A Prospective, Randomized, Controlled Trial Comparing Cytal® Wound Matrix 1-Layer to Standard of Care (SOC) in the management of Diabetic Foot Ulcers (DFUs)

Unique Protocol ID: CR2017-006

NCT03626623

Version 3.0

27 August 2019

A Prospective, Randomized, Controlled Trial Comparing Cytal® Wound Matrix 1-Layer to Standard of Care (SOC) in the management of Diabetic Foot Ulcers (DFUs)

CR2017-006

ACell, Inc.

6640 Eli Whitney Drive
Columbia, MD 21046

Version 3.0

27 August 2019

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABI	Ankle Brachial Index
AE	Adverse Event
Alb	Albumin
BMI	Body Mass Index
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CMP	Complete Metabolic Panel
CMS	Centers for Medicare and Medicaid Services
CRF	Case Report Form
DFU	Diabetic Foot Ulcer
DFS-SF	Diabetic Foot Ulcer Scale- Short Form
DM	Diabetes Mellitus
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
HbA1C	Glycated Hemoglobin Level
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
KATZ ADL	Katz Index of Independence in Activities of Daily Living
PAB	Pre-albumin
PAD	Peripheral Artery Disease
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SF	Short Form
SF-20	The SF-20® Health Survey
SID	Subject Identification Number
SOC	Standard of Care
UADE	Unanticipated Adverse Device Effect
VAS/VPS	Visual Analogue Scale or Visual Pain Scale

Intervention and treatment are used interchangeably for the purpose of this protocol.

Wound and ulcer are also used interchangeably for the purpose of this protocol.

PROTOCOL SUMMARY

Title: A prospective, randomized, controlled trial comparing Cytal® Wound Matrix 1-Layer to Standard of Care (SOC) in the management of Diabetic Foot Ulcers (DFUs)

Protocol Number: CR2017-006

Study Design: Multi-site, prospective, randomized study comparing Cytal® Wound Matrix 1-Layer (Group 1) to Standard of Care (Group 2) in the management of diabetic foot ulcers.

Subjects who meet the inclusion and exclusion criteria for the study will be randomized (2:1) to Group 1 or Group 2 using a permuted block randomization scheme.

Study DFUs will be treated up to 10 times in a 12 week period and must be located on a single foot.

Study Objectives:

Primary Objective

1. To compare the incidence of complete wound closure (defined as 100% epithelialization) by 12 weeks between randomized groups.

Secondary Objective(s)

1. To compare the rate of wound healing over time in cm^2/week between randomized groups.
2. To compare the time to complete wound closure between randomized groups.
3. To compare DFU recurrence after healing between randomized groups.
4. To compare subject reported outcomes between randomized groups.
5. To compare the intervention-emergent adverse event (AE) safety profile between randomized groups.

Health Economic Objectives

1. Perform a health economics and cost effectiveness analysis between randomized groups.

Endpoints:

Primary Endpoint

1. Measure incidence of complete wound closure (defined as 100% epithelialization) by 12 weeks between randomized groups.

Secondary Endpoint(s)

1. Measure changes in wound size, measured in cm^2 per week between randomized groups by 12 weeks.
2. Measure time to complete wound closure, in days, between randomized groups by 12 weeks.
3. Measure wound recurrence after healing is complete at 26 weeks and 52 weeks (6 & 12 months post intervention initiation) between randomized groups.
4. Changes in subject reported outcomes including quality of life and pain (quality of life surveys & visual analogue scale), pre-intervention to post-intervention between randomized groups over time and by 12 weeks.
5. Measure frequency of adverse events related to DFU including unexpected adverse device events or serious adverse experiences throughout the duration of the study and evaluate between randomized groups.

Health Economic Outcomes

1. Any changes from baseline in the prescription of narcotics related to the DFU(s) by subject and between randomized groups.
2. Any changes in ambulatory status (i.e. bed, wheel chair, walk w/ assistance, or walk independent) by subject and between randomized groups.
3. Any changes in “return to work/reported work status”, activities of daily living, or disability status by subject and between randomized groups.
4. Health economics analysis, as measured by direct costs as measured by total cost per subject stratified by healed vs. non healed DFU.

Population:

A maximum of 150 subjects who present to the participating Investigator(s) in the in-patient or out-patient facility with at least a single diabetic foot ulcer that is characterized as Wagner Grade 1 or Grade 2 will be enrolled and randomized. An interim assessment will be performed after 60 subjects complete the 12

week intervention and evaluation period or withdraw prematurely. The conditional power will be estimated by an independent biostatistician at the interim assessment to ensure the observed incidence of response is commensurate with the planning estimates. The biostatistician may recommend that enrollment be increased from the target population of 120 subjects (80 subjects in Group 1 vs. 40 subjects in Group 2) to a maximum of 150 subjects (100 subjects in Group 1 vs. 50 subjects in Group 2). Given the primary endpoint is dichotomous, subjects who withdraw prematurely (<12 weeks) from their randomized intervention assignment without complete wound closure will be considered in the analysis as not having met the definition for closure.

Study Criteria: **Inclusion Criteria:**

1. Provision of signed and dated informed consent form by subject or legally authorized representative.
2. Stated willingness to comply with all study procedures and availability for the duration of the study. Subject is able and willing to tolerate non-removable offloading device for the duration of the run-in and intervention phases of the study.
3. Subject is male or female and ≥22 years of age.
4. Subject has a clinical diagnosis of type 1 or type 2 diabetes.
5. Subject's current foot ulcer(s) has been present for ≥ 30 days and ≤ 365 days.
6. Subject's current foot ulcer(s), post-debridement is/are predominantly below the malleoli and on the plantar surface of the foot.
7. Subject's foot ulcer(s) must be Wagner Grade 1 or 2.
8. Post debridement, subject's ulcer(s) are free of necrotic debris and appear to be comprised of healthy, vascularized tissue.
9. All qualifying ulcers are ≥ 5cm away from any other ulcer on the same foot.
10. Subject's ulcer(s) surface area is ≥ 1cm^2 and ≤ 20cm^2 at randomization.
11. Subject's HbA1C reading is ≤10%.
12. Subject's Serum Creatinine ≤ 3.0mg/dL.
13. Subject has adequate circulation to the foot as measured by Ankle-Brachial Index (ABI) ≥ 0.7
14. Negative pregnancy test at randomization for women of

childbearing potential.

Exclusion Criteria:

1. Subject is pregnant, breastfeeding, or unwilling to practice birth control during participation in the study, if applicable.
2. Allergy or hypersensitivity to materials in porcine-based study products (per subject report) or personal preference.
3. Subject report of concurrent participation in another clinical trial that involves a drug or device.
4. The subject has any condition that, in the Investigator's opinion, would warrant exclusion from the study or prevent the subject from completing the study.
5. Subject has clinical evidence of gangrene on any part of the affected foot.
6. The subject's ulcer(s) is/are definitively due to a non-diabetic etiology, ulcers of arterial, venous stasis, pressure, radiation, traumatic, rheumatoid, vasculitis, collagen vascular disease, or other non-diabetic etiologies in the opinion of the Investigator.
7. Subject has unstable Charcot foot, Charcot foot with a bony prominence(s) or Charcot amputation.
8. Qualifying wound(s) is connected to another ulcer via a fistula.
9. Subject has one or more medical condition(s) including renal, hepatic, hematological, neurologic, or immune disease that in the opinion of the Investigator would make the subject an inappropriate candidate for this wound healing study.
10. Subject has or has had a malignant disease (other than basal cell carcinoma) that has not been in remission for at least five years.
11. Subject is receiving oral or parenteral corticosteroids, immunosuppressive or cytotoxic agents, or is anticipated to require such during the course of the study.
12. Subject has acute osteomyelitis of the affected foot.
13. Subject's ulcer(s) is accompanied by active cellulitis.
14. Subject has received growth factor or enzymatic therapy in the area of the wound, within 2 weeks of consent.
15. Subject has ever received radiation or radiologic implants in the area of the wound OR chemotherapy within the past 5 years.
16. Subject is allergic to any of the primary or secondary dressing

materials, including occlusive dressings and the adhesives on such dressings.
17. Subject's ulcer(s) surface area has decreased in size by > 30% during the run-in phase.
18. Subject's ulcer(s) surface area has increased in size by > 50% during the run-in phase.
19. Subject's ulcer(s) has tunnels or sinus tracts that cannot be completely debrided.
20. Subject has severe malnutrition as evidenced by albumin <2.0 g/dL.
21. Subject has a bleeding disorder that would make the subject unsuitable for serial bedside sharps or chemical cautery debridement.
22. Subject is on dialysis.
23. Any DFU(s) is actively infected and has not been treated for any clinically suspected infection prior to application of any product.

Number of Sites enrolling participants: 3-6

ACell Study Products: 1. Cytal® Wound Matrix 1-Layer

Participant & Study Duration: The maximum number of subjects expected to be enrolled and randomized in this trial is 150; the target sample size to detect a 25% difference between the 2 randomized intervention groups is 120 subjects. Subjects will undergo a 2 week run-in phase and a 12 week intervention phase. For wounds that are treated during the intervention phase and subsequently heal, subjects will be followed for 3 weeks to confirm complete epithelization. For healed wounds, additional study visits will be conducted at 6 and 12 months from intervention initiation to evaluate the state of healing or the reoccurrence of the DFU(s). The duration of participation for each subject will be up to a maximum of 54 weeks.

Eligible subjects will be recruited and enrolled, under the responsibility of the participating Investigators. The actual overall study duration or subject recruitment period may vary.

Statistical Methodology:

Baseline is defined as the observations recorded immediately prior to the application of study intervention. Continuous demographic parameters, such as the subject's age at the time of enrollment, will be summarized for the ITT population using descriptive statistics (N, mean, median, standard deviation, minimum and maximum value, and 95% two-sided confidence limits) and compared between groups using a two-sample t-test. Categorical demographic parameters, such as gender, will be summarized as a proportion of the ITT population and compared using a two-tailed Fisher's Exact test. Co-morbid risk factors will be summarized for the ITT population by intervention assignment and according to the type of variable (categorical, continuous) and compared between groups. Kaplan-Meier estimates for time-to-event analyses will be prepared based on the ITT population. Separate tables containing subject counts, percentages, and 95% binomial confidence limits will be prepared based on individual risk factors.

The primary efficacy analysis population will be subjects in the ITT population who have any wound measurement data following randomization. For data analyses, complete wound closure requires confirmation at 3 weeks (≥21 days) post clinically apparent wound closure.

The primary efficacy analysis will compare the proportion of subjects who achieve complete target ulcer closure within 12 weeks after the first study intervention using a random-effects generalized linear model specifying the distribution of the dependent variable as binomial. Statistical significance will be declared if the two-sided probability value is <0.05; tests for interaction will be performed at the 0.1 alpha level. Asymptotic confidence limits will be presented based on the normal approximation to the binomial distribution for differences in proportions. Exact confidence intervals will be used for all other presentations.

For the secondary efficacy endpoints, the change in the wound size, measured in cm^2, will be calculated on an intra-subject basis for each post-baseline measurement. The mean change per week

will be calculated for each subject and compared between the intervention and standard of care groups using a random-effects generalized linear model specifying the distribution of the dependent variable as continuous. The time to complete wound closure will be calculated in days from the date of the initial study intervention and compared between the intervention and standard of care groups using a log-rank test. Subjects who fail to achieve complete wound closure will be censored using the last date of examination in the study. Wound recurrence after healing is complete at 26 weeks and 52 weeks will be tabulated by intervention group and the proportions will be compared between intervention and standard of care groups using a 2-tailed Fisher's Exact test. Changes in subject reported outcomes will be summarized and compared between the intervention and standard of care groups based on the distribution of the data. A random-effects generalized linear model, specifying the distribution of the dependent variable based on the type of data, will be used to compare the results over time between the intervention and standard of care groups. All computations will be performed using SAS® (Version 9.4 or higher).

Adverse events will be coded according to the MedDRA system dictionary. The percentage of subjects experiencing adverse events will be summarized by body system and preferred term. Subject counts will be tabulated for all adverse events for the ITT population. Adverse events will also be tabulated for events that occurred following initial treatment application through final study visit. The incidence of subjects with 1 or more adverse events, and restricted to serious adverse events, will be summarized using counts and percentages. The incidence will be compared using a generalized linear model specifying the distribution as binomial.

The maximum number of subjects to be enrolled into this clinical investigation is 150 subjects; the target sample size is 120 subjects with a 2:1 randomization ratio to detect a difference in the proportion of responders of 25% with a power approaching 80% (assumes an asymptotic normal distribution and a normal approximation). A sample size re-estimation will be performed when 60 of the subjects have completed the 12 week intervention and evaluation period or withdrawn prematurely to establish the

primary endpoint results (confirmed complete wound closure or no confirmed wound closure).

1 KEY ROLES

Elizabeth A. Smith
Senior Director of Clinical Research
ACell Inc.
(443) 283–2768
elizabethsmith@acell.com

Tejal Gambhir
Clinical Research Manager
ACell, Inc.
(443) 283-7961
tejalgambhir@acell.com

Becky Seufert
Clinical Research Project Manager
ACell, Inc.
410-953-8549
rebeccaseufert@acell.com

Mike Anibaba
Clinical Research Associate
ACell, Inc.
(443) 283-2798
mikeanibaba@acell.com

Elizabeth Okeke
Clinical Research Assistant
ACell, Inc.
(443) 283-7960
elizabethokeke@acell.com

Cindy Mattingly
Clinical Data Manager
ACell Inc.
(410) 953-8539
cindymattingly@acell.com

Bruce C. Stouch, Ph.D.
Biostatistician
(610) 787-0742
BCSSTATS@aol.com

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION AND RATIONALE

Diabetes mellitus (DM) is a chronic disease that causes elevated glucose levels in the blood and, if left untreated, can lead to a number of short and long-term health complications. In 2015, the American Diabetes Association estimated 30.3 million Americans, or 9.4% of the population, are affected by this disease, with the incidence expected to dramatically increase over the next 25 years[1,11]. There has been much success in treating diabetes, which has led to improvements in the life expectancy of those affected by the disease[3]. However, the increased prevalence coupled with the extended amount of time people live with the disease has led to an increase in the number of diabetes-related complications[3]. These subjects are at risk for a number of health problems, with diabetic foot ulcers (DFUs) being one of the most common and serious complications, affecting roughly 15-25% of diabetes subjects in their lifetime[4,5,12].

Diabetic foot ulcerations present as an open wound or sore and are commonly located on the bottom of the foot or lower extremities of people with diabetes. The most common contributing factors leading to the development of DFUs are ischemic, neuropathic, or combined neuroishcemic abnormalities, peripheral artery disease (PAD), foot deformity, and minor trauma [3,5,12]. However, when the ulcer appears, the progression is often exacerbated by other risk factors and comorbidities such as gender, duration of diabetes longer than 10 years, advanced age of subjects, high Body Mass Index (BMI), retinopathy, diabetic peripheral neuropathy, glycated hemoglobin level (HbA1C), high plantar pressure, infections, and inappropriate foot self-care habits[12]. Regardless of etiology, DFUs require clinical treatment to prevent further progression of the ulcer and other sequelae that have the potential to result in severe and costly outcomes[8]. Due to the chronic nature of these wounds and the characteristically poor healing response, DFU(s) require extensive healing time and are a major source of morbidity and mortality in diabetes subjects, with a 3-year mortality rate of 28% and amputation rates 15 times higher than subjects without the disease[8,12]. These wounds are also a leading cause of hospitalization in diabetes subjects; it is estimated that 20% of hospitalizations for subjects with diabetes are due to complications associated with foot ulceration[8,12]. Aside from these complications, the development of DFUs can lead to physical and mental distress as well as productivity and financial losses that lower the quality of life of subjects[12].

The economic burden associated with people with diabetes is significant, with medical expenditures 2.3 times higher in subjects with diabetes compared to those without the

disease[6]. In 2012, the annual cost of diabetes was estimated to be $245 billion, which included health care expenditures and productivity losses[8]. A substantial amount of these expenditures were related to managing comorbidities and chronic complications such as DFU and lower-extremity amputation[8]. The current Standard of Care (SOC) for DFU(s) as highlighted in the literature includes debridement, pressure offloading, moist wound therapies, and adequate education and nutritional support. The incidence of closure for DFUs treated with SOC alone has been reported to be between 20-38%[9,10,13]. Thus, with the prevalence of diabetes and diabetes-related complications on the rise, there is a need for an intervention that has the ability to increase the incidence of DFU healing. Such an intervention may provide a cost-effective means of decreasing morbidity and mortality and improve the quality of life for subjects who suffer from diabetic foot ulceration(s). This clinical investigation will assess the efficacy of Cytal® Wound Matrix 1-Layer as a potential therapy for the management of chronic, non-healing DFUs compared to SOC over a 12 week period.

2.2 POTENTIAL RISKS AND BENEFITS

2.2.1 POTENTIAL RISKS

Cytal® Wound Matrix 1-Layer

Complications and reactions are possible with any soft tissue repair, including but not limited to: infection, increased chronic inflammation, allergic reaction, unexplained fever or chills, excessive redness, pain, or swelling.

2.2.2 POTENTIAL BENEFITS

Cytal® Wound Matrix 1-Layer

Cytal® Wound Matrix 1-Layer is constructed of a naturally-occurring biomaterial designed to manage wounds. The devices may be cut to size and must be hydrated prior to placement by immersion in room temperature sterile saline. The biomaterial is a resorbable extracellular matrix scaffold containing basement membrane which facilitates constructive remodeling of host tissue by integration.

3 OBJECTIVES

The primary objective of the study is:

1. To compare the incidence of complete wound closure (defined as 100% epithelialization) by 12 weeks between randomized groups.

The secondary objectives of the study are:

1. To compare the rate of wound healing over time in cm^2/week between randomized groups.
2. To compare the time to complete wound closure between randomized groups.
3. To compare DFU recurrence after healing between randomized groups.
4. To compare subject reported outcomes between randomized groups.
5. To compare the intervention-emergent adverse event (AE) safety profile between randomized groups.

The health economic objective of the study is:

1. Perform a health economics analysis between randomized groups.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

Multi-site, prospective, randomized study comparing Cytal® Wound Matrix 1-Layer (Group 1) to Standard of Care (Group 2) in the management of diabetic foot ulcers.

Subjects who meet the inclusion and exclusion criteria for the study will be randomized (2:1) to Group 1 or Group 2 using a permuted block randomization scheme.

Study DFUs will be treated up to 10 times in a 12 week period and must be located on a single foot.

The target number of subjects to be enrolled and randomized in this trial is 120, with 150 being the maximum number of subjects based on the interim sample size re-estimation. Subjects will undergo a 2 week run-in phase and a 12 week intervention phase. For wounds that are treated during the intervention phase and subsequently heal, subjects will be followed for 3 weeks to confirm complete epithelization. For healed wounds, additional study visits will be conducted at 6 and 12 months from intervention initiation to evaluate the state of healing or the reoccurrence of the DFU(s).

The duration of participation for each subject will be up to a maximum of 54 weeks.

Eligible subjects will be recruited and enrolled, under the responsibility of the participating Investigators. The actual overall study duration or subject recruitment period may vary.

4.2.1 PRIMARY ENDPOINT

Measure incidence of complete wound closure (defined as 100% epithelialization) by 12 weeks between randomized groups.

4.2.2 SECONDARY ENDPOINTS

The secondary endpoints of the study, comparing measurements between randomized groups, include:

1. Measure changes in wound size, measured in cm^2 per week between randomized groups by 12 weeks.
2. Measure time to complete wound closure, in days between randomized groups by 12 weeks.
3. Measure wound recurrence after healing is complete at 26 weeks and 52 weeks (6 & 12 months post intervention initiation).
4. Changes in subject reported outcomes including quality of life and pain (quality of life surveys & visual analogue scale), pre- intervention to post- intervention over time and by 12 weeks.
5. Measure frequency of adverse events related to DFU including unexpected adverse device events or serious adverse experiences throughout the duration of the study.

4.2.3 HEALTH ECONOMIC OUTCOMES

The health economic outcomes of the study include:

1. Any changes in the prescription of narcotics related to the DFU(s) by subject and between randomized groups.
2. Any changes in ambulatory status (i.e. bed, wheel chair, walk w/ assistance, or walk independent) by subject and between randomized groups.
3. Any changes in “return to work/reported work status”, activities of daily living, or disability status by subject and between randomized groups.
4. Health economics analysis, as measured by direct costs as measured by total cost per subject stratified by healed vs. non healed DFU.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form by subject or legally authorized representative.
2. Stated willingness to comply with all study procedures and availability for the duration of the study. Subject is able and willing to tolerate non-removable offloading device for the duration of the run-in and intervention phases of the study.
3. Subject is male or female and ≥22 years of age.
4. Subject has a clinical diagnosis of type 1 or type 2 diabetes.
5. Subject's current foot ulcer(s) has been present for ≥ 30 days and ≤365 days.
6. Subject's current foot ulcer(s), post-debridement is/are predominantly below the malleoli and on the plantar surface of the foot.
7. Subject's foot ulcer(s) must be Wagner Grade 1 or 2.
8. Post debridement, subject's ulcer(s) are free of necrotic debris and appear to be comprised of healthy, vascularized tissue.
9. All qualifying ulcers ≥ 5cm away from any other ulcer on the same foot.
10. Subject's ulcer(s) surface area is ≥ 1cm^2 and ≤ 20cm^2 at randomization.
11. Subject's HbA1C reading is ≤10%.
12. Subject's Serum Creatinine ≤ 3.0mg/dL.
13. Subject has adequate circulation to the foot as measured by Ankle-Brachial Index (ABI) ≥ 0.7.
14. Negative pregnancy test at randomization for women of childbearing potential.

5.2 PARTICIPANT EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Subject is pregnant, breastfeeding, or unwilling to practice birth control during participation in the study, if applicable.
2. Allergy or hypersensitivity to materials in porcine-based study products (per subject report) or personal preference.
3. Subject report of concurrent participation in another clinical trial that involves a drug or device.
4. The subject has any condition that, in the Investigator's opinion, would warrant exclusion from the study or prevent the subject from completing the study.

5. Subject has clinical evidence of gangrene on any part of the affected foot.
6. The subject's ulcer(s) is/are definitively due to a non-diabetic etiology, ulcers of arterial, venous stasis, pressure, radiation, traumatic, rheumatoid, vasculitis, collagen vascular disease, or other non-diabetic etiologies in the opinion of the Investigator.
7. Subject has unstable Charcot foot, Charcot foot with a bony prominence(s) or Charcot amputation.
8. Qualifying wound(s) is connected to another ulcer via a fistula.
9. Subject has one or more medical condition(s) including renal, hepatic, hematological, neurologic, or immune disease that in the opinion of the Investigator would make the subject an inappropriate candidate for this wound healing study.
10. Subject has or has had a malignant disease (other than basal cell carcinoma) that has not been in remission for at least five years.
11. Subject is receiving oral or parenteral corticosteroids, immunosuppressive or cytotoxic agents, or is anticipated to require such during the course of the study.
12. Subject has acute osteomyelitis of the affected foot.
13. Subject's ulcer(s) is accompanied by active cellulitis.
14. Subject has received growth factor or enzymatic therapy in the area of the wound, within 2 weeks of consent.
15. Subject has ever received radiation or radiologic implants in the area of the wound OR chemotherapy within the past 5 years.
16. Subject is allergic to any of the primary or secondary dressing materials, including occlusive dressings and the adhesives on such dressings.
17. Subject's ulcer(s) surface area has decreased in size by > 30% during the run-in phase.
18. Subject's ulcer(s) surface area has increased in size by > 50% during the run-in phase.
19. Subject's ulcer(s) has tunnels or sinus tracts that cannot be completely debrided.
20. Subject has severe malnutrition as evidenced by albumin <2.0 g/dL.
21. Subject has a bleeding disorder that would make the subject unsuitable for serial bedside sharps or chemical cautery debridement.
22. Subject is on dialysis.
23. Any DFU(s) is actively infected and has not been treated for any clinically suspected infection prior to application of any product.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Eligible subjects will be recruited and enrolled, under the responsibility of the participating Investigators.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

A subject may be discontinued from the study at any time if the subject, the Investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of *possible* reasons for study and/or intervention discontinuation (including but not limited to):

- Screen Failure/Subject does not continue to meet eligibility
- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Adverse Event that in the opinion of the Investigator would be in the best interest of the subject to discontinue study participation and/or study intervention
- Protocol deviation
- Lost to follow-up
- Sponsor request for early termination of study
- Subject death

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. Reasonable attempts will be made by the Investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents and the Case Report Form (CRF).

If a subject is withdrawn from intervention due to an AE, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

The Investigator (study staff) must make every effort to contact subjects who are lost to follow-up. Three (3) attempted contacts should be documented by research personnel prior to considering the subject lost to follow-up. Attempts to contact such subjects must be documented in the subjects' records (e.g. dates of attempted telephone contact).

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Although subjects may withdraw from the study at any time and for any reason, (or may be withdrawn at the Investigator's discretion), subject withdrawal should be avoided as much as reasonably possible. In any case, appropriate follow-up for endpoints should be continued. The reason for study discontinuation will be recorded on the subject's source document and CRF.

Subjects who do not continue to meet eligibility criteria following the 2 week run-in phase will be withdrawn from the study. The reason for study discontinuation will be recorded on the subject's source document and CRF.

Subjects who prematurely discontinue following initial treatment are not to be replaced.

Randomized subjects who terminate their clinical trial participation for any reason post randomization will not be replaced and their randomization number will not be re-assigned.

For subjects considered lost to follow-up, the CRF must be completed up to the last visit performed.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the Investigator, the Sponsor and the Institutional Review Board (IRB), as appropriate. If the study is prematurely terminated or suspended, the Investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the Sponsor and/or the IRB.

6 STUDY DEVICE

6.1 STUDY DEVICE DESCRIPTION

6.1.1 DEVICE DESCRIPTION

Cytal® Wound Matrix 1-Layer

Cytal® Wound Matrix 1-Layer is composed of a porcine-derived extracellular matrix known as urinary bladder matrix and is intended for the management of wounds including: partial and full-thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Mohs surgery, post-laser surgery, podiatric, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, skin tears), and draining wounds. The (individual) device is intended for one time use.

6.1.2 DEVICE CONFIGURATION

Cytal® Wound Matrix 1-Layer

The study devices are supplied in a fenestrated sheet configuration in sizes up to 10 cm x 15 cm and packaged in double peel-open pouches.

Standard of Care Products

Fibracol™
The standard of care primary dressing is standardized under this protocol to be Fibracol™. The devices are supplied in a sheet configuration in sizes up to 10 cm x 11 cm.

The standard of care secondary dressings used by treating Investigator which are part of his/her standard of care will be used per their approved labelling and as packaged per their manufacturer.

Please note: Silver impregnated products may not be used under this protocol as SOC treatments. Silver impregnated products may be used at the investigator's discretion when a patient presents with an emergent wound condition, such as a local wound infection. Products used must be documented on the AE CRF.

6.1.3 PRODUCT STORAGE AND ACCOUNTABILITY

Cytal® Wound Matrix 1-Layer should be stored in a clean, dry environment, between 15-35°C (59-95°F), in the unopened and undamaged package. The products should be protected from freezing temperatures, excessive heat, and high humidity.

Regular study device reconciliation will be performed to document study product assigned to each eligible participant, if applicable. This reconciliation will be logged on the Device Accountability Log, and signed and dated by the PI at the end of the study.

Fibracol™ should be stored per manufacturer's instructions.

6.1.4 RETURN OR DESTRUCTION OF STUDY DEVICE

All unused Cytal® Wound Matrix 1-Layer devices will be retrieved by the Sponsor, if applicable. A detailed Device Accountability Log and a Storage Attestation Form of the returned study product(s) will be provided to the Sponsor at the end of the study.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY SCHEDULE

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix I.

7.1.1 RUN-IN PERIOD (2 WEEKS) (DAY -14 AND -7)

1. The run-in period will be for two consecutive weeks (Day -14 and Day -7 +/-2 days) prior to intervention initiation. Subjects should return for two weekly visits during this period. All eligibility criteria are to be confirmed prior to randomization and intervention at Study Visit 1. Subjects are considered enrolled in the study once randomized. Confirmation of continued eligibility at treatment visit allows for subject randomization. Obtain informed consent from the potential subject, or legally authorized representative.
2. Assign subject a Subject ID (SID). Subject IDs will be assigned in consecutive order and cannot be re-used.
3. Collect demographic data including: date of birth, gender, race, and ethnicity only at Run-In Visit 1.
4. Document weight (pounds), height (inches), and calculated BMI. BMI will be calculated using the National Institutes of Health BMI calculator.
5. Collect blood sample(s) for albumin, pre-albumin, serum creatinine and Hemoglobin HbA1C (only required at one of the two Run-In visits).
6. Record ABI to confirm appropriate perfusion per Inclusion/Exclusion criteria (only required at one of the two Run-In visits).
7. Perform physical exam and evaluation needed to determine eligibility based on inclusion/exclusion criteria.
8. Record concomitant medication use (type, dose, frequency, duration, indication) for the following (including but not limited to): pain medication related to DFU, any anti-hypertensives, medication or supplements to control diabetes, nutritional supplements, and antibiotics.
9. Record the anatomic location of the wound(s), date of onset, and any previous interventions.
10. Record wound characteristics pre-debridement. Wound appearance and characteristics including but not limited to: presence of epibole, eschar, undermining,

tunneling, sinus tracts, slough, and/or adverse odor; Appearance of Exudate: serous, serosanguinous, purulent, and bloody.

11. Record Appearance of Wound Bed : Beefy Red, Red, Pink, White, or Yellow. If debridement is performed, record appearance of wound bed post-debridement.
12. Use the Silhouette system to capture at least one image per wound **pre-debridement at the first run-in visit only** (this is a reference picture and measurements will not be recorded).
    - Images should be captured in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). Refer to the SilhouetteConnect User Guide and the SilhouetteStar Quick Reference Guide for detailed instructions on how to use the Silhouette system.
13. Use the Silhouette system to capture at least one image per wound **post-debridement at the first and second run-in visit** and record the length, width, and depth of the diabetic foot ulcer(s) in centimeters (surface area, volume, and percent changes from baseline will be auto-calculated). If debridement is not performed, at least one image per wound must be captured and measured as noted above.
14. Treat the DFU(s) with SOC to include debridement (if necessary), moist wound therapy, the appropriate secondary dressing, and off-loading (per SOC) for 2 consecutive weeks prior to randomization. This 2 week run-in period is required to determine continued eligibility in the study. No advanced wound dressings (i.e. Fibracol™) should be used prior to the initiation of the treatment phase as SOC treatment.

7.1.2 BASELINE/RANDOMIZATION (STUDY VISIT 1)

Baseline/Randomization visit should occur 1 week following second run-in visit (+/- 2 days) for all subjects.

1. Verify and document inclusion/exclusion criteria continue to be met. Subjects whose DFU(s) surface area decreases in size by > 30% or increases by > 50% from the initial run-in visit will not be eligible for study intervention and will be considered a screen failure. Qualifying subjects will be randomized to either Group 1 or Group 2.
2. Record subject's employment status, disability status, ambulatory status, medical history (including duration of diabetes disease, past diabetes complications, and DFUs), and surgical history per available medical reports and/or patient self-report.
3. Record social information including alcohol and tobacco use.
4. Confirm concomitant medication use (type, dose, frequency, duration, indication) for the following (including but not limited to): pain medication related to DFU, any anti-hypertensives, medication or supplements to control diabetes, nutritional supplements, and antibiotics.
5. Perform urinary pregnancy test for women of childbearing potential.

6. Administer the SF-20 Health Survey, DFS-SF, Visual Analogue Scale for Pain (VAS), and KATZ ADL prior to intervention.
7. Record the following information for the eligible wound(s) (record wound characteristics pre-debridement, if debridement at this visit is deemed necessary by the investigator). Wound appearance and characteristics including but not limited to: Presence of epibole, eschar, undermining, tunneling, sinus tracts, slough, and/or adverse odor; Appearance of Exudate: Serous, serosanguinous, purulent, and bloody; Appearance of Wound Bed: Beefy Red, Red, Pink, White, or Yellow.
8. Record the following information for each of the eligible ulcer(s) (record size and classification post-debridement, if the ulcer(s) was debrided at this visit):
    - *If debridement is performed*: Use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image *post debridement and prior to product application.*. Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). Use SilhouetteConnect to trace one image in order to calculate and record the length, width, depth, surface area and volume.
    - *If debridement is **not** performed*: Use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image prior to product application. Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). Use SilhouetteConnect to trace one image in order to calculate and record the length, width, depth, surface area and volume.
    - Additional images beyond the requirements stated above may be taken however are not required under this protocol. Additional images should not be measured within ARANZ SilhouetteConnect.
    - Categorize the wound using the Wagner Classification System
9. If all eligibility criteria continue to be met, perform randomization and proceed with intervention procedures as outlined below, depending on randomization assignment. If there is more than one qualifying wound, randomize by subject so all wounds are in the same intervention group..

    Group 1 (ACell products) Procedures

    i. Wash the wound using saline prior to debridement, as needed.
    ii. Sharps debride the eligible diabetic foot ulcer(s) to remove all non-viable tissue, callus, epibole at wound edges, slough, and debris (at the physician's discretion). The wound margins should be excised to healthy bleeding tissue.
    iii. Wash the wound(s) once more with saline to remove remaining debris and confirm hemostasis is achieved, as needed.
    iv. Refer to section 7.1.2 Number 8 above for wound image and measurement instructions for the eligible wound(s) post-debridement.

v. Place an appropriately sized **Cytal® Wound Matrix 1-Layer** sheet (previously hydrated in sterile saline per the manufacturer's Instructions for Use) into the wound bed. The hydrated sheet should be cut to fit the interior of the wound, with no overlap onto healthy skin. Record appropriate wound care time inclusive of product preparation, debridement and dressing.
vi. Secure product with a non-adherent, contact layer.
vii. Apply a water-based surgical lubricant of physician choice as necessary to maintain a moist wound environment.
viii. Apply the appropriate secondary dressing based on wound characteristics and physicians discretion.
ix. Apply the OPTIMA® Diab boot with the appropriate insole kit based on subject weight and wound size and make the boot non-removable by using the locking system.
x. In cases of AEs/SAEs in the **ACell arm**, the PI may treat subjects with any other primary wound contact layer as per the Investigator's discretion **except** for any Fibracol™ devices, so as to not confound study arms and results.

Group 2 (Standard of Care Only) Procedures

i. Wash the wound using saline prior to debridement.
ii. Sharps debride the eligible diabetic foot ulcer(s) to remove all non-viable tissue, callus, epibole at wound edges, slough, and debris (at the physician's discretion). The wound margins will be excised to healthy bleeding tissue.
iii. Wash the wound(s) once more with saline to remove remaining debris and confirm hemostasis achieved.
iv. Refer to section 7.1.2 Number 8 above for wound image and measurement instructions for the eligible wound(s) post-debridement.
xi. Place an appropriately sized Fibracol™ sheet (per the manufacturer's Instructions for Use) into the wound bed. Record appropriate wound care time inclusive of product preparation, debridement and dressing.
v. Apply a water-based surgical lubricant of physician choice as necessary to maintain a moist wound environment.
vi. Apply the appropriate secondary dressing based on wound characteristics and physicians discretion.
vii. Apply the OPTIMA® Diab boot with the appropriate insole kit based on subject weight and wound size and make the boot non-removable by using the locking system.

viii. In cases of AEs/SAEs in the **SOC arm**, the PI may treat subjects with any other primary wound contact layer per the Investigator's discretion **except** for Cytal ® Wound Matrix 1-Layer, so as to not confound study arms and results.

10. Record wound-related AEs and all SAEs as reported by participant or observed by the Investigator. In cases of AEs/SAEs in either study arm, use of oral antibiotics, topical antimicrobials, or the use of silver infused primary contact layers is acceptable. For subjects in the ACell arm, use of any Fibracol™ devices **is prohibited** per protocol so as to not confound study arms and results. For subjects in the SOC arm, use of Cytal ® Wound Matrix 1-Layer **is prohibited** so as to not confound study arms and results. Corresponding documentation should be included in submitted AE reporting as appropriate.
11. Provide instructions to subject regarding maintenance of adequate nutrition and diabetes control as per SOC.
12. Provide instructions to subject regarding off-loading. The boot should not be removed between visits. Relevant information provided by the manufacturer of the boot may be found in the Appendix of this protocol.
13. Confirm date of next visit with the subject.

7.1.3 STUDY VISITS (2-13 and confirmation visits)

Study visits at which the wound(s) is evaluated should occur every 7 days (± 2 days) for all subjects.

1. Record written documentation of off-loading per protocol requirements (compliance to off-loading instructions).
2. Record wound-related AEs and all SAEs as reported by subject or observed by Investigator. In cases of AEs/SAEs in either study arm, use of oral antibiotics, topical antimicrobials, or the use of silver infused primary contact layers is acceptable. For subjects in the ACell arm, use of any Fibracol™ devices **is prohibited** per protocol so as to not confound study arms and results. For subjects in the SOC arm, use of Cytal ® Wound Matrix 1-Layer **is prohibited** so as to not confound study arms and results. Corresponding documentation should be included in submitted AE reporting as appropriate.
3. Record any changes to activities of daily living, employment, disability, and primary ambulatory status. For confirmation visits, this is required at the third and final confirmation visit only.
4. If the subject is unable to work due to their DFU(s), record whether or not the subject could be released to return back to work independent of their current work status based on the status of the wound and physicians discretion.

5. Confirm concomitant medication use for the following (including but not limited to): pain medication use relating the DFU(s), any anti-hypertensives, medications or supplements to control diabetes, nutritional supplements, and antibiotics, if applicable.
6. Administer VAS for pain-overall body pain and DFU foot pain prior to intervention weekly, as appropriate.
7. Record weight (pounds) and BMI at wound healing.
8. Collect blood samples for Hemoglobin HbA1C at **Visit 13** and Albumin at **Visit 3, Visit 6, Visit 9 and Visit 13**. For confirmation visits, collect blood sample at the third and final confirmation visit only.
9. Record ABI to confirm appropriate perfusion PRN for non-healing wounds.
10. Administer DFS-SF at the **last closure confirmation visit**.
11. Administer SF-20 Health Survey and the Katz ADL prior to intervention at **Visit 7 & 13** and at the third and final confirmation visit if the wound heals prior to **Visit 13.**
12. Record the following information for all treated wound(s) pre-debridement, if debridement at this visit is deemed necessary by the Investigator. Wound appearance and characteristics including, but not limited to: Presence of epibole, eschar, undermining, tunneling, sinus tracts, slough and/or adverse odor; Appearance of Exudate: Serous, serosanguinous, purulent, and/or bloody; Appearance of Wound Bed: Beefy Red, Red, Pink, White, or Yellow.
13. Record the following information for all treated wound(s) post-debridement, if debridement at this visit is deemed necessary by the Investigator:
    a. Use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image and record measurements including length, width, and depth of the diabetic foot ulcer(s) in centimeters (surface area, volume, and percent changes from baseline will be auto-calculated). Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). If debridement is not performed, capture at least one wound image and record measurements as noted.
    b. If wound(s) is healed, Use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image of the healed wound area.
    c. Additional images beyond the requirements stated above may be taken however are not required under this protocol. Additional images should not be measured within ARANZ.
14. Proceed with treatment procedures as outlined below (Note, a maximum of 10 treatments within the 12 week period or until wound has completely closed, whichever occurs first, is allowed. These applications may be made per the discretion of the Investigator.)
    a. Group 1 Procedures (ACell Products)

i. Do not remove or disrupt any residual ACell product (Group 1) when preparing the wound bed for reapplication.
ii. Minimal “spot” sharps debrides and gentile saline washes may be performed at subsequent treatment visits as determined by the investigator.
iii. Apply weekly applications of Cytal® Wound Matrix 1-Layer until wound closure or up to, and including, week 12, whichever occurs first. Record appropriate wound care time inclusive of product preparation, debridement and dressing if additional study devices are applied.
iv. Secure product with a non-adherent, primary contact layer.
v. Apply a water-based surgical lubricant of physician choice as necessary to maintain a moist wound environment.
vi. Apply the appropriate secondary dressing based on wound characteristics and physicians discretion.
vii. Apply the OPTIMA® Diab boot with the appropriate insole kit based on subject weight and wound size and make the boot non-removable by using the locking system.
viii. In cases of AEs/SAEs in the **ACell arm**, the PI may treat subjects with any other primary wound contact layer as per the Investigator’s discretion **except** for any Fibracol™ devices, as to not confound study arms and results.

b. Group 2 Procedures (SOC Only)

i. Minimal “spot” sharps debrides and gentile saline washes may be performed at subsequent intervention visits as determined by the investigator.
ii. Place an appropriately sized Fibracol™ sheet (per the manufacturer’s Instructions for Use) into the wound bed Record appropriate wound care time inclusive of product preparation, debridement and dressing as appropriate. Apply a water-based surgical lubricant of physician choice as necessary to maintain a moist wound environment.
iii. Apply the appropriate secondary dressing based on wound characteristics and physicians discretion.
iv. Apply the OPTIMA® Diab boot with the appropriate insole kit based on subject weight and wound size and make the boot non-removable by using the locking system.
v. In cases of AEs/SAEs in the **SOC arm**, the PI may treat subjects with any other primary wound contact layer per the Investigator’s

discretion **except** for Cytal ® Wound Matrix 1-Layer, so as to not confound study arms and results.

15. Confirm date of next visit. Visits should be completed every 7 days (± 2 days). If wound re-opens at one of the closure confirmation visits, resume weekly study visits and treatment as appropriate for a maximum of 10 treatments in a 12 week period.
    a. **If complete wound healing is observed by, or prior to, Visit 13:** Subject will return for **3 consecutive visits** to confirm complete wound closure and to perform the procedures outlined above, sections 1-11 as applicable.
    For example, if the wound heals at Visit 13, subject is to return for Study Visit 14, 15, and 16; if wound heals at Visit 8, subject is to return for Study Visit 9, 10, and 11. The maximum number of study visits in this phase of the study is 16 and is dependent on wound healing. Subjects will be evaluated for DFU recurrence.
    b. **If treatment is administered at Visit 13:** Subjects will complete an Unscheduled visit to evaluate treatment effect. If healing is noted at the Unscheduled visit, subject should return for 3 confirmation visits.

7.1.4 FINAL STUDY VISITS (WEEK 26 AND 52 ±2 WEEKS)

The Final Study Visits will occur 26 and 52 weeks (± 2 weeks) after the initial intervention visit (Day 0). The Final Study Visits are required ONLY for subjects with at least 1 healed wound noted following treatment during the intervention phase.

1. Record weight (pounds) and BMI.
2. Record any changes to subject's employment status, disability status, ambulatory status, medical history, and surgical history.
3. Record changes to concomitant medication use for the following (including but not limited to): pain medication use relating the DFU(s), any anti-hypertensives, medications or supplements to control diabetes, nutritional supplements, and antibiotics, if applicable.
4. Administer SF-20 Health Survey, DFS-SF, VAS for pain (overall body pain and DFU foot pain), and KATZ ADL.
5. Record diabetes and/or wound-related AEs and all SAEs as reported by participant or observed by Investigator.
6. Collect blood samples for Hemoglobin HbA1C and albumin.
7. Record off-loading as applicable.
8. Record ABI to confirm perfusion PRN for non-healing wounds..
9. Record if there has been recurrence at the original anatomical location of the wound.
10. If recurrence is noted following Visit 13, record the following information:

a. Wound appearance and characteristics including, but not limited to: Presence of epibole, eschar, undermining, tunneling, sinus tracts, slough and/or adverse odor; Appearance of Exudate: Serous, serosanguinous, purulent, and/or bloody; Appearance of Wound Bed: Beefy Red, Red, Pink, White, or Yellow
b. Using the SilhouetteStar and SilhouetteCentral, obtain at least one wound image and measurements including length, width, and depth of the diabetic foot ulcer(s) in centimeters (surface area, volume, and percent changes will be auto-calculated). Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). If debridement is performed, images must be post-debridement.
c. If wound(s) remains healed, use the SilhouetteStar and SilhouetteConnect system to capture at least one wound image of the healed wound area.
d. Additional images beyond the requirements stated above may be taken however are not required under this protocol. Additional images should not be measured within ARANZ.

7.1.5 UNSCHEDULED VISIT

Unscheduled visits may occur at any time between Study Visit 1 and Visit 13, in the event of an adverse event (i.e. recurrence, infections, etc.) related to the original, qualifying DFU(s) up to week 52; or in the event that product is applied at Visit 13. Reasons for an Unscheduled Visit include, but are not limited to: diabetic foot ulcer events, assessment or follow-up of adverse events, etc.

1. Document reason for the unscheduled visit.
2. Review and record any changes to subject's employment status, disability status, ambulatory status, medical history, and surgical history, if applicable.
3. Record changes to concomitant medication use for the following (including, but not limited to): pain medication use relating the DFU(s), any anti-hypertensives, medications or supplements to control diabetes, nutritional supplements, and antibiotics, if applicable.
4. Record diabetic and wound-related AEs and all SAEs as reported by participant or observed by Investigator.
5. As applicable:
    a. Confirm closure or record if there has been a recurrence at the original anatomic location of the diabetic foot ulcer(s).
    b. Record wound appearance and characteristics including but not limited to: Presence of epibole, eschar, undermining, tunneling, sinus tracts, slough, and/or adverse odor; Appearance of Exudate: Serous, serosanguinous,

purulent, and bloody; Appearance of Wound Bed: Beefy Red, Red, Pink, White, or Yellow.

c. Using the SilhouetteStar and SilhouetteCentral, obtain at least one wound image and record measurements including length, width, and depth of the diabetic foot ulcer(s) in centimeters (surface area, volume, and percent changes will be auto-calculated). Capture images in a manner where no identifiable information is contained in the photograph (i.e. tattoos, full body, etc.). If debridement is performed, images must be post-debridement.
d. Record ABI to confirm perfusion PRN for non-healing wounds.

6. Record actions taken to treat the wound(s), if applicable.

7.1.6 MISSED VISIT

If a subject misses a visit, the site is to make every effort to have the subject return as soon as possible for the missed visit. Once the subject is seen, he/she is to return to his/her original weekly visit schedule (window +/- 2 days). For example:

- If a subject was seen regularly on Mondays but missed a scheduled Monday visit and came in on Wednesday, he/she should return the next Monday to maintain his/her weekly Monday Visit.
- If the same subject was not able to come in until Friday, the subject would still come in the next Monday to maintain the weekly schedule.

7.1.7 DESCRIPTION OF SYSTEMS/OUTCOME MEASUREMENTS

Wagner Ulcer Classification System

GRADE	LESION
0	No open lesions; may have deformity or cellulitis
1	Superficial diabetic ulcer (partial or full thickness)
2	Ulcer extension to ligament, tendon, joint capsule, or deep fascia without abscess or osteomyelitis
3	Deep ulcer with abscess, osteomyelitis, or joint sepsis
4	Gangrene localized to portion of forefoot or heel
5	Extensive gangrenous involvement of the entire foot

The SF-20® Health Survey (SF-20)

The SF-20 is a 20-item questionnaire used to assess generic health outcomes from the subject's perspective. Generic subject-reported outcome (PRO) measures like the SF-

20 assess general health and well-being [or health-related quality of life (HRQOL)], including the impact of any and all illnesses on a broad range of functional domains. The SF-20 consists of a subset of 20 items from the SF-36® Health Survey (SF-36), covering the same eight domains of health outcomes, including physical functioning, role functioning, mental health, current health perceptions, and pain.

Diabetic Foot Ulcer Scale- Short Form (DFS-SF)

The DFS-SF is a 29-item questionnaire is a specific instrument designed to assess the impact of diabetic foot ulcers and their intervention on quality of life in people with diabetes. The short form is based on the original Diabetic Foot Ulcer Scale; this questionnaire reduces the burden of a lengthy survey on subjects and has been shown to have good internal consistency, reliability, construct validity, and demonstrated sensitivity to ulcer healing[14].

Visual Analogue Scale for Pain (VAS)

The visual analogue scale or visual analog scale (VAS) is a psychometric response scale which can be used in patient-based outcome questionnaires. It is a measurement instrument for subjective characteristics or attitudes that cannot be directly/objectively measured. The VAS is anchored by two verbal descriptors, one for each symptom extreme measured from the zero mark, indicating “no pain” (score of 0) to the mark of 10 on the 10-cm line, indicating “pain as bad as it could be” or “worst imaginable pain.” Respondents are asked to report current pain intensity or pain intensity in the last 24 hours.

When responding to a VAS item, respondents specify their level of agreement to a statement by placing a vertical line perpendicular to the VAS line at the point that represents their pain intensity. After the patient has marked, using a ruler, the score is determined by measuring the distance (mm) on the 10-cm line between the “no pain” anchor and the patient’s mark.

Katz Index of Independence in Activities of Daily Living (KATZ ADL)

The Katz Index of Activities of Daily Living, formerly referred to as the Katz Activities of Daily Living Scale[15], is a validated measure used to quantify independence in activities of daily living. These activities include bathing, dressing, toileting, transfers, feeding, and continence. The Katz ADL is a 6-item questionnaire where each item is dichotomized as either having a score of zero (i.e. dependence) or one (i.e. independence). A score of 2 or less indicates a dependent subject, 3-5 for partially dependent and 6 for independent.

ARANZ Medical Silhouette

Silhouette® is an easy to use wound imaging, 3D measurement, and documentation system using non-invasive laser technology. As an FDA approved medical device, Silhouette has been proven to provide accurate, precise, and repeatable wound assessments for all phases of clinical research in more than 70 studies globally.

- SilhouetteStar™ + SilhouetteConnect™: The SilhouetteStar camera is used with the SilhouetteConnect application software for fast and easy imaging, 3D wound measurement and clinical notes capture. SilhouetteStar connects via USB to a computer with SilhouetteConnect installed.
- SilhouetteCentral™ (central server) is an integrated electronic wound information management system that enables trial managers to review, report, securely share, and analyze data collected by Silhouette point-of-care devices.

7.2 CONCOMITANT MEDICATIONS

At the discretion of the Investigator, the subject may be administered any necessary medications, provided such medications are not listed under the study exclusion criteria. Concomitant medications that will be documented in the subject's source documents and CRFs at all of the intervention and follow-up visits as well as recorded on the Medications Log include but not limited to: pain medication use relating the DFU(s), any anti-hypertensives, medications or supplements to control diabetes, nutritional supplements, and antibiotics, if applicable.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse Event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related.

Adverse events of interest under this protocol are those to be considered related to diabetes or the DFU. They include but are not limited to:

- Infection
- Increased chronic inflammation
- Allergic reaction
- Unexplained fever or chills
- Excessive redness
- Pain – excessive or exacerbated
- Swelling - excessive or exacerbated

- Non-characteristic wound odor not associated with product degradation (odor or suspected infection of wound)
- Pus drainage from the treated wound
- Serosanguinous drainage from the wound- excessive or exacerbated
- Bleeding- excessive or exacerbated
- Hematoma
- Seroma
- Diabetes related, specify
- Diabetic foot ulcer – newly developed or worsening
- Necrotic Tissue-excessive or exacerbated
- Gangrene
- Wound dehiscence
- Osteomyelitis
- Cellulitis
- Periwound maceration
- Wound recurrence (after closure)
- Hypergranulation of treated wound(s)
- Amputation
- Death

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. All SAEs are required to be collected following randomization through the final study visit for this study.

8.1.3 DEFINITION OF UNANTICIPATED ADVERSE DEVICE EFFECT (UADE)

Unanticipated adverse device effect is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

The Investigator will be asked to assess the severity of the AE using the following categories:

Mild: Events require minimal or no intervention and do not interfere with the participant's daily activities.

Moderate: Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: Events interrupt a participant's usual daily activity and may require systemic drug therapy or other intervention. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 RELATIONSHIP TO STUDY AGENT

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

Definitely: The relationship of the AE and the study device or the study procedure can definitely be established.

Probably: While a clear relationship to the study device or to the study procedure cannot be established, the AE is associated with an expected AE or there is no other medical condition or intervention, which could explain the occurrence of such an event.

Possibly: There is no clear relationship between the AE and the study device or study procedure; however, one cannot definitely conclude that there is

no relationship.

Unrelated: There is no relationship between the AE and the study device or study procedure. This may include but is not limited to the incident being an expected outcome of a previously existing or concurrent disease, concomitant medication or procedure the subject experienced.

8.2.3 EXPECTEDNESS

The Investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study products.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits or upon review by a study monitor. All AEs (per Investigator discretion) that are deemed 'Possibly', 'Probably' or 'Definitely' related to the development or healing of the DFU(s) and those including local and systemic reactions not meeting the criteria for an SAE, will be captured on the appropriate CRF. Information to be collected includes, but is not limited to, event description, date of onset, clinician's assessment of severity, relationship to study products, and date of resolution/stabilization of the event. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time following initial treatment application through final study visit. At each study visit, the Investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

Adverse Events will be documented on the appropriate Case Report Form (CRF) as the Investigator learns of the event. All AEs, not serious in nature, will be reviewed by the Sponsor during scheduled Interim Monitoring Visits (IMVs). The Investigator will follow all AEs until adequate resolution is achieved or until AE returns to baseline status, at which point the AE would be considered resolved. The IRB should be notified of all AEs according to their notification policies.

All AEs will be reported to ACell Quality Assurance and reported under the appropriate regulatory consideration.

8.4.2 SERIOUS ADVERSE EVENT (SAE) REPORTING

In the case of an SAE, the Investigator must immediately notify (within 1 working day of becoming aware of the event) the study Sponsor (contact information is provided in **Section 1, Key Roles).** The IRB must also be notified according to their notification policies.

All SAEs will be followed until satisfactory resolution or until the site Investigator deems the event to be chronic or the appearance to be stable. Other supporting documentation of the event may be requested by the study Sponsor and should be provided as soon as possible. The study Sponsor will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the Sponsor's initial receipt of the information.

8.4.3 UNANTICIPATED ADVERSE DEVICE EFFECT (UADE) REPORTING

The study Investigator shall submit to the Sponsor and to the reviewing IRB as soon as possible, but in no event later than 10 working days after the Investigator first learns of the effect. The Sponsor contact information is provided in **Section 1, Key Roles**. The study Sponsor is responsible for conducting an evaluation of an UADE and shall report the results of such evaluation to the FDA, all reviewing IRB(s) and Investigator(s) within 10 working days after the Sponsor first receives notice of the effect.

This study is not being conducted under an IDE; therefore, Sponsor reporting timelines may differ from those defined above.

8.4.4 REPORTING OF PREGNANCY

If a female subject becomes pregnant during the trial, she must be followed until the outcome of the pregnancy is known.

If pregnancy occurs, the Investigator must contact the Sponsor immediately for further instruction. Both the detection and the outcome of the pregnancy must be reported to the Sponsor.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with Good Clinical Practice (GCP), and with applicable regulatory requirement(s).

A Clinical Monitoring Plan will describe in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

A Statistical analysis Plan (SAP) will be written and signed-off prior to the first subject being enrolled in the study. The SAP will include a comprehensive description of the statistical methods and analyses to be included in the final study report. Any change to the analysis methods described in the protocol will require an amendment only if it changes a principal feature of the protocol. Any other change to the analysis methods described in the protocol, and the justification for making the change, will be described in the SAP.

Tabulations of summary statistics, graphical presentations, and statistical analyses will be performed using SAS software version 9.4. The statistical analyses will be based on data pooled across subject and wound characteristics and presented in aggregate; a separate series of analyses will retain specific subject and wound characteristics found to be significant covariates in response. All subset summary tables and data listings will be sorted by subject. The pre-procedure observations will be used as the baseline value for calculating post-procedural changes from baseline.

Statistical significance will be declared if the two-sided probability value is <0.05; tests for interaction will be performed at the 0.1 alpha level. Asymptotic confidence limits will be presented based on the normal approximation to the binomial distribution for differences in proportions. Exact confidence intervals will be used for all other presentations. All computations will be performed using SAS® (Version 9.4 or higher).

10.2 STATISTICAL HYPOTHESES

Primary Efficacy Endpoint(s):

The primary endpoint of the study is the incidence of complete wound closure by 12 weeks between Group 1 and Group 2. The comparison between the randomized groups will be based on the overall incidence of complete closure.

Ho: Incidence of complete wound closure by 12 weeks in Group 1 = Incidence of complete wound closure by 12 weeks in Group 2

Ha: Incidence of complete wound closure by 12 weeks in Group 1 ≠ Incidence of complete wound closure by 12 weeks in Group 2

Secondary Efficacy Endpoint(s):

The first secondary endpoint is the change in wound size, measured in cm^2 per week. The null and alternative hypotheses are presented below.

Ho: Change in the wound surface area in Group 1 = Change in the wound surface area in Group 2

Ha: Change in the wound surface area in Group 1 ≠ Change in the wound surface area in Group 2

The second secondary endpoint is the time to complete wound closure, in days, between Group 1 and Group 2. The null and alternative hypotheses are presented below.

Ho: Median time to complete closure in Group 1 = Median time to complete closure in Group 2

Ha: Median time to complete closure in Group 1 ≠ Median time to complete closure in Group 2

The third secondary endpoint is the incidence of wound recurrence after healing is complete at 26 weeks and 52 weeks (6 & 12 months post intervention initiation). The null and alternative hypotheses are presented below.

Ho: Incidence of wound recurrence after healing at 26 weeks in Group 1 = Incidence of wound recurrence after healing at 26 weeks in Group 2

Ha: Incidence of wound recurrence after healing at 26 weeks in Group 1 ≠ Incidence of wound recurrence after healing at 26 weeks in Group 2

Ho: Incidence of wound recurrence after healing at 52 weeks in Group 1 = Incidence of wound recurrence after healing at 52 weeks in Group 2

Ha: Incidence of wound recurrence after healing at 52 weeks in Group 1 ≠ Incidence of wound recurrence after healing at 52 weeks in Group 2

The fourth secondary endpoint is the changes in subject reported outcomes (quality of life surveys & visual analogue scale), pre- intervention to post- intervention. The null and alternative hypotheses are presented below.

Ho: Change in the subject reported outcomes in Group 1 = Change in subject reported outcomes in Group 2

Ha: Change in the subject reported outcomes in Group 1 ≠ Change in the subject reported outcomes in Group 2

The fifth secondary endpoint is the incidence of adverse events (serious and non-serious) related to DFU throughout the duration of the study and compared between randomized groups. The null and alternative hypotheses are presented below.

Ho: Change in the overall incidence of DFU-related adverse events in Group 1 = Change in the overall incidence of DFU-related adverse events in Group 2

Ha: Change in the overall incidence of DFU-related adverse events in Group 1 ≠ Change in the overall incidence of DFU-related adverse events in Group 2

Ho: Change in the incidence of serious DFU-related adverse events in Group 1 = Change in the incidence of serious DFU-related adverse events in Group 2

Ha: Change in the incidence of serious DFU-related adverse events in Group 1 ≠ Change in the incidence of serious DFU-related adverse events in Group 2

Health Economic Outcome(s):

There are 4 specific outcomes that will be summarized in this clinical investigation. The summary statistics that will be calculated will facilitate a comparison of the point estimates and variability. No formal hypothesis testing will be conducted to compare the 2 groups. However, the summary statistics and appropriate confidence intervals will be presented in a graphical format to examine the differences between the intervention and standard of care groups.

A summary of the 4 health economic outcome parameters is presented below.

- Summary of the changes in the prescription of narcotics related to the DFU(s) by subject and between randomized groups
- Summary of the changes in ambulatory status (i.e. bed, wheel chair, walk w/ assistance, or walk independent) by subject and between randomized groups
- Summary of the changes in “return to work/reported work status”, activities of daily living, or disability status by subject and between randomized groups
- Summary of the direct costs as measured by total cost per subject stratified by healed vs. non-healed DFU

10.3 ANALYSIS DATASETS

Safety Population - The Safety population will include all subjects receiving any investigational product. The safety population will be used for the safety analysis.

Intention-to-Treat (ITT) Population - The ITT population will include all subjects in the Safety population with at least one post-baseline efficacy data point. The ITT population will be used for the primary efficacy analysis.

Per Protocol (PP) Population - The PP population will consist of subjects in the ITT population who did not have major protocol violations. The review of protocol violations, which will be defined in the statistical analysis plan (SAP), will be performed and signed off prior to study database lock. The primary efficacy analysis and the secondary analysis will be conducted on the PP population as part of a sensitivity analysis.

Specific algorithms for imputing missing or partially missing dates will be discussed in the Statistical Analysis Plan. Derived data will be identified in the individual subject data listings. Imputed data for dates will not be incorporated into the case report form datasets. Imputed data for dates will be used in the preparation of the derived datasets. All recorded data will be presented in the individual data listings.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

The observed data will be used for analysis. Specific algorithms for imputing missing or partially missing dates will be discussed in the Statistical Analysis Plan. Derived data will be identified in the individual subject data listings. Imputed data for dates will not be incorporated into the case report form datasets. Imputed data for dates will be used in the preparation of the derived datasets. All recorded data will be presented in the individual data listings.

The pre-procedure observations will be used as the *baseline* value for calculating post-procedural changes from baseline.

Continuous demographic parameters, such as the age of the subject at the time of enrollment, will be summarized for the ITT population using descriptive statistics (N, mean, median, standard deviation, minimum and maximum value, and 2-sided 95% confidence limits). Categorical demographic parameters, such as gender, will be summarized as a proportion of the ITT population using Clopper-Pearson 2-sided 95% confidence limits.

Adverse events will be coded according to the MedDRA system dictionary. The percentage of subjects experiencing adverse events will be summarized by body system and preferred term. Subject counts will be tabulated for all adverse events for the ITT population. Adverse events will also be tabulated for events that occurred following initial treatment application through final study visit. Tabulated subject counts will be presented as a proportion of the ITT population with 95% binomial confidence intervals.

10.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary efficacy analysis population will be subjects in the ITT population who have any wound measurement data following randomization. For all statistical and data analyses, wound measurement data and confirmation of wound closure will be obtained from an independent wound core lab or clinical assessor based on the photograph of the wound and the assessment of the investigator (closed vs. not closed). For data analyses, complete wound closure requires confirmation at 3 weeks(≥ 21 days) post clinically apparent wound closure. The date of complete wound closure for those that have confirmed closure will be the date that it was clinically apparent (up to and including 12 weeks post Randomization Day).

The primary efficacy analysis will compare the proportion of subjects who achieve complete target ulcer closure within 12 weeks after the first study intervention using a random-effects generalized linear model specifying the distribution of the dependent variable as binomial. Results from this analysis are expected to approach the test statistic used to derive the sample size estimates.

10.4.3 ANALYSIS OF THE SECONDARY AND TERTIARY ENDPOINT(S)

For the secondary efficacy endpoints, the change in the wound size, measured in cm^2, will be calculated on an intra-subject basis for each post-baseline measurement. The mean change per week will be calculated for each subject and compared between the intervention and standard of care groups using a random-effects generalized linear

model specifying the distribution of the dependent variable as continuous. The time to complete wound closure will be calculated in days from the date of the initial study intervention and compared between the intervention and standard of care groups using a log-rank test. Subjects who fail to achieve complete wound closure will be censored using the last date of examination in the study. Wound recurrence after healing is complete at 26 weeks and 52 weeks will be tabulated by group and the proportions will be compared between intervention and standard of care groups using a 2-tailed Fisher's exact test. The time to wound recurrence will also be compared between the intervention and standard of care groups. Changes in subject reported outcomes will be summarized and compared based on the distribution of the data. A random-effects generalized linear model, specifying the distribution of the dependent variable based on the type of data, will be used to compare the results over time between the intervention and standard of care groups.

The incidence of adverse events related to DFU recorded during the course of the study will be totaled by intervention group and compared between randomized groups using a generalized linear model and specifying the distribution as binomial. A separate tabulation will be prepared comparing the overall incidence of all adverse events, and restricted to serious adverse events.

The first health economic outcome parameter to be summarized is the change from baseline in the prescription of narcotics related to the DFU(s) by subject and between randomized groups. The summary statistics will be calculated using the daily dosage converted into morphine equivalent units, averaged over successive 7-day intervals from the time of study enrollment until week 52. The average 7-day changes will be summarized by group assignment.

The second health economic outcome parameter is the intra-subject change in ambulatory status (i.e. bed, wheel chair, walk w/ assistance, or walk independent). The distribution of subjects by ambulatory status (improved, no change, worse) at 12, 26 and 52 weeks will be summarized by group. The 2-sided 95% exact binomial confidence intervals will be summarized by ambulatory status category and treatment group. The time to a change in status (improved status and worsening status) will also be summarized using K-M estimates and compared between the intervention and standard of care groups using a log-rank test.

The third health economic outcome includes work status, disability status, and activities of daily living. The change in "return to work/reported work status" or disability status as defined by the investigator's evaluation of the subject's ability to return to work related to their DFU status will be derived for each subject and compared between randomized groups. It is expected a measureable proportion of the subjects may not be employed at the time of enrollment for reasons not related to

their DFU. Shift tables will be prepared by intervention and standard of care only assignment based on sequential 30 day intervals to categorize the proportion of subjects who transition from not working because of the DFU to working following study intervention for the DFU. Separately, the time to return to work will be summarized using descriptive statistics and Kaplan-Meier estimates. Finally, the investigator will be asked to provide the date of the examination where the subject is cleared to return to work, independent of the subject's actual work status. Using this date, the number of days from the date of enrollment will be summarized by intervention assignment using descriptive statistics and summarized by intervention group using Kaplan-Meier estimates. This tabulation will include all subjects, independent of the subject being employed prior to enrollment into the study.

The scores from the Activities of Daily Living questionnaire will be summarized each time the questionnaire is administered. The actual scores, and the changes from scores recorded at baseline, i.e., prior to study intervention, will be summarized using descriptive statistics and compared between the intervention and standard of care only groups using an analysis of variance test.

The fourth health economic outcome is the total direct cost per DFU by intervention and standard of care only group assessed during the treatment period. The total intra-subject direct cost will include the wound care time (physician time, nursing time, procedural time, application of products dressing time, application of off-loading device, etc.) and will be modeled over sequential 30 day intervals culminating at the end of the intervention phase; additional factors that are known predictors of healing, along with specific data about the size, depth, and location of the ulcer will be added to the model to derive an estimated direct intervention cost.

10.4.4 SAFETY ANALYSES

Safety will be assessed by physical examinations, clinical laboratory tests and collection of AEs as outlined in the Schedule of Events. All summaries of AEs will be based on intervention-emergent adverse events, which will be coded according to the MedDRA system dictionary. The number and percentage of subjects experiencing adverse events will be summarized by body system organ class and description. Subject counts will be tabulated for all intervention- emergent adverse events for the ITT population. Adverse events will also be tabulated for events that occurred following initial treatment application through final study visit. Summaries by maximum severity and relationship to the study intervention will also be provided. SAEs and AEs leading to discontinuation from the study will be presented by system organ class and preferred term. Tabulated subject counts will be presented as a proportion of the ITT population with 95% binomial confidence intervals.

Adverse events will be coded using MedDRA. Verbatim description of the adverse events and the MedDRA System Organ Class (SOC) and Preferred Term (PT) for all AEs will be contained in the subject data listings. A separate listing sorted by MedDRA SOC and PT will include all verbatim descriptions associated with each SOC/PT category. All reported AEs will be included in by-subject AE listings. A separate listing will be created with all the distinct levels of SOC, Preferred Terms and the verbatim investigator description reported in the study. Sorting will be alphabetically by SOC, Preferred Term and then verbatim description.

The incidence of AEs will be presented using counts and percentages of subjects with AEs and tabulated by SOC and PT. SOC will be sorted alphabetically and PT within SOC will be sorted by descending frequency based on the incidence across subjects overall. If a subject has multiple occurrences (start and stop) of an event associated with a specific SOC or PT within a SOC, a subject will only be counted once in the incidence count for the SOC or PT within SOC respectively.

The following specific summary tables may be generated:

- Overall incidence of adverse events
- Incidence of AEs by SOC overall and by PT within each SOC
- Incidence of AEs related to Intervention by SOC overall and by PT within each SOC
- If a subject reports two or more adverse events that code to the same PT, the event with the maximum relationship will be included in the table. AEs with a missing relationship will be assumed to be intervention related.
- Incidence of AEs by Maximum Severity, SOC overall and by PT within each SOC
- If a subject reports two or more adverse events that code to the same PT, the event with the maximum severity will be included in the table. AEs with a missing severity will be assumed to be severe.
- Incidence of AEs occurring in ≥ 5% of subjects in at least one intervention group by SOC overall and by PT within each SOC
- Incidence of intervention-related AEs occurring in ≥ 5% of subjects in at least one intervention group by SOC overall and by PT within each SOC
- Incidence of Serious AEs by SOC overall and by PT within each SOC
- Incidence of AEs leading to treatment discontinuation by SOC overall and by PT within each SOC

Deaths, other SAEs, and AEs leading to treatment discontinuation, dose interruption, or dose reduction will be listed including the intervention group and dosing information (if applicable), start and stop dates/times of the AE, and days on study relative to the day of exposure to the study product.

10.4.5 ADHERENCE AND RETENTION ANALYSES

The number and proportion of subjects eligible for and compliant with each follow-up examination will be presented. Subjects who withdraw from the study will be tabulated with reasons for withdrawal. The primary presentation of the results for the ITT population will be based on the observed data with imputation for missing endpoint data using the last recorded observation. Compliance and device duration will be summarized using descriptive statistics (N, mean, median, standard deviation, minimum and maximum value, and 2-sided 95% confidence limits).

10.4.6 BASELINE DESCRIPTIVE STATISTICS

Baseline is defined as the observations recorded prior to the application of study intervention. Continuous demographic parameters, such as the subject's age at the time of enrollment, will be summarized for the ITT population using descriptive statistics (N, mean, median, standard deviation, minimum and maximum value, and 95% two-sided confidence limits) and compared between groups using a two-sample t-test. Categorical demographic parameters, such as gender, will be summarized as a proportion of the ITT population and compared using a two-tailed Fisher's exact test. Co-morbid risk factors will be summarized for the ITT population by intervention assignment and according to the type of variable (categorical, continuous) and compared between groups. Kaplan-Meier estimates for time-to-event analyses will be prepared based on the ITT population. Separate tables containing subject counts, percentages, and 95% binomial confidence limits will be prepared based on individual risk factors.

10.4.7 PLANNED INTERIM ANALYSES

A sample size re-estimation will be performed when 50% of the target population or 60 subjects have completed the intervention / evaluation period (confirmed complete wound closure or no confirmed wound closure by Visit 13) of the study to derive the primary endpoint results.

10.4.7.1 SAFETY REVIEW

The clinical investigator will be responsible for monitoring the safety of the subjects on an ongoing basis.

10.4.7.2 EFFICACY REVIEW

Efficacy will be based on the results from the final analysis of the primary endpoint.

10.4.8 ADDITIONAL SUB-GROUP ANALYSES

All sub-group analyses will be described in the SAP.

10.4.9 MULTIPLE COMPARISON/MULTIPLICITY

There is a single primary endpoint and no multiple testing will be performed.

10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

The method for presenting the individual response data will presented in the SAP.

10.4.11 EXPLORATORY ANALYSES

All exploratory analyses will be described in the SAP.

10.5 SAMPLE SIZE

The maximum number of subjects to be enrolled into this clinical investigation is 150 subjects; the target sample size is 120 subjects, based on a difference in the proportion of responders of 25% and a power approaching 80%.

Estimates were prepared based on the primary endpoint considering a type 1 error rate of 5%. A 25% difference in the incidence of response was used to estimate the statistical power over a range of sample sizes from 100 to 150 subjects. To address concerns regarding the reliability of the estimates of intervention effectiveness, an adaptive mid-course sample size re-estimation procedure will be performed when 50% of the subjects have completed the 12 week study or withdrawn prematurely. The data for the re-estimation of sample size will be unblinded for the independent biostatistician; the sample size will be increased based on promising conditional power; therefore, there will be no inflate of the type I error[2].

Table 1 presented below contains 6 scenarios considering total sample sizes from 100 to 150 subjects.

Table 1: Power Estimates based on a Type 1 Error Rate of 5% and a 2:1 Randomization Ratio (active: control)

Scenario	Power	Total Sample Size	Sample Size / Active Intervention	Sample Size / Control	Incidence of Closure / Active Intervention (%)	Incidence of Closure / Control (%)
1	0.700	100	67	33	45%	20%
2	0.740	110	73	37	45%	20%
3	0.787	120	80	40	45%	20%
4	0.818	130	86	44	45%	20%
5	0.844	140	93	47	45%	20%

6	0.874	150	100	50	45%	20%

With a target sample size of 120 subjects, if the difference in the incidence of response was 25% with exactly 45% of the subjects in Group 1 responding and 20% of the subjects in Group 2 responding, the power would be just under 80%. The re-estimation of the sample size by the independent biostatistician is designed to ensure adequate power for the final analysis if the results are promising at the interim assessment.

10.6 MEASURES TO MINIMIZE BIAS

The methods for assessing bias will be described in the SAP.

10.6.1 RANDOMIZATION

Subjects will be randomized using a permuted block randomization scheme in a 2:1 ratio of Cytal to SOC.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Principal Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a subject number and initials.

It is the responsibility of the Investigator to maintain adequate and accurate eCRFs designed by the Sponsor to record all observations and other data pertinent to the clinical investigation. All paper documentation should be completed in their entirety in a neat, legible manner to ensure accurate interpretation of data; black or blue ink should be used to ensure the clarity of the reproduced copy of all documentation. Should a correction be made, the information to be modified must not be overwritten. The corrected information will be transcribed by the authorized person next to the previous

value, initialed and dated, and the corrected documentation should be verified again by the monitor.

The handling of the data by the Sponsor after review of the eCRFs may generate additional requests to which the Investigator is obliged to respond by confirming or modifying the data questioned.

Participating site(s) will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of the Sponsor or designee, and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to: hospital records, clinical and office charts, laboratory notes, memoranda, participants' memory aids or evaluation checklists, pharmacy records, recorded data from automated instruments, , and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

The SF-20 Health Survey, DFS-SF, and VAS for Pain will be considered source documents and also act as the original CRFs.

12 QUALITY ASSURANCE AND QUALITY CONTROL

Quality Control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol and GCP.

The site will provide direct access to all trial related materials, including source data/documents, electronic medical records (if applicable), CRFs, and reports for the purpose of monitoring and auditing by the Sponsor and/or the IRB.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The Investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 LAWS AND REGULATIONS

This clinical study will be conducted in compliance with all national laws and regulations of the countries in which the clinical trial is performed, as well as any applicable guidelines. The trial will be registered on www.clintrials.gov and on other sites, as appropriate.

13.3 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.4 INFORMED CONSENT PROCESS

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB approved and the participant will be asked to read and review the document. The Investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

If a consent document is revised due to changes in study procedures, subjects who were enrolled prior to the change, but are affected by the change, will be informed of the

changes and will sign the amended consent document. If a consent document is revised due to changes in the risks or safety of the study, all active participants must sign the revised consent.

13.5 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating Investigator(s), their staff, the Sponsor and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

The study monitor, other authorized representatives of the Sponsor, and/or representatives of the IRB may inspect all documents and records required to be maintained by the Investigator for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the local IRB and Institutional regulations.

14 DATA HANDLING AND RECORD KEEPING

14.1 STUDY RECORDS RETENTION

All study documents (subject files, signed informed consent forms, Study Regulatory Binder, etc.) must be kept secured for a period of two years following completion of the study. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

14.2 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the participant, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

All protocol deviations/violations should be documented using the Protocol Deviations/Violations CRF and submitted to the IRB according to their reporting guidelines.

14.3 PUBLICATION AND DATA SHARING POLICY

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

15 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the device industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

16 LITERATURE REFERENCES

1. American Diabetes Association (ADA). (2017). Statistics about diabetes: Overall numbers, diabetes and prediabetes. Retrieved from: *http://www.diabetes.org/diabetes-basics/statistics/*

2. Chen, Y. N., DeMets, D. L., Lann, K. K. (2004). Increasing the sample size when the unblended interim result is promising. *Statistics in Medicine, 23*(1), 1023-1038.

3. Dumville, J. C., O'Meara, S., Deshpande, S., & Speak, K. (2013). Hydrogel dressing for healing diabetic foot ulcers (review). *Cochrane Database of Systematic Reviews, 7*(1), 1-52. doi: 10.4002/14651858.CD009101.pub3.

4. Gordon, K. A., Lebrun, E. A., Tomic-Canic, M., & Kirsner, R. (2012). The role of surgical debridement in healing of diabetic foot ulcers. *SKINmed, 10*(1), 24-26.

5. Iraj, B., Khorvash, F., Ebneshahidi, A., & Askari, G. (2013). Prevention of diabetic foot ulcer. *International Journal of Preventative Medicine, 4*(3), 373-376.

6. Margolis, D. J., Malay, S., Hoffstad, O. J., Leonard, C. E., MaCurdy, T., Tan, Y., Molina, T., Lopez de Nava, K., & Siegel, K. L. (2011). Economic burden of diabetic foot ulcers and amputations: Data points #3. Data Points Publication Series [Internet]. Rockville, MD: Agency for Healthcare Research and Quality (US).

7. Margolis, D. J., Kantor, J., Berlin, J. A. (1999). Healing of diabetic neuropathic foot ulcers receiving standard treatment. *Diabetes Care, 22*(5), 692-695.

8. Rice, J. B., Desai, U., Cummings, A. K., Birnbaum, H. G., Skornicki, M., & Parson, N. B. (2014). Burden of diabetic foot ulcers for Medicare and private insurers. *Diabetes Care, 27*(1), 651-658.

9. Veves, A, Sheehan, P., Pham, H. T. (2002). A randomized, controlled trial of Promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers. *Archives of Surgery, 137*(7), 822-827.

10. Veves, A., Falanga, V., & Armstrong, D. G. (2001). Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: A prospective randomized multicenter clinical trial. *Diabetes Care, 24*(2), 290-295.

11. Wu, L., Norman, G., Dumville, J. C., O'Meara, S., Bell-Syer, S. E. (2015). Dressing for treating foot ulcers in people with diabetes: An overview of systematic reviews (review). *Cochrane Database of Systemic Reviews, 7*(1), 1-62.

12. Yazdanpanah, L., Nasiri, M., Adarvishi, S. (2015). Literature review on the management of diabetic foot ulcer. *World Journal of Diabetes, 6*(1), 37-53.

13. Zelen, C. M., Orgill, D. P., Serena, T., Galiana, R., Carter, M. J., DiDomenico, L. A., Keller, J., Kaufman, J., & Li, W. W. (2017). A prospective, randomized, controlled, multicenter clinical trial examining healing rates, safety and cost to closure of an acellular reticular allogenic human dermis versus standard of care in the treatment of chronic diabetic foot ulcers. *International Wound Journal, 14*(2), 307-315. doi: 10.1111/iwj.12

14. Bann, C. M., Fehnel, S. E., & Gagnon, D. D. (2003). Development and validation of the diabetic foot ulcer scale-short form (DFS-SF). Pharmacoceconomics, 21(17), 1277-1290.

15. Bann, C. M., Fehnel, S. E., & Gagnon, D. D. (2003). Development and validation of the diabetic foot ulcer scale-short form (DFS-SF). *Pharmacoceconomics*, 21(17), 1277-1290.

16. Katz, S., Ford, A.B., Moskowitz, R.W., Jackson, B.A., & Jaffe, M.W., 1963. Studies of illness in the age. The index of ADL: a standardized measure of biological and psychological function. *Journal of the American Medical Association, 185,* 914–919.

APPENDIX I: SCHEDULE OF EVENTS

Parameters	Run-In Visit 1 Day -14 (+/- 2 days)	Run-In Visit 2 Day -7 (+/- 2 days)	V1 Enrollment/ Randomization Day 0 (+/- 2 days)	V2 7 days (+/- 2 days)	V3 14 days (+/- 2 days)	V4 21 days (+/- 2 days)	V5 28 days (+/- 2 days)	V6 35 days (+/- 2 days)	V7 42 days (+/- 2 days)	V8 49 days (+/- 2 days)	V9 56 days (+/- 2 days)	V10 63 days (+/- 2 days)	V11 70 days (+/- 2 days)	V12 77 days (+/- 2 days)	V13 84 days (+/- 2 days)	Confirmation Closure 1	Confirmation Closure 2	Confirmation Closure 3	Final Visit 1 26 Weeks (+/- 2 weeks)	Final Visit 2 52 Weeks (+/- 2 weeks)
Informed Consent	X																			
Assign Subject ID	X																			
Inc/Exc Criteria	X	X	X																	
Demographics	X																			
Employment/ Disability/Ambulatory			X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X
Height, Weight[1], BMI	X																		X	X
Med/Surg/Social History			X																	
Con Meds Log	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Wagner Ulcer Classification			X																	
Serum Creatinine	X																			
HbA1C Draw	X														X				X	X
Pre-Albumin Draw	X																			
Albumin Draw	X				X			X			X				X			X	X	X
Randomization			X																	
Pregnancy Test			X																	
AEs/SAE Eval			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ABI Eval[2]	X		X																	
Product Application			10 Applications in a 12 Week (84 day) Period																	
DFS-SF Admin			X															X	X	X
KATZ-ADL Admin			X						X						X			X	X	X
SF-20 Admin			X						X						X			X	X	X
VAS Admin			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Offloading Instruction and Documentation			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Closure Assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Recurrence Assessment																X	X	X	X	X

[1]Record weight and BMI at study visit wound healing is initially observed.

[2]Record ABI PRN for non-healing wounds. Laboratory tests as well as ABIs performed during the Run In period may be used at Study Visit 1 for confirmation of eligibility.

.

Closure Confirmation Visit 1 – Within 7 days to 91 Days Post Initial Product App **After** Healing

Closure Confirmation Visit 2 – Within 14 days to 98 Days Post Initial Product App **After** Healing

Closure Confirmation Visit 3 – Within 21 days to 105 Days Post Initial Product App **After** Healing

APPENDIX II: STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH E6, the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 812.

The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Sponsor and documented approval from the Institutional Review Board, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Signature of Principal Investigator	**Date (mm/dd/yy)**
Printed Name	
Name of Institution	

APPENDIX III: OPTIMA DIAB BOOTS USER'S GUIDE

See attachment.