SAP: TTCRNE-1501: A Multicenter, Open Label Phase 2 Pilot Trial of Subjects with Complex Non-healing Diabetic Foot Ulcers Treated with Standard Care plus Cryopreserved Umbilical Cord Allograft (TTAX01)

NCT03230175

Version 1.0 (08Nov2017)

STATISTICAL ANALYSIS PLAN

Protocol No.:	**TTCRNE-1501**
Protocol Title:	**A Multicenter, Open Label Phase 2 Pilot Trial of Subjects with Complex Non-healing Diabetic Foot Ulcers Treated with Standard Care plus Cryopreserved Umbilical Cord Allograft (TTAX01)**
Drug/Product Name:	**TTAX01**
Indication:	**Diabetic Foot Ulcers**
Sponsor:	**TissueTech, Inc.** **7300 Corporate Center Drive, Suite 700** **Miami, Florida 33126**
Version:	**Final (1.0)**
Amendment(s):	**None**
Date:	**November 8, 2017**

[redacted]

[redacted]

[redacted]

STATISTICAL ANALYSIS PLAN

ABBREVIATIONS AND DEFINITIONS

AE	Adverse Event
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
BS	Baseline
CI	Confidence Interval
CMH	Cochran-Mantel-Haenszel Test
CRF	Case Report Form
DFU	Diabetic Foot Ulcers
EOS	End of Study
EOT	End of Treatment
ET	Early Termination
HRQoL	Heath-Related Quality of Life
IWRS	Interactive Web-Based Response System
ITT	Intention to Treat
LOCF	Last Observation Carried Forward
LS	Least Square
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetics
PP	Per protocol
SAE	Serious Adverse Event
SD	Standard Deviation
SC	Standard Care
TEAE	Treatment Emergent Adverse Event
WC	Worst-Case

1 INTRODUCTION

1.1 Trial Objectives

The primary objective of this study is to examine the safety and estimate the efficacy of TTAX01 plus standard care (SC) in achieving complete wound closure of complex non-healing diabetic foot ulcers (DFU) with high risk factors of [1] ulcer depth indicating exposed bone, tendon, muscle, and/or joint capsule and [2] clinical suspicion of osteomyelitis.

This document describes the statistical analysis methods and data presentations for the data analyses of Protocol TTCRNE-1501. Related documents to this SAP are the study protocol and case report form (CRF). Data analysis will be based on the final dataset(s) provided by Sponsor or designee Data Management group. The study database(s) includes all of the CRF-based clinical data and all of central laboratory data.

1.2 Background Information

One of the most prevalent complications of diabetes is the diabetic foot ulcer in the lower extremity. Foot ulceration is the most common single precursor of lower extremity amputations among persons with diabetes and is a precursor, especially the non-healing foot ulcers, to approximately 85% of the lower extremity amputations within this population.

Three major risk factors, including (1) ulcer depth, (2) infection, and (3) ischemia, have been recognized to complicate non-healing foot ulcers leading to limb amputation. Contiguous spread of any infection of adjacent soft tissue into the bone of the foot may complicate the ulcer, predisposing the patient to risk of osteomyelitis.

Cell- or tissue-based skin substitutes are able to initially cover the non-healing foot ulcer while the cellular or tissue components may secrete or provide growth factors or structures that stimulate the host's cells, thereby promoting healing activity. Currently marketed cell- or tissue-based treatment therapies include Apligraf® and Dermagraft®, with both products combining allogeneic human epidermal and dermal cells.

TissueTech, Inc. is developing a tissue-based skin therapy (code named as TTAX01). TTAX01 is a cryopreserved human umbilical cord (UC) product aseptically processed with the patented CRYOTEK® process in compliance with current Good Tissue Practices (cGTP) from donated human placenta tissues following healthy, live, caesarian section, full-term births after determination of donor eligibility and placenta suitability. The CRYOTEK® process inactivates the cell activity in the tissue[1] to eliminate the possibility of graft rejection while retaining the natural properties. In clinical, this cryopreserved human UC product has been used for the treatment of chronic non-healing wounds.

This Phase II single-arm and open-label pilot trial will focus on the subgroup of diabetic foot ulcers that are defined as complex with the ulcer depth showing exposed bone, tendon, muscle and/or joint capsule, as well as infection as indicated by clinically suspicious osteomyelitis defined by positive probe-to-bone and positive radiographic (X-ray, MRI, or bone scan) of adjacent bone necrosis.

2 STUDY DESIGN

2.1 Rationale

Cell- or tissue-based skin substitutes are able to initially cover the wound while the cellular or tissue components may secrete or provide growth factors or cellular structures that stimulate or support the host's cells, thereby promoting healing activity. TTAX01 is a tissue-based skin therapy for treatment of DFU.

2.2 Description of Trial Design

This is a multi-center, Phase 2, open-label and single-arm pilot study of the safety and efficacy with TTAX01 plus SC treatment in subjects with a complex diabetic foot ulcer (DFU), ranging in size from 1.0 o 10.0 cm^2, with exposed bone, tendon, muscle and/or joint capsule and clinically suspicious osteomyelitis defined as positive probe to bone (PTB) and positive radiographic (x-ray, MRI, or bone scan) evidence of adjacent bone necrosis.

The study consists of a screening visit and two periods, including a 16-week open-label treatment period with an Initial Procedure Visit/baseline and 16 weekly visits (Visit 01 through Visit 16), and a 4-week confirmatory assessment period of closed wound with 2 weekly visits (Visits 17 and 18) and a bi-weekly visit (Visit 19, which also serves as the end-of-treatment visit for the efficacy evaluation).

Following the screening visit, approximately 36 subjects meeting entry criteria will be enrolled in the study at the Initial Procedure Visit (Visit BS/baseline) to receive the care for the index ulcer of TTAX01 plus SC for 16 weeks (Visits 01 thru 16). At each visit, all subjects will receive the SC treatment, and will receive the TTAX01 application on the baseline visit (Visit BS) or following the last surgical sharp debridement and bone resection, if a staged surgical procedure is performed; and, subjects will receive the TTAX01 application(s) on the subsequent visits (Visits 01 thru 15) per the guidelines of TTAX01 treatment described in details in the study protocol.

Subjects' identified index ulcer will be assessed over the course of the treatment visits (Visit BS and Visits 1 through 16) or until their ulcers close. Subjects whose ulcers have closed, as assessed by the investigator and verified by an independent blinded evaluator using the images collected via the electronic wound imaging and measurement device, prior to the 16^{th} week of treatment (or Visit 15) will stop treatment and return for two consecutive weekly visits (i.e., Visits 17, and 18) to verify if the target ulcer remains closed at these two visits following the initial observation of ulcer closure, which is defined as the complete ulcer closure in this study. Two weeks after the 2^{nd} confirmatory visit (Visit 18), subjects with index ulcer remained as closed at Visits 17 and 18 will return and complete an End of Treatment Period visit (Visit 19). Subjects whose ulcers have not closed at the end of the 16-week treatment (i.e., Visit 16) or whose ulcers have re-opened during the confirmatory visits (Visits 17 and 18) will move on directly to complete the assessments of Visit 19. Visit 19 will serve as the study's End of Treatment (EOT) visit for the treatment and ulcer closure confirmatory period and will also serve as study exit visit.

For those subjects who are enrolled and discontinue from the treatment prior to Visit 16 due to any reasons other than target ulcer closure, Visit 19 will serve as the early termination (ET) visit.

The total duration of study starting from enrollment to the end of the treatment period is 16 weeks, during which time adverse events and ulcer-related complications, including limb amputation are assessed.

The status and size of the index ulcer will be assessed for each subject at screening visit, initial visit (Visit BS), Visits 01 thru 15, Visit 16 (for the ulcer assessment only), Visits 17 and 18 (confirmation of complete ulcer closure). At the initial visit (Visit BS), eligible subjects will receive ulcer care of TTAX01 plus SC performed by the Investigator or a designated physician at the clinic unit. The test article consists of validated inner clear pouch that is placed inside an outer foil pouch that is pre-packaged. The open-label treatment period consists of 16 weeks, during which the subject will return to the clinic for 16 visits (Visits 01 to 16). At each visit of Visit BS and Visits 01 to 15, all subjects will receive the SC treatment and will receive the TTAX01 application on the Initial Visit (Visit BS) or following the last surgical sharp debridement and bone resection, if a staged surgical procedure is performed; and, subjects will receive the TTAX01 application(s) on the subsequent visits (Visits 01 thru 15) per the guidelines of TTAX01 treatment described in details in the study protocol.

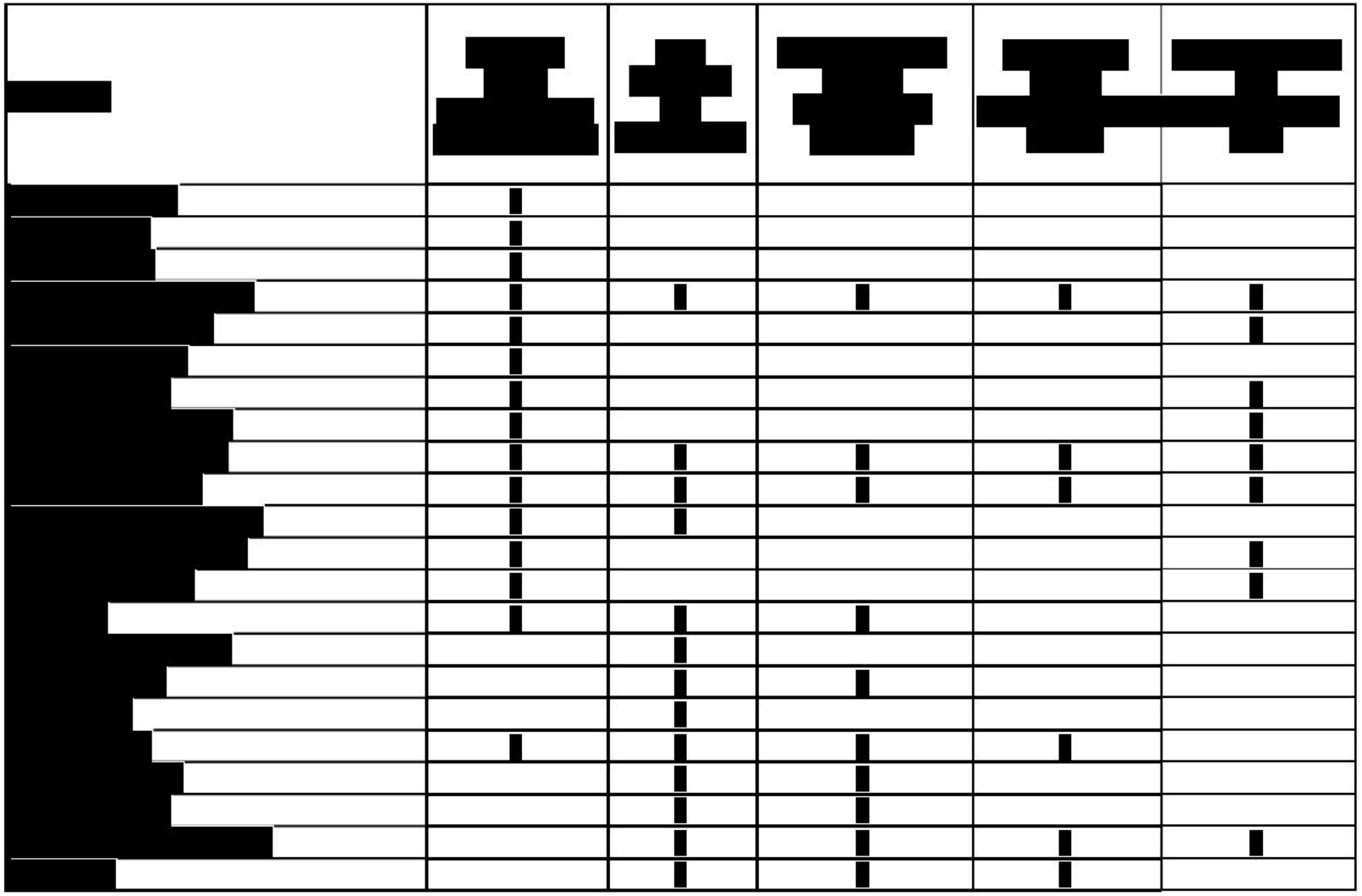
2.3 Schedule of Assessments

The complete schedule of assessments for this study is shown in table below. With reference to the Initial Procedure Visit (Visit BS), study visits are allowed to have a window of ±2 days for the weekly treatment and confirmatory visits (Visits 01 thru 19).

At Screening, eligible subjects will be assessed for ulcer measurements (i.e., size, perimeter, and depth) and duration of the index ulcer. At the initial visit (Visit BS/baseline), eligible subjects will receive the ulcer care of TTAX01 plus SC. At each of the study visits from Visit 01 through Visit 19, the subject's ulcer measurements (i.e., size, perimeter, and depth) will be assessed, evaluated, and images collected via the electronic wound imaging and measurement device. All of the subjects will receive SC treatment at the clinic at Visit BS and Visits 01 thru 18, and will receive the TTAX01 application at Visit BS or at the last surgery for a staged surgical procedure, and will receive the TTAX01 application(s) at subsequent visits of Visits 01 thru 15 per the guidelines of TTAX01 treatment described in details in the study protocol.

The assessment schedule over the entire study is outlined in the table below.

[redacted]



[redacted]

2.4 Randomization

This is an open-label study with single-treatment arm. All eligible subjects will be enrolled to receive the treatment. No randomization will be performed.

2.5 Sample Size Estimation

[redacted]

2.6 Efficacy and Safety Measurements

In this study, primary efficacy will be assessed using the proportion of subjects with complete ulcer closure over the 16-week treatment period. Secondary efficacy endpoints will include the number of days to complete ulcer closure over the 16-week treatment period, the proportion of subjects with ulcer closure at each of the 16 treatment weeks. The index ulcer will be measured at screening visit and baseline visit (Visit 01) and at each of the post-baseline visits from Visit 02 through Visit 19.

Safety will be assessed through subject early terminations, adverse events, physical examination, clinical laboratory tests (hematology, chemistry), and concomitant medications.

2.6.1 Primary efficacy measure

[redacted]

2.6.2 Secondary efficacy measure

The secondary efficacy endpoints are listed below:

- [redacted]

2.6.3 Safety measures

Adverse events are recorded at each visit. A physical examination is performed at screening visit and Visit 19.

The clinical biochemistry and hematology laboratory tests are conducted at screening visit and at Visit 19. Additional laboratory tests are collected at screening visit. Laboratory tests are analyzed by a central laboratory and include the following parameters:

Other safety measures of interesting include as follows:

[redacted]

2.6.4 Other measures

Prior treatment for DFU and concomitant medications taken over the course of the study will be recorded throughout the study. Concomitant medications associated with the treatment of the index ulcer will be specifically noted as such.

2.7 Drug Concentration and PK Measurements

No drug concentration measures will be taken in this trial.

2.8 Handling of Missing, Incomplete, and Repeat Data

[redacted]

Primary Efficacy Endpoint: Confirmed complete ulcer closure

[redacted]

Secondary Efficacy Endpoint: Time to complete ulcer closure

[redacted]

Secondary Efficacy Endpoint: Complete ulcer closure at each treatment week:

[redacted]

Secondary Efficacy Endpoint: Ulcer and granulation tissue measures at each treatment visit

[redacted]

Safety Endpoints:

[redacted]

2.9 Statistical Methods

[redacted]

[redacted]

[redacted]

2.9.1 Primary efficacy analysis

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2.9.2 Secondary efficacy analysis

[redacted]

2.9.3 Planned Interim Analysis

No interim analysis is planned.

2.9.4 Safety analysis

Treatment Exposure

Frequencies of exposure to the study treatment will be calculated based on the number of [redacted]

[redacted]

Early Termination

[redacted]

Adverse Events

Adverse events will be coded for preferred terminologies using Medical Dictionary for Regulatory Activities (MedDRA). AE will be grouped into pre-treatment AE and treatment-emergent AE as well as post-treatment AE and will be reported separately, based upon the start date of the events, the start date of the treatment, and the start date of the observational follow-up period.

Adverse events will be defined as the pre-treatment AEs if they occur prior to the treatment start date; as the treatment-emergent AEs if they occur on or after the treatment start date and prior to the observational follow-up start date; and as the post-treatment AEs if they occur on or after the observational follow-up start date.

Frequency of the treatment-emergent AEs will be calculated for each body system (or system organ class), by preferred terminology, by post-debridement ulcer size group, for number of subjects and percentage reporting the event. The severity of the adverse events and the relationship to the investigational product will be summarized for each body system and preferred terminology by group. Withdrawals due to adverse events will be summarized for each body system and preferred terminology by group and will be listed with demographic variables for individual subjects.

Proportion of subjects who experience ulcer-related complications (e.g., life-threatening, infection-related sepsis complications, recurrent osteomyelitis, development of gangrene) will be analyzed by group.

Proportion of subjects who experience recurrent limb amputation (i.e., full amputation involving the index ulcer anatomic location) will be analyzed by group, respectively, for subjects with and without pathologically confirmed osteomyelitis

Time in number of days to the first amputation on the target limb or foot or death, whichever occurs first, will be analyzed by group, using Kaplan-Meier survival analysis.

Serious adverse events (SAE) (including deaths) will be listed for individual events.

Narratives will be provided for all deaths, non-fatal serious adverse events, and subjects withdrawn due to adverse events.

Physical Examination

[redacted]

Laboratory Assessments

[redacted]

2.9.5 Other analysis

The concomitant medications, including those for target ulcers and non-target ulcers, will be [redacted]

3 STUDY POPULATION

3.1 Definition of Subject Populations

All subjects who have procedures completed at Visit BS are considered study participants. The following subject populations are defined to assess the safety and efficacy of TTAX01 with SC.

Safety Population

The safety population is defined as all subjects who are enrolled and who have received at least one treatment of the investigational product.

Intention-to-treat (ITT) Population

This population is defined as all subjects who are enrolled to receive treatment. The ITT population is the primary population for efficacy evaluation in this study. The ITT population will be identified and finalized before the database is locked.

Per-Protocol (PP) Population

This population is defined as sub-population of the ITT population. The PP population is the secondary population for efficacy evaluation in this study. The detailed criteria of the PP population will be provided in a separate “Data Standards” document completed prior to data base lock.

3.2 Screening and Enrollment

An enrollment summary including the number of subjects screened, enrolled, and completed treatment will be presented for all screened subjects and by site (Table 1.1.1). The number of subjects enrolled (i.e., study participants or ITT population) will be summarized for each post-debridement ulcer size group and overall (Table 1.1.2). The number of subjects treated (i.e., the safety population) will be summarized for each post-debridement ulcer size group and overall (Table 1.1.3). The number of treated subjects terminating from treatment will be summarized for each group (Table 1.1.4).

3.3 Population Demography

For ITT and safety populations, descriptive summaries of demography and baseline characteristics will be presented for each group and overall to establish baseline comparability (Tables 1.2.1 to 1.2.2).

Demography includes age in years (continuous and categorical 18 to 39, 40 to 49, 50 to 59, 60 to 69, 70+), gender, race, ethnicity, weight, and height. Demography will also be listed for all study participants (Listing 1).

3.4 Disease Characteristics and Prior Treatment

For ITT and safety populations, descriptive summaries of baseline index ulcer characteristics will be presented for each group and overall to establish baseline comparability (Tables 1.3.1 to 1.3.2).

The size and duration and other characteristics of index ulcer will be listed for all subjects (Listing 1).

Primary therapy used for treatment of DFU prior to participating in the study will be listed for all subjects (Listing 1).

3.5 Medical History

Medical history will be listed in CRF data tabulation.

3.6 Disposition of Study Participants

Subject disposition including completion and discontinuation along with CRF-based termination reason will be summarized for ITT population and safety population (Tables 1.4.1 to 1.4.2). Subjects' early termination details will be listed for all subjects (Listing 2).

Subjects' TTAX01 treatment and date will be listed for all subjects enrolled (Listing 3).

3.7 Protocol Deviations and Violations

A summary of protocol deviation and violations will be provided for ITT population (Table 1.5.1). Subjects who are enrolled to receive treatment in the study and included in the ITT population, but do not have post-baseline status of ulcer closure will be listed for subjects enrolled (Listing 4).

Subjects who are included in the ITT population, but not included in the PP population due to either protocol violations or other documented reasons will be listed for all subjects excluded from the PP population (Listing 5). Major protocol violations may include the following:

- The subject fails to meet all inclusion/exclusion criteria and receives no waiver for entering into the study;
- The subject is terminated for protocol violations.

4 EFFICACY EVALUATION

4.1 Datasets Analyzed

The ITT population is defined as the primary efficacy population, and the PP population as the secondary efficacy population. Both populations will be identified and finalized before the database is locked. The definitions of the two populations are provided in Section 3.1.

4.2 Treatment Compliance

The compliance rate of SC treatment will be summarized by treatment group and demographic parameters for the ITT and safety populations (Tables 1.5.2 and 1.5.3).

4.3 Data Missing and Imputation

Summary of the observed data missing and imputation performed will be reported for index ulcer closure status, the primary efficacy measure, of the ITT population in Table 1.6.1.

4.4 Baseline Measures

Descriptive statistics of the index ulcer area, depth, and volume at baseline will be summarized by treatment for the ITT and safety populations (Tables 1.3.1 and 1.3.2).

4.5 Efficacy Results and Tabulations

4.5.1 Results of primary efficacy analysis

Descriptive and inferential statistics on the ITT population of the proportion of subjects with complete ulcer closure over the treatment period will be presented in Table 2.1.1.

For the PP population, the results of the same proportion analysis on the number of subjects with complete ulcer closure will be presented in Table 2.1.2.

The by-site summary of descriptive statistics of the number of subjects with complete ulcer closure will be presented in Table 2.1.3 for pooled sites and in Table 2.1.4 for original sites.

Individual subjects' primary efficacy data (i.e., complete ulcer closure), ulcer area measure and ulcer closure status at each visit, with and without imputation, will be listed by treatment for the ITT population (Listing 6).

4.5.2 Results of secondary efficacy analyses

Descriptive and inferential statistics on the ITT population of the number of days to complete ulcer closure will be presented in Table 2.3.1 for Cox regression analysis. The resultant probability of subjects with index ulcer open over the 16-week treatment period and estimated median time to ulcer closure will be reported in Table 2.3.2. For the PP population, the results of the same Cox regression analysis on the number of days to complete ulcer closure will be presented in Table 2.3.3 and Table 2.3.4.

Descriptive and inferential statistics on the ITT population of the proportion of subjects with complete ulcer closure for each post-baseline treatment week will be presented in Table 2.1.1.

Descriptive and inferential statistics on the ITT population of percent change of index ulcer parameters (ulcer area, depth, and volume) and percent of granulation tissue coverage at each treatment visit will be presented in Tables 2.4.1 thru 2.4.4.

5 SAFETY EVALUATION

For safety evaluation, the safety population as defined in Section 3.1 will be utilized to conduct safety analyses among treatment groups.

5.1 Extent of Drug Exposure

Exposure to study medication will be tabulated by treatment in Table 3.1.1 for total number of SC treatments received and in Table 3.1.2 for TTAX01 application received, using the following categories: 1-2 applications, 3-4 applications, 5-6 applications, 7-8 applications, 9-

10 applications, 11-12 applications, 13-14 applications, and 15-16 applications. The tabulations will be done for the safety population, and for man and woman, separately.

Individual subjects' treatment exposure (applications) will be listed by treatment regimen for the safety population (Listing 3).

5.2 Early Termination

Early termination will be categorically summarized for the ITT population in Table 1.5.1, and for the safety population in Table 1.5.2. Subjects' study termination details will be listed (Listing 2).

5.3 Adverse Events

An overall summary of AE reported in this study will provided in Table 4.1.1 for study participants and in Table 4.1.2 for safety population. The incidences of subjects reporting pre-treatment AE will be reported in Table 4.2.1 for the pre-randomization period and in Table 4.2.2 for the treatment-emergent AE by post-debridement ulcer size group. Those treatment-emergent AE by preferred terminology that are reported by more than 3% of subjects in any treatment will be tabulated in Table 4.2.3.

Incidences of subjects reporting treatment-emergent AE will also be tabulated by onset time Table 4.3.1.

The severity of the treatment-emergent AE and the relationship to the investigational product will be summarized in Table 4.4.1.

Treatment-emergent AE associated with early terminations and serious AE will be summarized by preferred term in Table 4.4.2 and Table 4.4.3.

Details of AE associated with both index ulcer and non-index foot ulcers will be listed for each subject (Listing 7)

Details of AE associated with early terminations will be listed for each subject (Listing 8). SAE, including deaths, will be listed for each event (Listing 9).

Narratives will be presented for all deaths, non-fatal serious adverse events, and subjects withdrawn due to adverse events.

5.4 Physical Examination

Abnormal findings of physical examination will be recorded as adverse events in this study, if so determined by the investigator.

Qualitative findings in terms of abnormal, normal, and not done at both screening and end-of-treatment visits will be tabulated by group in Table 5.1.1 for the safety population.

5.5 Clinical Laboratory Evaluation

Descriptive statistics of laboratory parameters will be presented by group at the screening and end-of-treatment visits for the safety population in Tables 6.1.1 to 6.1.3 for hematology and chemistry as well as other laboratory tests, respectively.

Qualitative findings in terms of abnormal low, normal, and abnormal high at both screening and end-of-treatment visits will be tabulated by group in Tables 6.2.1 to 6.2.2 for the safety population for hematology and chemistry, respectively.

Any clinically meaningful abnormalities observed during the study will be listed for hematology (Listing 10) and chemistry (Listing 11), as well as pregnancy test (Listing 12).

5.6 Concomitant Medication

Number and percent of subjects who take pre-baseline concomitant medications will be reported in Table 7.1.1. Number and percent of subjects who take post-baseline concomitant medications will be reported by group in Table 7.1.2.

Number and percent of subjects who take pre-baseline concomitant medications for ulcer treatment will be reported in Table 7.2.1 for index ulcer and in Table 7.2.2 for non-index ulcer. Number and percent of subjects who take post-baseline concomitant medications for ulcer treatment will be reported by group in Table 7.3.1 for index ulcer and Table 7.3.2 for non-index ulcer.

6 REFERENCES

Study protocol.

7 List of Statistical Table Shells

Statistical table shells are provided after the text portion. Table shells are presented only for those tables with a unique format. Tables with duplicate formats are indicated in the list of tables below.

8 List of Key Listing Shells

Listing shells are provided after the statistical table shells.

9 List of Figures

10 List of CRF Data Tabulations