>
<td>Severe: red to dark red</td>
</tr>
<tr>
<td><strong>Induration</strong></td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td><strong>Tenderness (sign)</strong></td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td><strong>Pain (symptom)</strong></td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td><strong>Local warmth</strong></td>
<td>Same</td>
<td>Mildly Increased</td>
<td>Moderately Increased</td>
<td>Severely Increased</td>
</tr>
<tr>
<td>(relative to uninfected contralateral foot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each of the 7 parameters is scored from 0 to 3. The scores are then totaled to generate the score for the DFI-General Parameters.

Table 3. Diabetic Foot Infection – Wound Size Score

<table>
<thead>
<tr>
<th>Size (cm²)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>≥1-2</td>
<td>1</td>
</tr>
<tr>
<td>&gt;2-5</td>
<td>3</td>
</tr>
<tr>
<td>&gt;5-10</td>
<td>6</td>
</tr>
<tr>
<td>&gt;10-30</td>
<td>8</td>
</tr>
<tr>
<td>&gt;30</td>
<td>10</td>
</tr>
</tbody>
</table>

Score the wound size according to Table 3. Add the DFI - Wound Size Score and the DFI - General Parameters Score for the Total DFI Score. The individual DFI scores as well as the Total Diabetic Foot Infection Score should be noted in the subject’s source documentation and will also be captured in the CRF.

5.2.5 Microbiologic Assessment

The target ulcer will be cultured and samples obtained at baseline (Enrollment Visit - Day 0) and at all other study visits at which the investigator estimates that at least 0.05 gm of culturable material can be collected without significant disruption in the healing process.
Microbiological response will be assessed during data analysis according to the following definitions:

- Complete Response (pathogens not detectable or no available material to culture);
- Microbiologic Failure (Detection of one or more of the original pathogens present at Day 0);
- Colonization (Detection of new pathogens not detected at Day 0);
- Unevaluable (less than 3 days of study treatment or subject lost to follow-up).

In addition to the Central Lab findings; findings from the Research Lab may also be used in the analysis.

Corynebacterium, Propionibacterium, or Bacillus species will not be considered as infecting organisms, unless one of these 3 species is isolated in pure culture.

Specimens are to be obtained by wound biopsy technique (Appendix D) after wound cleaning and debridement. The sample is then placed into transport media and shipped to a central laboratory for quantitative analysis, species identification, and antibiotic susceptibility testing. In the event the site does not collect a sample, the reason why the procedure was not performed should be documented in the subject’s chart and the CRF.

After central laboratory analysis, the remainder of the sample will be frozen and batch shipped to the University of Arizona for further testing. Subject samples sent to the University of Arizona will be processed to extract DNA for sequencing. While DNA sequencing is being used in this study, the focus is not on the genetic information in the subject’s DNA; instead the sequence of interest is that of the bacteria infecting the subject. Total DNA will be isolated from samples, resulting in purified DNA sequence from both bacterial and human cells. For the purposes of this study, the human DNA is considered a contaminant, and will be selectively removed from the sample prior to sequencing. However, the purification of the bacterial DNA to remove contaminating human DNA is not perfect, and some human DNA will be sequenced (representing < 1% of the data). The contaminating human sequences will be removed from the dataset during data processing and will not be included in the data analysis. Furthermore, the DNA sequence information generated at the University of Arizona will be unlinked from any personally identifiable information. Data analysis will be limited solely to the bacterial sequence present in the subject’s wound and will entail identification of the species present, estimates of their abundance, and taxonomic classification based on 16s rRNA sequence. By removing human DNA prior to sequencing and not including any contaminating human sequence in the analysis, this study will not generate any useable or personally identifiable information about the subject’s genome thereby protecting the subject’s privacy and preventing inadvertent discovery based on their genome.

5.2.6 Clinical Laboratory

Clinical laboratory values will be compared to those obtained at the initial visit from Protocol PGN-1300 as part of the safety assessment for the study.

Clinical Laboratory specimens (blood, urine) will be collected at the final visit according to the instructions provided by LabConnect. The values to be assessed are as follows:
Clinical Chemistry:

Albumin, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, Bicarbonate, BUN, Calcium, Chloride, Cholesterol, Creatinine, CK, GGT, Glucose, LDH, Magnesium, Phosphorus, Potassium, Sodium, Total Protein, Triglycerides, Uric Acid, Globulin

Hematology:

WBC, RBC, Hemoglobin, Hematocrit, Platelet, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Abs Neutrophils, Abs Lymphocytes, Abs Monocytes, Abs Eosinophils, Abs Basophils

Urinalysis:

pH, Specific gravity, Bilirubin, Blood, Protein, Glucose, Ketones, Nitrites, Leukocyte esterase, Urobilinogen, Microscopic elements, White blood count, Red blood count

5.3 STUDY VISITS

5.3.1 Enrollment Visit (Day 0)

Before completing any procedures or assessments required for the Enrollment Visit that were not already completed as part of final Treatment Visit in protocol PGN-1300, informed consent should be obtained.

The subject’s complete medical history, concomitant medications, and demographics from protocol PGN-1300 will be reviewed, updated if needed, and entered in the CRFs for this trial. Unresolved adverse events from protocol PGN-1300 will also be added to the Medical History CRF for protocol PGN-1300X.

Subjects entering PGN-1300X on the same day as completing their last Treatment Visit in PGN-1300 do not need to repeat any procedures already conducted. Data from the procedures or assessments that were completed as part of the subject’s last Treatment Visit in protocol PGN-1300 will also be entered into the Enrollment Visit CRF for this trial.

The following procedures should be performed (either as part of the last Treatment Visit in protocol PGN-1300 or as part of the Enrollment Visit in PGN-1300X):

- Complete Physical
- Vital Signs
- Urine Pregnancy Test (females of childbearing potential)
- Wound Cleaning / Debridement
- Clinical Response
- Wound Assessment (DFI Scoring)
- Wound Measurement
- Wound Photography
- Microbiology Collection
For a subject to be eligible to be enrolled, they must have a Total Diabetic Foot Infection Score of at least 3. The DFI-General Parameter score must be at least 2 and the DFI-Wound Size Score must be at least 1.

After the Investigator has determined that a subject meets the eligibility criteria and the enrollment is complete, a copy of the completed Subject Eligibility CRF must be faxed to the Arkios (859.317.5990) on the day of enrollment. Arkios will acknowledge receipt of the fax via fax or email response. The Unique Subject Identification number assigned during the subject’s participation in protocol PGN-1300 will be used for this trial. No additional subject numbers will be assigned.

5.3.1.1 IP Dispensing, Wound Dressing, and Study Product Application

Once the subject is determined to be eligible, study product will be dispensed to the subject. Subjects will be instructed to apply the study product to their ulcer twice daily (morning and evening). Proper changing of the dressing will be demonstrated to the subject and the first treatment with the study product will take place at the Day 0 visit in the office to ensure that adequate instruction and demonstration is provided to the subject.

The first dressing will be applied by a study site professional who will not be conducting clinical assessments. **THOSE SITE PERSONNEL CONDUCTING CLINICAL ASSESSMENTS MUST REMAIN BLINDED AND THEREFORE SHOULD NOT BE INVOLVED WITH PRODUCT APPLICATION OR WOUND DRESSING CHANGES TO MAINTAIN THE BLIND.**

The details of the procedure will be explained to the study subject. At subsequent treatment visits, changing of the dressing and application of the treatment will be done by the subject under observation by site staff (not conducting clinical assessments) to ensure adequate understanding of the procedure. The subject should be instructed to bring their product with them to all subsequent study visits. If the subject forgets their study product, another jar may be assigned to the subject and retained on site for use at future visits. The subject’s initials and the date assigned should be noted on the jar. This additional jar should not be given to the subject to take home. It should be stored separately from unassigned study product but in accordance with the storage requirements per protocol.

Wound dressings need to be changed twice per day (usually in the morning and again before bedtime). The fresh dressing should be applied to a thoroughly cleaned wound. The contact layer is a sterile gauze pad that has been cut to fit inside the wound. Lay the cut gauze pad on a clean, hard surface. Hold the gauze in place with a clean finger. Using a sterile tongue depressor, remove a small amount of treatment gel from its container and spread it onto the gauze. Only use enough treatment gel to provide a thin layer (~the thickness of a dime) of gel over the entire surface of the gauze pad.

Place the gel-coated side of the gauze pad in contact with the wound. When the gauze pad is placed in the wound, if the pad does not fill the wound to the surface of the wound, cut additional sterile gauze pads to the size of the wound and place in the wound until the wound cavity has been filled. Then place a gauze pad that is larger than the wound over the wound and secure to the skin with tape.

This fresh dressing should remain in place approximately 12 hours before being changed. At dressing change, remove the cover dressing and if necessary the additional layers of gauze in
the wound above the contact layer. Gently lift the contact layer to determine how adherent it is to the wound surface. If the contact layer is adherent, soak the gauze in warm saline or water for several minutes before removing it.

5.3.2 Treatment Visits

Day 3 (±1), Day 10 (±1) and Day 14 (±1) will be considered the Treatment Visits.

The following procedures/assessments are performed at each Treatment Visit:

- Vital Signs
- Wound Cleaning / Debridement (as required)
- Clinical Response
- Wound Assessment (DFI Scoring)
- Wound Measurement
- Wound Photography
- Microbiology Collection (if at least 0.05g can be obtained)
- Wound Dressing and Study Product Application (by subject / under observation)
- Adverse Event collection
- Concomitant Medications

Subjects will return to the investigational site for their first treatment visit on Day 3 (±1) and may return for subsequent Treatment Visits on Day 10 (±1) and Day 14 (±1) depending on their clinical response to treatment. The Day 14 Visit or the visit at which the subject discontinues early will be the End of Treatment Visit.

The investigator will immediately discontinue the subject and consider the initiation of an appropriate, alternative (non-study) therapy in the following instances:

- Clinical Treatment Failure at any visit - one or more signs or symptoms of the infection are worsening or the overall signs and symptoms of infection have not changed since the previous visit, or
- Recurrence at any visit - previously cured or improved infection shows worsening of signs or symptoms of infection, or
- Partial Response (at the Day 14 Visit) - most, but not all, signs and symptoms of infection have improved or resolved.

The following visit windows will be allowed:

- Visit Day 3: + 1 day
- Visit Day 10: ± 1 day
- Visit Day 14: ± 1 day

When utilizing visit windows, the site must be able to adhere to the shipping and processing requirements for the microbiology sample.

At each visit, the dressing will be removed using great care to avoid disrupting the wound site. The wound site should be gently cleaned using saline irrigation and patted dry. Subjects should follow the same procedure when changing the dressing at home.
At each visit, the wound site should be evaluated, photographed, and measured. Healing of the wound is defined as full epithelialization with absence of wound drainage.

5.3.3 Two Week Follow-up Visit

The Two Week Follow-up Visit should be scheduled to occur two weeks (± 1 day) after the End of Treatment Visit.

The following procedures/assessments are performed at the Two Week Follow-up Visit:

- Complete Physical
- Vital Signs
- Blood and Urine Sample for Clinical Laboratories
- Clinical Response
- Wound Assessment (DFI Scoring)
- Wound Measurement
- Microbiology Collection (if at least 0.05g can be obtained)
- Photography
- Adverse Event collection
- Concomitant Medications

All subjects will return for the Two Week Follow-up Visit for final efficacy and safety assessments.

Subjects will return for the Two Week Follow-up Visit approximately two weeks after the Day 14 Visit for final efficacy and safety assessments (unless discontinuing from the trial to begin an alternative [non-study] treatment or in circumstances requiring immediate withdrawal). For subjects choosing an alternative (non-study) treatment or in circumstances requiring immediate withdrawal, the final Two Week Follow-up Visit procedures should be conducted at the End of Treatment Visit.
### 5.3.4 Schedule of Evaluations

<table>
<thead>
<tr>
<th></th>
<th>ENROLLMENT</th>
<th>TREATMENT VISITS</th>
<th>FOLLOW-UP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 3 (+1)</td>
<td>Day 10(^1) (±1)</td>
</tr>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History &amp; Demographics</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete Physical</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital Signs</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Clinical Laboratory Safety Measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPT (females-childbearing potential)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound Cleaning / Debridement</td>
<td>X</td>
<td>X(^2)</td>
<td>X(^2)</td>
</tr>
<tr>
<td>Clinical Response</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Wound Assessment (Total DFI Score)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Wound Measurement</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Wound Photography</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Microbiology Collection</td>
<td>X</td>
<td>X</td>
<td>X(^3)</td>
</tr>
<tr>
<td>Study Product Dispensed</td>
<td>X</td>
<td>X(^2)</td>
<td>X(^2)</td>
</tr>
<tr>
<td>Adverse Event Notation</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Concomitant Medication Notation</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

\(^1\) If was not a clinical treatment failure or recurrence at the previous visit, visit is performed
\(^2\) If needed
\(^3\) If at least 0.05g culturable material can be obtained without significant disruption to the healing process
5.4 CRITERIA FOR DISEASE EVALUATION

The target ulcer will be evaluated for:

- Clinical Response
- Wound Assessment (DFI Scoring)
- Microbiologic Response (as part of data analysis)

5.5 RECORDING OF ADVERSE EVENTS AND CONCOMITANT MEDICATIONS

All adverse events occurring while the subject is receiving protocol therapy and during the follow-up period will be recorded. During the follow-up period, resolution of adverse events present at the final treatment day as well as any new adverse events will be recorded. All medications taken while the subject is receiving protocol therapy will be recorded. Completion of any medication regimen ongoing during the treatment period will also be recorded.

5.6 CONCOMITANT MEDICATIONS

All medications necessary for the subject's well-being will be allowed with the exception of those noted in the Exclusion Criteria. All concurrent medication use must be documented in the subject's chart.

The use of local anesthesia for wound biopsies is allowed and should be documented as a concomitant medication.

6 ADVERSE EVENTS

6.1 GENERAL INFORMATION

An adverse event (AE) is any untoward medical occurrence or clinical investigation in a subject administered a pharmaceutical product that does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign, symptom, or disease temporally associated with the use of a study product, whether or not it may be considered to be related to the study product. This includes any side effect, injury, toxicity, or sensitivity reaction, and may include a single symptom or sign, a set of related symptoms or signs, or a disease. An AE may also be any laboratory abnormality judged to be clinically significant by the Investigator or Subinvestigator(s).

Throughout the course of the study, every effort should be made to remain alert to possible AEs. Subjects should be encouraged to report AEs spontaneously or in response to general, non-directed questioning. In the event of an AE, the first concern should be for the safety of the subject. If necessary, appropriate medical intervention should be provided, and the study product discontinued (Section 3.3 / 7.2).

For each AE reported by the subject, the Investigator should obtain all information required to complete the AE CRF including the following: description, start date and time, stop date and time (or noted as ongoing), severity, seriousness, relationship to study product, action taken, and outcome.
Hospitalizations or procedures that are scheduled prior to enrollment in the study must be recorded in the subject’s medical history but will not constitute a serious AE (SAE – see below).

6.2 RECORDING OF ADVERSE EVENTS

All AEs, regardless of severity, seriousness, or presumed relationship to study product, must be recorded using medical terminology in the source document and on the CRF. Whenever possible, a diagnosis should be given when signs and symptoms are due to common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as “upper respiratory infection”). Investigators must record their opinion concerning the relationship of the AE to study therapy on the AE CRF.

Table 4. Adverse Event Grading

<table>
<thead>
<tr>
<th>Severity</th>
<th>Grade</th>
<th>Scale</th>
<th>Further Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>Mild</td>
<td>Awareness of sign, symptom, or event but easily tolerated</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Moderate</td>
<td>Discomfort enough to cause interference with usual activity and may warrant intervention</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Severe</td>
<td>Incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Life-threatening</td>
<td>Immediate risk of death</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Seriousness</th>
<th>Grade</th>
<th>Scale</th>
<th>Further Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Not serious</td>
<td>All other AEs not meeting the criteria below will be graded as &quot;Not Serious&quot;.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Serious</td>
<td>A serious AE (SAE) is any untoward medical occurrence that at any dose results in or is:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• death</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• life-threatening</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• insubject hospitalization or prolongation of existing hospitalization</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• persistent or significant disability/incapacity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• a congenital anomaly/birth defect.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• an event that may require intervention to prevent any one of the other outcomes listed above (based on medical judgment).</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Hospitalizations or procedures that are scheduled prior to enrollment in the study must be recorded in the subject’s medical history, and do not constitute an SAE.

<table>
<thead>
<tr>
<th>Expectedness</th>
<th>Grade</th>
<th>Scale</th>
<th>Further Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Expected</td>
<td>All other AEs not meeting the criteria below will be considered &quot;Expected&quot;.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Unexpected</td>
<td>An unexpected AE is any AE that is not identified in nature, severity, or frequency in the current Investigator’s Brochure or product information.</td>
<td></td>
</tr>
</tbody>
</table>

| **Relationship to the Administration of the Study Product** |
|---|---|---|
| 0 | Not related | Applies to those AEs that, after careful medical consideration at the time they are evaluated are clearly and incontrovertibly due to extraneous causes (disease, environment). |
| 1 | Possibly related | Applies to those AEs which, after careful medical consideration at the time they are evaluated appear unlikely to be related to administration of the study product, but a relationship cannot be ruled out with certainty |
| 2 | Probably related | Are felt with a high degree of certainty to be related to administration of the study product (i.e., follows reasonable temporal sequence, could not be reasonably explained by known characteristics of subject’s clinical state, environmental or toxic factors. |

| **Study Product Action Taken In Response** |
|---|---|---|
| 0 | None | |
| 1 | Study product interrupted | |
| 2 | Study product discontinued | |

| **Outcome** |
|---|---|---|
| 0 | Recovered without sequelae | Sequelae are defined as any disorder or pathological condition that results from preceding disease or accident. |
| 1 | Recovered with sequelae | |
| 2 | Not yet recovered | Not yet recovered indicates that the subject has not reached a stable clinical endpoint. |
| 3 | Death | Death indicates that the subject died. |
| 4 | Unknown | Unknown means that information is unavailable at the time of reporting incident or that subject was lost to follow-up. |

**6.3 REPORTING SERIOUS OR UNEXPECTED ADVERSE EVENTS**

ANY SERIOUS OR UNEXPECTED CLINICAL EVENT, INCLUDING DEATH DUE TO ANY CAUSE THAT OCCURS DURING THIS STUDY, WHETHER OR NOT RELATED TO THE STUDY TREATMENT, MUST BE REPORTED IMMEDIATELY (WITHIN 24 HOURS) TO THE SPONSOR OR THEIR DESIGNATED REPRESENTATIVE. This verbal or faxed report must be followed by a written report signed by the investigator within 48 hours. Written notification should include SAE start and stop dates and times, study product dosage and treatment history, description of SAE with applicable laboratory test results, list of concomitant medications, relatedness of study product to SAE, autopsy report (if applicable), and any other relevant information such
as medical history. A reporting form and general instructions for documenting event is provided in the Study Binder.

An SAE Report should be prepared containing as much available information concerning the event as possible so that a written report can be filed with appropriate regulatory authorities. Any SAE follow-up information requested by the Sponsor or the Sponsor’s designee should be provided in a timely manner. All investigators will be notified of the occurrence of product-related serious and unexpected AEs. The Investigator must notify the relevant Institutional Review Board (IRB)/Ethics Committee of any product related SAEs.

<table>
<thead>
<tr>
<th>Contact in Case of an SAE:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gary Pekoe, PhD</td>
</tr>
<tr>
<td>Tel: 757 618-2300 (cell)</td>
</tr>
<tr>
<td>Fax: 859 317-5990</td>
</tr>
</tbody>
</table>

6.4 FOLLOW-UP OF ADVERSE EVENTS

All AEs (both serious and non-serious) must be followed until resolution or until a stable clinical endpoint is reached. All measures required for AE management and the ultimate outcome of the AE must be recorded in the source document.

7 TERMINATION AND SUBJECT DISCONTINUATION

7.1 STUDY OR SITE TERMINATION

A study may be halted or a site may be terminated if the Sponsor, Investigator, Clinical Monitor, or FDA officials discover conditions arising during the study that warrant such action. This action may be taken after consultation among the appropriate parties.

The following are examples of conditions that may halt the study:

- The discovery of an unexpected, serious, or unacceptable risk to the subjects enrolled;
- A decision by the Sponsor to suspend or discontinue product evaluation or development.

The following are examples of conditions that may result in site termination:

- Failure of the Investigator to enroll subjects into the study at an acceptable rate;
- Failure of the Investigator to comply with the protocol;
- Failure of the Investigator to comply with pertinent FDA regulations;
- Submission by the Investigator of knowingly false data.

Study termination and follow-up will be performed in compliance with 21 CFR 312.50 and 21 CFR 312.56.
7.2 SUBJECT DISCONTINUATION

All subjects will be encouraged to complete the study although a subject may voluntarily withdraw from the study at any time. A subject may be discontinued from the study at any time by the Investigator or the Sponsor for the following reasons:

- If a subject suffers an AE that, in the judgment of the Investigator, Sponsor, or Medical Monitor, presents an unacceptable risk to the subject;
- If a subject develops an intercurrent illness or complication that is not consistent with the protocol requirements or justifies withdrawal from the study;
- If the subject’s ulcer is considered a Clinical Treatment Failure, Recurrence, or Partial Response (Day 14) according to the protocol definition;
- If the subject withdraws consent;
- If the subject develops an infection (other than that under study and requiring systemic antibiotic treatment).

The Investigator should immediately discontinue a subject from the study if it is learned at any time during the course of treatment that their eligibility for the trial has changed.

If a subject is discontinued or withdraws from the study, the subject must have a final close-out evaluation. All procedures required for the Two Week Follow-up Visit should be conducted if possible. Procedures required for the last evaluation should be performed at the time of subject discontinuation and the Investigator must document the reason for subject discontinuation or withdrawal on the subject discontinuation page of the CRF. A follow-up phone call will be made to the subject 4 weeks after the date of discontinuation or withdrawal in order to determine resolution of any outstanding AEs or to record any new AEs. All AEs will be followed up by phone or in person until resolution or until a stable clinical endpoint is reached.

In the event a subject discontinues due to an AE, the Investigator must notify the Project Director or Medical Monitor by telephone within 48 hours of discontinuation (see cover page of Protocol for contact information).

8 DATA COLLECTION, PROCESSING AND ANALYSIS

8.1 DATA COLLECTION AND PROCESSING

The site will complete the Subject Eligibility CRF to verify that each subject meets the inclusion/exclusion criteria for this study. This CRF will be provided to Arkios (via fax or email) the day of enrollment.

All subjects screened will be documented on a site specific Subject Identification Code List that is retained only by the site to link subjects to their participation in the trial. The log will capture the following information: Investigator’s Name, Site Number, Protocol Number, Subject’s Name, unique Subject ID, Subject’s date of birth, Subject’s gender.

Subject enrollment will be documented in a screening and enrollment log at the study site that will contain screening and enrollment information for both protocol PGN-1300 and PGN-1300X. The log will be completed electronically and printed when updated. This log will capture the following information: Investigator’s name, Investigative site number,
Unique Subject ID, and date of enrollment. This log will be provided to Arkios at regular intervals.

A case report form (CRF) will be provided for each study subject. Data collected through the completion of experimental procedures required by this protocol will be recorded in the subject's chart as source documentation. All source documentation should be accurate, legible, contemporaneous, original, and attributable. Appropriate data will then be transcribed onto the CRF. All laboratory results (safety and microbiology) will be collected via data transfer directly from labs conducting the analysis and will not be recorded on the CRFs.

All entries to the CRF must be made in indelible black or blue ink. All corrections must be made by drawing a single line through the error and entering the correct data as close as possible to the original entry without obscuring any data. The correction should be initialed and dated by the person making the correction. The Investigator remains responsible for the accuracy and adequacy of all data entered on CRFs.

Data will be monitored as described in Section 9.6. Under direction of the clinical monitor, CRFs will be retrieved and further processed for data entry and analysis. A copy of each CRF page must remain at the investigative site in the appropriate subject's CRF binder.

Upon further data processing, queries may be generated and sent to the Investigator for clarification or correction. The Investigator will address any queries and forward resolutions as directed by the clinical monitor.

8.2 STATISTICAL ANALYSIS

8.2.1 Description of Statistical Methods

This is an open-label, non-randomized, non-controlled, multi-site follow-up study to evaluate the safety and efficacy of topical PluroGel PN for up to 14 days in subjects with mild diabetic foot ulcer infections who failed treatment, had a recurrence, or partial response (Day 14) in the companion double-blinded (evaluator-blind), randomized, vehicle-controlled study preceding it. The primary efficacy outcome will be complete clinical response at the Two-Week Follow-up Visit. Safety outcomes will be subject disposition, AEs, development of drug resistance, clinical laboratory parameters, physical examination findings, and vital signs.

8.2.2 Analysis Populations

8.2.2.1 Per Protocol Population

The Per Protocol Population will be all subjects with who were not replaced and who did not have any major protocol violations.

8.2.2.2 All Treated Population

The All Treated Population will be all subjects who received treatment.
8.2.2.3 Efficacy Populations

The Primary Efficacy Population will be all subjects in the All Treated Population with who have at least one baseline efficacy parameter recorded. The secondary efficacy population will be the Per Protocol Population. In addition, the All Treated populations from both the open-label trial and the double-blind, randomized trial preceding it may be pooled for a combined analysis. Additional details will be provided in a comprehensive Statistical Analysis Plan (SAP) that will be place prior to data analysis.

8.2.2.4 Safety Population

The Safety Population will be the All Treated Population.

8.2.3 Randomization

All subjects will receive PluroGel PN on an open-label basis.

8.2.4 Sample Size and Power

Sample size will be dependent on the number of treatment failures in the preceding double-blind, randomized trial.

8.2.5 General Statistical Analysis Methods

There will be no comparison group for the open-label trial; therefore, statistical analyses will not be applicable. Any comparisons involving combined data from both the open-label and preceding double-blind, randomized trial will be fully detailed in the SAP.

All data collected will be listed. Tables will show data summarized by study visit. Data from unscheduled visits will be listed but not summarized or analyzed. Subjects with incomplete data will be included in summaries for which they have data available. Categorical variables will be summarized as frequencies and percentages in each category. Continuous variables will be summarized as numbers of subjects, means, standard deviations, medians, and ranges. All programs for data output and analyses will be written in SAS® version 9.2 or higher (SAS Institute, Inc., Cary, NC).

8.2.6 Baseline Characteristics

Baseline characteristics will be listed and summarized and will include inclusion/exclusion criteria, basic demographics (e.g., age, sex, race/ethnicity), height/weight, medical history and baseline screens (e.g., pregnancy).

8.2.7 Study product Dispensation and Compliance

Details of study product dispensation and compliance will be listed. Compliance, daily dose by study week, and duration of treatment will be summarized.

8.2.8 Concomitant Medications

Concomitant medications will be coded to generic terms using the World Health Organization Drug Dictionary (WHOdd). The listing will include dosing date, WHOdd drug class, WHOdd preferred drug name, reported drug name, start and stop date, and whether the drug was taken during study product treatment. The summary table will consist of numbers and percentages of subjects reporting each WHOdd drug class and preferred name within
drug class.

8.2.9 Efficacy Analysis

Efficacy parameters will be listed for all subjects and summarized for subjects in the efficacy population(s). The primary efficacy analysis will be descriptive only; i.e., there will be no statistical testing. The primary efficacy outcome will be complete clinical response, as described in Section 3.1.1 above, in the Primary Efficacy Population at the Two-Week Follow-up Visit.

Secondary efficacy outcomes will be:

- Complete or partial clinical response in all efficacy populations at all post-baseline visits other than the Two-Week Follow-up Visit.
- Change in microbiological response in all efficacy populations at all post-baseline visits.
- Change in Total DFI Score in all efficacy populations at all post-baseline visits.
- Rate of Recurrence at the Two Week Follow-up Visit

8.2.10 Safety Analysis

Safety parameters will be listed for all subjects and summarized for subjects in the Safety Population. Safety analysis will involve the examination of incidence and reasons for discontinuation; incidence of AEs and their relationship to the study product; development of drug resistance; and changes in clinical laboratory results, vital signs, and physical examination findings. Each of these outcomes will be detailed in the SAP.

9 STUDY ADMINISTRATION

9.1 INFORMED CONSENT

Written informed consent must be obtained from each subject or their guardian in accordance with FDA regulations set forth in Part 50 of Title 21 of the Code of Federal Regulations. Informed consent must be obtained prior to any study related procedures being performed.

The subject and person explaining informed consent must sign the current IRB-approved version of the consent form. A copy of the signed consent form will be given to the subject. The date that consent was obtained will be recorded on the CRF as well as in the subject's chart.

A copy of the IRB-approved version of the consent form will be provided to the Sponsor. Original signed consents must be maintained at the site and be made available for inspection, as appropriate.

A sample consent form is provided separately from protocol.

9.2 STUDY DOCUMENTATION

A study binder must be maintained at the investigative site and must contain the documents identified in Appendix A. A final signed copy of the Investigator Agreement should be
retained separately from the study binder. The Sponsor or its representative will provide the initial Study Binder to the site.

According to Federal Regulations (21 CFR 312), all records related to this clinical trial must be retained by the Investigator for at least 2 years after the last approval of a marketing application and until there are no pending or contemplated marketing applications OR until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. The Sponsor will inform the Investigator as to when these documents no longer need to be retained. These documents must be stored in a safe location and be available in the event of a regulatory audit.

Study records that must be retained include, but are not necessarily limited to: subject charts, CRFs, product disposition records, essential documents identified in Appendix A, and study reports.

9.3 CONFIDENTIALITY AND PUBLICATION

The anonymity of subjects participating in this study must be maintained. Subjects will be identified by their assigned subject number which includes their initials (e.g. 02-XYZ-001) in all written communications between the Investigator and Sponsor. Documents that are not submitted to the Sponsor and that identify the subject (i.e. signed informed consent) will be made available to the Sponsor or regulatory authorities for inspection, but will be maintained in confidence.

All study related information provided by the Sponsor to the Investigator and not previously published, including but not limited to the study product identity, the Investigator's Brochure, the study protocol, verbal and written communication, CRFs, assay methods and scientific data, will be considered confidential. In addition, all information developed during the conduct of the clinical investigation of the study agent is also considered confidential. Neither the Investigator nor any of his/her employees or agents shall disclose or use this information for any purpose other than the performance of the clinical study. Such information shall remain the confidential and proprietary property of the Sponsor, and disclosure to others will be limited to other physicians who are conducting studies with the same study product, the IRB and the FDA except by prior written permission of the Sponsor or its agents. At such time that information becomes widely and publicly available through no fault of the Investigator, the obligation of nondisclosure toward that particular information will cease.

Initial publication of the results of this study will be of a cooperative nature that may include authors representing the Sponsor, Investigator(s), and collaborating scientists. Independent publications by involved individuals may follow. At least 60 days prior to expected submission to the intended publisher or meeting committee, the Investigator must submit a copy of the desired presentation (oral or written) or publication manuscript to the Sponsor. This review period may be shortened upon mutual consent where circumstances require expeditious review. The Sponsor reserves the right to require modification of any publication, presentation or use by the Investigator if such activity may jeopardize a patent application, an existing patent or other proprietary rights. The Sponsor shall determine order of authorship of any publication combining all clinical results of this trial.
9.4 INSTITUTIONAL REVIEW BOARD

The final protocol and proposed informed consent document must be submitted to an Institutional Review Board (IRB) that is constituted and operates in accordance with Part 56 of Title 21 of the Code of Federal Regulations. The IRB will provide the Investigator with a written decision regarding the conduct of the study at that site and a copy of the document will be forwarded to the Project Director. The study will not be initiated and no subjects will be enrolled until the appropriate documentation of IRB approval of the study protocol and the informed consent has been received.

Substantive modifications to the protocol will be submitted to the IRB for approval (Section 9.8). These modifications may be implemented only after IRB written approval has been received and forwarded to the Project Director. Administrative changes to the protocol such as a change that has no effect on the conduct of the study or risk to the subject should be submitted to the IRB for review, but formal approval is not required.

Any other written information that will be given to the study subjects as well as any advertisements for subject recruitment, if used, must be submitted to the IRB for approval prior to implementing these documents.

The Investigator will make appropriate and timely reports to the IRB as required by applicable government regulations and IRB policy. In addition to progress reports, all known information regarding serious and unexpected AEs, whether observed at their clinical site or at another site participating in a clinical investigation with the study product, will be reported to the IRB. It is the Sponsor and/or its representative’s responsibility to inform the Investigator of serious and unexpected events observed at other investigational sites.

It is the Investigator’s obligation to provide the Sponsor and/or its representatives with copies of all study-related correspondence with the IRB in a timely fashion and to retain originals in a file. This IRB correspondence file will be made available as requested to appropriate representatives for monitoring or quality assurance review and to FDA representatives during site audits.

9.5 ETHICAL STUDY CONDUCT

This study is to be conducted in accordance with the ethical principles that originate in the Declaration of Helsinki.

9.6 STUDY MONITORING

So that the study may be adequately monitored, the Investigator will cooperate in providing the Sponsor’s representatives with all study documents (e.g. subject charts and study files) and responding to inquiries that may arise as a result of the document review.

Review of these documents will usually occur during a routine monitoring visit, but may also be required during a visit by a quality assurance auditor. The Investigator will also provide access to these records to FDA representatives if and when requested. The Sponsor reserves the right to terminate the study if access to source documentation of work performed in this study is denied to the Sponsor or FDA representatives.
9.7 QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor or designee will assure the accuracy of data, the selection of qualified Investigators, appropriate study centers and review protocol procedures with the Investigators and associated personnel prior to the study and during periodic monitoring visits. The Sponsor or a designee will review CRFs for accuracy and completeness during on-site monitoring visits and after their return from the clinical site. Any discrepancies will be resolved with the Investigator as appropriate.

The study will be monitored by the Sponsor or its representatives as required by the FDA using the following methods:

- frequent telephone contacts
- periodic site visits
- review of original subject records, CRFs, product accountability and storage, and general study documentation.

9.8 PROTOCOL AMENDMENTS

Neither the Investigator nor the Sponsor will modify this protocol without obtaining the concurrence of the other. All protocol amendments must be signed and dated by the Investigator prior to implementation of the amendment. The Sponsor will submit protocol modifications to the FDA and other Regulatory Agencies as required. The Investigator is responsible for notifying the IRB of changes. Substantive changes will require IRB approval, such as changes in experimental procedures that affect subject safety, changes in dosage or study treatment, changes in assessment parameters, or changes in subject eligibility criteria.

In situations requiring a departure from the protocol, the Investigator or other physician in attendance will contact the Sponsor or designee by fax or telephone. If possible, this contact will occur before implementing any departure from protocol. In all cases, contact with the Sponsor or designee must be made as soon as possible in order to discuss the situation and agree on an appropriate course of action. The CRF and source document must describe any departure from the protocol and the circumstances.
## APPENDIX A  ESSENTIAL STUDY DOCUMENTS

<table>
<thead>
<tr>
<th>DOCUMENT</th>
<th>LOCATION</th>
<th>COLLECTION TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>The following documents should be maintained by the site and will be obtained for the sponsor regulatory files as indicated during the trial.</td>
<td>ARKIOS</td>
<td>SITE</td>
</tr>
<tr>
<td>Confidentiality Agreement</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Investigator Agreement (filed separately)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Investigator Financial Disclosure</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>IRB Membership Roster</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>IRB Approval Letter for Protocol</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>IRB Approval for Informed Consent Form (ICF) and Revision(s)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>IRB-Approved ICF and Revision(s)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Completed 1572 or Equivalent and Revision(s)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Signed Protocol and Revision(s)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Investigator’s Brochure and Revision(s)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Sample Case Report Forms and Revision(s)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Decoding Procedures for Blinded Trials</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Master Randomization List</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>CV &amp; Medical Licenses (Investigator/Subinvestigator)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Laboratory Certification (and updates)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Laboratory Normal Ranges (and updates)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Laboratory Director’s CV</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Retention Sample Records</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Pre-Study Visit Report</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Trial Initiation Monitoring Report</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Monitoring Reports</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Signed Protocol Amendment(s)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>IRB Approval Letter for Protocol Amendment(s)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Serious Adverse Event (SAE) Reports (all subjects)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>IRB Notifications of SAEs (site-specific subjects)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Interim/Annual Reports from Investigator to IRB</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Final Report by Investigator/Institution to IRB</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Site Personnel Signature Log</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>IRB Close-Out Letter</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Subject Identification Code List</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Subject Screening/Enrollment Log</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Monitor Sign-In Log</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Investigational Product Inventory Log</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Investigational Product Shipment Documentation</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Correspondence (study-related)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Telephone Log</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Note: x signifies that information should be provided whenever there is an update
APPENDIX B  PROCEDURE FOR DIGITAL PHOTOGRAPHY

Preparation

- For the sake of consistency, try to use the same camera, flash, lighting, and subject position.
- Position the subject so there is a seamless background (rich blue is the preferred color) and no facial features are identifiable.
- Set your camera resolution to at least 640 x 480 pixels (higher is better).
- With a marker (a Sharpie provides good legibility) print the subject’s enrollment number, initials and date on an adhesive strip/ledger that can be place on the subject’s foot for identification purposes.
- Assure that the entire site is in your viewfinder and that extraneous objects are not (e.g. subject gowns, crumbled blue pads, loose sheets).
- Take your shots after focusing on the printed information.

Photography

- Post-debridement photos should be taken at each visit.
- The file naming convention should identify the protocol number, PI last name, subject unique identifier, visit date (yyyy.mm.dd), and visit type.
- Try to keep a fairly consistent image size (relative to the viewfinder or LCD screen) for a particular subject.
- Strongly consider using your camera’s macro setting, if it has one. The macro setting enables you to take close photos in focus (see your individual camera’s instructions, but in general the macro setting is a “flower” icon).
- Get as close to the wound as possible while maintaining focus, keeping the identification strip/ledger in the frame.

Transferring Digital Images

Photos should be transferred to Arkios upon request. These may be transferred in a variety of formats: uploading to our site, burning a CD, or e-mailing.
EXAMPLES OF DIGITAL WOUND PHOTOGRAPHY

CORRECT

![Correct Image](image1)

Neutral background
Image size is good – fills most of the photo
Wound identified with subject’s initials, enrollment number, and date.

INCORRECT

![Incorrect Image](image2)

Focus on wound is pretty good but can see subject’s face in the background
Wound is not in focus and does not have any identifying information (subject initials, date, etc)
APPENDIX C  IDSA DIABETIC FOOT INFECTION DEFINITIONS

The Infectious Disease Society of America defines a mild diabetic foot infection as mild, moderate, or severe based on the following guidelines:

Infection present, as defined by the presence of at least 2 of the following items:

- Local swelling or induration
- Erythema
- Local tenderness or pain
- Local warmth
- Purulent discharge (thick, opaque to white or sanguineous secretion)

**Mild**
Local infection involving only the skin and the subcutaneous tissue (without involvement of deeper tissues and without systemic signs as described below). If erythema, must be \( >0.5 \) cm to \( \leq 2 \) cm around the ulcer. Exclude other causes of an inflammatory response of the skin (eg, trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, venous stasis)

**Moderate**
Local infection (as described above) with erythema \( >2 \) cm, or involving structures deeper than skin and subcutaneous tissues (eg, abscess, osteomyelitis, septic arthritis, fasciitis), and no systemic inflammatory response signs (as described below)

**Severe**
Local infection (as described above) with the signs of systemic inflammatory response signs (SIRS), as manifested by \( \geq 2 \) of the following:

- Temperature \( >38^\circ\text{C} \) or \( <36^\circ\text{C} \)
- Heart rate \( >90 \) beats/min
- Respiratory rate \( >20 \) breaths/min or \( \text{PaCO}_2 \text{ <32 mm Hg} \)
- White blood cell count \( >12\,000 \) or \( <4000 \) cells/\( \mu\text{L} \) or \( \geq 10\% \) immature (band) forms

**Diabetic Foot Infection-General Parameters Score (Items Comprising the Diabetic Foot Infection-General Parameters Score and the Method for Scoring Each)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purulent discharge</td>
<td>Absent = 0  Present = 3</td>
</tr>
</tbody>
</table>

**Signs and symptoms of inflammation**

<table>
<thead>
<tr>
<th></th>
<th>Absent = 0  Mild = 1 Moderate = 2 Severe = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpurulent discharge</td>
<td></td>
</tr>
<tr>
<td>Erythema</td>
<td></td>
</tr>
<tr>
<td>Induration</td>
<td></td>
</tr>
<tr>
<td>Tenderness</td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td></td>
</tr>
<tr>
<td>Local warmth</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX D     MICROBIOLOGY PROCEDURES

Tissue and Biopsy Sample Collection:

- Using aseptic technique, collect sufficient tissue (avoiding necrotic areas). The use of local
  anesthesia is acceptable and should be documented as a concomitant medication. Collect 3 to 4
  mm biopsy samples when possible (a 4 mm biopsy punch will be provided). If the ulcer area is
  too small for a 4 mm biopsy punch, use an alternative sterile method (eg curettage) to collect as
  much tissue as possible without causing disruption of the healing process. If a minimum of 0.05g
  can’t be obtained, the wound should be considered as “no available material to culture”.
- Place in a sterile container provided in the LabConnect specimen collection kit. Keep tissues
  moist with a small amount of sterile saline to preserve organism viability.
- Transport samples under refrigerated conditions using the LabConnect specimen collection kit
  and following the provided instructions.

Tissue and Biopsy Sample Processing (Quantitative Culture):

- Record the weight of the specimen.
- For soft tissues, use the Stomacher lab blender to grind tissues.
- If the tissue is hard (e.g. bone, skin) add it to 0.5 ml of broth culture fluid (BHI) and vortex for
  20-30 seconds.
- After grinding or vortexing, remove sample from bag or tube using a sterile pipette.
- Using a .001 UL calibrated inoculation loop, place a drop of specimen onto each BAP, CHOC
  agar plate, BHI broth. Streak the plate to obtain isolated colonies.
- Prepare a gram stain by placing a drop of specimen on a slide and spreading it to make a thin
  preparation.
- An aliquot of the remainder of the specimen (~500µl) will be stored at LabConnect at -80ºC for
  future testing at the University of Arizona.
- If sufficient specimen is available, a portion will be saved in the refrigerator for up to 7 days for
  possible further testing.

Isolation, Identification and Susceptibility Testing and Reporting:

- Plates should be screened daily for growth. If growth is noted in the BHI broth, a gram stain
  should be performed and the broth sub-cultured to the appropriate media. If growth is noted on
  the original plates, subcultures should be performed and identification & susceptibility panels set
  up from pure isolates.
- Plates that were sub-cultured from the BHI should be examined for purity and for different
  colony types. Identification and susceptibility panels should be set up on these isolates.
  Susceptibilities are performed on all organisms that may grow from the quantitative tissue
  cultures. The only drugs tested for susceptibilities for this Clinical Trial are Nitrofurantoin and
  Polymixin B. These two drugs are to be tested for any and all organisms that grow for this
  clinical trial.
- All specimens should be kept for 72 hours before being discarded. If all media is negative after
  72 hours, the culture should be signed out "NO GROWTH".
Calculation of Final Colony Count:

- Count the number of colonies on the plate; for the cultures that have 10, 100, and 1000 dilutions choose the plate that has between 30 and 300 colonies.
- For Tissues that weighed \( \geq 0.1g \) use the formula: Colony Count \( \times 10 \times 1000 \times \) dilution of plate that you counted colonies from divided by weight of the tissue.
- For tissues that weighed < 0.1g use the formula: Colony Count \( \times 10 \times 1000 \) divided by weight of the tissue.

Results and Interpretation

- Report total count per gram of tissue. Identify and perform antimicrobial susceptibility testing on organisms when count is > \( 10^5 \) organisms / gram.
- The presence of > \( 10^5 \) organisms per gram of tissue is alleged to represent bacterial infection as opposed to colonization of the sample.