Study Protocol: 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)

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Version 1.1 (05Feb2018)

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)

Protocol Number: TTCRNE-1501

IND Sponsor: TissueTech, Inc.

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
1.1	Updated study population to 36 patients with approximately 12 sites	Adjusted study population based on the projected number of clinical sites to participate, and to enroll 3 patients/site
3.0, 8.1.1	Updated safety endpoint to include proportion of subjects who experience subsequent limb amputation	Updated safety endpoint to distinguish initial amputation completed at the Initial Procedure Visit and any subsequent limb amputation that would discontinue the subject per section 7.2
5.2	Removed previous exclusion #1 'the subject has another ulcer within 3cm of the index ulcer Removed previous exclusion #10 'The subject has tested positive for HIV or has AIDS'	Since there is no worsening criterion of the index ulcer, any ulcer within close proximity to the index ulcer would be surgically addressed at the Initial Procedure Visit. Allowing patients with HIV or AIDS since there are no contraindications with TTAX01

	Added sepsis definition to previous exclusion #20, which is now exclusion #18	Added sepsis criteria to ensure site consistency when evaluating new patients
6.1.2, 8.1.2	Added clarity that TTAX01 should be surgically fixed by either sutures or staples Added TTAX01 must be fenestrated	Provided additional means to surgically fix TTAX01 with staples TTAX01 must be fenestrated to avoid possible seromas or hematomas after tissue placement on the woundbed
8.1.2, 8.1.3, 8.1.4, 8.1.5	Allowing total contact casts for off-loading	Some sites use total contact casts as their standard care so allowing both full length boot or total contact cast for off-loading methods
8.4.1, 8.4.2, 8.4.3.2, 8.4.6	Updated adverse event (AE) definition, reporting AEs, updating related terminology, updated Medical Monitor email	Provided clarity on AE definition, reporting of AEs in the electronic data capture system, and updated medical monitor email for SAE
9.4.7	Updated sub-group analysis by race	Updated sub-group analyses per FDA recommendation

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

Investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of this trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is screened or enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

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)

Study Description: It is hypothesized that application at 4-week intervals of the human placental umbilical cord tissue TTAX01 to the surface of a well debrided, complex diabetic foot ulcer will, with concomitant management of infection, result in a higher proportion of wounds showing complete healing within 16 weeks of initiating therapy. This open label pilot study provides a framework for a larger, controlled study. The purpose for conducting this study is to evaluate the functionality of the protocol, and to obtain an estimate of product safety and efficacy when applied according to the protocol instructions, and measured according to the stated endpoints.

Objectives: Primary Objective: 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 with high risk factors of [1] ulcer depth indicating exposed bone, tendon, muscle, and/or joint capsule and [2] clinical suspicion of osteomyelitis.

Summary of Secondary Objectives: To examine alternative measures of wound healing, progress towards healing, and healing among stated subsets, as well as to estimate the influence of treatment on rates of amputation.

Endpoints: Primary Endpoint: Proportion of subjects with complete wound healing by Treatment Week 16

Secondary Endpoints: Various associated measures such as time to heal, proportion healed by week, and wound area reduction.

Study Population: Approximately 36 adults aged 18 years and older, with diabetes mellitus types 1 or 2, and non-ischemic, non-dorsal diabetic foot ulcer measuring 1-10 cm^2 inclusive with evidence of exposed bone, tendon, muscle and/or joint capsule and presumptive evidence of osteomyelitis.

Phase: 2

Description of Sites/Facilities Enrolling Participants: Approximately twelve sites specializing in care of diabetic foot ulcers, including inpatient hospital and outpatient based wound practices to include podiatric office practices, wound care centers, dermatology and vascular surgery clinics.

Description of Study Intervention:	All subjects will receive aggressive debridement at baseline, followed by standard care supplemented with TTAX01, a cryopreserved human umbilical cord product derived from human placental tissue. Test article is applied and surgically fixed in direct contact with the wound surface, at 4 week intervals unless healing is evident, or when replacement of dislodged material is necessary.
Study Duration:	Estimated time from first subject eligible and enrolled to completion of statistical analysis is 18 months.
Participant Duration:	The maximum time in study for any subject will be 21 weeks, with 1 week in screening and assuming wound healing occurs at week 16 followed by 2 weeks of observation for confirmation of closure, and a final exit visit 14 days later.

1.2 SCHEMA

Figure 1. Flow diagram

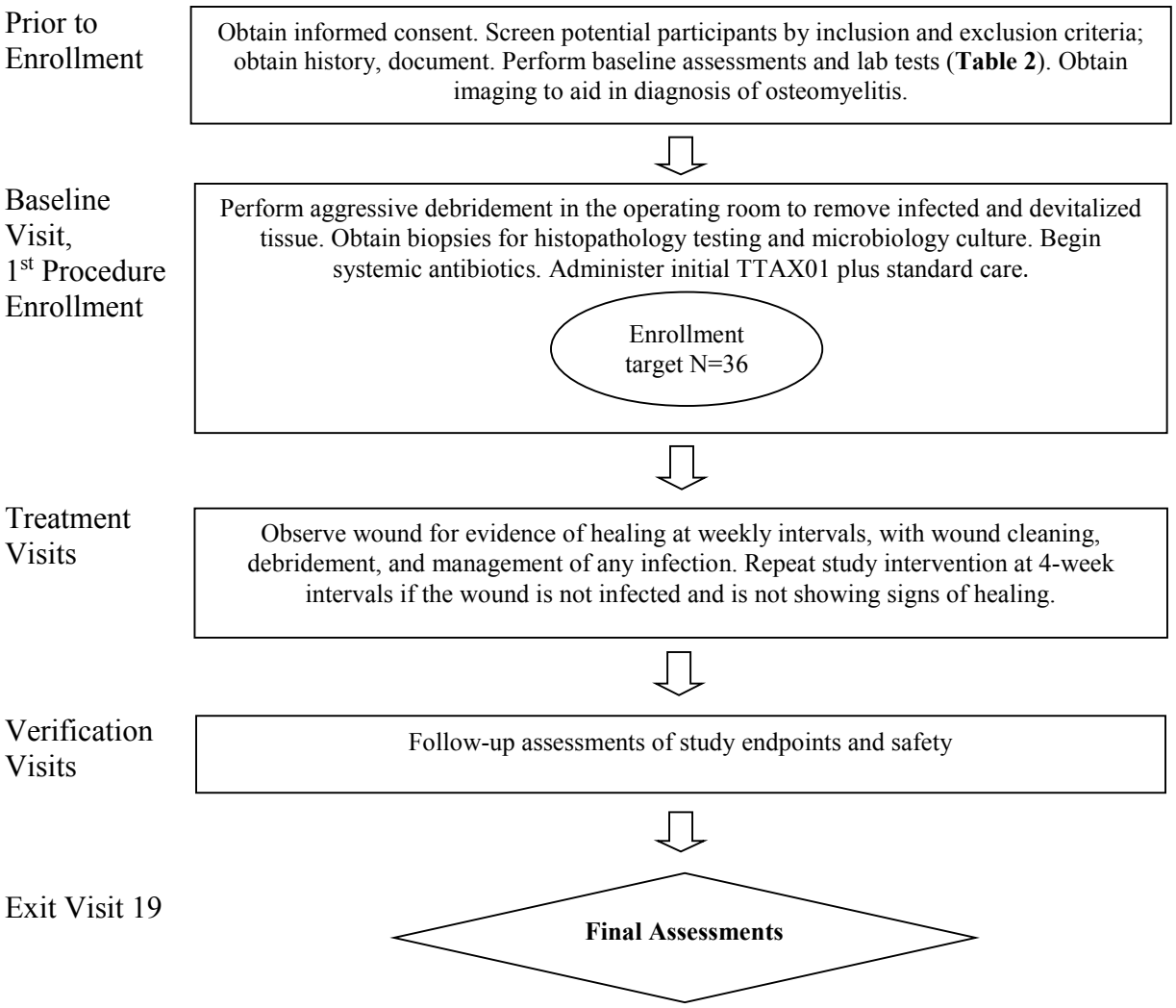

Figure 2. Process diagram

Visit SCR — **Screening**

- Total estimated screened n=47 (30% screen failure rate)
- Obtain informed consent, screen potential participants by inclusion and exclusion criteria
- Obtain history, document
- Obtain baseline laboratory and imaging studies for osteomyelitis
- Initiate empiric systemic antibiotics

BL/Initial Procedure — **Baseline debridement, Qualification**

- Perform aggressive debridement in the operating room, obtain biopsies for histopathology and culture
- Verify that subject meets all criteria for enrollment
- Apply first TTAX01

Visits 1-16 (7±2 d) — **Evaluations and re-treatments**

- Observe wound weekly for healing or stalled, infected
- Re-treat at 4 week intervals if not infected, not healing
- Perform weekly cleaning and debridement; observe and interview for adverse events
- If the wound has closed, proceed to Visit 17. If the wound remains open at Visit 16, proceed to Visit 19

Visits 17, 18 (7±2 d) — **Follow-up assessments of study endpoints and safety**

- For subjects whose wound has closed, determine again if the wound remains healed or has re-opened

Visit 19 (14±2 d) — **End of Study Exit Visit**

- Verify wound healing, resolution of AEs, assess vascular perfusion, obtain final lab tests (**Table 2**)

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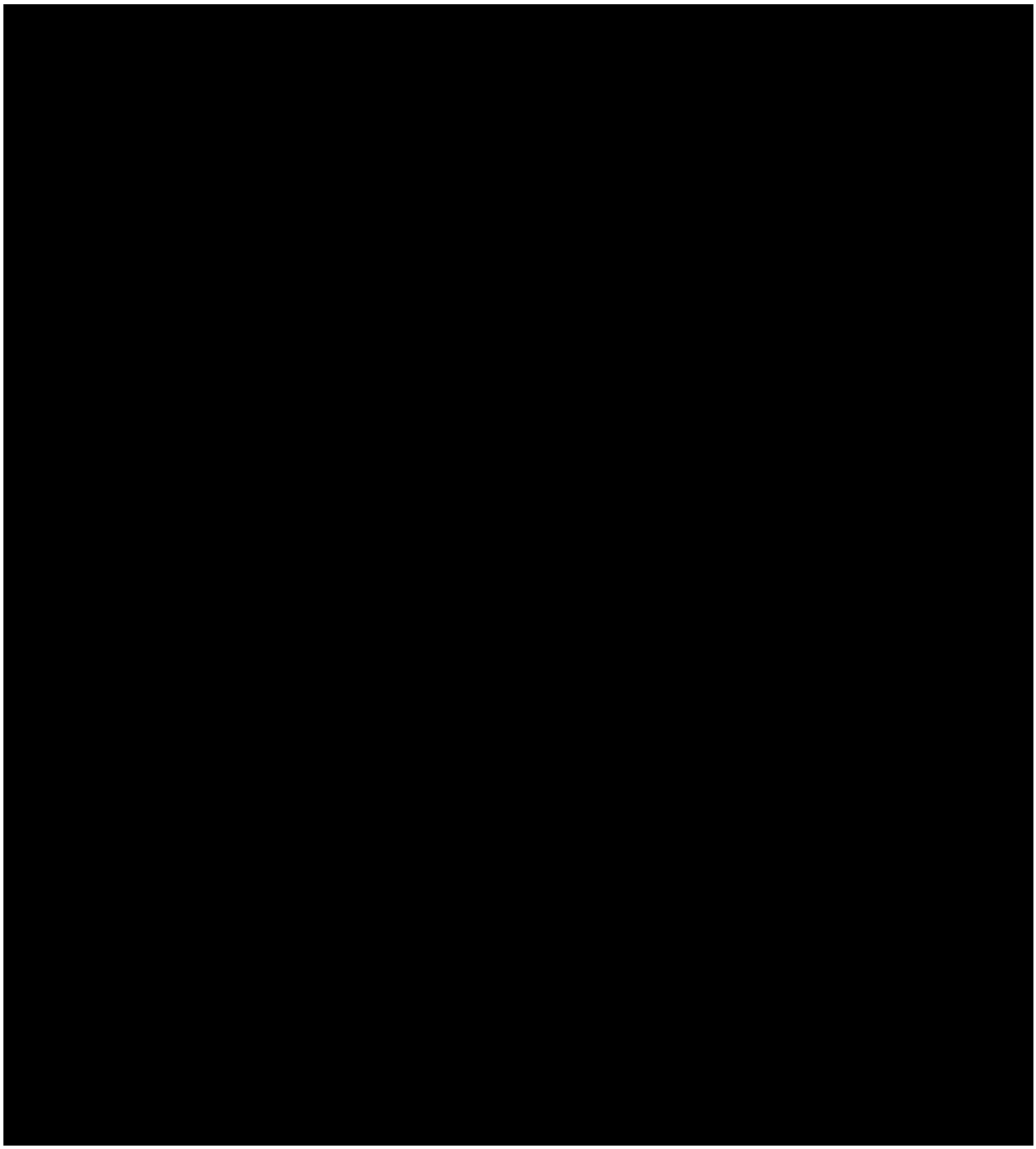
1.3 SCHEDULE OF ACTIVITIES (SOA)



2 INTRODUCTION

2.1 STUDY RATIONALE

According to the United States (US) Centers for Disease Control, there were 29.1 million Americans of all ages or 9.3% of the population in 2014 who had diabetes (1) either diagnosed or undiagnosed, a figure that has increased by fivefold since 1980 (2). An estimated 21 million persons were diagnosed with the disease, while an additional 8.1 million remain undiagnosed (1). By 2050, as many as 1 out of every 3 adults in the US could have diabetes if the trend continues (2).

One of the most prevalent complications of diabetes is the diabetic foot ulcer. Diabetic persons have approximately 25% risk of developing a foot ulcer in their lifetime (3) with an estimated annual incidence rate of 0.5-3.0% (4-8). When the ulcer is non-healing, the dermal first line of defense is compromised for a prolonged period and the patient is susceptible to tissue loss that leads to limb amputation. Indeed, foot ulceration is the most common single precursor of lower extremity amputations among persons with diabetes and is a precursor to approximately 85% of the lower extremity amputations within this population (3, 9-20). In the US, diabetes has been the most common underlying cause of non-traumatic lower extremity amputations (21, 22); more than 60% of non-traumatic lower extremity amputations occur in diabetic persons (1).

Over 73,000 non-traumatic lower-limb amputations are performed in the US for people with diabetes annually (1). Unfortunately, after one major lower extremity amputation, the 5-year survival rate is estimated to be 50% (14, 15), worse than those of most malignancies and second only to that of lung cancer (14, 16). Moreover, once amputation occurs, 50% of the patients will develop an ulcer in the contralateral limb within 5 years (15). For amputation survivors, day-to-day functioning is greatly impaired. Many cannot walk, with or without the use of a cane or walker. A study found that in 2010, 22.8% of patients undergoing amputation of a lower extremity in the US were readmitted to the hospital within 30 days, the highest rate of re-admission among the procedures considered in the study (23). Moreover, even with the best of medical care, amputation and its aftermath are traumatic experiences that can be expected to produce depression as the patient copes with the social and financial consequences of disfigurement and loss of function. Collectively, one can envision a grave picture of the seriousness of the complex non-healing foot ulcers that carry these high risks that may lead to amputation in this country and worldwide.

Three major risk factors, i.e., [1] ulcer depth, [2] infection, and [3] ischemia, have been recognized to complicate non-healing foot ulcers leading to limb amputation (17). The first risk factor is “ulcer depth”. This clinical trial will enroll patients with the ulcer depth exhibiting exposed bone, tendon, muscle and/or joint capsule. It has been well known that these deep ulcers with extensive tissue loss are at high risk for infection-related ulcer complications including osteomyelitis (24).

The second risk factor is "infection". While infection is not often implicated in the pathway leading to ulceration, it is a major risk factor in the causal pathway to amputation (25, 26). Contiguous spread of any infection of adjacent soft tissue into the bone of the foot will complicate the ulcer, predisposing the patient to risk of osteomyelitis (18),(19). Foot ulcers accompanied by limb-threatening infections such as osteomyelitis have reported amputation rate as high as 51% (27-29). Indeed, the presence of an infection is a major predisposing factor for diabetic foot amputations, as 85% of these amputations are preceded by an infection (21, 30, 31). The risk of amputation increases by four times when the foot ulcer is complicated by osteomyelitis compared to soft tissue infection alone (32).

The third risk factor is "ischemia". Critical limb ischemia (CLI) has been used to denote a subgroup of patients with a threatened lower extremity primarily due to chronic ischemia. Foot ulcers complicated by ischemia have been reported to have an amputation rate of between 17-23% and a mortality rate of 33% (33, 34). Because CLI will compromise wound healing and because the standard of care calls for vascular consultation as well as potential revascularization procedures to address limb ischemia (17), clinical trials conducted under the Sponsor's IND exemption will only enroll those patients with diabetic foot ulcer in which the vascular perfusion status is adequate to support healing.

Collectively, the Inclusion and Exclusion criteria stated in this protocol allow us to enroll patients with a chronic diabetic foot ulcer that carries the severity of Wagner Grade 3 or worse (on a scale of 0 to 5 based on Wagner Classification System). This open label pilot study is intended to examine the operational aspects of a proposed Phase 3 pivotal trial, including ease of compliance with various decision points in the protocol (e.g., antibiotic use, debridement in the operating room, whether to treat with TTAX01 or withhold treatment), and to provide a refined estimate of treatment response.

2.2 BACKGROUND

Although new advances in wound care products include advanced skin substitutes and recombinant growth factors such as platelet-derived growth factor, none of these products are indicated for treating complex wounds presenting with osteomyelitis. In addition, the vast majority have not been demonstrated to be safe or effective in the treatment of complex non-healing ulcers that have a depth exhibiting exposed bone, tendon, muscle, and/or joint capsule. Apligraf® and Dermagraft® are not indicated for ulcers with exposed bone, tendon, muscle, and/or joint capsule and are contraindicated for use on clinically infected ulcers or manifesting ischemia (e.g., Ankle Brachial Index (ABI) less than 0.60) or infection (e.g., osteomyelitis). Exposure of bone, tendon, muscle, and/or joint capsule, ischemic wounds (e.g., ABI less than 0.60), clinically infected ulcers and osteomyelitis are the exclusion criteria in clinical trials of various advanced skin substitutes approved for chronic foot ulcers of various etiologies. Only Theraskin® carries indication for use in exposed tendon, joint and/or bone. It should be noted that the approval of Theraskin® is supported by a retrospective observational study or clinical case studies (35-37) rather than a prospective randomized and controlled clinical trial. In one study of 188 patients

(38), only 2.14% of the ulcers extend to the tendon or capsule and only 0.5% were ulcers with exposed bone. Additionally, it should also be noted that Theraskin® is not indicated for ulcers with severe infection including osteomyelitis.

Of note, a limited number of randomized and controlled clinical trials are available to support the use of hyperbaric oxygen therapy for wound healing (but not resolving infection) (39-42). Although some studies have demonstrated that the negative pressure therapy may improve healing of diabetic foot ulcers, especially after wide debridement or partial amputation (43-45), there is limited high-level evidence to support widespread utilization, especially in an infected wound (46, 47). In fact, both hyperbaric oxygen therapy and negative pressure therapy are *not* recommended by the Infectious Disease Society of America panel to treat DFU with infection (3). Consequently, hyperbaric oxygen therapy and negative pressure therapy are excluded from the Standard Care (SC) provided in this protocol.

Amniotic membrane (AM) tissue has long been recognized as having unique wound healing, anti-scarring, and anti-inflammatory properties in various indications including dermal wounds (for reviews see ref.(48-50)). The Sponsor has received several research grants from the National Institutes of Health (NIH) to identify a novel matrix component termed the HC-HA/PTX3 complex from both AM and umbilical cord (UC). This complex is formed by a covalent linkage between hyaluronan (HA) and heavy chain 1 (HC1) of inter-α-trypsin inhibitor (IαI) (51, 52) that may then be tightly associated with pentraxin 3 (PTX3) to form the HC-HA/PTX3 complex (53).

[redacted]

Many currently available therapies are targeted to treat specific aspects of a condition, for example, silver dressings are intended to specifically manage infection and platelet-derived growth factors (PDGFs) are intended to stimulate angiogenesis. Furthermore, nearly all of the aforementioned advanced skin substitutes were understood to require "engraftment" or "graft take" to promote wound healing. For example, the percentages of "graft take" as an indicator of successful application has been reported for Apligraf® (61). TTAX01's mechanism of action is quite unique in exerting multi-modal actions including anti-inflammatory, anti-scarring and regenerative effects in different types of cells. The mechanism of action for TTAX01 does not depend on engraftment.

In summary, the aforementioned non-clinical data suggest that TTAX01 employs a novel mechanism of action to promote wound healing.

A single center, retrospective study of cryopreserved UC showed the clinical efficacy of promoting healing of 33 complex foot ulcers in 31 patients, of which 27 of the 33 ulcers showed depth indicating exposed bone, tendon, muscle, and/or joint capsule as well as histopathologically-confirmed osteomyelitis via bone biopsy (62).

These 31 patients included 26 males and 5 females with an age of 58.3 ± 12.9 years. The majority of patients treated were Caucasian (12/31) or African-American (10/31). Overall, these patients presented with multiple co-morbidities leading to the development of chronic, non-healing ulcers. Among the most significant co-morbidities were diabetes (26/31), hypertension (23/31), peripheral vascular disease (16/31), renal failure (12/31), and coronary artery disease (9/31). All 31 patients had histopathological evidence of osteomyelitis and carried a number of risk factors such as ischemia (i.e. gangrene (n=17), ischemic ulcers (n=24), previous partial amputation on the study leg (n=9), and previous revascularization attempt (n=1)) and infection (including osteomyelitis (n=33) and cellulitis (n=7)). The average ulcer size was 15.6 ± 17.7 cm^2 (0.4 - 74 cm^2).

Six of the 33 ulcers were lost to follow up during the course of the treatment including 1 patient who died of causes unrelated to product treatment. During the follow-up period, 26 of the remaining 27 wounds (96.3%) achieved complete wound closure. The overall clinical efficacy is reflected by complete healing in 22/27 (81%) at 24 weeks and 96% (26/27) at 44 weeks. For the 26 wounds that completely healed, the average time to wound closure was 16.0 ± 9.3 weeks (range: 4 – 44 weeks) by one application in 21 wounds and 2 applications in the remaining 5 wounds, with the second application being applied from 4 – 10 weeks after the initial application. The patient with the non-healing wound went on to receive a below the knee amputation due to complications related to the wound and other co-morbidities, resulting in an amputation rate of 3%.

In summary, the above data support the feasibility of conducting a Phase 2 multiple-center open label pilot trial to examine TTAX01's efficacy in promoting healing of complex non-healing diabetic foot ulcers with high risk factors of [1] ulcer depth indicating exposed bone, tendon,

muscle and/or joint capsule and [2] clinical suspicion of osteomyelitis, so as to reduce the likelihood of ulcer-related complications including limb amputation.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The major risk associated with use of human tissue as a temporary, non-engrafting therapeutic is the transmission of infectious agents. (63) This risk is minimized though careful donor screening and Good Tissue Practices. Decellularization removes any risk of potential graft vs. host reactions. A review of the literature indicates that various cell and tissue based products tested as therapeutics for non-healing wounds show no differences in adverse event profile compared with standard care (SC). Two ongoing controlled trials of NEOX® CORD 1K® for treatment of DFU show no pattern of treatment emergent adverse events associated with use of the product (NCT02166294, NCT02707406). The procedures required by the protocol are standard practice in the care of diabetic foot ulcers.

2.3.2 KNOWN POTENTIAL BENEFITS

Three published retrospective case series provide an estimate of the complete healing rate when treating chronic DFU with NEOX® CORD 1K®, a marketed HCT/P 361 product identical to the test article TTAX01, with the exception that TTAX01 will be released against identity, potency and purity specifications put in place under Good Manufacturing Practices.

Ref	Patients	Baseline wound area	Number of applications	Complete healing	Mean time to heal
(64) Retro case series	31 patients, 33 wounds; complex DFU with osteomyelitis	15.6 ± 17.7 cm^2	1.24 ± 0.44	26/33 (78.8%)	16.0 ± 9.3 weeks
(65) Retro case series	29 patients, 32 wounds; DFU	10.6 ± 2.15cm^2	1.68 ± 0.18	28/32 (87.5%)	13.8 ± 1.95 weeks
(66) Retro case series	57 patients, 64 wounds of the lower extremity	6.85 ± 16.29 cm^2	3.43 ± 2.42	51/64 (79.7%)	5.53 ± 3.93 weeks

The anticipated immediate benefit is healing of the open diabetic foot ulcer. The associated long term benefit is expected to be a reduced risk of amputation. In the published experience of Caputo (64), only one of 15 planned amputations was necessary following intervention with NEOX® CORD 1K®.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The standard care provided in this protocol is based on guidelines and recommendations from experts in the field of diabetic foot ulcer care. The initial aggressive debridement in an operating room is considered best practice for the type of wounds being studied, which extend to deep subdermal structures and include evidence of associated osteomyelitis. All investigators are skilled

and experienced in the debridement techniques required by the protocol. Phlebotomy is performed twice, at baseline and end of study. The test article is made to the same exacting Good Tissue Practices standards as the commercialized NEOX® CORD 1K®, with additional specifications for release under Good Manufacturing Practices. There have been no reported cases of infectious agent transmission in more than 15,500 patients treated with either NEOX®/CLARIX® 100 (trade name for amniotic membrane products) or NEOX®/CLARIX® CORD 1K® (trade name for umbilical cord products), nor have any remarkable adverse events or serious adverse events been reported related to these products.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To examine the safety and estimate the efficacy of TTAX01 plus standard care (SC) in achieving complete wound healing of complex non-healing diabetic foot ulcers with high risk factors of [1] ulcer depth indicating exposed bone, tendon, muscle, and/or joint capsule and [2] clinical suspicion of osteomyelitis	Proportion of subjects with complete wound healing over the 16-week treatment period, defined as complete epithelialization with no drainage and no need for a dressing, confirmed at two visits, two weeks apart, and a biweekly end of treatment visit for a confirmatory duration of 4 weeks	Per FDA 2006 Guidance on endpoints for wound healing trials
Secondary		
To examine additional efficacy endpoints related to wound healing	• Time in number of days to complete wound closure over the 16-week treatment period, starting from enrollment to the time of the initial observation of wound closure	Time to heal is an alternative endpoint per the 2006 Guidance
	• Proportion of subjects with complete wound closure at each of the 16 treatment weeks	Typical alternative to Cox proportional hazard
	• Percent change in the wound surface area (cm^2) at each visit from baseline for assessing the rate of wound closure in area	Wound area reduction may allow surgical closure
	• Percent change in the wound volume (cm^3) at each visit from baseline for assessing the rate of wound closure in volume	Alternate view of healing progress
	• Percent change in the wound depth (cm) at each visit from baseline for assessing the rate of wound closure in depth	Alternate view of healing process
	• Percent of index wound covered by the granulation tissue at each visit for assessing the rate of wound closure in granulation tissue	Alternate view of wound healing process
	• Proportion of subjects achieving complete wound closure in subjects with pathologically confirmed osteomyelitis	Subset analysis
To examine the effect of therapy on ulcer-related complications (safety)	• Proportion of subjects who experience ulcer-related complications (e.g., life-	Relevant safety measures

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	threatening, infection-related sepsis complications, recurrent osteomyelitis, development of gangrene) • Proportion of subjects who experience subsequent limb amputation (i.e., full amputation involving the index ulcer anatomic location) among 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.	Therapy may reduce amputation rate
Tertiary/Exploratory		
To identify unanticipated problems with the protocol or study procedures.	n/a	n/a

4 STUDY DESIGN

4.1 OVERALL DESIGN

The hypothesis under study is that one or more applications of TTAX01 to the wound surface of a well debrided, complex diabetic foot ulcer managed with appropriate antibiotic therapy will result in a higher probability of complete healing than would be expected from management with standard care alone. In this open label Phase 2 pilot trial, all subjects will receive the intervention over a maximum period of 16 weeks. Eligible consenting subjects will undergo a baseline aggressive debridement in the operating room to remove infected and devitalized bone and soft tissue. A six week course of systemic antibiotics will be used to resolve baseline infection. TTAX01 will be applied to the debrided wound bed at baseline, and if healing is not evident, it will be applied again at 4 week intervals. At each weekly visit the wound will be further debrided as necessary.

To reduce bias in the ascertainment of complete closure, one independent observer will review the image obtained from a wound measurement device (eKare inSight™) in each case where the Investigator makes a determination of closure. Discordant opinions will be adjudicated by a third independent reviewer who will examine multiple additional images taken at various angles to the wound surface.

Bias will be further controlled by distributing enrollment over approximately twelve clinical sites. Because all subjects will receive the intervention, there will be no stratification on variables known to influence the probability of healing (e.g., wound size, wound duration, gender). No interim analysis is planned, but an ongoing review of protocol compliance may lead to amendments where it is determined that additional clarity is needed. It is expected that operational issues with this protocol will be worked through in anticipation of designing a similar, pivotal Phase 3 trial.

Consenting subjects who qualify for enrollment will undergo an aggressive surgical debridement at the baseline visit, including biopsies of bone for histology and microbiologic testing at the start

and completion of debridement. Systemic antibiotics will be given empirically, with adjustments made on the basis of culture and sensitivity results. New or recurrent infections will be managed with additional debridement and adjustment or addition of appropriate systemic antibiotics. The test article, TTAX01, will be surgically fixed using sutures or staples to the debrided wound bed at baseline and again at 4 week intervals over the 16-week treatment period for wounds that do not show evidence of healing. For wounds that do show evidence of healing, additional applications of TTAX01 will be withheld, based on observations from retrospective case series (refer to Section 2.3.2)

Subjects whose wounds close prior to 16 weeks will move directly to a 2-week confirmation of closure. Subjects whose wounds have not closed by the end of 16 weeks will exit the trial.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The prospective, multi-center design of this trial should serve to reduce selection and ascertainment bias compared with retrospective case series reported by individual clinicians. The design is expected to yield a more accurate estimate of response rates for the population under study, which can be used for statistical power calculations of a larger controlled trial. The open label design allows the Sponsor to immediately consider the relationship between treatment emergent adverse events and the test article, against the background of anticipated adverse events in this population.

4.3 JUSTIFICATION FOR DOSE

4.4 END OF STUDY DEFINITION

A subject is considered to have completed the study if he or she has completed the End of Treatment Visit 19, shown in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

Adult subjects aged ≥18 year-old with reasonably controlled diabetes mellitus and a complex non-healing DFU with high risk factors of [1] ulcer depth indicating exposed bone, tendon, muscle, and/or joint capsule and [2] clinical suspicion of osteomyelitis will be recruited for this trial. Assuming a screen failure rate of 30%, 47 potential subjects may be screened to enroll 36.

5.1 INCLUSION CRITERIA

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

1. The subject has signed the informed consent form
2. The subject is male or female, at least 18 years of age inclusive at the date of Screening
3. The subject has confirmed diagnosis of Type I or Type II diabetes
4. The subject's index ulcer is located on the plantar surface, inter digital, heel, or lateral or medial surface of the foot
5. The subject has an index ulcer with visible margins having an area $\geq$1.0 cm^2 to $\leq$ 10.0 cm^2 when measured by the electronic measuring device at Screening
6. The subject's index ulcer extends beyond the dermis, into subcutaneous tissue with evidence of exposed bone, tendon, muscle and/or joint capsule
7. The subject presents with history, signs or symptoms leading to a clinical suspicion of osteomyelitis in the opinion of the Investigator supported by positive Probe to Bone (PTB) and any of the following: radiographic (x-ray, Magnetic Resonance Imaging (MRI), or bone scan) or evidence of bone necrosis
8. The subject has an ABI $\geq$ 0.7 to $\leq$ 1.3 or TcPO2 $\geq$ 40 mmHg on the dorsum of the affected foot, or Great Toe Pressure $\geq$ 50 mmHg
9. The subject is under the care of a physician for the management of Diabetes Mellitus
10. The subject is willing to return for all mandatory visits as defined in the protocol
11. The subject is willing to follow the instructions of the trial Investigator

5.2 EXCLUSION CRITERIA

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

1. The subject's index ulcer is primarily located on the dorsal surface of the foot
2. The subject's index ulcer can be addressed by primary closure through the completion of the initial or staged surgical procedure
3. The subject has a glycated hemoglobin A1c (HbA1c) level of > 12%
4. The subject has a serum albumin level $\leq$ 2.0 g/dL
5. The subject has a white blood cell count < 2.0 $x10^9$/L, neutrophils < 1.0 $x10^9$/L, or platelets < 100 $x10^9$/L
6. The subject has been on oral steroid use of > 7.5 mg daily for greater than seven (7) consecutive days in 30 days before Screening

7. The subject has been on parenteral corticosteroids, or any cytotoxic agents for seven consecutive days in the period of 30 days before Screening
8. The subject is currently taking the type 2 diabetes medicine canagliflozin (Invokana™, Invokamet™, Invokamet XR™)
9. The subject has malignancy or a history of cancer, other than non-melanoma skin cancer, in five years before Screening
10. The subject is pregnant
11. The subject is a nursing mother
12. The subject is a woman of child-bearing potential who is unwilling to avoid pregnancy or use an appropriate form of birth control (adequate birth control methods are defined as: topical, oral, implantable, or injectable contraceptives; spermicide in conjunction with a barrier such as a condom or diaphragm; IUD; or surgical sterilization of partner).
13. The subject is unable to sustain offloading as defined by the protocol
14. The subject has an allergy to primary or secondary dressing materials used in this trial
15. The subject has an allergy to Amphotericin B, Dulbecco's Modified Eagle Medium (DMEM) or glycerol
16. The subject's index ulcer is over an acute Charcot deformity
17. The subject has had previous use of NEOX®, CLARIX®, or TTAX01 applied to the index ulcer
18. Per Investigator's discretion the subject is not appropriate for inclusion in the trial, e.g., undergoing surgical treatments listed in the protocol or the subject currently has sepsis, i.e., life-threatening organ dysfunction caused by a dysregulated host response to infection

5.3 LIFESTYLE CONSIDERATIONS

Subjects are expected to maintain ongoing care and management of their diabetes under the supervision of a qualified diabetologist, endocrinologist or internist with appropriate specialty training and experience. The need for offloading of the wound requires compliance with daily wearing of the offloading device, which may limit ambulation to some degree.

5.4 SCREEN FAILURES

Screen failures are defined as potential subjects who consent to participate in the trial but are not subsequently entered. Screen failure information will be captured to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial because of an abnormal lab test result, wound size out of range, current treatment with an excluded medication or inadequate

perfusion to the ulcerated foot may be rescreened ONE TIME. Rescreened participants will be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Potential study subjects will be recruited primarily from the clinical practice of the participating study sites. Investigators will share with their clinical staff the inclusion and exclusion criteria, which may also be provided by the Sponsor on a laminated card. Any signage or advertising at the clinics relating to this trial will have been previously submitted to and approved by the relevant IRB.

Broader advertising through newspaper, radio, or fliers or posters in adjacent healthcare facilities will only be used if recruitment falls substantially below the expected rate of one screened subject per site per month. All such advertising will have been previously submitted to and approved by the relevant IRB.

Enrolled subjects will receive a nominal stipend to offset travel and meal costs for the duration of their participation. The actual amount will depend upon IRB approval, but in no case will it be intended to create an incentive to ignore the risks associated with participation in the trial. Additional details are found in a separate recruitment and retention plan document.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

6.1.2 DOSING AND ADMINISTRATION

TTAX01 will be applied directly to the wound surface and surgically fixed with sutures or staples. A single layer of the test article should cover the entire open surface of the wound. TTAX01 may overlap onto adjacent healthy tissue and must be fenestrated prior to or after fixture. The material is to be applied once every 4 weeks unless the wound shows evidence of healing, in which case product will be withheld; or, if the test article has been accidently dislodged within 1-week post application, it may be replaced at the subsequent treatment visit. Wounds showing evidence of new or recurrent soft tissue infection must undergo debridement and receive antibiotic therapy

before considering another application of TTAX01. Wounds showing new or recurrent clinical suspicion of osteomyelitis must establish the diagnosis of osteomyelitis and if positive, repeat the Initial Procedure Visit, including reapplication of TTAX01.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The Investigator shall be responsible for ensuring the records adequately document the disposition of all TTAX01 received by the site for the trial. Documentation includes review of shipment papers to confirm accurate receipt, and disposition of all product received by site. Any product used on a trial subject should be documented both in the subject record and on the product accountability log. Any unused product and/or product past expiration will be returned to the Sponsor following product accountability completed by the Sponsor.

Participation by a pharmacy or drug repository unit is not mandated, however, the test article must be maintained in a secured (locked) environment with limited access to ensure accountability.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

[redacted]

6.2.3 PRODUCT STORAGE AND STABILITY

[redacted]

6.2.4 PREPARATION

Handling Instructions for the test article are as follows:

1. If frozen, allow TTAX01 to sit at controlled room temperature in its original unopened packaging for at least 5 minutes.
2. Open the outer foil peel pouch and present the clear inner peel pouch to the sterile field using aseptic techniques.
3. Open the inner clear peel pouch to retrieve the TTAX01.
4. Secure the TTAX01 on the wound bed.

6.3 MEASURES TO MINIMIZE BIAS

The trial is open label, with no blinding of the site staff. All eligible subjects will be enrolled into a single active treatment arm. The determination of wound closure will be made by the Investigator based on visual and tactile assessment of the wound. High quality photographic images will be taken at each visit, from an angle and distance recommended for optimum computerized calculation of wound area. In the event that the Investigator determines that a wound is completely healed, several additional images will be acquired at different angles in order to observe surface reflection of light from newly formed epithelium, and the absence of exudate. An independent reviewer will examine the first photographic image in order to make a determination of wound closure. If this opinion is concordant with the Investigator's opinion, the wound will be recorded as healed. If the independent reviewer finds reason to disagree with the Investigator, a third independent reviewer will examine all of the photographs for the purpose of breaking the tie.

6.4 STUDY INTERVENTION COMPLIANCE

The Investigator is responsible for ensuring that the treatment and follow-up procedures are followed as laid out in this protocol, unless a deviation is needed to protect the health and welfare of a subject. Such deviations should promptly be reported to the Sponsor or their designee and may also require reporting to the IRB.

Protocol compliance by the Investigator and site staff will be monitored by routine site visits conducted by the Sponsor, the frequency of which will be determined by any findings of issues as outlined in a separate detailed monitoring plan. All subjects will be provided with instructions which may include written materials on how to off-load correctly. Subject compliance will be based on observation of the offloading device at each visit for evidence of wear, by queries regarding excluded concomitant medications / therapies, and queries regarding dosing of any antibiotics. No blood testing or other test procedure will be utilized for determination of compliance. Diaries will not be used.

6.5 CONCOMITANT THERAPY

Systemic anti-microbials may be used as prescribed. Wound cleansing with a neutral, non-irritating and non-toxic solution is recommended at the discretion of the Investigator. Sterile saline, non-ionic cleanser, or hypochlorous acid are recommended.

Canagliflozin, a type 2 diabetic medication, also referred to as Invokana, Invokamet, and Invokamet XR, is not permitted throughout the duration of the screening, treatment and confirmation of closure periods.

Concomitant therapies for treating the index ulcer other than those defined in the protocol will not be permitted during the treatment and confirmation of closure period. They include topical antibiotics, antiseptic agents including hydrogen peroxide, acetic acid, chlorhexidine, povidone/iodine, and cetrimide, enzymes, negative pressure wound therapy, hyperbaric oxygen, growth factors, living skin, dermal substitutes, silver-containing products, amniotic membrane or umbilical cord products or other advanced biological therapies, or revascularization procedures (e.g., endoscopic perforator surgery, superficial venous ablation, endovenous laser ablation, valvuloplasty, free flap transfer with microvascular anastomoses), and Achilles tendon lengthening.

6.5.1 RESCUE MEDICINE

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Given that the manufacturing method of the test article is similar to the commercial product NEOX® CORD 1K®, which has an outstanding safety record, there is no *a priori* expectation of needing to suspend or terminate the trial for unexpected adverse events.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

All subjects have the right to discontinue the trial at any time. The Investigator may also withdraw a subject from the trial at any time if they deem it medically necessary. The reason for discontinuation should be documented and trial staff should attempt to bring the subject in for an Early Termination Visit and perform all applicable assessments as appropriate listed under the End of Treatment Period Visit (Visit 19) for discontinuations that occur during the Treatment Period.

Subjects may be discontinued from the trial for the following reasons:

- Adverse Event (including illness)

- Subject withdrawal of consent
- The Sponsor or Investigator terminates the trial
- Lost to follow-up
- Product-related > Grade 2 skin or systemic allergic reaction
- Ulcer-related complications (i.e., life-threatening, infection-related sepsis complications)
- Subsequent limb amputation (i.e., full amputation involving the index ulcer anatomic location)

In the event of a subject's withdrawal, the Investigator will promptly notify the medical monitor and will make every effort to complete all procedures at either the End of Treatment Period Visit. Even if removed subjects have been removed from the trial or if an adverse event remains ongoing, Investigators should follow up with subjects as per their standard medical practice.

Discontinued subjects will not be replaced. Simultaneous screening at multiple sites may result in an allowable modest over-enrollment.

7.3 LOST TO FOLLOW-UP

If a subject is lost to follow-up, a minimum of three documented contact attempts including one certified letter should be in the records. If there is not contact made by the subject after sending the certified letter, the next of kin and the physician responsible for managing the subject's diabetes should be contacted to obtain information about the subject's current health status.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 STUDY ACTIVITIES BY VISIT

8.1.1 SCREENING PERIOD (WITHIN -7 DAYS FROM INITIAL PROCEDURE VISIT)

[Purpose]: To determine the trial eligibility by assessing all inclusion/exclusion criteria based on the medical information available, clinical assessment, clinical laboratory testing, imaging studies and interview with subject.

Each subject who enters Screening will be assigned a subject ID number for traceability. The subject ID will consist of a 2-digit site number and a 3-digit subject number (i.e. 01-001, etc.).

Any subject who signs an informed consent, but fails to meet the required eligibility criteria is considered to be a Screen Failure. Screen Failure subjects should have their demographic information captured with the reason for screen failure specified. If a subject fails to meet all criteria after two screening attempts, the subject may not be enrolled into the trial. A new informed consent is required for each screening attempt. All procedures, excluding qualifying lab and

vascular perfusion tests obtained within 30 days of re-screening, must be repeated for each screening attempt.

All subjects will be screened for trial eligibility using the following procedures. All procedures must be completed prior to the Initial Procedure Visit:

8.1.2 INITIAL PROCEDURE VISIT (VISIT BL/BASELINE)

[Purpose]: To perform surgical procedures and to assign eligible subjects, after surgical sharp debridement and post-debridement measurement procedures, to receive the intervention.

The Initial Procedure Visit will occur within (7) seven days of the Screening Visit. In addition to the interventional treatment, subject will receive standard care consisting of surgical sharp debridement in the Operating Room (OR), resection of necrotic bone, bone biopsy for microbiology and histopathology, wound lavage, wound dressing, off-loading, and systemic antibiotics.

The first application of TTAX01 must be made during the Initial Procedure Visit following the surgical debridement, or at the last surgery for a staged surgical procedure.

8.1.3 TREATMENT PERIOD [VISIT 01 THROUGH VISIT 15 (7 +/- 2 DAYS)]

[Purpose]: To monitor safety and wound healing, adjust antibiotic regimens, and administer treatment. At each visit of Visits 01 through 15, if the wound is found to be open, the listed procedures will be performed for the subject.

If the index ulcer is observed to be closed, the subject will continue immediately to Treatment Period Visit 16 and complete all of the procedures listed under Visit 16.

If the subject will discontinue from treatment at any of these visits, the subject should move immediately to the end of Treatment Period Visit (Visit 19) and complete all of the procedures listed under Visit 19.

Figure 3. Treatment if there is no SSTI observed

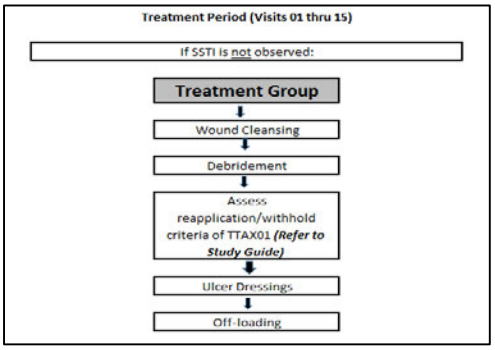


Figure 4. Treatment if SSTI is observed without recurrence of clinical suspicion of osteomyelitis

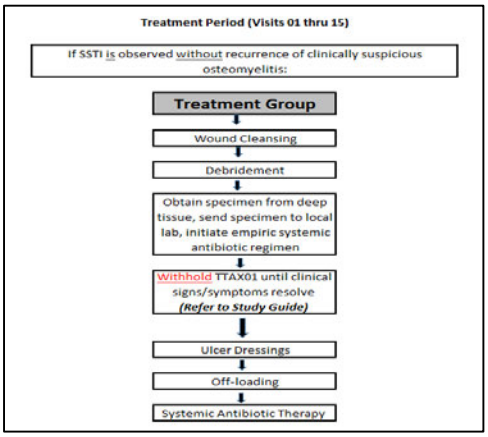
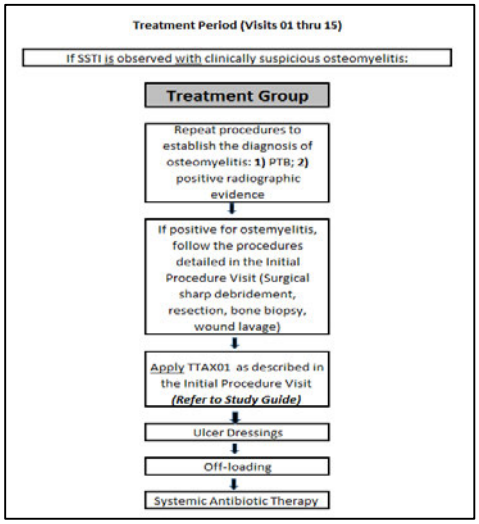


Figure 5. Treatment if SSTI is observed with clinical suspicion of osteomyelitis



8.1.4 TREATMENT PERIOD [VISIT 16 (7 +/- 2 DAYS)]

[Purpose]: To document the index ulcer status of open versus closed.

If a subject's wound is judged to have not closed at this visit the subject will not complete the procedures for this Visit, but will move immediately to complete the End of Treatment Visit (Visit 19) procedures.

If the index ulcer is observed to be closed prior to or at this visit, the following procedures will be completed and the subject will be instructed to return for the Confirmatory Period Visit 17 in (7 +/- 2 days).

8.1.5 CONFIRMATORY PERIOD [VISIT 17 AND VISIT 18 (7 +/- 2 DAYS)]

[Purpose]: To confirm complete wound healing for those who demonstrate wound closure during Treatment Period by two consecutive visits, one week apart, providing a total of two observations two weeks apart.

If a subject's ulcer is judged to have re-opened at this visit the subject will not complete the procedures for this Visit, but will complete the End of Treatment Period Visit (Visit 19) procedures.

- [redacted]

8.1.6 END OF TREATMENT PERIOD VISIT (VISIT 19) (14 +/- 2 DAYS)

[Purpose]: To document the patient status if they complete the entire treatment period or if they are withdrawn from the study during this period.

- [redacted]

8.2 EFFICACY ASSESSMENTS

At each of the Treatment Period visits (1 – 16), the Investigator will make a determination as to whether the wound has healed completely. If complete healing is observed, the subject will be scheduled to return in one week for Visit 17. During that week, the Investigator must confer with the independent reviewers to assure concordance of opinion regarding closure. Should it happen that the independent reviewers judge the wound to be unhealed, the subject will be rescheduled to attend the next regular Treatment Period visit instead of Visit 17.

8.3 SAFETY AND OTHER ASSESSMENTS

Safety is evaluated at each visit by examination of the lower extremity to which test article is being applied, and through both spontaneous and elicited reporting of adverse events.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 DEFINITION OF ADVERSE EVENTS (AE)

An Adverse Event is any untoward medical occurrence temporally associated with the use of an investigational medicinal product, whether or not considered causally related to that product. An AE can be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease. The Investigator, or his/her designee, will document AEs. AE data will be entered onto the appropriate source and electronic case report form (eCRF). Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA).

All AEs should be followed during the trial period. The Investigator should use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events. Event outcome at resolution or at time of last follow up will be recorded as event resolved, resolved with sequelae, ongoing at discontinuation, or death. Investigators should continue to follow up events of concern outside of the trial per their standard of care. The trial database may be finalized in these cases with events noted as ongoing.

The Investigator should consider adverse events both as they relate to the investigational product and as they relate to the procedures involved in the trial such as debriding tissue. The Investigator is responsible for determining initial relationship, expectedness, and severity of adverse events.

All adverse events must be reported regardless of whether or not they are considered to be related to the test article.

8.4.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A serious adverse event (SAE) is defined by federal regulation as any AE occurring at any dose that results in any of the following outcomes: death, life-threatening AE, requires hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

8.4.3 CLASSIFICATION OF AN ADVERSE EVENT

8.4.3.1 SEVERITY OF EVENT

The severity of all AE, will be assessed by the Investigator and should be classified as mild, moderate, or severe. Severity will be graded according to the following definitions:

- **Mild:** The subject experiences awareness of symptoms but these are easily tolerated or managed without specific treatment.
- **Moderate:** The subject experiences discomfort enough to cause interference with usual activity, and/or the condition requires specific treatment.
- **Severe:** The subject is incapacitated with inability to work or do usual activity, and/or the event requires significant treatment measures.

8.4.3.2 RELATIONSHIP TO STUDY INTERVENTION

The relationship of the event to the investigational product should be determined by the Principal Investigator according to the following criteria:

Not Related: The event is most likely produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs, and does not follow a known response pattern to the trial product which makes a causal relationship unlikely.

Related: The event follows a temporal relationship or known response pattern to the trial product, and cannot be reasonably explained by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs.

8.4.3.3 EXPECTEDNESS

As with the use of any human tissue, the possibility of infectious agent transmission cannot be completely eliminated although all screening and microbial testing results were satisfactory for the tissue and tissue donor. Possible significant adverse events include microbial infection and transmission of viral disease.

Expected adverse events non-related to the product are those post-surgical conditions consistent with any dermal debridement and excoriation of an ulcer. Potential adverse events for all research subjects in this trial intrinsic to the nature of DFUs: bleeding, hematoma, cellulitis, pain, deteriorating ulcer exudation, erythema, edema, infections including deep tissue infections (osteomyelitis), and occurrence of new ulcers.

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

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis) confirmed by the Principal Investigator,

and date of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship.

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

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

The Investigator will record all reportable events with start dates occurring any time after informed consent is obtained until the final study visit. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Event outcome at resolution or at time of last follow up will be recorded as "event resolved", "resolved with sequelae", "ongoing at discontinuation", or "death".

8.4.5 ADVERSE EVENT REPORTING

Disease Related Events expected in this population (angina, neuropathy, nephropathy, urinary tract infection, erectile dysfunction, retinopathy, hypoglycemic events) may be reported by the investigator if they differ in nature or frequency from what is expected, or if they appear to have a causal relationship to the test article. All adverse events must be reported in the subject's source documents, and recorded on an Adverse Event Case Report Form regardless of whether or not they are considered to be related to the test article.

8.4.6 SERIOUS ADVERSE EVENT REPORTING

In the event of any SAE reported or observed during the trial, whether or not attributable to the trial product, the Investigator, or designee, shall report the event to the medical monitor within 24 hours being made aware of the event. An initial report should be made with the understanding that it may be lacking in details.

A Serious Adverse Event Form must be completed for all serious adverse events and submitted within 24 hours of the Investigator's knowledge of the event and to the Institutional Review Board/Independent Ethics Committee, according to their reporting requirements. When new significant information is obtained as well as when the outcome of an event is known, the Investigator must provide this information as soon as it becomes available. Depending on the nature of the adverse event, copies of the subject's medical records as well as results of any relevant laboratory tests performed maybe required to be submitted. If the subject was hospitalized, a copy of the discharge summary should be forwarded as soon as it becomes available. In certain cases, a letter from the Investigator that summarizes the events related to the case may be required.

Serious adverse events must be reported using the eCRF system (preferred), or via telephone or email, to the following representative within 24 hours of the Investigator's knowledge of the event:

SAE reports shall be entered into the eCRF and hospital records will be submitted to:

[redacted]

The study Sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. In addition, the Sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the Sponsor determines that the information qualifies for reporting.

8.4.7 REPORTING EVENTS TO PARTICIPANTS

All important changes to the risks associated with TTAX01 will be conveyed to study subjects through updated consent forms. Final results of the study will be provided to each investigator, who will then share individual results with study subjects.

8.4.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.4.9 REPORTING OF PREGNANCY

Pregnancy occurring during the study is not an adverse event, but it should be brought to the attention of the sponsor as soon as possible. The continuation of a subject who becomes pregnant will be determined on a case by case basis.

8.5 UNANTICIPATED PROBLEMS

8.5.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The fundamental goal of this pilot protocol is to identify unanticipated problems with the protocol itself (e.g., clarity, contradictions) and with study procedures. Monitoring of the study will focus on the identification of such problems. Unanticipated Problems which place participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized may result in placing the study on hold, or study termination.

8.5.2 UNANTICIPATED PROBLEM REPORTING

Investigators should report all unanticipated problems to the sponsor as soon as they are identified.

8.5.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Not applicable.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

This pilot study will utilize descriptive statistics in the analysis and reporting of observed outcomes, to be described in greater detail in a separate Statistical Analysis Plan.

9.2 SAMPLE SIZE DETERMINATION

It is expected that 47 potential subjects will be screened in order to find 36 who qualify for this trial (screen failure rate 30%). The target number 36 is selected based on the law of large numbers, which holds that sample sizes less than 25 are more likely to produce skewed distributions of observed values, while sample sizes greater than 25 should generate a bell shaped distribution of observed values whose mean approaches the expected value as sample size increases.

The dropout rate is expected to be ~10%. As numbers between 25 and 30 should yield reasonably equivalent estimates of efficacy, there is no plan to replace dropouts.

9.3 POPULATIONS FOR ANALYSES

The Intent-to-Treat (ITT) population will consist of all enrolled subjects. The ITT population is the primary efficacy population and will be used to conduct all analyses on primary and secondary efficacy endpoints.

The Per Protocol (PP) population is a subset of subjects in the ITT and is the secondary efficacy population. The PP population will be defined as all qualified and treated subjects meeting inclusion and exclusion criteria and completing the study treatment as planned in the protocol, with no major protocol deviations during the 16-week Treatment Period. Major protocol deviations will be determined by the trial clinical team prior to database lock. Subjects found to have major protocol deviations will be excluded from the PP population.

The Safety Population includes all subjects who underwent treatment, regardless of protocol compliance. The Safety Population will be used for the analysis of safety endpoints.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Categorical data will be presented as percentages. Continuous data will be presented as means ± standard deviation, median and range.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

[redacted]

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

[redacted]

9.4.4 SAFETY ANALYSES

Safety measures will be reported as summary statistics during treatment, with shift tables provided for laboratory measures taken at baseline and end of study. Adverse events will be coded using the version of MedDRA available at the start of the trial. Each AE will be counted once for each subject unless it resolves and recurs, in which case it may appear as multiple AEs. Severity, frequency, and relationship of AEs to study intervention will be presented by System Organ Class (SOC) and preferred term groupings.

Listings will be provided for discontinuations, deviations, compliance, AEs leading to discontinuation, and AEs related to the index ulcer. AE listings will include severity and relationship to test article, as well as actions taken.

Tables will be provided summarizing reasons for screen failure, reasons for premature discontinuation, protocol deviations, subject compliance, treatment emergent AEs (TEAEs), TEAEs of interest, TEAEs by severity, SAEs, and AEs leading to discontinuation.

[redacted]

9.4.5 BASELINE DESCRIPTIVE STATISTICS

[redacted]

9.4.6 PLANNED INTERIM ANALYSES

Not applicable.

9.4.7 SUB-GROUP ANALYSES

[redacted]

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual subject data will be listed by measure and time point.

9.4.9 EXPLORATORY ANALYSES

[redacted]

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting any study procedure. IRB-approved consent forms will be provided to the IND, and will contain at a minimum the following information:

(a) That the trial involves research.

(b) The purpose of the trial.

(c) The trial treatment(s) and the probability for random assignment to each treatment.

(d) The trial procedures to be followed, including all invasive procedures.

(e) The subject's responsibilities.

(f) Those aspects of the trial that are experimental.

(g) The reasonably foreseeable risks or inconveniences to the subject and, when applicable, to an embryo, fetus, or nursing infant.

(h) The reasonably expected benefits. When there is no intended clinical benefit to the subject, the subject should be made aware of this.

(i) The alternative procedure(s) or course(s) of treatment that may be available to the subject, and their important potential benefits and risks.

(j) The compensation and/or treatment available to the subject in the event of trial-related injury.

(k) The anticipated prorated payment, if any, to the subject for participating in the trial.

(l) The anticipated expenses, if any, to the subject for participating in the trial.

(m) That the subject's participation in the trial is voluntary and that the subject may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the subject is otherwise entitled.

(n) That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the subject or the subject's legally acceptable representative is authorizing such access.

(o) That records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential.

(p) That the subject or the subject's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the trial.

(q) The person(s) to contact for further information regarding the trial and the rights of trial subjects, and whom to contact in the event of trial-related injury.

(r) The foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated.

(s) The expected duration of the subject's participation in the trial.

(t) The approximate number of subjects involved in the trial.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Only authorized trial staff should obtain consent and the most currently approved IRB consent form must be used.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

The trial may be terminated at any time by the Sponsor if serious side effects occur, if the Investigator does not adhere to the protocol, or if, in the Sponsor's judgment, there are no further benefits to be achieved from the trial. In the event that the clinical development of the investigational product is discontinued, the Sponsor shall inform all trial Investigators/institutions and regulatory authorities.

To ensure subject safety, the following approach is included to allow suspension or termination of the trial should a serious unforeseen risk arise: in the event that there are 2 reports of the same serious, unexpected and related adverse event, the Medical Monitor will within 5 calendar days of notification of the second event make a recommendation to Sponsor senior management whether to enhance surveillance, suspend enrollment, halt all further exposures, terminate the trial or continue without change.

If suspended, the trial may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA.

10.1.3 CONFIDENTIALITY AND PRIVACY

Subject confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or the FDA may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

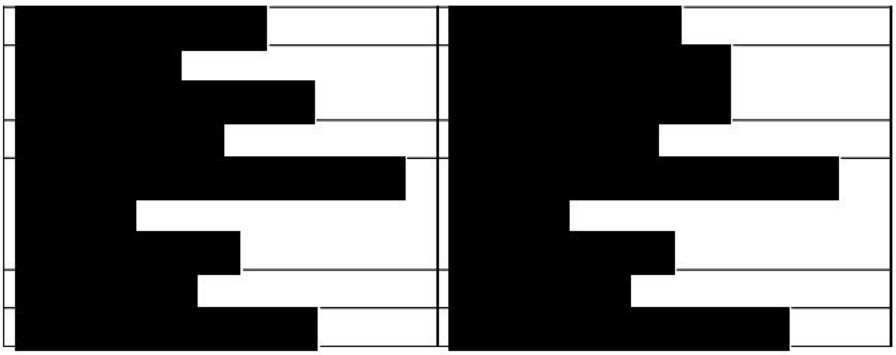
The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, or sponsor requirements.

Study subject research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the sponsor or a subcontracted data management service. This will not include the participant's contact or identifying information. Rather, individual subjects and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites will be secured and password protected.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

This protocol does not include storage of any biological samples for future testing. All study data will remain de-identified and kept securely by the sponsor or by a qualified contracted vendor, for at least the period of time required by FDA.

10.1.5 KEY ROLES AND STUDY GOVERNANCE



10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of an internal Data Monitoring Committee, chaired by the Sponsor's Chief Medical Officer. To ensure subject safety, the following plan is included to allow suspension or termination of the trial should a serious unforeseen risk arise.

- In the event that there are 2 reports of the same serious, unexpected and related adverse event, the Medical Monitor will convene the internal Data Monitoring Committee (DMC) within 5 calendar days of notification of the second event for the purpose of determining

whether to enhance surveillance, suspend enrollment, halt all further exposures, terminate the trial or continue without change.

[redacted]

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s). A separate clinical monitoring plan will be developed by the Sponsor.

The clinical monitor will periodically inspect all eCRFs, trial documents, and research facilities associated with this trial at mutually convenient times during and after completion of the trial. The monitoring visits provide the Sponsor with the opportunity to evaluate the progress of the trial; verify the accuracy and completeness of eCRFs; ensure that all protocol requirements, applicable FDA regulations, and Investigator's obligations are being fulfilled; and resolve any inconsistencies in the trial records. This includes inspection of all documents and records that are required to be maintained by the Investigator, including but not limited to medical records (office, clinic, or hospital) for the patients in this trial. The names and identities of all research patients will be kept in strict confidence and will not appear on eCRFs.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

TissueTech, Inc. or its designee shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the trial is conducted and data are generated, documented, and reported in compliance with the protocol, GCP, and applicable regulatory requirements. Specifically, this trial shall be conducted in accordance with the provisions of the Declaration of Helsinki, FDA regulations 21 CFR 50, 54, 56, and 312.50, and the ICH guidelines on GCP (ICH E6).

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

During the trial, the Investigator will maintain adequate records for the trial, including medical records, records detailing the progress of the trial for each patient, laboratory reports, eCRFs, signed informed consent forms, investigational product disposition records, correspondence with the IRB/IEC, AE/SAE reports, and information regarding patient discontinuation and completion of the trial.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. Refer to the eCRF completion guidelines for eCRF completion, correction, and transmission procedures. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Hardcopies of study visit worksheets may be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered via eDC into iMedNet, a 21 CFR Part 11-compliant data capture system provided by MedNet Solutions. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

All records and documents pertaining to the trial including, but not limited to, data and source documents, will be maintained by the Investigator for a period of 10 years after completion of the trial or for a period of at least 2 years following the removal of an IND, whichever is longer. If the Investigator retires, relocates, or for any other reason withdraws from responsibility of keeping the study records, custody must be transferred to the Sponsor or to a person who will accept responsibility and is approved by the Sponsor. In order to avoid any possible errors, the Investigator will contact the Sponsor prior to the destruction of any trial records. The Investigator will promptly notify the Sponsor in the event of accidental loss or destruction of any trial records.

10.1.9.3 PROTOCOL DEVIATIONS

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

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations. The Investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by the Sponsor and an appropriate IRB, except when necessary to eliminate imminent hazards to the patient or when the change(s) involve only logistical or administrative aspects of the trial. A deviation may result in the patient having to be withdrawn from the trial, rendering that patient non-evaluable.

10.1.9.4 PUBLICATION AND DATA SHARING POLICY

Agreements regarding publication and data sharing are found in the individual Clinical Trial Agreements between the Sponsor and each Investigator. At a minimum, the trial and its results will be posted on ClinicalTrials.Gov.

10.1.9.5 CONFLICT OF INTEREST POLICY

Each investigator will be asked to complete a financial disclosure prior to the initiation of this study, and one year following database lock.

10.2 ADDITIONAL CONSIDERATIONS

None.

10.3 ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISO	International Organization for Standardization
ITT	Intention-To-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Standard Care
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
SSTI	Skin & Soft Tissue Infections
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.0	01JUN2017	Original issue	
1.1	24Jan2018	Amendment #1	Updates to the protocol were made to improve the implementation and execution of the pilot trial across all clinical sites

11 REFERENCES

1. [redacted]

13. [redacted]

26. [redacted]

39. [redacted]

52. [redacted]

53. [redacted]

12 SPONSOR SIGNATURES

Study 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)
Study Number:	TTCRNE- 1501
Amendment Version Date:	05 February 2018

This clinical trial protocol was subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

13 INVESTIGATOR SIGNATURE

Study 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)
Study Number:	TTCRNE- 1501
Amendment Version Date:	05 February 2018

By my signature, I confirm that my staff, and I have carefully read and understand this protocol, and agree to comply with the conduct and terms of the trial specified herein. In particular, I/we have agreed to:

1. Abide by all obligations stated in the Clinical Trial Agreement (Contract)
2. Maintain confidentially and assure security of all confidential documents such as the protocol, product information documents, final trial reports, manuscript drafts, unpublished data, correspondence, etc.
3. Assure access by the Sponsor representatives to original source documents and medical records.
4. Obtain Institutional Review Board approval of trial, any amendments to the trial, recruitment advertisements, informed consent document, and periodic re-approval as required.
5. Keep the Institutional Review Board and Sponsor informed of serious adverse events and periodically report status of the trial to them as required.
6. Obtain written informed consent from each participant or his/her legal representative.
7. Make immediate reports of serious adverse events (SAE) to the Sponsor or designee.
8. Cooperate fully with any trial-related Good Clinical Practice audit as performed by the Sponsor's representatives.
9. Abide by manuscript preparation/authorship guidelines established at the outset of the trial.

Investigator's Signature: _____

Investigator's Name (please print): _____

Date: _____