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Statistical Analysis Plan

Prospective, Multi-Center, Randomized Controlled Trial Evaluating the Use of Primatrix Dermal Repair Scaffold for the Management of Diabetic Foot Ulcers

Protocol Number: T-PMXDFU-01 (Version 5.0)

Integra LifeSciences Corporation
311 Enterprise Drive
Plainsboro, NJ 08536 USA

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Protocols No: T-PMXDFU-01

Prepared By:

Qian Mao

27-JAN-2021

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Signature Date

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The undersigned have approved this Statistical Analysis Plan for use in this study.

Signature: Joshua J Dilworth **Date:** 27-JAN-2021

SignNow e-signature ID: 0add56f8a8...
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Joshua Dilworth DD/MMM/YYYY
Manager, Clinical Research
Integra LifeSciences Corporation

Signature: YI Arnold **Date:** 27-JAN-2021

SignNow e-signature ID: 4640d930e5...
01/27/2021 17:53:26 UTC

Yi Arnold DD/MMM/YYYY
Snr. Manager, Medical Affairs
Integra LifeSciences Corporation

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Document Revision Number: 1.1 change history

Original	Revision	Justification
Version 1.0 established on 03AUG2020	See changes below.	Changes made to fix errors identified.
• Rate of complete wound closure as assessed by computerized planimetry A point estimate will be presented along with the exact 95% confidence interval based on the binomial distribution.	• Rate of complete wound closure as assessed by computerized planimetry	Deleted section was not errantly copied from other section but was not deleted in the past.
At the begininig of the study, site 109 had randomization issues due to data entry delay to the EDC. 3 subjects were not randomized to their original treatment assignments per randomization date/time. A sensitivity analysis will be conducted to assess the impact on the randomization issue on the primary endpoint using the primary efficacy analysis population.	At the begininig of the study, site 109 had randomization issues due to data entry delay to the EDC. Eight (8) subjects were not randomized to their original treatment assignments per randomization date/time. A sensitivity analysis will be conducted to assess the impact on the randomization issue on the primary endpoint using the primary efficacy analysis population.	Typo, it should be 8 subjects in total from stie 109, not 3.
Treatment groups will be compared with respect to the incidence of Adverse Events (all and TEAEs) and the proportion of subjects withdrawing from the study for Adverse Events by reason of withdrawal. Comparisons will be made overall, by body system and by preferred term and will be further categorized by severity and by study drug relationship.	Treatment groups will be compared with respect to the incidence of Adverse Events (all and TEAEs) and the proportion of subjects withdrawing from the study for Adverse Events by reason of withdrawal. Comparisons will be made overall, by body system and by preferred term and will be further categorized by severity and by study device relationship.	Primatrix is device, not drug, therefore, the study drug relationship has been changed to "Study device relationship"

1. LIST OF ABBREVIATIONS

Abbreviation	Term
ABI	Ankle/Brachial Index A comparison of the systolic blood pressure at the ankle compared to that obtained in the right arm pressure. The systolic pressure obtained in the ankle is divided by that obtained in the arm.
Active Treatment Group	Subjects randomized to the Active Treatment Group will receive PriMatrix Dermal Repair Scaffold dressed with a non-adherent contact layer, a bolster of saline moistened gauze (if necessary to maintain intimate contact between PriMatrix and ulcer bed) 0.9% sodium chloride gel as required to maintain a moist wound environment, a non-adherent foam dressing, an outer elastic gauze wrap, a self-adherent wrap, and provided an off-loading/protective device appropriate to the location of the ulcer. Wound dressings will be changed weekly at the study site.
AE	Adverse Event (As further defined in Section 5.1)
BMI	Body Mass Index
CFR	Code of Federal Regulations
Complete Wound Closure	100% re-epithelialization of the ulcer surface without detectable exudate, confirmed on 2 consecutive study visits 1 week apart
Control Treatment Group	Subjects randomized to the Control Treatment will receive Standard of Care consisting of 0.9% Sodium Chloride gel applied to the wound as required to maintain a moist wound environment, a non-adherent foam dressing, an outer gauze wrap, a self-adherent wrap, and an off-loading/protective device appropriate to the location of the ulcer. Dressings will be changed daily by the subject. Wound dressings and instructions for wound care will be provided to the subject.
DFU	Diabetic Foot Ulcer, a foot wound that is a result of the body's response to an injury given the altered immunologic, neurological, and vascular state of the diabetic. These wounds are hard to heal and often are chronic in nature.
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FDA	Food and Drug Administration
Full-Thickness	Refers to the depth of a wound in which both the epidermis and dermis are lost.
GCP	Good Clinical Practices
HbA_1c	Glycosylated Hemoglobin

Abbreviation	Term
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IEC	Independent Ethics Committee
Integra	Integra LifeSciences Corporation
IRB	Institutional Review Board
ITT	Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MRU	Medical Resource Utilization
Partial-Thickness	Refers to the depth of a wound involving a portion of the dermal layer of the skin and the entire epidermal layer of the skin.
PEA	Primary Efficacy Analysis
PP	Per Protocol
QC	Quality Control
SAE	Serious Adverse Event (As further defined in section 5.1)
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SoC	Standard of Care
SD	Standard Deviation
Sharp Debridement	The surgical removal of all devitalized tissue involving the use of a knife, curette or blade.
SOP	Standard Operating Procedures
SV	Screening Visit
$TcPO_2$	Transcutaneous Oxygen Pressure
TV	Treatment Visit
USA	United States of America

2. INTRODUCTION

This document provides a detailed description of the statistical methods and procedures to be implemented during the analysis of the clinical study. The methods and procedures are intended to support the generation of study report, including detailed descriptions of the populations and methodologies, as well as summary tables, listings and graphics.

This statistical analysis plan (SAP) is based on Version 5.0 of the Protocol # T-PMXDFU-01.

3. STUDY OBJECTIVES

The objective of this study is to evaluate the efficacy of PriMatrix Dermal Repair Scaffold in the management of diabetic foot ulcers (DFUs) in subjects with diabetes mellitus and without significantly compromised arterial circulation in comparison to Standard of Care (SoC) treatment. In addition, to the efficacy data, safety associated with the management of DFU, will be evaluated, as well as medical resource utilization (MRU).

3.1. Primary Efficacy Endpoint

The primary efficacy endpoint for the study is the incidence of complete wound closure of the study ulcer, as assessed by the investigator, during the 12-week treatment phase, and confirm one week later.

3.2. Secondary Efficacy Endpoints

The secondary endpoints include:

- Proportion of subjects with complete wound closure of the study ulcer during the 12-week treatment phase as assessed by computerized planimetry, during the treatment phase
- Time to complete wound closure, as assessed by the investigator.
- Time to complete wound closure, as assessed by computerized planimetry.
- Rate of wound closure as assessed by computerized planimetry.

3.3. Exploratory Endpoints

1) Medical resource utilization associated with treatment of DFU and related complications, including:

   - Interference of activities of daily living
   - Interference of paid work (full-time or part-time)/school
   - Physician office visits
   - Emergency room visits
   - Hospitalizations
   - Home health nursing visits
   - Procedures
   - Medication regimens to treat complications (analgesics, antibiotics, etc.)
   - Diagnostic tests, imaging, etc.
   - Treatment of adverse events
   - Use of durable medical equipment (off-loading device/protective, crutches, wheelchair, etc.)
   - Dressing regimen and materials

2) Wound closure status at the 4-week follow-up visit

3.4. Safety Endpoints

Safety will be assessed through reports of AEs and SAEs, including incidence of all AEs related to the study treatment, study ulcer or study procedures and all serious adverse events.

4. STUDY DESIGN

This is a multicenter, randomized, open-label, parallel-group clinical trial designed to establish the superior efficacy of PriMatrix over that of Control treatment in the management of DFUs in subjects with DM and adequate arterial circulation. Study enrollment will be paused once 50 subjects (25 in each treatment arm) have been enrolled in order to do an early data review. There will also be an interim analysis after 120 subjects have completed their study requirements.

Treatment Groups

- Active Treatment – PriMatrix Dermal Repair Scaffold + SoC
- Control Treatment – SoC

Study Phases

- Screening Phase

  After the informed consent process is complete, medical history will be collected and clinical assessments will be performed to determine subject eligibility for the study. If the required eligibility criteria are met, the subject will begin the two-week screening phase. During this phase, the subject will receive the control treatment as defined in Section 5. During the two-week screening phase, the investigator will determine if the subject continues to meet all eligibility criteria, and if confirmed, the subject will be randomized and enter the treatment phase.

- Treatment Phase

  Subjects will be randomized to receive the Active or Control Treatment in a 1:1 scenario (details can be found in Section 8.4). Efficacy evaluations during this phase include weekly investigator assessments of wound closure and planimetric evaluations of ulcer area. Safety evaluations will include AE assessments at each visit. Additionally, collection of medical resource utilization (MRU) data will occur. The randomized study treatment will be administered for up to 12 weeks or until the study ulcer has closed, as assessed by the investigator.

  At the end of the treatment phase, subjects, all of whom will be considered treatment phase completers, will be assigned to one of two outcomes:

  - Subjects with confirmed closed ulcers will be considered treatment successes.

  - Subjects with open ulcers at the end of the treatment phase will be considered treatment failures.

  Unscheduled Visits (i.e., those not specifically scheduled per protocol) are at the discretion of the investigator. If the unscheduled visit is for treatment of the target ulcer, data for that visit will be collected and reported on the Unscheduled Visit eCRF page. For detailed information, see Figure 1 and Table 3 below.

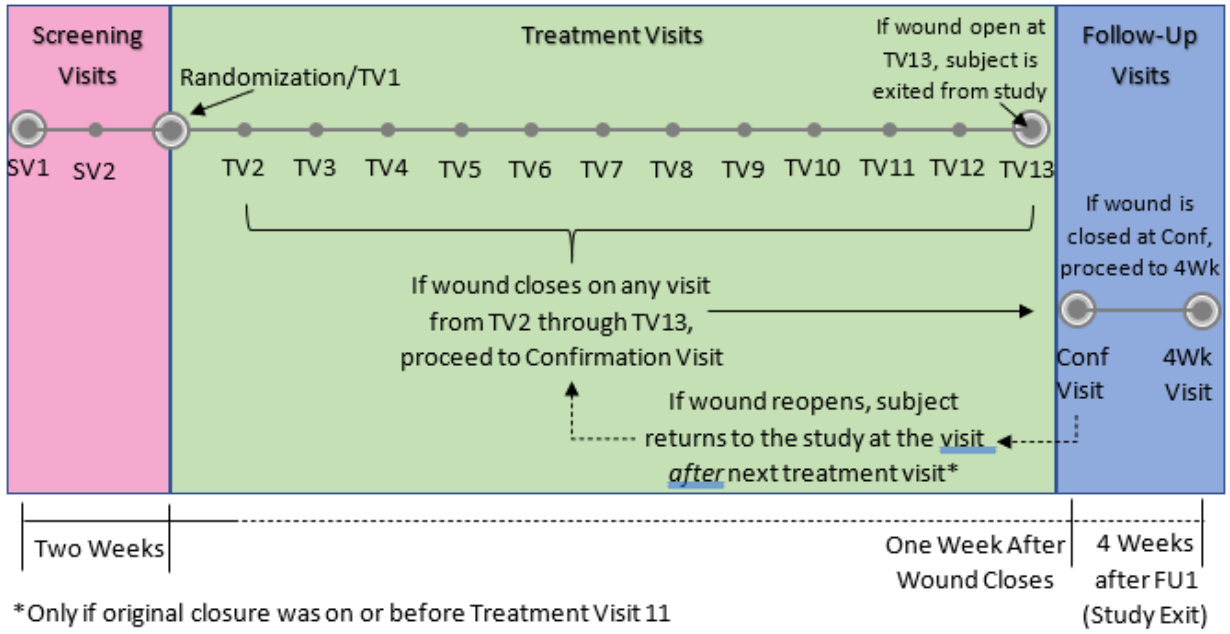
- Follow-up Phase

  Subjects whose ulcer is confirmed closed at the Confirmation Visit (i.e., 1 week after closure is observed) will return to the clinic 4-weeks later for a follow-up visit (28 ± 3 days) to evaluate if the ulcer has remained closed.

5. STUDY PROCEDURE

The flow chart and schedule for this study are as follows. Additional information concerning the conduct of the study can be found in the protocol.

5.1. Study Flow Chart



5.2. Schedule of Events

Procedure	Screening Phase		Treatment Phase													Follow-Up Phase	
	SV 1 -14±3d	SV 2 -7±3d	Randomization/ TV[3] 1 (Day 0)	TV 2 7±3d	TV 3 14±3d	TV 4 21±3d	TV 5 28±3d	TV 6 35±3d	TV 7 42±3d	TV 8 49±3d	TV 9 56±3d	TV 10 63±3d	TV 11 70±3d	TV 12 77±3d	TV 13 84±3d	Conf Visit 7±3d After Closure	4-Week 28±3d After Confirm.
Inf. Consent/HIPAA	X																
Inclusion/Exclusion	X	X	X														
MRU Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Demographics	X																
Medical History	X																
Physical Exam	X																
Vascular Perfusion	X																
Ulcer Assessments[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ulcer Photography	X[5]	X[6]	X[4,7]	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	X[8]	X[8]
Ulcer Debridement	X	X[9]	X	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]			
Ulcer Measurement	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Control Treatment	X	X	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]			
Treatment Compliance	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Offloading[11]	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Concomitant Meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ulcer Size Reduction			X														
Randomization			X														
Active Treatment			X[12]	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]			
Closure Assessment			X	X	X	X	X	X	X	X	X	X	X	X	X[14]	X	X

[1] Only to be used for a visit that falls out of range of one of the other visit windows. If the proposed Unscheduled Visit falls within the next visit window, use that visit instead of documenting an unsched
[2] See important information in section 6.2.5 for guidance on Unscheduled Visits after wound closure.
[3] "TV" = Treatment Visit
[4] Study Ulcer Assessments include: 1) location 2) duration 3) size 4) exudate 5) infection 6) measurement of ulcer area
[5] Collect pre- and post-debridement images
[6] Collect pre-debridement image; if performing debridement, collect post-debridement image
[7] Post-application image required if randomized to PriMatrix
[8] Image of the closed wound area to be taken
[9] Debride, if necessary
[10] If randomized to the Standard of Care cohort
[11] Not required for dorsal wounds; required for plantar ulcers or lateral ulcers that would benefit from offloading
[12] If randomized to the Active Treatment and if necessary; see Section 5.17 for guidelines on applying PriMatrix.
[13] Optional application of PriMatrix. See Section 5.17 for guidelines on applying or re-applying PriMatrix
[14] If wound is not healed at Treatment Week 13, Investigator will document why, in his opinion, the wound did not heal.

6. SAMPLE SIZE DETERMINATION AND RATIONALE

This is a randomized study with two treatment groups. Assuming:

- 46% of DFUs receiving PriMatrix close by 12 weeks (84 days).
- 26% of DFUs receiving with Standard of Care close by 12 weeks (84 days).

Overall 2-sided Type I Error rate (alpha) of 0.05. Under the above assumptions, 102 subjects per treatment group will be required to meet the Type I error rate of 0.05 and 80% power; for a total

of 204 subjects for the study. As the treatment duration is short, the discontinuation rate is predicted to be around 15%. To accommodate the potential discontinuations, 240 subjects will be randomized (120 per treatment group) to this trial. The subjects will be randomized to the treatment groups in a 1:1 ratio.

A single interim analysis when 50% of subjects complete the trial, using group-sequential methods and Pocock alpha spending function, where the hypotheses will be tested at a two-sided alpha of 0.031 at the interim analysis and at a two-sided alpha of 0.028 at the final analysis.

7. RANDOMIZATION

The randomization will be stratified by center and will use mixed blocks of 2 and 4 with a 1:1 ratio to ensure even distributions of Active and Control subjects at each site. Trial ulcer size (area) at the time of randomization (2 strata: 1 cm^2 to $\leqslant$3 cm^2 vs. >3 cm^2 to 12 cm^2) will also be used as a stratification factor to ensure balance between treatment groups in this stratum.

8. BLINDING

Due to the nature of this trial and the product, the subjects, Investigators and personnel involved in this trial cannot be blinded. The assessment of complete wound closure, determination of ulcer area and wound closure parameters will be determined by both the Investigator and by computerized planimetric analysis of 3D digital photographs of the DFU using the 3D LifeViz Micro camera system and DermaPix software developed by QuantifiCare.

9. INTERIM ANALYSIS FOR SAMPLE SIZE ADJUSTMENT

9.1. Interim Prediction for Early Success

A single interim analysis will be performed when 50% of subjects complete the study. By employing a group-sequential design with a Pocock alpha spending function, the following decisions will be made at the interim analysis with respect to the efficacy outcome:

1) Stop the study upon demonstrating statistically significant superiority of the Active Treatment (PriMatrix Dermal Repair Scaffold) to the Control Treatment (Standard of Care) if the two-sided p-value testing the stated hypotheses is $\leq$ 0.031 and the difference is in favor of the Active Treatment (PriMatrix Dermal Repair Scaffold).

2) Stop the study upon demonstrating futility of the Active Treatment (PriMatrix Dermal Repair Scaffold) to the Control treatment (Standard of Care) if the two-sided p-value

testing the stated hypotheses is ≤ 0.031 and the difference is in favor of the Control Treatment (Standard of Care).

3) Continue the study if the two-sided p-value testing the stated hypotheses is > 0.031.

With this design and assuming 46% of DFUs managed with the Active Treatment achieve complete wound closure by 12 weeks and 26% achieve complete wound closure when managed with the Control Treatment, the probability of stopping early due to showing superiority with the Active Treatment (PriMatrix Dermal Repair Scaffold) is 0.479. If the decision at the interim analysis is to continue the study, the hypotheses at the end of the study will be tested at a two-sided alpha level of 0.028 to maintain an overall two-sided alpha of 0.05.

Further, conditional power will be calculated at the interim analysis for predicting the final study success. In case more data is needed, sample size re-estimation will be considered in order to meet the final study objective.

Conditional Power

Conditional power: the probability of statistical significance at the completion of the study given the data obtained so far. From Jennison and Turnbull (2000) pages 205 to 208, the general upper one-sided conditional power at stage k for rejecting a null hypothesis about a parameter θ at the end of the study, given the observed test statistic, Z_k , is computed as

$$P_{uk}(\theta) = \Phi\left(\frac{Z_k\sqrt{I_k} - z_{1-\alpha}\sqrt{I_K} + \theta(I_K - I_k)}{\sqrt{I_K - I_k}} \right),$$

the general lower one-sided conditional power at stage k is computed as

$$P_{lk}(\theta) = \Phi\left(\frac{-Z_k\sqrt{I_k} - z_{1-\alpha}\sqrt{I_K} - \theta(I_K - I_k)}{\sqrt{I_K - I_k}} \right),$$

and the general two-sided conditional power at stage k is computed as

$$P_k(\theta) = \Phi\left(\frac{Z_k\sqrt{I_k} - z_{1-\alpha/2}\sqrt{I_K} + \theta(I_K - I_k)}{\sqrt{I_K - I_k}} \right) + \Phi\left(\frac{-Z_k\sqrt{I_k} - z_{1-\alpha/2}\sqrt{I_K} - \theta(I_K - I_k)}{\sqrt{I_K - I_k}} \right),$$

where

θ = the parameter being tested by the hypothesis

k = an interim stage at which the conditional power is computed ($k = 1, \ldots, K-1$)

K = the stage at which the study is terminated, and the final test computed

Z_k = the test statistic calculated from the observed data that has been collected up to stage k

I_k = the information level at stage k

I_k = the information level at the end of the study
$1-z_\alpha$ = the standard normal value for the test with a type I error rate of α.
For a test of two proportions with null hypothesis H0: $P_1 = P_2$, where P_1 *and* P_2 are the population proportions in groups 1 and 2, respectively, under the alternative hypothesis, these components are computed in Chang (2008) pages 70 and 71 as

p_{jk} is the sample proportion for group j, estimating P_j at stage k
I_k is the estimated information from the sample at stage k
N_{jk} is the sample size in group j at stage k
N_j is the final sample size in group j

Computing conditional power requires you to set P_1 *and* P_2. Their values can come from the values used during the planning of the study, from similar studies, or from estimates made from the data that has emerged.

In addition, an early data review will be performed when 50 patients reach the primary endpoint. The purpose of this review is to ensure the study execution is as originally planned, and therefore, no alpha spending is involved. The review will be involved only blinded data for a purpose of data cleaning and checking. And no statistical tests will be computed.

9.2. Sample Size Re-Estimation

For sample size re-estimation purposes, conditional power will be calculated at the interim analysis for predicting the probability of the final success. In case that additional data information is needed, appropriate sample size re-estimation will be considered to ensure sufficient data to evaluate the study objectives at the end of the study.

The statistical power at the time of the interim analysis will be calculated using the conditional power approach [Chen et al., 2004]). The formula is listed as following:

$$CP(\tau, Z) = \Phi\{Z_\tau/\sqrt{\tau(1-\tau)} - Z_\alpha/\sqrt{(1-\tau)}\}$$

where:

- $CP(\tau, Z)$ is the conditional power at the interim analysis
- $\Phi\{.\}$ is the cumulative distribution function of a standard Normal distribution
- Z_α is the upper αth quintile for standard Normal distribution (i.e. $Z_{0.05}$)
- τ is the information fraction at the interim analysis

$$\tau = (n_t + n_c)/(N_t + N_c)$$

where:

- N_t is the sample size in the treatment group

- N_c is the sample size in the control group
- n_t is the number of subjects in the treatment group at the time of interim analysis
- n_c is the number of subjects in the control group at the time of interim analysis

- Z_τ is Z-score at the information fraction of τ. For binary endpoints, the Z-score will be obtained from the following formula:
  - Superiority (primary efficacy)

$$Z_\tau = \frac{\hat{p}_t - \hat{p}_c}{SE} = \frac{\hat{p}_t - \hat{p}_c}{\sqrt{\hat{\sigma}_t^2/n_t + \hat{\sigma}_c^2/n_c}}$$

where: $\hat{p}_t$ is the estimated mean value in the treatment group at the time of interim analysis, and $\hat{p}_c$ is the estimated mean value in the control group at the time of interim analysis.

The following decision rules will be followed:

a. If the conditional power is larger than 50%, the sample size may be adjusted upward and no Type I error adjustment will be made to the final analysis [Chen et al., 2004].

b. If the conditional power is less than 50% then the Type I error rate will be inflated and increase the sample size will be necessary. The total sample size M required to achieve conditional power of CP by assuming the current treatment trend continues is obtained by solving the following equation:

$$Z_{CP} = \frac{Z_\tau/\sqrt{r/M} - Z_\alpha}{\sqrt{(1 - r/M)}}$$

where:

- M: required samples size to achieve the desired CP (i.e., 80%)
- $\Phi(Z_{CP})$ is the cumulative standard normal distribution evaluated at Z_{CP}
- $r = n_T + n_C$ is total number of subjects at the time of interim analysis

10. ANALYSIS POPULATIONS

10.1. Intent-to-Treatment Population

The Intent-to-Treat (ITT) population is defined as all subjects randomized and received treatment after randomization. The ITT population will be used for the analysis of exploratory and safety endpoints.

10.2. Per Protocol Population

The Per Protocol (PP) population is defined as all randomized subjects who were not associated with a major protocol violation. This population will be identified before the database lock. The PP analysis of primary and secondary endpoints will be considered supportive.

10.3. Safety Population

The Safety Population is defined the same as ITT. This population will be used for the analysis of safety parameters.

10.4. Primary Efficacy Population

Due to COVID-19 restrictions on clinical trial operation at many sites, and the reality that the study did not experience the anticipated 15% "Lost to Follow Up" figures assumed, a decision was made to terminate screening/enrollment prior to reaching the full enrollment of 240. As a result, 5/21/2020 was determined as the date for determing the primary analysis set. To accommodate for the schedule of events within the protocol, it was determined that subjects randomized by 3/3/2020 will be included in the PEP. All randomized subjects within the PEP will have enough time to go through the 12 weeks treatment phase by 5/21/2020.

The PEP will be used for the analysis of primary and secondary endpoints.

11. DESCRIPTION OF EFFICACY ENDPOINTS AND ANALYSES

The analyses of the primary efficacy endpoint will be performed using the PEP. The sensitivity analysis of the primary endpoint will be performed for the PP population. All secondary efficacy endpoints will be performed using the PEP. Safety analysis will be based on the safety population.

11.1. Primary Efficacy Endpoint Analysis

The primary efficacy endpoint for the study is the incidence of confirmed complete wound closure of the study ulcer, as assessed by the investigator, during the 12-week treatment phase, and confirm one week later.

The primary efficacy analysis will be conducted on the PEP. The proportion of subjects with confirmed closed ulcers during the Treatment Phase of the study, as assessed by the Investigator, will be compared using the Cochran–Mantel–Haenszel (CMH) test. For the primary efficacy analysis, missing values will be imputed using last observation carried forward.

The clinical study will be conducted under a common protocol for each investigational site with the intention of pooling the data for analysis. The Breslow-Day test will be used to assess the homogeneity of the primary endpoint across study sites. Sites with enrollment less than 6 will be combined to a new study site for testing. If the Breslow-Day test concludes lack of homogeneity (at a two-sided alpha level of 0.15), additional exploratory analyses will be performed to characterize the differences in results among sites.

A point estimate of the incidence will be presented along with its associated exact 95% confidence interval based on the binomial distribution for Active Treatment and Control Treatment patients respectively.

The complete wound closure as assessed by the Investigator will be listed for each patient.

In addition, subgroup analyses of the primary endpoint will be performed for age, sex, race, region, and other groupings if appropriate.

11.1.1 Timing of Primary Analysis

The primary efficacy analysis will be conducted when all patients who were randomized on or before 03 March 2020 completes up to 12 weeks of treatment and follow up (see primary efficacy population of Section 10.4). Based on this enrollment cut-off date, 207 patients will be included in the PEP.

The safety analysis will be performed on safety population. . The results will be generated based on final database lock.

11.2. Secondary Endpoint Analysis

To maintain the trial-wise Type I error rate at 0.05, a closed test procedure will be used for the secondary endpoints. For efficacy analyses, the first date of assessment of 100% re-epithelialization of the wound surface without detectable exudate or visible non-epidermal tissue will be used.

The order of the endpoints to be assessed will be as follows:

- Proportion of subjects with confirmed complete wound closure of the study ulcer during the 12-week Treatment Phase as assessed by computerized planimetry, during the Treatment Phase

  A point estimate will be presented along with the exact 95% confidence interval based on the binomial distribution.

- Time to complete wound closure, as assessed by the Investigator.

  The endpoint will be summarized by descriptive statistics. In addition, duration of time to complete wound closure/healed will be analyzed as a time-to-event endpoint. The survival curves will be estimated using the Kaplan-Meier method and compared using the log-rank test.

  The derivations of time to complete wound closure/healed will be listed for each patient.

- Time to complete wound closure, as assessed by computerized planimetry.

  The endpoint will be summarized by descriptive statistics. In addition, duration of time to complete wound closure/healed will also be analyzed as a time-to-event endpoint. The survival curves will be estimated using Kaplan-Meier method and compared using the log-rank test.

  The derivations of time to complete wound closure will be listed for each patient.

- Rate of complete wound closure as assessed by computerized planimetry

11.3. Exploratory Endpoint Analysis

An exploratory analysis will be performed to assess costs associated with the management of DFUs for the active and control groups using MRU data and standardized direct and in-direct unit costs. Additionally, wound closure status at the 4-week follow up visit will be evaluated.

11.4. Prognostic Factors Assessments

For the ITTpopulation, a covariate analysis will be conducted to assess the impact of various prognostic factors on ulcer closure and to demonstrate the robustness of the primary efficacy analysis. Prognostic factors (i.e., covariates) will be included if they are found to be contributing factors (i.e., the individual covariate p-value is less than 0.05). Potential prognostic factors to be considered include:

Baseline ulcer size	Diabetes type
Age of ulcer at baseline	Race
Gender	Nicotine Use
Recorded HbA1c	Baseline BMI
Ulcer volume	Ulcer location

Additional planned analyses include the use of Analysis of Covariance (ANCOVA) models which incorporate consideration of stratification factors (i.e., site and baseline ulcer area) to compare reduction in study ulcer size between the two treatment groups and Kaplan-Meier analysis to assess and describe the differences in the time to wound closure in the two treatment groups.

11.5. Treatment Failures

Subjects who are withdrawn or discontinue during the Treatment Phase and/or who do not achieve complete wound closure during the Treatment Phase will not be replaced and will be considered treatment failures.

11.6. Missing Data

For efficacy evaluation data points, the last available observation (LOCF) will be used. In the analyses using the planimetry data, the last available planimetric value will be used for discontinued subjects. All subjects discountinued during the treatment period and before 100% wound closure during the treatment phase of the study will not be replaced and will be considered treatment failures for the primary and secondary endpoints evaluations.

12. DESCRIPTION OF OTHER VARIABLES AND ANALYSES

12.1. General Statistical Considerations

All collected study data will be presented in subject data listings. Statistical analyses will be performed using SAS® for Windows, version 9.2 or later. Descriptive statistics (n, mean, standard deviation, median, minimum and maximum) will be calculated by treatment group for continuous variables. Frequencies and percentages will be presented by treatment group for categorical variables.

12.2. Study Subjects

12.2.1. Discontinuations and Protocol Deviation

The disposition of all subjects who sign an ICF will be provided. The numbers of subjects screened, randomized, completed, and discontinued during the study, as well as the reasons for all post-randomization discontinuations will be summarized for each treatment group, for all centers combined and for each center separately. Disposition and reason for study discontinuation will also be provided as a by-subject listing.

Protocol Deviation data will be listed for each patient.

12.2.2. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized by treatment group using appropriate descriptive statistics. These variables include: Age of Ulcer at baseline, Gender, Race, Ethnic Origin, Substance Use, Baseline Ulcer Size, Diabetes Type, Nicotine Use, Recorded HbA1c, Baseline BMI, Ulcer Volume, and Ulcer Location. Meaningful differences between the treatment groups will be further explored to evaluate the impact, if any, on the study results.

Physical examination (including vitals) will be performed and data will be summarized using descriptive statistics.

12.2.3. Medical History

Medical History data will be listed for each patient.

12.3. Medical Resource Utilization

Data for below questions will be summarized using descriptive statistics by visit.

Since your last scheduled study visit, have you had any of the following visits related to DFU identified as the study ulcer?

1. Since the last scheduled study visit OR DFU related office visit, how much time have you or a care-giver (paid or unpaid e.g., family-member, friend, neighbor) spend on caring for the dressing changes on your study wound?
2. Since the last scheduled study visit OR DFRU related office visit, please answer the following questions related to off-loading:

3. Since the last scheduled study visit OR DFU related office visit, have you developed any NEW ulcers?
4. Since the last scheduled study visit OR DFU related office visit, have you purchased or rented any of the following equipment?
5. Since the last scheduled study visit OR DFU related office visit, how much have you had to spend out-of-pocket on the following items related to the treatment of your study wound?
6. Do you have government or private health care insurance?
7. Since the last scheduled study visit OR DFU related office visit, how much has the study ulcer limited your ability to do the following activities?
8. Since the last scheduled study visit OR DFU related office visit, please provide details about how your study ulcer has affected your participation in usual daily activities (e.g., school, work or other daily activities.

An exploratory analysis will be performed by an approved external vendor to assess costs associated with the management of DFUs for the active and control groups using medical resource utilization data and standardized direct and in-direct unit costs.

12.4. Ulcer Assessment

Data for below questions will summarized by visit using descriptive statistics

- Ulcer Exudate Assessment
- Ulcer Infection Assessment
- Was picture of the study ulcer taken prior to debridement per protocol
- Ulcer Area
- Ulcer Size Reduction
- Is the Ulcer wound surface 100% re-epithelialized?
- Is the Ulcer wound surface without detectable exudate?
- Is the Ulcer wound surface without visible non-epidermal tissue?
- Extent of Ulceration
- Location of Study Ulcer
- Exudate Type
- Exudate Amount
- Ulcer Infection Assessment

- Ulcer Photography: Pre-Debridement
- Ulcer Measurement: Pre-Debridement.

12.5. Ulcer Treatment Procedure

Data for below questions will be summarized using descriptive statistics by visit.

- Sharp Debridement
- Ulcer Measurement
- PriMatrix Re-application
- Ulcer Photography
- PriMatrix Outer Dressings,
- Standard of Care Treatment,
- Dressing Supplies

12.6. Safety Analysis

Safety will be assessed through reports of adverse events and serious adverse events. Adverse Events related to the device, study ulcer and/or study procedures and all Serious Adverse Events will be recorded.

All safety parameters will be summarized descriptively by treatment. No inferential statistics are planned.

12.7. Adverse Events

Adverse Events will be coded using the MedDRA Medical Dictionary. AE's are defined as events with an onset after screening visit 1. Treatment Emergent AE's (TEAE) are defined as events with an onset on or after the randomized treatment. TEAEs will be summarized by treatment group, System Organ Class, and preferred term. The following TEAE summaries will be provided:

- TEAEs by severity grade
- TEAEs by relationship to study treatment.

In addition, separate summaries of Serious Adverse Events, and Adverse Events resulting in discontinuation of study treatment will be presented.

The safety population will be used for the safety analyses. Subjects will be counted only once for subject-based analysis. If the same event changes in severity for a subject, the worst severity will be reported.

An Adverse Event is considered treatment emergent if (1) it was not present at Screening Visit 1, and it is not a chronic condition that is part of the subject's medical history; or (2) it is present at Screening Visit 1 or as part of the subject's medical history, but the severity or frequency increases during treatment.

Treatment-emergent adverse events (TEAE) will be summarized for each treatment group by body system and preferred term. The severity of all adverse events will be determined by the investigator's judgment and classified as mild, moderate or severe.

Treatment groups will be compared with respect to the incidence of Adverse Events (all and TEAEs) and the proportion of subjects withdrawing from the study for Adverse Events by reason of withdrawal. Comparisons will be made overall, by body system and by preferred term and will be further categorized by severity and by study device relationship.

12.8. Supportive Analysis

To assess the robustness of the primary efficacy analysis results, supportive analysis will be conducted using the PP population. Statistical methodology for the supportive analyses will be the same as that of the primary analysis, except for the analysis population used. The PP population will be used for the supportive analysis while PEP will be used for the primary efficacy analysis.

13. STATISTICAL METHODS AND ISSUES

The statistical analysis will be performed by the Clinical Biostatistics Department and/or an approved external vendor.

13.1. Randomization

At the beginig of the study, site 109 had randomization issues due to data entry delay to the EDC. Eight (8) subjects were not randomized to their original treatment assignments per randomization date/time. A sensitivity analysis will be conducted to assess the impact on the randomization issue on the primary endpoint using the primary efficacy analysis population.

13.2. Methods of Analysis

13.2.1. Efficacy Endpoints

Confidence interval for proportions:

The method used to compute the two-sided 95% confidence interval (CI) for a single proportion will be the 'exact' method of Clopper and Pearson. For two proportions, the two-sided 95% CI for the proportion difference will be calculated based on the Z-test with continuity correction.

13.2.2. Standard calculations

The following calculations will be used:

- Baseline: Last non-missing value on/before randomization date.
- Age=int ((date of ulcer at baseline – birth date + 1) / 365.25).
- Height (cm)
- Weight (kg)
- BMI (kg/m^2)
- Clinical Trail Duration:
    - For subjects completing the study: (Four Week Follow-up visit date)-(randomization date)+1
    - For subject withdrawals: (date of withdrawal)-(randomization date)+1
- AE date: (date of onset)-(randomization date)+1
- AE duration: (date of onset)-(randomization date)+1
- Days to complete wound closure: This metric will only be calculated for subjects who achieve investigator-assessed complete wound closure:
    - (Date of the first of two successive 100% epithelialization assessment) – (randomization date) +1
- Days to complete wound closure, as assessed by computerized planimetry: The metric will only be calculated for subjects who achieve planimetric-based complete wound closure during the Treatment Phase. Time to first healing will be used for the analysis

- (Date of the first of two successive planimetric readings of 0cm^2) – (randomization date) +1

- Rate of wound closure

  Rate (% healed/week) = 7*[(Baseline wound size)- (Post-baseline wound size)] / [(Baseline wound size) *(days in trial)]

13.2.3. Other Variables

Discontinuations

The difference between treatments, both overall and for each primary reason, will be compared by using a 2-sided Fisher's exact test.

Demographic and Baseline Characteristics

Comparisons between treatment groups of continuous variables will be made by using a one-way analysis of variance with treatment as a factor. For categorical variables, the Fisher's exact test will be used. All tests will be 2-sided and performed at the 0.05 level of significance.

Time-to-Event Data

Time-to-event endpoints including time-to-complete-wound-closure/healed will be evaluated using the Kaplan-Meier method. The log-rank test will be used to compare the distributions between two treatment groups, stratified by the ulcer size (1 cm^2 to $\leqslant$3 cm^2 vs. >3 cm^2 to 12 cm^2).

Rate of Wound Closure, as Assessed by Computerized planimetry

Analysis of Covariance (ANCOVA) will be used to compare the rate of wound closure (as defined in section 13.2.2 for the two treatment groups. The stratification factor, ulcer size at the time of randomization, will be used in this analysis. Other covariate, as outlined in section 11.4, may also be incorporated in the model, if their presence is statistically significant (i.e. p-value $\leq$0.05).

Safety Data

The primary safety endpoint for this study is any severe device-related complication where relationship to the device was assessed by the clinical event committee possible, probably, or definite. Fisher's exact test will be used to compare incidence rates of serious device-related complications as well as for any device-related complication, and for specific complications between the treatment group and the control group.

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