Study Title	A PHASE I/II CLINICAL TRIAL TO DETERMINE SAFETY AND FEASIBILITY OF USING AN ACELLULAR AMNIOTIC FLUID APPLICATION TO EXPEDITE HEALING IN CHRONIC WOUNDS
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Statistical Analysis Plan

Protocol Title: A Phase I/II Clinical Trial to Determine Safety and Feasibility of Using an Acellular Amniotic Fluid Application to Expedite Healing in Chronic Wounds

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Contents

Abbreviations

Abbreviation	Definition
CRF	Case Report Form
CW3	Closed Wound at Visit 3 (population)
CW4	Closed Wound at Visit 4 (population)
CW5	Closed Wound at Visit 5 (population)
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
ITT	Intent-To-Treat
MI	Multiple Imputation
mVSS	modified Vancouver Scar Scale
pAF	processed Amniotic Fluid
PP	Per-Protocol
(S)AE	(Serious) Adverse Event
SAFETY	Safety Population
SAP	Statistical Analysis Plan
SOC	Standard of Care

1 PREFACE

1.1 Purpose of SAP

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the protocol: A Phase I/II Clinical Trial to Determine Safety and Feasibility of Using an Acellular Amniotic Fluid Application to Expedite Healing in Chronic Wounds.

The structure and content of this SAP provides sufficient detail to meet the requirements and standards set by the Data Coordinating Center (DCC).

1.2 Auxiliary/Other Documents

The following documents were reviewed in preparation of this SAP:

- Protocol (including intended revisions): A Phase I/II Clinical Trial to Determine Safety and Feasibility of Using an Acellular Amniotic Fluid Application to Expedite Healing in Chronic Wounds
- Case Report Forms (CRFs) for the pAF for the Treatment of Chronic Wounds protocol

The reader of this SAP is encouraged to read the protocol for details on the conduct of this study, and the operational aspects of clinical assessments.

The purpose of this SAP is to outline the planned analyses to be completed for the pAF for the Treatment of Chronic Wounds trial. The planned analyses identified in this SAP will be included in future study abstracts and manuscripts. Also, exploratory analyses not necessarily identified in this SAP may be performed. Any post hoc, or unplanned, analyses not explicitly identified in this SAP will be clearly identified as such in any published reports from this study.

It is possible that, due to updates or identification of errors in specific statistical software discussed in this SAP, the exact technical specifications for carrying out a given analysis may be modified. This is considered acceptable as long as the original, prespecified statistical analytic approach is completely followed in the revised technical specifications.

2 STUDY OBJECTIVES AND OUTCOMES

2.1 Study Objectives

2.1.1 Primary Objective

The primary objective of the pAF for the Treatment of Chronic Wounds trial is:

1. To determine the safety of using processed amniotic fluid (pAF) to treat patients with chronic wounds.

2.1.2 Secondary Objective

The secondary objective of the pAF for the Treatment of Chronic Wounds trial is:

1. To determine efficacy by examining a reduction in wound size after application of pAF compared to Standard of Care (SOC).

2.2 Study Outcomes

2.2.1 Primary Outcome

There is one primary outcome, which pertains to safety: whether or not the subject has any post-randomization serious adverse events (SAEs) related to study treatment up to and including the final visit (Visit 5).

Eligibility for Primary Analysis The primary outcome is to be evaluated in subjects who are randomized. Randomization does not occur until Visit 2 to make sure a wound is not randomized if the subject has become ineligible between Visit 1 and Visit 2. The inclusion/exclusion criteria from baseline should be reevaluated as feasible (without needing to perform additional lab tests). A wound is also considered ineligible for randomization if it has reduced in area by at least 50% with standard of care since Visit 1. To permit an efficient eligibility assessment work-flow at Visit 2, clinician judgment may be used to ascertain if the wound has reduced in area by at least 50% since Visit 1.

2.2.2 Secondary Outcome

The secondary outcome pertains to efficacy: the percentage reduction in wound area at Visit 5 relative to Visit 2.

Eligibility for Secondary Analysis The secondary outcome is to be evaluated in subjects who are randomized.

2.2.3 Exploratory Outcome(s)

Exploratory outcomes include:

- Wound closure at Visit 5
- Wound recurrence at Visit 5
- Percent reduction in wound area at Visits 3, 4 (relative to Visit 2)
- Percent reduction in maximal wound depth at Visits 3, 4, 5 (relative to Visit 2)
- Change in wound-associated pain at Visits 3, 4, 5 (relative to Visit 2)
- Hypertrophic scar formation at Visit 5 (graded with modified Vancouver Scar Scale)
- Wound infection at Visit 4 (per wound culture exceeding the threshold)

Eligibility for Prespecified Exploratory Analyses The prespecified exploratory outcomes are to be evaluated in subjects who are randomized. Recurrence of wounds at Visit 5 will be assessed only in subjects with wound closure prior to Visit 5. It is possible that additional exploratory analyses will be conducted, and such analyses may have different eligibility criteria.

2.2.4 Safety Outcome(s)

The primary outcome of this study is related to safety: the occurrence of any post-randomization SAEs related to study participation, up to and including the final visit. In addition, other safety outcomes evaluated (from randomization up to and including the final visit) include the following:

- Occurrence of any post-randomization SAEs (regardless of relatedness to study participation)
- Occurrence of any post-randomization adverse events (AEs)
- Occurrence of any post-randomization AEs related to study participation
- Occurrence of any post-randomization unexpected AEs

3 STUDY DESIGN AND METHODS

3.1 Overall Study Design

The pAF Wound trial is a two-arm, randomized controlled trial to assess the safety and efficacy of using processed amniotic fluid (pAF) to treat chronic wounds. The largest qualifying wound per subject will be assessed at the initial visit. At Visit 2 (about 6 weeks later), if the

wound has not decreased by at least 50% in area, the patient's wound is to be randomized to either standard of care or the injection of pAF. The randomized treatment is given to the randomized wound at Visit 2 and at Visit 3 (about six weeks after Visit 2). Thereafter, two additional visits (Visit 4 about six weeks after Visit 3, Visit 5 about 3 months after Visit 4) are used to collect further information. At all study visits, non-randomized wounds are to receive standard of care treatment.

3.2 Method of Treatment Assignment and Randomization

Subjects will be randomized into two groups at a 1:1 ratio. The randomization will employ random block sizes of two, four, or six subjects to limit the ability to predict the next subject's treatment prior to randomization. It is not guaranteed that exactly 30 subjects will be randomized to each arm due to random block sizes and the unlikely event of administering backup randomization for a subject. There may be as few as 28 in the pAF arm (32 in the standard of care–SOC–arm) or as many as 32 in the pAF arm (28 in the SOC arm). Because of the small number of overall patients and the anticipated slow accrual rate, stratification by subject characteristics will not be used in the randomization procedure.

3.2.1 Delivery of Randomization and Emergency Backup

The randomization treatment assignment will be generated by a DCC faculty statistician and uploaded to a REDCap instance. At Visit 2, if a subject is still eligible to have the largest qualifying wound randomized to treatment, the treatment assignment will be delivered using the randomization module in REDCap. Backup randomization envelopes will be available to the research coordinator(s) and/or other authorized study staff, to be used only when technical difficulties preclude use of the web-based randomization.

3.2.2 Handling of Incorrect Randomization in Study Analyses and Reports

The Intention-to-Treat (ITT) principle will be used, in that subjects will be analyzed in the arm to which they were randomized. Assuming that misrandomization is infrequent, no further adjustments are planned for misrandomizations beyond applying the ITT principle.

3.3 Treatment Masking (Blinding)

This is an unblinded trial, but the person interacting with the imaging software is to be blinded to treatment arm to avoid bias between arms for purposes of measuring the secondary outcome. This is because wound area calculations used by the software rely on an individual manually outlining the wound within the imaging software.

3.4 Study Intervention Compliance

As part of the work-flow for assessments at Visits 2–5, the subject will be asked if any investigational wound care has been received since the last study visit that is external to that described in the protocol. Subjects who received investigational wound care between Visits 1 and 2 are not to be randomized as the protocol does not allow such individuals to receive any pAF injections during the study. Subjects randomized to the pAF arm who receive external wound care between Visits 2 and 3 are not eligible to receive pAF at Visit 3. Follow-up data at Visits 2–5 should still be collected on all individuals who were randomized to a treatment arm at Visit 2, regardless of study intervention compliance. The only exceptions to the desire to follow-up are for individuals who die or formally withdraw during the study.

4 STUDY SUBJECTS AND ANALYSIS POPULATIONS

4.1 Eligibility

The inclusion and exclusion criteria for the pAF for the Treatment of Chronic Wounds Trial are described in the protocol. Enrolled subjects may be randomized to a treatment arm at Visit 2 if still eligible and if the wound area has not decreased by ≥ 50% since Visit 1. The determination of whether the wound has decreased by ≥ 50% since Visit 1 may be per the rectangular approximation to the area at Visit 1 and at Visit 2 or per clinician assessment, even if subsequent analysis (i.e., wound size per the imaging software) would yield a different conclusion.

4.2 Populations for Analyses

4.2.1 Eligible and Approached for Consent Population

The CONSORT-like diagram will begin with its most general population being those who met all eligibility criteria and who were approached for consent by study staff.

4.2.2 Enrolled Population

All subjects who meet all inclusion criteria, none of the exclusion criteria, have the appropriate consent, and are enrolled into the trial at Visit 1 will be considered part of the enrolled population. That is, the enrolled population consists of the subset of the eligible and approached population who provide consent and are enrolled into the trial at Visit 1.

4.2.3 Intention-to-Treat Population

The Intention-to-Treat (ITT) population consists of all enrolled subjects who at Visit 2 are randomized to have the study wound be treated with pAF or SOC.

In order to be randomized, the wound area must not have decreased by at least 50% at Visit 2 relative to Visit 1. Because of the infeasibility of having adjudication of imaging-software-derived wound areas in real time at Visit 2, the rectangular approximation to the wound area and/or clinician judgment will be used for the Visit 1 and Visit 2 areas to determine if the Visit 2 wound area is more than half of the Visit 1 wound area. Furthermore, the study inclusion/exclusion criteria are to be reassessed at Visit 2 to ensure the subject is still eligible for the study. Receiving investigational treatment to the wound outside of the study between Visits 1 and 2 render the subject ineligible to be randomized.
Even if a ≥ 50% reduction in wound size by Visit 2 causes the subject to be ineligible for randomization of one wound to pAF/SOC, the subject will still be considered part of the enrolled population. Likewise, even if a subject is not randomized because, e.g., out-of-study investigational wound care was received after Visit 1 and before Visit 2, the subject is still considered part of the enrolled population.

There is no requirement that the subject receive the randomized treatment (e.g., that pAF be injected into the wound of somebody randomized to the pAF treatment arm) to be part of this population. Primary and secondary analyses (and exploratory analyses, unless explicitly noted otherwise) comparing the arms are to be performed according to the arm to which the subject was randomized, even if this differs from the actual treatment received.

4.2.4 Per-Protocol Efficacy Population

The Per-protocol (PP) population consists of all subjects from the ITT population who meet both of the following conditions:

- The subject did not receive out-of-study investigational wound care between enrollment and the earlier of Visit 5 and the time when Visit 5 should have occurred; and
- The subject received the randomized treatment at Visit 2 and, if applicable, at Visit 3. Treatment at Visit 3 is applicable unless the wound was healed at the time when Visit 3 was to have occurred or the subject had a study-related SAE by the time of Visit 3 that precluded treatment administration. Study-related is defined as having a classification of "probably related" or "possibly related."

4.2.5 Closed Wound Efficacy Populations

Several analyses are only applicable among those whose wound closes between Visits 2 and 5. The closed wound at Visit 3 (CW3) population consists of all subjects from the ITT

population whose wound is closed at Visit 3. The closed wound at Visit 4 (CW4) and closed wound at Visit 5 (CW5) populations are analogously defined.

4.2.6 Safety Population

The safety population (SAFETY) coincides with the ITT population. The SAFETY population admits comparisons between arms.

An additional population for assessing safety will be limited to ITT participants randomized to the pAF treatment arm who received at least one pAF injection during the course of the study.

5 GENERAL ISSUES FOR STATISTICAL ANALYSES

5.1 Analysis Software

Analysis will be performed using SAS® Software version 9.4 or later whenever possible. Other software packages, including R and StatXact®, may be used for particular specialized procedures. IVEware is the preferred software for creating the study's multiply imputed data sets.

5.2 Methods for Withdrawals, Missing Data, and Outliers

The applicability of some measures will depend on whether or not the wound is (or has previously) closed. Values that are not observed because they are not applicable will be excluded from the pertinent analyses, rather than regarded as missing. Values that are not directly obtained but are implied by other measurements will use the implied value.

Multiple imputation (MI) will be applied for analyses of the secondary outcome. Using MI leverages partial information and attempts to reduce bias in final inferences that could result from a naive complete-case analysis. A minimum of ten data sets will be generated. Chained equations will be used along with variable selection; for imputing missing values for a given variable, the input variables will be limited to those that increase the R^2 value by at least 0.01. If feasible, MI will be conducted separately within each treatment arm. The pool of variables for the imputation process includes: wound area per the imaging software at Visits 1, 2, 3, 4, and 5; treatment arm if infeasible to impute by treatment arm; and indicators for comorbidities. The following comorbidities will be considered: diabetes, peripheral vascular disease, and immunosuppression. Imputation will exclude individuals

who were not randomized.

Visit measurements obtained outside the permissible window may or may not be used in the imputation model. We will determine the closest visit number to assign the out-of-window visit per the protocol's timing for follow-up visits. The out-of-window measurement will be used for the determined visit number in the case that the visit number does not already have a valid measurement (and if there are multiple out-of-window candidate measurements, the one closest to the protocol timing will be used). Otherwise, the out-of-window measurement will be ignored.

Multiple imputation may also be used for exploratory outcomes, as appropriate.

We do not plan to use different methods for missing data due to withdrawal compared with data missing for other reasons.

5.3 Multiple Comparisons and Multiplicity

There is one primary endpoint (occurrence of post-randomization study-related SAEs on or before Visit 5), which is not intended to be tested between arms. Instead, descriptive summaries and confidence intervals will be constructed by study arm. There is one secondary endpoint (percent reduction in wound area at Visit 5 compared to Visit 2), which will be tested at $\alpha = 0.05$. The additional analyses are regarded as exploratory and therefore no adjustments are indicated for the multiplicity of testing; each will use $\alpha = 0.05$.

5.4 Planned Subgroups, Interactions, and Covariates

The primary and secondary outcomes will be analyzed within the following subgroups; these analyses will be considered exploratory even though they are prespecified. Determination of subgroups is per Visit 1 assessment, unless stated otherwise.

- Sex (male, female)
- Race (white, non-white)
- Ethnicity (Latinx, non-Latinx)
- Diabetes Mellitus or high A1C (diagnosis or A1C ≥ 6.5, no diagnosis and A1C < 6.5)
- Use of immunosuppressive medications (yes, no)
- Known malignancy other than non-metastatic basal or squamous cell carcinoma (yes, no)
- Nutritional status (albumin level < 3.0, ≥ 3.0)

- Blood coagulation conditions (PT/INR > 1.0 or PTT > 15, PT/INR ≤ 1.0 and PTT ≤ 15)
- Venous vascular disease affecting the wound area (yes, no)
- Arterial vascular disease affecting the wound area (yes, no)
- Visit 1 oxygen saturation (< 90%, ≥ 90%)
- Concomitant anticoagulation medication (yes, no)
- Injury type/disease etiology: (ulcer, trauma, post-surgical non-healing wound, other)

For the subgroup analyses, if a subject's subgroup cannot be ascertained or is not compatible with any of the stated options, the individual will be excluded from the analysis of that subgroup.

5.5 Derived and Computed Variables

The definitions of key derived variables will be provided in a separate dataset specifications document. For example, age will be calculated as the integer portion of

$$\frac{\text{Date of informed consent} - \text{Date of birth}}{365.25}.$$

Primary and secondary outcome definitions are described in Section 7.1.

All derived variables used in analyses will be dual-coded by a second statistician to promote quality and minimize error; see also Section 5.6.

5.6 Independent Review

All statistical analyses for primary reporting of trial results will be independently verified through dual programming. Two statisticians will each program all datasets and analyses for the DSMB reports and the primary manuscript(s) and the results will be compared. This process will begin at the analysis design stage and will continue through writing of abstracts and manuscripts for primary analyses.

Additional analyses for secondary abstracts or manuscripts will use a second statistician conducting at least code review, if not dual programming.

6 INTERIM ANALYSES

6.1 Frequency of and Timepoints for Interim Analysis

The only planned interim analyses are for reports to be provided to the Data and Safety Monitoring Board (DSMB). The frequency of the interim analyses for the DSMB will be per the DSMB charter.

The DSMB will be provided with summaries of adverse events (AEs) and serious adverse events (SAEs); some of the AE and SAE summaries coincide with safety outcome analyses described in Section 7.6.1. Adverse events will be summarized as to their seriousness, expectedness, relatedness to study, and severity. Overall summaries of the ITT population may be provided for the open session of DSMB meetings, but summaries by treatment arm for the ITT population will be limited to the closed session for interim analyses. Even overall summaries might be limited to the closed session of interim DSMB reports to promote the blindedness of study results for the principal investigator until all patients have had time for all study-prescribed follow-up.

The closed session will also present adverse event summaries for the subset of ITT subjects who were randomized to the pAF treatment arm AND received at least one study-administered pAF injection.

6.2 Stopping Rules for Interim Efficacy Analysis

There is no stopping rule for interim analysis of the secondary efficacy outcome, either for demonstrated efficacy or for futility. The study is not adequately powered to spend alpha early.

6.3 Subgroups in the Interim Analysis

No subgroup analyses are planned for the interim analysis. Nonetheless, the DSMB has the prerogative to request such subgroup analyses as they deem appropriate.

6.4 Blinding in the Interim Analysis

Any interim analyses for the DSMB reports will be limited by only including summaries by treatment arm in the closed session. Attendance at the closed session will be limited to the DSMB members and the DCC biostatisticians. Actual treatment designations will be used in the closed session report.

7 PLANNED ANALYSES

7.1 Description of Subject Characteristics

The primary results will include a summary of the following baseline measures, by randomized treatment arm and overall:

- Sex (male, female)
- Race (white, non-white)
- Ethnicity (Latinx, non-Latinx)
- Age
- Diabetes mellitus diagnosis or A1C ≥ 6.5 (yes, no)

Additional measures may also be included.

7.2 Primary Outcome Analysis

The primary outcome is whether or not the subject has any post-randomization study-related SAEs while on study, up to and including the Visit 5 checkpoint (or the last permitted time for Visit 5, if Visit 5 was not conducted). The primary analysis of the primary outcome implicitly adopts a last-observation-carried-forward approach, in that if such an SAE is not identified it will be assumed there was no such SAE.

Safety will be assessed by descriptive reporting of the incidence proportion of study-related SAEs in each study group, with upper one-sided 95% binomial exact confidence intervals (equivalently, lower one-sided 95% binomial exact confidence intervals for the proportion without any study-related SAEs). No statistical hypothesis test is planned to assess safety, which is consistent even with large Phase III trials.

7.2.1 Additional Analyses of Primary Outcome

The primary analysis of the primary outcome implicitly adopts a last-observation-carried-forward approach. As a sensitivity analysis, it will be assumed all randomized subjects without follow-up through Visit 5 had a study-related post-randomization SAE.

The primary endpoint of presence/absence of study-related SAEs will be summarized in the ITT population for the primary analysis, but it will also be separately summarized among the subset of individuals randomized to the pAF arm who received at least one dose of pAF during the study. An exact one-sided 95% binomial confidence interval will be calculated for the proportion in this subpopulation who had at least one study-related SAE.

7.3 Secondary Outcome Analysis

The secondary outcome is the percentage reduction in wound area at Visit 5 relative to wound area at Visit 2. Because reduction in wound area at Visits 3 and 4 are exploratory outcomes, the general definition for deriving percent reduction in wound area at Visit k relative to Visit 2 is

$$1 - \frac{\text{Visit } k \text{ wound area}}{\text{Visit 2 wound area}} \times 100\%,$$

with all measurements expressed as the area determined through the imaging software. Wound area will be reported in (cm^2), although for purposes of the percent reduction the units are immaterial. The percent reductions will be summarized overall and by assigned treatment arm for the ITT population, using means and standard deviations or medians and interquartile ranges, as appropriate given the observed skewness and presence of extreme outliers.

The inclusion criteria limit the study to wounds which are difficult to heal, explaining why patients are not randomized if the wound area shrinks significantly between Visits 1 and 2. Therefore, any greater reduction in wound size with pAF than with SOC will suggest efficacy of pAF for wound treatment. To describe a trend in a statistical hypothesis testing framework, the following two-sided hypothesis will be assessed:

$$H_0 : \mu_{pAF} = \mu_{SOC} \text{ versus } H_1 : \mu_{pAF} \neq \mu_{SOC}.$$

Note that μ_{pAF} (μ_{SOC}) represents the average percent reduction in wound area at Visit 5 compared to Visit 2 for the pAF (SOC) treatment.

To provide an intuitive effect size, percentage change from Visit 2 will be computed at Visits 3, 4, and 5 (approximately 6, 12, and 25 weeks after randomization). If there are study visits outside of the visit's ± 1 week window, all percentages will be divided by the number of elapsed days since Visit 2, so the outcome is expressed as a percentage change per day.

If the change in wound size at Visit 5 follows a reasonably symmetric distribution within each treatment arm, then a two-sided, two-sample, unpooled t-test will be performed to compare the mean percentage reductions at Visit 5 between arms for the ITT population. If the distribution is very asymmetrical in one or both arms, a Wilcoxon rank sum test will be used. In either case, Rubin's rules will be used to combine inferences across the multiply imputed data sets and the test will be declared statistically significant if the two-sided p-value is ≤ 0.05.

Only the wound randomized to treatment is considered for the secondary outcome. Other wounds are to receive standard-of-care and measurements on other wounds are only applicable, if at all, to exploratory analyses. See the protocol for further details.

7.3.1 Additional Analyses of Secondary Outcome

The primary analysis of the secondary outcome explicitly adopts a multiple imputation approach. Two sensitivity analyses will be conducted in which missing values are replaced with the lowest (highest) observed value in the assigned treatment arm.

7.4 Exploratory Outcome(s) Analyses

7.4.1 Wound Area Reduction at Visits 3, 4

The percent reduction in wound area at Visit 3 (4) relative to Visit 2 will be summarized overall and by assigned treatment arm for the ITT population. If there are study visits outside of the visit's ±1 week window, all percentages will be divided by the number of elapsed days since visit 2, so the outcome is expressed as a percentage change per day. The summary will include means and standard deviations or medians and interquartile ranges, as appropriate.

If the change in wound size at Visit 3 (4) follows a reasonably symmetric distribution within each treatment arm, then a two-sided, two-sample, unpooled t-test will be performed to compare the mean reductions at Visit 3 (4) between arms for the ITT population. If the distribution is very asymmetrical in one or both arms, a Wilcoxon rank sum test will be used.

7.4.2 Wound Area Reduction at Visits 3, 4, 5, per rectangular wound area approximation

The secondary outcome relies on wound images that are compatible for use with the imaging software. However, there is also interest in assessing reduction in wound area for wound areas calculated with the rectangular approximation. The area per rectangular approximation at visit k, $RectangularApprox_k$, is defined as:

$$RectangularApprox_k \equiv MaxWidth_k \times MaxLength_k,$$

where $MaxWidth_k$ and $MaxLength_k$ are the maximal width and length (in cm), at Visit k, respectively.

As an exploratory outcome, the percent reductions per rectangular approximation at Visits 3, 4, and 5 relative to Visit 2 will be analyzed in the same manner as for the percent reductions per imaging software. Each analysis of the rectangular wound area approximations is considered an exploratory analysis to gauge the sensitivity of the secondary outcome analysis.

7.4.3 Wound Closure at Visit 5

An exploratory endpoint is whether or not the randomized wound is closed at Visit 5. Fisher's exact test will be used to test for an association between treatment arm and whether the wound is closed at Visit 5. This will be assessed for the ITT population. In the principal analysis of this outcome, only participants with a nonmissing Visit 5 would closure status will be included. If feasible, multiple imputation will be used to fill in missing wound closure statuses, with candidate variables in the imputation model possibly including variables such as treatment arm, wound closure status at visits 3 and 4, wound area at Visit 1 per the imaging software, and percent reduction in wound area from Visit 1 to Visit 2.

7.4.4 Wound Closure Prior to Visit 5

Another exploratory endpoint is whether or not the randomized wound was closed at Visit 3 and/or Visit 4, regardless of whether it later recurred. This will be determined by having the wound area per the imaging software assessment be equal to 0. The number and percentage of wounds closed prior to Visit 5 will be summarized, overall and by treatment arm, for the ITT population.

7.4.5 Wound Recurrence at Visit 5

This exploratory endpoint is limited to those from the ITT population whose randomized wound closed prior to Visit 5. Among this subset, the number and percent whose wound was open at the time of Visit 5 will be reported overall and by treatment arm. If the denominator is not zero, the corresponding percentage will also be displayed, overall and by treatment arm.

7.4.6 Hypertrophic Scar Formation at Visit 5

This exploratory endpoint is measured by the modified Vancouver Scar Scale (mVSS) total score and is applicable to those from the ITT population whose randomized wound is closed at Visit 5. Among this subset, the mean and standard deviation (or median and interquartile range) will be summarized overall and by treatment arm for the applicable subjects with nonmissing mVSS total scores.

7.4.7 Wound Depth Reduction

The maximal depth of the wound is scheduled to be measured at each study visit. The percent reduction in the maximal depth at Visits 3, 4, and 5 relative to Visit 2 will be calculated as (1-(maximal depth at Visit k)/(maximal depth at Visit 2))× 100% for $k = 3, 4, 5$. The percent reduction for these visits will be summarized overall and by treatment arm in the ITT population. The summaries for each visit number (3, 4, and 5) will use means and

standard deviations or medians and interquartile ranges, as appropriate, and will be limited at each timepoint to observations with nonmissing values. As for the wound area reduction, if any measurements are obtained outside of the permissible follow-up windows the wound depth reductions will be standardized by dividing by the number of days since Visit 2. As a sensitivity analysis, multiple imputation may be attempted to fill in missing wound depths, with summaries of the completed data sets provided, overall and by treatment arm.

7.4.8 Change in Wound-associated Pain

The wound-associated pain level is scheduled to be measured at each study visit using a numerical rating scale: 0–10, with higher numbers indicating more pain. The change in wound-associated pain at Visits 3, 4, and 5 relative to Visit 2 will be calculated as (pain at Visit k − pain at Visit 2) for $k = 3, 4, 5$. The change will be summarized overall and by treatment arm in the ITT population at timepoints 3, 4, and 5. The summaries will use means and standard deviations or medians and interquartile ranges, as appropriate, and will be limited at each timepoint to observations with nonmissing values. As a sensitivity analysis, multiple imputation may be attempted to fill in missing wound depths, with summaries of the completed data sets provided, overall and by treatment arm.

7.4.9 Wound Infection at Visit 4

An exploratory endpoint is whether or not the randomized wound is infected at Visit 4. Version 3.00 of the protocol stated that "incidence of wound infection as determined by wound culture assessment from Visit 4" will be descriptively summarized by treatment arm, but is open to interpretation as to how wound infection is defined. Wounds that are closed at a particular visit will be assumed to not have an infected status at that visit. For open wounds, the principal determination will be based on having a culture level of at least 2+ per the laboratory's metric AND having the appearance of an infection, as indicated by a database element asking whether the wound appears to be infected; if there is not a valid wound culture result for the visit then the database element will be used to determine the outcome. As sensitivity analyses, two other endpoint definitions will be considered: one ignoring the database element about the wound appearing infected, and the other ignoring the laboratory metric in favor of the database element.

The number and percentage of wounds with wound infection at Visit 4 will be summarized, overall and by treatment arm. The summaries will be limited to the ITT subjects with observable wound infection status at Visit 4. The presence of wound infection at Visit 4 will be modeled with ordinary or exact logistic regression, as appropriate, with adjustment for infection status at Visit 1 and treatment arm. The two-sided p-value of the treatment effect will be compared with $a = 0.05$ for the principal analysis of this exploratory endpoint, as well as for the sensitivity analyses mentioned in the preceding paragraph.

7.5 Technical Approaches for Subgroup Analyses

For each subgroup, the primary outcome and secondary outcome will be analyzed in separate models with a main effect for subgroup level, a main effect for treatment arm, and the subgroup × treatment interaction effect. The interaction effects are the targets for the subgroup analyses. For the primary outcome, exact logistic regression or (ordinary) logistic regression will be used, as appropriate. For the secondary outcome, quantile regression for the median or linear regression for the mean, as appropriate, will be used.

Because of the modest sample size and large number of subgroups, the exploratory nature of these analyses is especially important to emphasize. If any subgroup analyses are not feasible to conduct (e.g., no participants have the primary outcome), the infeasible subgroup analyses may be omitted.

7.6 Safety Analyses

7.6.1 Formal Safety Outcome(s)

In addition to the primary outcome (post-randomization, study-related SAEs by the time of Visit 5), there are several exploratory safety outcomes.

- Whether the subject experienced any post-randomization SAEs while on study (regardless of relatedness to study participation)
- Whether the subject experienced any post-randomization, study-related AEs while on study.
- Whether the subject experienced any post-randomization unexpected AEs while on study.

The primary outcome is whether or not the subject has any post-randomization study-related SAEs while on study, up through and including the Visit 5 checkpoint (or the last permitted time for Visit 5, if Visit 5 was not conducted). Safety will be assessed by descriptive reporting of the incidence proportion of study-related SAEs in each study group and the confidence intervals as described in Section 7.2.

Any Post-randomization SAEs The number and percentage of subjects with at least one post-randomization SAE (up through the Visit 5 checkpoint, or through the last permitted time for Visit 5, if Visit 5 was not conducted), will be summarized overall and by treatment arm for the ITT population. Because this is an ITT analysis, a note will be provided along with the summary if there are any subjects randomized to the SOC group who receive one or more pAF injections in the wound during the course of the study. If applicable, the note would state how many individuals in the SOC arm had pAF administered while on study as

well as how many such individuals had any post-randomization SAEs while on study.

As a supplementary analysis, the number and percentage of subjects with at least one post-randomization SAE will be calculated for the pAF arm among the subset of the ITT population that received at least one study administration of pAF.

Any Post-randomization Study-related AEs The number and percentage of subjects with at least one post-randomization study-related AE (up through the Visit 5 checkpoint, or through the last permitted time for Visit 5, if Visit 5 was not conducted), will be summarized overall and by treatment arm for the ITT population. Note that AEs also include SAEs. Because this is an ITT analysis, a note will be provided along with the summary if there are any subjects randomized to the SOC group who receive one or more pAF injections in the wound during the course of the study. If applicable, the note would state how many individuals in the SOC arm had pAF administered while on study as well as how many such individuals had any post-randomization study-related SAEs while on study.

In addition, the number and percentage of subjects with at least one post-randomization study-related AE will be calculated for the pAF arm among the subset of the ITT population that received at least one study administration of pAF.

Any Post-randomization Unexpected AEs The number and percentage of subjects with at least one post-randomization unexpected AE (up through the Visit 5 checkpoint, or through the last permitted time for Visit 5, if Visit 5 was not conducted), will be summarized overall and by treatment arm for the ITT population. Note that AEs also include SAEs, and that expectedness is to be noted for each recorded AE. Because this is an ITT analysis, a note will be provided along with the summary if there are any subjects randomized to the SOC group who receive one or more pAF injections in the wound during the course of the study. If applicable, the note would state how many individuals in the SOC arm had pAF administered while on study as well as how many such individuals had any post-randomization unexpected AEs while on study.

In addition, the number and percentage of subjects with at least one post-randomization unexpected AE will be calculated for the pAF arm among the subset of the ITT population that received at least one study administration of pAF.

7.6.2 Adverse Events

The adverse event summaries described for the interim DSMB reports will also be created after time has lapsed for all study-related data to have been acquired within the permitted

time windows. The final AE summaries may be made available to the principal investigator and study sponsor.

8 SAMPLE SIZE DETERMINATION

A sample size of n = 30 patients per group (total n = 60) is expected, though this may differ slightly because of the randomization procedure. Thirty participants in the pAF group with no study-related serious adverse events will provide a lower bound of the 95% one-sided exact binomial confidence interval for safety of 90% and provide enough evidence to move on to the next phase of study.

The power calculation depends on several assumptions:

- That 30 subjects are assigned to each treatment arm and that the secondary outcome is observed for all 60 subjects;
- That each subject has wound closure at Visit 5 (i.e., 100% wound area reduction relative to Visit 2) with probability $p_{SOC} = 0.2$ or $p_{pAF} = 0.5$ in the SOC and pAF arms, respectively; AND
- That for the remaining subjects in each arm, the wound area reduction percentage is uniformly distributed between 0% and 100%.

Using the Wilcoxon rank sum test (in this scenario, the response is very skewed in the pAF treatment arm), the Monte Carlo estimate of power is approximately 54%.

The trial has only modest power to detect statistically significant efficacy of pAF, but this is not unexpected for a phase I/II trial. More importantly, the sample size is sufficiently large to support the safety of moving on to the next phase of study if no study-related SAEs occur in the pAF arm, and the next phase of study would be appropriately powered for assessing efficacy.

pAF_in_Wounds_SAP_v_1_0

Final Audit Report 2021-03-11

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