CLINICAL RESEARCH PROTOCOL

TEST ARTICLE:	TTAX01
STUDY NUMBER(S):	TTAX01-CR003
PROTOCOL(S) TITLE:	A Multicenter Phase 3 Trial of Biotherapy using Cryopreserved Human Umbilical Cord (TTAX01) for Late Stage, Complex Non-healing Diabetic Foot Ulcers (AMBULATE DFU)
IND NUMBER:	16942
SPONSOR:	TissueTech, Inc. 7300 Corporate Center Dr STE 700 Miami, FL 33126
ORIGINAL PROTOCOL DATE:	08 Aug 2019
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STATEMENT OF COMPLIANCE

This 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 (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)

Investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of this trial will be required to complete Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to an 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

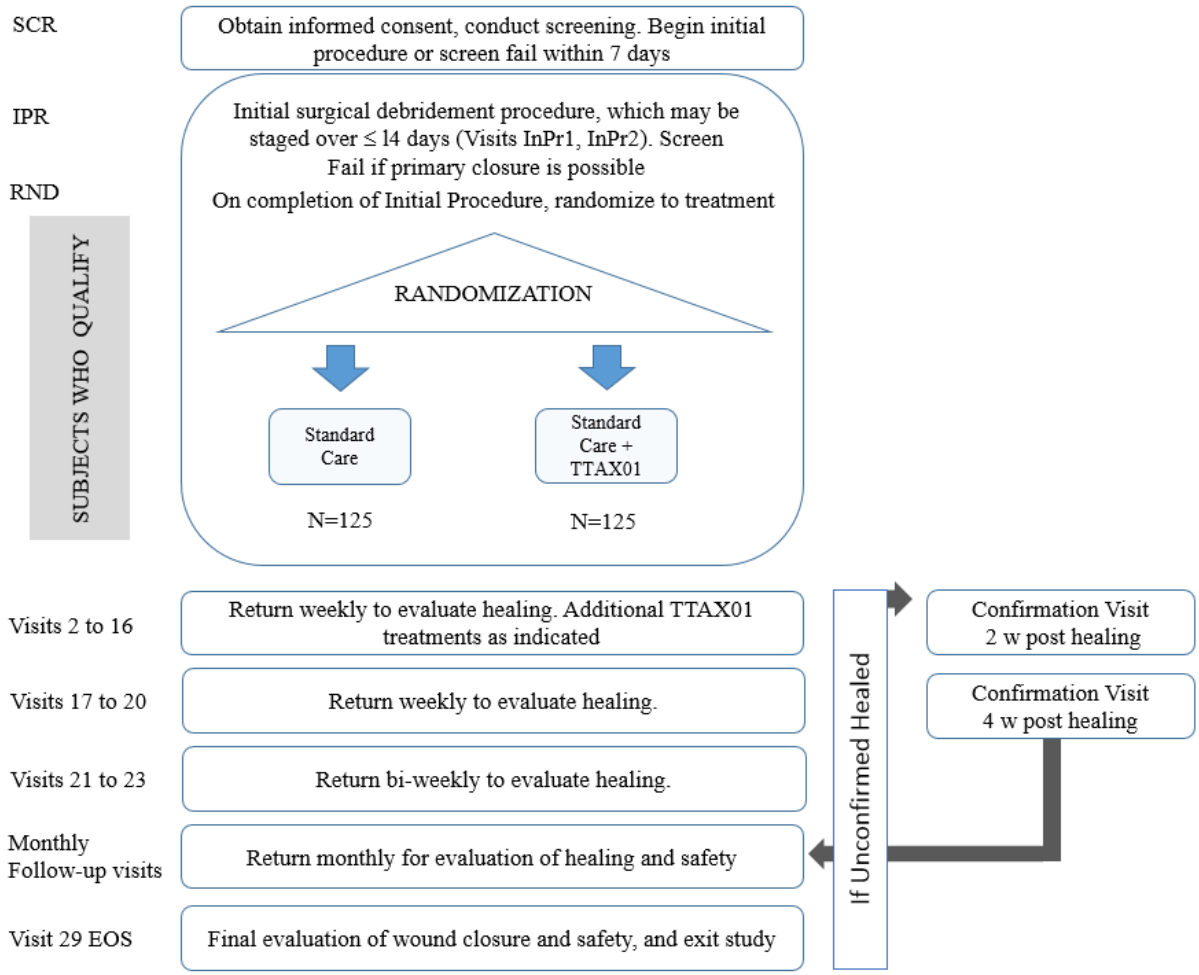
1.1 Study Synopsis

Title:	A Multicenter Phase 3 Trial of Biotherapy using Cryopreserved Human Umbilical Cord (TTAX01) for Late Stage, Complex Non-healing Diabetic Foot Ulcers (AMBULATE DFU)
Study Description:	It is hypothesized that application at 4-week or greater intervals of the human placental umbilical cord tissue TTAX01 to the surface of a well debrided, complex diabetic foot ulcer (DFU) will, with concomitant management of infection, will result in a higher rate of wounds showing complete healing within 26 weeks of initiating therapy, compared with standard care alone. This confirmatory Phase 3 study examines a population of diabetic foot ulcer patients having adequate perfusion, with or without neuropathy, and a high suspicion of associated osteomyelitis in a complex, high grade wound.
Objectives:	**Primary Objective**: To determine whether treatment of high grade DFU with standard care plus TTAX01 results in a higher probability of achieving complete wound closure over 26 weeks, compared with standard care alone. **Secondary Objectives**: To compare differences between treatment groups in proportions of wounds healed through 50 weeks, long term durability of closure, limb preservation, and patient reported mobility (lower extremity function) at study end.
Endpoints:	**Primary Endpoint**: Time from baseline to initial observation of healing, over 26 weeks, where healing has been confirmed at two consecutive visits each two weeks apart. **Secondary Endpoints**: Proportions of wounds healed by Week 50, proportions of re-ulcerations by Week 50, proportions of minor and major amputations by Week 50, total score on a lower extremity function quality of life measure.
Study Population:	A total of 250 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.
Phase:	3

Description of Sites/Facilities Enrolling Participants:	Approximately 20 United States and 3 Taiwanese sites specializing in care of diabetic foot ulcers, including hospital or outpatient based wound practices such as podiatric office practices, wound care centers, dermatology and vascular surgery clinics. Each site will have access to inpatient or outpatient surgery facilities for the accomplishment of initial surgical debridement.
Description of Study Intervention:	Subjects randomized to the control and intervention arms will receive aggressive debridement at baseline, followed by 6 weeks of systemic antibiotics plus standard wound care, including off-loading. Subjects randomized to the intervention arm will also receive 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 no shorter than 4 week intervals or when replacement of dislodged material is necessary, unless progress towards healing is evident.
Study Duration:	The entire study is expected to last for approximately 28 months, from first subject enrolled to last subject completed.
Participant Duration:	Each subject will be in the study for 50 weeks following randomization.

1.2 Study Schematic

Study schematic showing the overall flow of visits, and the main procedures carried out at the various visits. Monthly follow-up visits begin after confirmation of healing, or after the first 26 weeks for wounds not healed by then.



1.3 Schedule of Activities (SoA)

Parameter ↓	Screening	Initial Procedure (Baseline)	Randomization to Treatment	Treatment Period Visits 2-16 7 ± 2 days	No TTAX01 Weekly Visits 17-20 7 ± 2 days	Observation Period Visits 21-23 14 ± 2 days	Long-term Obs.Period Visits 24-28 (g) 28 ± 4 days	End of Study Visit 29 28 ± 7 days	*Confirmation Visits* (f) *14 ± 2 days*
Informed consent	X								
Demographics	X								
Medical history	X								
Concom medications	X	X		X	X	X	X	X	*X*
Physical examination	X							X	
Radiography, PTB	X (h)								
Neuropathy Test	X								
Vascular Perfusion test	X								
Wound photograph (a)	X	X		X	X	X	X	X	*X*
Wound assessment	X	X		X	X	X	X	X	*X*
Incl/exclusion criteria	X	X							
Laboratory assessments	X							X	
Pregnancy Test (b)	X							X	
Debridement (c)		X		X	X	X	X		
Bone Biopsy		X							
Randomization (d)			X						
Antibiotics	as needed	as needed	X	X	as needed	as needed	as needed		as needed
Standard Care	X	X	X	X	X	X	X		*X*
Apply Treatment (e)			X	X					
Other Care Allowed							X		
AE assessment		X		X	X	X	X	X	*X*
Off-loading		X	X	X	X	X	X		*X*
PROMIS and CWIS	X							X	

a. Wound photography will be done at every visit post debridement (if debridement is performed).
b. For women of childbearing potential.
c. Debridement of open wounds may be performed to remove callus, macerated, necrotic/devitalized/non-viable tissue and purulence.
d. Randomization procedures should be completed during surgery once it has been determined the subject will not be primarily closed and subsequent staged surgery is not needed. If the surgery is to be staged the randomization procedure should be completed during surgery at the last staged surgical procedure.
e. In the Treatment Group TTAX01 must be applied during surgery following Randomization, or if the surgery is staged, during the last surgery for a staged surgical procedure following Randomization. Additional applications of TTAX01 may be needed during the Treatment Period based on the retreatment algorithm provided in the Appendix and Study Guide.
f. For subjects whose wounds achieve initial closure at any visit.
g. All subjects with open wounds through Visit 21. Any subject who achieves confirmed closure will move to monthly follow-up following Visit C2.
h. Plain radiographs, MRI, radionuclide or labeled white blood cells bone scan obtained within 30 days of screening are acceptable.

2 INTRODUCTION

2.1 Study Rationale

This trial is designed as a confirmatory study of the benefits and risks of TTAX01 when used in the treatment of Wagner Grades 3 and 4 DFU. Experience with the use of a cryopreserved umbilical cord (UC) product in treating such wounds, both prior to this IND and under this IND, has indicated that a frequency of application of no shorter than every 4 weeks is associated with better than expected outcomes. Although treatment cannot be blinded, a "standard care only" arm is included to control for the benefits of aggressive baseline debridement combined with aggressive (6 weeks systemic) antibiotics. Current treatment guidelines indicate that aggressive debridement plus 1-2 weeks of antibiotics, or, minor debridement plus 6 weeks of antibiotics, would produce equivalent outcomes, although the evidence is not strong. By utilizing both maximum debridement and maximum antimicrobial therapy, the standard care described in this protocol may result in healing rates somewhat superior to current standard practice.

The design of this confirmatory study is matched to the design of the Phase 2 efficacy study TTCRNE-1501, with the exception of extending the primary endpoint from 16 to 26 weeks, and utilizing a proportional risk analysis rather than a landmark analysis. This design consideration is based on analysis of previous studies (see Background section), and a desire to fold data from every visit into the primary analysis, rather than generating an excessive number of secondary endpoints.

2.2 Background

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 infection and tissue loss that can lead 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 (1, 3, 9-19). In the US, diabetes has been the most common underlying cause of non-traumatic lower extremity amputations (20, 21); 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, 22, 23), worse than those of most malignancies and second only to that of lung cancer (15). Moreover, once amputation occurs, 50% of the patients will develop an ulcer in the contralateral limb within 5 years (14). 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 (24). 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.

Three major risk factors - [1] ulcer depth, [2] infection, and [3] ischemia - have been recognized to complicate non-healing foot ulcers leading to limb amputation (16). 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 is well known that these deep ulcers with extensive tissue loss are at high risk for infection-related ulcer complications including osteomyelitis (25).

The second risk factor is "infection", which is a major risk factor in the causal pathway to amputation (26, 27). Contiguous spread of any infection of adjacent soft tissue into the bone of the foot will complicate the ulcer with osteomyelitis (17),(18). Foot ulcers accompanied by limb-threatening infections such as osteomyelitis have reported amputation rate as high as 51% (28-30). 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 (20, 31, 32). The risk of amputation increases by four times when the foot ulcer is complicated by osteomyelitis compared to soft tissue infection alone (33).

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% (34, 35). 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 (16), this protocol will only enroll those patients in whom the vascular perfusion status is adequate to support healing.

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.

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) (36-39). Although some studies have demonstrated that the negative pressure therapy may improve healing of diabetic foot ulcers, especially after wide debridement or partial amputation (40-

42), there is limited high-level evidence to support widespread utilization, especially in an infected wound (43, 44). In fact, both hyperbaric oxygen therapy and negative pressure therapy are *not* recommended by the Infectious Diseases Society of America (IDSA) panel on treating 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.(45-47)). 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 (hyaluronic acid, HA) and heavy chain 1 (HC1) of inter-α-trypsin inhibitor (48, 49) that may then be tightly associated with pentraxin-3 (PTX3) to form the HC-HA/PTX3 complex (50).

HC-HA/PTX3 is a unique active matrix component that may contribute to AM and UC's clinical efficacy of delivering anti-inflammation and anti-scarring effects and plays an important role in regenerative wound healing (reviewed in ref.(51)). This complex was first discovered in the cumulus-oocyte complex that surrounds ovulated oocytes to ensure female fertility. Unlike the process of ovulation where HC-HA/PTX3 is produced under pro-inflammatory stimulation, HC-HA/PTX3 is constitutively synthesized by human amniotic epithelial and stromal cells during pregnancy (48, 49). HC-HA/PTX3 purified from cryopreserved AM can exert anti-inflammatory and anti-scarring effects well associated with the tissue. Moreover, such an anti-inflammatory effect extends from innate immune responses by facilitating apoptosis of stimulated neutrophils and polarizing macrophages to the pro-resolution M2 phenotype (52), and by suppressing activation of Th1 and Th17 lymphocytes to downregulate alloreactive immune responses as observed in the murine orthotopic corneal transplantation model (53). In addition to the observed anti-inflammatory and anti-scarring effects, HC-HA/PTX3 has also been shown to maintain quiescence of stem cells in the corneal

limbal niche (54, 55), suggesting its clinical usefulness in expanding the stem cell pool to promote regenerative healing in wounds that are under the threat of non-resolving inflammation.

Based on the identification of HC-HA/PTX3 as one key active substance in the mode of action, a series of nonclinical experiments have been conducted which demonstrate that this complex is present in TTAX01 processed by the CRYOTEK® method (56, 57). Results have shown that compared to AM, and to UC prepared by other manufacturing processes, TTAX01 contains significantly higher amounts of high molecular weight HA and HA-containing active matrix component (56).

Additional details of nonclinical studies are found in the Investigators Brochure.

Experience with a marketed 361 HCT/P version

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 exposed bone, tendon, muscle, and/or joint capsule as well as histopathologically-confirmed osteomyelitis via bone biopsy (58). 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, the most significant being diabetes (26/31), hypertension (23/31), peripheral vascular disease (16/31), renal failure (12/31), and coronary artery disease (9/31). Additional risk factors for non-healing included gangrene (n=17), local ischemia (n=24), and cellulitis (n=7). Previous partial amputation on the study leg had occurred in 9 cases. 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. Through the follow-up period, intent to treat analysis showed 24 patients (24/31, 77.4%) and 26 wounds (26/33, 78.8%) achieved complete wound closure. Among patients who were not lost, 26 of 27 wounds (96.3%) achieved

complete wound closure. 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%.

Two additional published studies provide evidence for high rates of healing with the 361 HCT/P product (Table 2.2-1).

Table 2.2-1. Published studies of cryopreserved UC in the treatment of lower extremity wounds

Ref	Patients	Baseline wound area	Number of applications	Complete healing	Mean time to heal
(58) 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
(59) 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
(60) 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

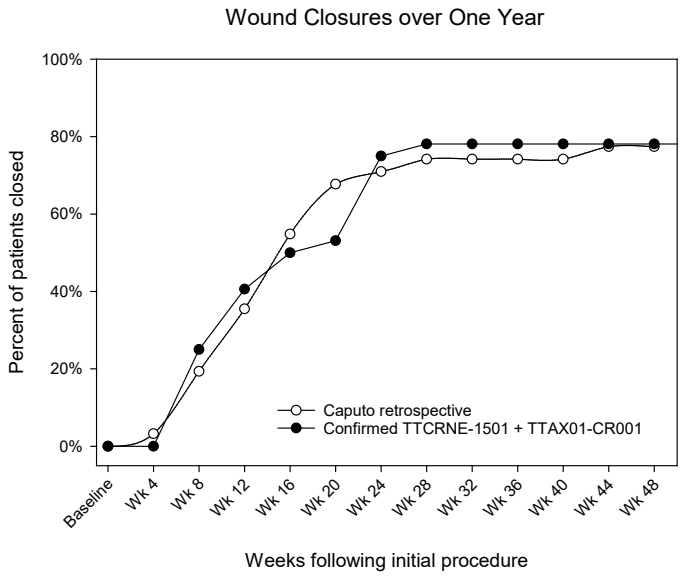
Phase 2 studies of TTAX01 in DFU

Study **TTCRNE-1501** was an open label trial of TTAX01 in a population meeting the same inclusion and exclusion criteria as described in this protocol (n = 32). The study design allowed treatment through the end of 15 weeks, with the final visit being at the end of 16 weeks. As with the current protocol, aggressive surgical removal of infected tissue and bone was performed at baseline, and systemic antibiotics were prescribed for 6 weeks. In combination with the use of systemic antibiotics, the application of TTAX01 to well debrided Wagner Grade 3 and 4 DFU, at intervals of 4 weeks or longer, resulted in a high proportion of confirmed

healed wounds (50%) within 16 weeks from initial application. An average of 1.5 applications (median 1, range 1 to 3) were made in those who achieved complete healing, which occurred at an average time of 12.8 ± 4.3 weeks. Overall, 16 of 32 (50%) achieved complete healing in the Intent to Treat (ITT) analysis, while 15 of 25 (60%) achieved confirmed healing in the per protocol (PP) analysis. Among subjects whose ulcer was recurrent at enrollment, 80% achieved confirmed healing. The average reduction from baseline in wound area was 91% by the end of 16 weeks for all wounds, closed or open; among the 16 who did not achieve protocol defined confirmed closure within 16 weeks, 5 achieved ≥ 99% wound area reduction by the 'end of study' visit. No deaths or major amputations occurred during the course of the trial, and no adverse events were found attributable to TTAX01. Two subjects experienced recurrent or persistent osteomyelitis requiring minor amputations.

Study **TTAX01-CR001** provided follow-up out to one year for subjects in study TTCRNE-1501, with 30 of 32 (94%) consenting to participate. Wound closures continued to occur during follow-up in a pattern that very closely matched to the retrospective study published by Caputo (58) (see Figure 2.2-1, initial observed closure data).

Figure 2.2-1. Comparison plots of retrospective and prospective studies



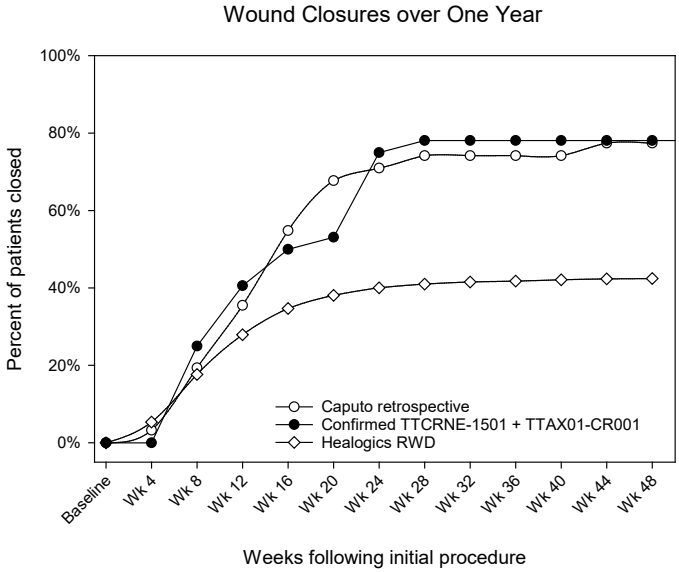
"Real World" data

Healogics, Inc. is the largest provider of advanced wound care services in the United States, treating more than 330,000 chronic wounds patients annually across more than 650 wound centers throughout the United States through a managed clinical care pathway. Through its proprietary, wound-specific Electronic Medical Record, Healogics has captured patient, wound, treatment, outcomes and other data at both the wound and product levels in a controlled care pathway on over 1.3 million wounded patients. As a result, Healogics now maintains the largest database of longitudinal wound care outcomes in the world, with research-ready clinical data representative of the real world setting.

The Healogics database contains 778,321 patients who presented for care of one or more diabetic foot ulcers. Matching the wound location, size, lack of Charcot deformity and Wagner classification grade to protocol TTCRNE-1501 entrance criteria, 25,954 patients were identified as matching the essential characteristics of the TTAX01 study subjects. Of these, 74% presented with Wagner 3, and 26% with Wagner 4 grade wounds. In the TTAX01 trial all advanced therapies such as topical silver dressings, negative pressure wound therapy, collagen dressings and hyperbaric oxygen therapy were prohibited. By contrast, these are among the therapies that were utilized at the discretion of each treating Healogics physician. This would be expected to bias outcomes in favor of the Healogics patients.

Among the set of 25,954 patients, there were 6,498 with records of a course of therapy for a single foot ulcer and disposition known over the course of one year. Of these, 34.7% (2,255) achieved healing within 16 weeks, with only 42.5% achieving closure by the end of one year. This proportion is in good agreement with data in the United States Wound Registry, another large real world database. In Figure 2.2-2, the healing rates from the Healogics database are shown in comparison with the plots from Figure 2.2-1.

Figure 2.2-2 Real World data for healing of Wagner 3 and 4 DFU (Healogics)



In summary, the above data support the conduct of a Phase 3 confirmatory trial.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

The major risk associated with use of human tissue, even as a temporary, non-engrafting therapeutic, is the transmission of infectious agents. This risk is minimized through careful donor screening, Good Tissue Practices, and aseptic current Good Manufacturing Practices (cGMP) processing. 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 SC alone. Experience with a 361 HCT/P version of TTAX01 (NEOX CORD) in treating DFU indicated that weekly application to large wounds may have been associated with a slightly higher rate of local tissue infection. This issue was not seen in Phase 2 testing at the lower frequency of every 4 weeks. Phlebotomy is performed twice, at baseline and end of study, which carries a minor risk of discomfort and possible hematoma. There have been no reported cases of infectious agent transmission in more than 53,000 distributed units of

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. The protocol itself carries a small risk of delaying healing by virtue of excluding a range of alternative therapies, however, none of these is of proven benefit in healing the high grade ulcers under study.

2.3.2 Known Potential Benefits

The high frequency of visits and the uniform application of prescribed procedures are expected to produce an increased number of wound closures in both groups (the "placebo effect"). The additional anticipated immediate benefit from TTAX01 is a more rapid and higher proportion of healing of the open diabetic foot ulcer. The associated long term benefits are expected to be a reduced risk of recurrent osteomyelitis and of major amputation.

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 surgical 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. The test article is made to Good Tissue Practices standards as well as Good Manufacturing Practices.

2.3.3 Assessment of Potential Risks and Benefits

The balance of risks and benefits appears favorable. An opportunity to add other therapies is provided following the first 8 weeks on study if certain criteria are met. Weekly visits for evaluation of both safety and efficacy serve to limit risk.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine whether treatment of high grade DFU with standard care plus TTAX01 results in a higher probability of achieving complete wound closure over 26 weeks, compared with standard care alone.	Time from baseline to initial observation of healing, over 26 weeks, where healing has been confirmed at two consecutive visits each two weeks apart.	The endpoint complies with FDA guidance. Dropouts should be minimal during the first 26 weeks, but may increase thereafter. Analysis will use a Cox proportional hazard model
Secondary		
To compare differences between treatment groups in proportions of wounds healed through 50 weeks	Proportions of wounds healed by Week 50	This endpoint provides insight into longer term benefits of the intervention
To compare long term durability of closure between treatment groups	Proportions of wounds that re-ulcerate by Week 50	May provide insight into the quality of healing
To compare limb preservation between treatment groups	Proportions of minor (portions of the foot) and major (ankle, above or below the knee) amputations following the initial procedure by Week 50	Each amputation risks loss of mobility, with consequent increased mortality
To compare patient reported mobility (lower extremity function) between groups.	Total score on PROMIS Health Organization Neuro-QoL “Lower Extremity Function” at Study End.	The tool is an NIH validated measure of patient reported outcomes
Safety		
To examine Quality of Life endpoints related to therapeutic intervention	Total score of quality of life assessment determined by Cardiff Wound Impact Schedule (CWIS) at Study End	
Proportion of subjects experiencing adverse events, by group	Spontaneously reported and elicited adverse events, coded in MedDRA	

4 STUDY DESIGN

4.1 Overall Design

The hypothesis under study is that one or more applications over 16 weeks 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 controlled Phase 3 trial, all subjects will receive standard care throughout the entire study, with the option to add one or more additional therapies if healing is inadequate (≤ 50% reduction in area) after 8 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 following recommendations from the Infections Diseases Society of America (IDSA) guidelines. For subjects randomized to the treatment group, TTAX01 will be applied during the surgical procedure to the debrided wound bed at baseline, and if healing is not evident, it will be applied again at intervals no shorter than 4 week or when replacement of dislodged material is necessary. At each visit the wound will be further debrided as necessary for subjects in both groups.

Surgical debridement at the baseline visit will include biopsies of bone for histology and microbiologic testing, at the start and completion of debridement. This debridement may involve resection of necrotic bone and performance of minor amputations. Systemic antibiotics may be given empirically from the first Screening visit, 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. For subjects randomized to the treatment group, the test article, TTAX01, will be fixed with sutures, staples or both to the debrided wound bed at baseline and again at no less than 4 week intervals over the 16 week treatment period for wounds that do not show evidence of healing. For wounds in this group that do show evidence of healing, additional applications of TTAX01 will be withheld, week by week.

Subjects whose wounds close at any time during the trial will move to a series of two consecutive confirmation of closure visits, two weeks apart, then continuing with monthly observational visits until the end of the study, unless the first observation of closure is at the final visit, in which case their participation in the study will extend out to 54 weeks, moving to a series of two consecutive confirmation of closure visits, two weeks apart.

4.2 Scientific Rationale for Study Design

The standard two-group parallel design is consistent with the CONSORT statement, being generally agreed to generate valid outcomes data when conducted with appropriate rigor. The inability to blind the treatment is a shortcoming, overcome in large part by the objective nature of the endpoint measurement, the use of multiple investigative sites, and the additional use of blinded, third party reviewers.

4.3 Justification for Dose

Dosing quantity is determined by the natural thickness of the product, while dosing frequency is informed by the clinical trials summarized in the Background section.

The form of the test article is suitable for application directly to the surface of the wound, surgically fixed with sutures or staples. Ideally, the test article will cover the entire wound surface. Dosage is understood to be a function of the thickness of the test article, from which intermediary molecules such as HC-HA/PTX3 should diffuse into the adjacent tissue.

4.4 End of Study Definition

A subject is considered to have completed the study if he or she has completed the final long term observation visit (Visit 29), 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. The majority of subjects (220) will come from US sites, with an additional 30 expected to be enrolled at three sites in Taiwan.

5.1 Inclusion Criteria

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

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, lateral or medial surface of the foot
5. The subject has an index ulcer with visible margins having an area ≤ 12.0 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 ≥ 0.7 to ≤ 1.3 or TcPO2 ≥ 40 mmHg on the dorsum of the affected foot, or Great Toe Pressure ≥ 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 continues to meet any of the following criteria in a maximum of two screening attempts 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 contralateral major amputation of the lower extremity
4. The subject has a glycated hemoglobin A1c (HbA1c) level of > 12% †
5. 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
6. The subject has been on parenteral corticosteroids, or any cytotoxic agents for seven consecutive days in the period of 30 days before Screening
7. The subject is currently taking the type 2 diabetes medicine canagliflozin (Invokana™, Invokamet™, Invokamet XR™)
8. The subject has malignancy or a history of cancer, other than non-melanoma skin cancer, in five years before Screening
9. The subject is pregnant
10. The subject is a nursing mother
11. 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).
12. The subject is unable to sustain off-loading as defined by the protocol
13. The subject has an allergy to primary or secondary dressing materials used in this trial
14. The subject has an allergy to glycerol

15. The subject's index ulcer is over an acute Charcot deformity
16. The subject has had previous use of NEOX®, CLARIX®, or TTAX01 applied to the index ulcer
17. 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

† An HbA1c obtained within 3 months of screening is acceptable to determine eligibility, or, HbA1c can be tested at a local laboratory at the time of screening, or, the HbA1c result from the central laboratory testing of safety labs can be used.

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 off-loading of the wound requires compliance with daily wearing of the off-loading 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. 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 may only be used if prior review and approval is granted by the Sponsor. All such advertising will be submitted to and approved by the relevant IRB prior to use.

All 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 Administration

6.1.1 Study Intervention Description

TTAX01 is a cryopreserved human umbilical cord product derived from donated human placental tissue following healthy, live, caesarian section, full-term births after determination of donor eligibility and placenta suitability. TTAX01 is manufactured by TissueTech Inc. utilizing a proprietary CRYOTEK® process, which devitalizes the living cells but retains the natural structural and biological characteristics relevant to this tissue. TTAX01 is aseptically processed in compliance with current Good Tissue Practices (cGTP). TTAX01 will be manufactured in various sizes, stored in a medium of lactated Ringer's/glycerol (1:1).

6.1.2 Dosing and Administration

All subjects in the treatment group and the control group will receive sponsor-approved SC, which follows the guidelines developed by the Clinical Practice Guideline Diabetes Panel of the American College of Foot and Ankle Surgeons through the consensus of current clinical practice and review of the clinical literature for managing diabetic foot ulcers in general (9). The trial also adopts SoC that follows clinical practice guidelines proposed by the IDSA for diagnosis and treatment of diabetic foot infections in 2012 (3) as well as by the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine in 2016 (10) for managing diabetic foot ulcer presenting with clinical concerns of infection.

Standard care is defined in this protocol to consist of the following:

- Debridement;
- Wound cleansing, using sterile saline, a non-ionic cleanser or a hypochlorous solution. Antiseptic agents including hydrogen peroxide, acetic acid, chlorhexidine, povidone/iodine, and cetrimide should not be used. Topical antimicrobials and silver dressings are also prohibited;

- Primary wound dressings including a non-adherent, standard foam pad with or without a hydrogel beneath the dressing, or and alginate dressing, as is appropriate to maintain a moist wound environment while managing exudate;
- A secondary retention bandage appropriate to the amount of wound exudate, so as to avoid maceration of the peri-wound skin;
- Off-loading device appropriate to the location of wound with full length boot or total contact cast (unless not appropriate where a substitute off-loading device can be made with sponsor approval); and,
- Appropriate use of systemic antibiotics.

TTAX01 will be applied directly to the wound surface and retained with sutures or staples. A single layer of the test article should cover the entire open surface of the wound. If more than one piece is needed, they may overlap, and in any case the material may overlap onto adjacent healthy tissue. The initial application of TTAX01 must occur during the surgical procedure. The umbilical cord is comprised of essentially two tissue layers: the amniotic (or epithelial) layer and the sub-amniotic (or mesenchymal) layer. The inner sub-amniotic layer will have an irregular appearance, where the tissue surface appears dull and rough following dissection and removal of the umbilical cord vessels and Wharton's Jelly. In contrast, the amniotic or epithelial layer has a smooth glossy appearance. While the product can be routinely applied using either side, it has been hypothesized that the epithelial layer may provide a more optimal surface for the new epithelial cells to move across when re-epithelialization is occurring in the wound environment.

As such, it is preferred (but not required) that the product be applied with the rough irregular surface placed directly in contact with the well-debrided wound bed surface while the smooth and glossy surface (side) should be facing the outside of the wound bed. The material is to be applied at intervals no shorter than every 4 weeks unless replacement of a dislodged piece is necessary, or the wound shows evidence of healing, in which case dosing is suspended. Wounds showing evidence of new or recurrent soft tissue infection or osteomyelitis must

undergo debridement and receive antibiotic therapy before placing another dose of the test article.

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 the 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, but only following product accountability completed by the Sponsor clinical site monitors.

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

TTAX01 is shipped frozen in a validated medium of lactated Ringer's/glycerol (1:1). The final product packaging consists of validated inner clear pouch placed inside an outer foil pouch. The labeling of the final product container meets the regulatory requirements set forth in 21 CFR 1271 and follows the GS1-128 standard which is in compliance with the Unique Device Identification (UDI) ruling by the FDA. An additional label containing the statement, "Caution: New Drug- Limited by Federal Law to Investigational Use" will be added to the final product container labeling, product insert, and other applicable labeling materials. The final products will be shipped to users in a validated shipper box (i.e., Nanocool™ boxes). The shipment packaging configuration has been validated by performing thermal test per ISTA-7D and transit testing per ISTA 3A.

The final product is labeled with the following information:

- Product Name and Description
- Product Reference No.
- Serial No.
- Expiration Date
- Lot No./Donor ID No.
- Production Identifier Barcode and No.
- Item Identifier Barcode and No.
- Quantity
- Content
- Cautions and Warnings
- Manufacturer Information
- Company Names
- "Caution: New Drug- Limited by Federal Law to Investigational Use"

The product insert contains the following information:

- Product Description
- Indications
- Precaution Statements
- Warning Statements
- Instructions of Use
- Storage Instructions
- Donor Eligibility and Summary of Records
- Allocated space to affix final product serial number label

The shipping container is labeled with the following information:

- Shipping Label with Addresses
- Alert label- Time limit for storage, packing time and personnel initials and
- Donated human tissue statement
- Perishable statement

6.2.3 Product Storage and Stability

The investigational product shall be stored as specified by the Sponsor and in accordance with Good Clinical Practice and regulatory requirements as applicable. Product access shall be limited to authorized trial personnel. Product should be maintained according to the conditions as outlined in Table 6.2.3-1.

Table 6.2.3-1: Investigational Product Storage Conditions

Location and temperature	Use After Receipt
Unopened insulated shipping container	Within the expiration date printed on outer shipping box
-20°C ± 10°C (-22°F → 14°F) Example: ultra-low temperature freezer or standard freezer	Within the expiration date printed on product packaging

6.2.4 Preparation

Handling Instructions for the test article are as follows:

- If frozen, allow TTAX01 to sit at room temperature in its original unopened packaging for at least 5 minutes.
- Open the outer foil peel pouch and present the clear inner peel pouch to the sterile field using aseptic techniques.
- Open the inner clear peel pouch to retrieve the TTAX01, taking note of the difference in appearance between the smooth side and the rough side.
- TTAX01 exposed to room temperature for up to 6 hours may be returned to cold temperature storage as long as the packaging remains unopened and intact.

6.3 Measures to Minimize Bias – Randomization and Blinding

Subjects will be randomly assigned to one of two groups, by study, using a predetermined block size. Stratification based on wound size post debridement at baseline (post debridement

wound size <= 8.0 cm^2 vs. wound size > 8.0 cm^2) will help to avoid spurious results driven by an imbalance in distribution of variables within this parameter which has a known correlation with probability of healing. Gender and wound duration are expected to distribute normally without the need for stratification. Bias will be further controlled by distributing enrollment over twenty or more clinical sites.

Due to the nature of the test article, the trial is open label, with no blinding of the site staff. The determination of wound closure will be made by the Investigator based on visual and tactile assessment of the wound. To reduce bias in the ascertainment of closure, one independent blinded reviewer 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 second independent blinded reviewer who will examine multiple additional images taken at various angles to the wound surface.

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 or 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 off-loading 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

In this study, the term Concomitant Medication is inclusive of all medications and therapies.

The following is excluded throughout the entire study:

- Canagliflozin, a type 2 diabetic medication, also referred to as Invokana, Invokamet, and Invokamet XR

The following are excluded through W26 (Visit 23):

- Topical antibiotics and antiseptic agents including hydrogen peroxide, acetic acid, chlorhexidine, povidone/iodine, and cetrimide
- Enzymes, negative pressure wound therapy, hyperbaric oxygen, growth factors and tissue products containing growth factors (e.g., Oasis™), living skin, cellular products (e.g., Apligraf™, Dermagraft™), amniotic membrane and umbilical cord products
- Powdered collagen dressings (e.g., Cellerate™), formulated collagen dressings (e.g., Excellagen™)
- Hyaluronic acid products (e.g., Hyalomatrix™)
- Revascularization procedures (e.g., endoscopic perforator surgery, superficial venous ablation, endovenous laser ablation, valvuloplasty, free flap transfer with microvascular anastomoses)
- Achilles tendon lengthening

The following therapies are excluded before Visit 9 (Day 57), but allowed thereafter if the wound area is ≤50% closed relative to the post debridement wound measurement obtained prior to randomization:

- Silver-containing products (e.g., Aquacel Ag™, Mepilex Ag™, Acticoat™)
- Collagen dressings (e.g., Integra Omnigraft™, Promogran Prisma™, Puracol™, Fibracol™)

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.

6.6 Rescue Medication

There are no prescription drugs, biologics or medical devices specifically labeled for use in DFU involving exposed tendon, joint capsule or bone with osteomyelitis. All subjects must have adequate vascular supply in order to qualify for this study, therefore hyperbaric oxygen therapy is unwarranted. Refer to Section 6.5 for information regarding additional therapies that may be used after Day 57.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Discontinuation from applications of TTAX01 does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by this protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, which is believed to be causally related to TTAX01, the Investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

Temporary discontinuation of TTAX01 could also occur in the event of a clinical hold imposed by FDA, or if the Sponsor discovers a deviation in product manufacturing or release which could pose a threat to the well-being of study participants.

7.2 Participant Discontinuation / Withdrawal from the Study

All subjects have the right to discontinue their participation in 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 Study Visit for discontinuations that occur during the Trial 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 (CTCAE criteria)
- Ulcer-related complications (i.e., life-threatening, infection-related sepsis complications)
- 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 the End of Study 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 no 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

Each subject who enters Screening will be assigned a subject ID number for traceability. The subject ID will consist of a 3 digit site number and a 3 digit subject number (i.e. 001-001, 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. Re-screening is allowed when a failed criterion can be expected to change naturally or with minimal intervention. 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 labs, radiographic imaging, and vascular perfusion tests obtained within 30 days of re-screening, must be repeated for each screening attempt.

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.

NOTE: It is possible for the Screening Visit, the Initial Procedure Visit (unstaged), and the Randomization Visit to occur all on the same day.

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

- Obtain informed consent, documenting the consenting process
- Obtain Demographics
- Screen the subject against protocol Inclusion and Exclusion criteria
- Review the Medical/Index Ulcer/Foot History, general systems, and present illnesses, including the duration of the index DFU and treatments that have been administered. At minimum, medical history should include significant co-morbidities, diabetes

diagnosis, and important medical events (e.g., amputations, Charcot deformity, etc.) that have occurred within the last five years. Index ulcer history includes location, prior treatments and duration.

- Record medications currently being taken by the subject. Concomitant Medications will be recorded in the subject's source document, but should only be recorded in the eCRF for the following:
  - All current and past treatments for the index ulcer only
  - Concomitant medications used to treat the index ulcer (e.g., systemic antibiotics for an index ulcer infection) and the peri-ulcer area (such as treatment for erythema, irritation, itching), together with the indication for the medication
  - Concomitant medications used to treat AE or SAE that arise during the screening period.

- Perform physical examination, including vital signs (i.e., heart and respiratory rates, blood pressure, and temperature while subject is seated) and collect height and weight. The exam shall include the following systems:
  - Eyes, ears, nose, throat
  - Lungs/thorax
  - Heart/cardiovascular system
  - Skin and mucosa
  - Nervous system
  - Lymphatic system
  - Endocrine/metabolic
  - Musculoskeletal system
- Assess criteria for evaluating sepsis-related organ failure using the quick sepsis-related organ failure assessment score. If ≥ 2 of the following criteria are met, the subject would be excluded (e.g., respiratory rate ≥ 22 breaths/min; altered mentation, systolic blood pressure ≤ 100 mmHg).

- Assess neuropathy of the foot with the index ulcer using the 5.07 Semmes-Weinstein monofilament 10 gram wire test on 10 areas of the foot. A subject's foot will be considered absent from protective sensation if 2 out of the 3 questions are answered incorrectly. If the subject is missing any of these areas (e.g., due to amputation), then the remaining areas present must be tested.
- Assess vascular perfusion by any of the following: ABI, $TcPO_2$, or Great Toe Pressure.
- Obtain photograph and measurement of the surface area, depth and volume of the index ulcer using the provided electronic wound imaging and measuring device
- Perform PTB to verify ulcer depth exhibiting exposed bone, tendon, muscle and/or joint capsule
- Obtain non-invasive imaging to examine clinical suspicion of osteomyelitis based on plain radiographs, MRI, or bone scan. Plain radiographs, MRI, radionuclide or labeled white blood cells bone scan obtained within (30) thirty days of the Screening Visit can be used to support the clinical suspicion of osteomyelitis.
- Collect specimens for safety laboratory tests ("safety labs") as listed in Table 8.1.1-1, which will be sent out to a central laboratory.
- If a HbAlc measurement is not available within 3 months of screening, the HbA1c result from the central laboratory testing of safety labs can be used if subject will not be randomized on the same day as screening; or, collect a blood specimen now for HbA1c testing at a local laboratory.
- Record any AE that occur during the Screening period, both in source documents and the eCRF.

Table 8.1.1-1. Central Laboratory Tests

Hematology: • Complete Blood Count (CBC) • Hematocrit (Hct) • Hemoglobin (Hgb) • Mean corpuscular hemoglobin (MCH) • Mean corpuscular hemoglobin concentration (MCHC) • Mean corpuscular volume (MCV) • Platelet count • Red blood cell (RBC) count • White blood cell (WBC) count with differential • Red cell distribution width (RDW) • Mean platelet volume (MPV) Pregnancy Test: • Urine (local) or serum human chorionic gonadotropin (hCG) (only for females of childbearing potential)	Serum Chemistry: • Albumin (ALB) • Alkaline phosphatase (ALP) • Alanine aminotransferase (ALT) • Aspartate aminotransferase (AST) • Blood urea nitrogen (BUN) • Calcium (Ca) • Chloride (Cl) • Creatinine • Gamma-glutamyltransferase (GGT) • Glucose • Lactate dehydrogenase (LDH) • Phosphorus • Potassium (K) • Sodium (Na) • Total bilirubin • Hemoglobin A1c (HbAlc) Urinalysis*: • Color • Appearance • Specific gravity • pH • Protein • Glucose • Ketones • Occult blood • Leukocyte esterase • Nitrite • Bilirubin • Urobilinogen.

Refer to the laboratory manual for the collection of bloods, storage, and shipping information.

*: If protein, leukocyte, occult blood, or nitrites are positive, microscopic examination is performed.

- Administer the PROMIS Lower Extremity Function questionnaire and the CWIS (examples shown in the Appendix)

If the subject qualifies and can begin the initial procedure on the same day as screening, then proceed directly to the Initial Procedure (refer to Section 8.1.2). Otherwise,

- Apply wound dressing including a non-adherent, standard foam pad or alginate dressing (e.g., hydrogels, foam dressings, and alginates) that is appropriate for the amount of drainage, and a secondary bandage to ensure a moist ulcer environment, which adequately controls exudate and avoids maceration of the surrounding skin
- Empiric, systemic antibiotic therapy may be started at this time if clinically indicated, in advance of biopsy for culture at the initial procedure visit. Topical antibiotics should not be administered
- Schedule the Initial Procedure Visit (may be the same day as Screening Visit or within (7) seven days once all eligibility criteria have been confirmed)

8.1.2 Initial Procedure Visit(s) (Baseline)

[Purpose]: To perform surgical procedures aimed at removing devitalized tissue

The Initial Procedure Visit may occur after all screening eligibility criteria have been confirmed. It may occur on the same day as screening, but must occur within (7) seven days of the Screening Visit. All subjects will undergo standard aggressive therapy consisting of surgical sharp debridement in the OR, resection of necrotic bone, bone biopsy for microbiology and histopathology, and wound lavage. It is advised to bring the TTAX01 product to the OR if it is expected that the surgical procedures can be completed without staging, in case the subject is randomized to that treatment group. Randomization will take place if primary closure is not possible and further surgery is not needed.

- Perform surgical sharp debridement in the OR, to remove:
    - infectious agents and biofilms (purulence),

- all debris, eschar, callus and macerated non-viable tissue from the wound base, and
- Dead (suprabasal epidermis), scarred (elevated/edematous) and necrotic/macerated tissue from the wound edge.

- Resect necrotic bone. Surgical Resection will be performed to remove as much of the necrotic bone detected by the radiographic evidence as is appropriate.
  - If the Investigator notes the following: (1) severe clinical signs of infection, (2) acute lymphangitis, or (3) the wound severity requires a subsequent surgical procedure to remove any remaining non-viable or unhealthy tissue, the surgical procedure should be staged, and randomization will be held until the final staged procedure.
  - In this instance, the interval between the Initial Procedure Visit and subsequent final staged procedure should be ≤ 14 days.
  - During this period, the wound bed should be managed with SC alone and no prohibited treatments should be used as detailed in Section describing Concomitant Therapy.
- Obtain bone biopsies for microbiology and histopathology. A biopsy to obtain specimens from the (a) resected bone containing suspected osteomyelitis (labeled 'dirty bone') and the (b) clean margin (labeled 'clean bone') will be submitted to the local laboratory for microbiology (culture and sensitivity) and histopathology examination.
- At the same time, obtain tissue harboring suspected infection and send for microbiology (culture and sensitivity).
- Perform wound lavage using sterile saline solution with or without antibiotics.
- If the wound can be closed by primary intention at this point, proceed to do so and the subject will exit the study as a Screen Failure.

Otherwise,

- Obtain post-debridement photograph and measurement of the surface area and volume of the index ulcer using the provided electronic wound imaging and measuring device. This is needed for randomization.

- Proceed with Randomization (refer to Section 8.1.3), unless a staged procedure is necessary, in which case do not randomize. Instead, provide the subject with appropriate wound dressing and off-loading and schedule them to return for completion of this Initial Procedure.

8.1.3 Randomization

[Purpose]: To randomly assign eligible subjects to a treatment group, with stratification, and initiate therapy.

Obtain the treatment group assignment using the computer-based randomization tool.

TREATMENT GROUP ONLY:

- Apply TTAX01 deep below the surface of the surrounding skin to cover and be in contact with the exposed tissue at the base of the wound, rough side down, and surgically fixed by sutures or staples. TTAX01 must be fenestrated prior to applying the secondary dressing. The initial application will occur as part of the surgical procedure.
    - Multiple pieces of TTAX01 may be used if required to cover the entire wound bed.
    - Obtain post-application photograph of TTAX01 using the provided electronic wound imaging and measuring device

ALL SUBJECTS (BOTH GROUPS):

- Apply wound dressing including a non-adherent, standard foam pad or alginate dressing (e.g., hydrogels, foam dressings, and alginates) that is appropriate for the amount of drainage, and a secondary bandage to ensure a moist wound environment, which adequately controls exudate and avoids maceration of the surrounding skin.
- Continue, adjust, or initiate an appropriate systemic antibiotic regimen. Topical antibiotics should not be administered. A definitive therapy will be guided by the microbiological results based on bone biopsy. An Infectious Diseases specialist may be

consulted to determine the appropriate antibiotic regimen to proceed with if the pathology report confirms the 'clean margin' is free of osteomyelitis. **At least six (6) weeks of systemic antibiotic therapy is required in this protocol**. If the initially chosen antibiotic is changed based on culture and sensitivity results, the total length of all antibiotic treatment will remain at 6 weeks.

Provide an off-loading device appropriate to the location of the wound with full length boot or total contact cast (unless not appropriate, where a substitute off-loading device can be made per the physician's suggestion with sponsor approval) with off-loading instructions. The substituted choice of off-loading modality should be based on the wound's location, the presence of any associated peripheral artery disease, the presence and severity of infection, and the physical characteristics of the subject.

Schedule the next Treatment Period Visit in one week (7 ± 2 days).

8.1.4 Treatment Period (Visits 2– 16; 7 ± 2 days)

[Purpose]: To monitor safety and wound healing, adjust antibiotic regimens, and administer treatment. At each visit of Visits 2 through 16, the listed procedures will be performed for the subject based upon status of the wound.

If the subject will discontinue from TTAX01 treatment at any of these visits, the subject should continue with the scheduled visits through the end of the study in order to observe wound status and general safety. If the subject will discontinue from the trial (drop-out), move immediately to the End of Study Visit (Visit 29) and complete all of the procedures listed.

Beginning at Visit 9 (Day 57), if the wound is not ≥ 50% reduced in wound area versus the post debridement measurement obtained at the Initial Procedure Visit prior to randomization, the Investigator may begin to utilize adjunctive therapies as explained in Section 6.5.

If at any Treatment Visit the index ulcer is observed to be closed, perform the following procedures:

- Assess adverse events
- Review concomitant medications
- Assess subject compliance regarding off-loading since the last visit
- Obtain photographs of the closed ulcer using the electronic wound imaging and measuring device
- Observe the wound – if it is fully epithelialized with no drainage or need for a dressing, record it as closed.

The next visit scheduled will serve as the first consecutive confirmation visit in 14 ± 2 days **and will begin the confirmation of closure procedures** (refer to Section 8.1.9).

If the wound remains open, conduct the following procedures:

- Assess adverse events
- Review concomitant medications. These will be recorded in the subject's source document, but should only be recorded in the eCRF for the following:
  - All current and past treatments for the index ulcer only
  - Concomitant medications used to treat the index ulcer (e.g., systemic antibiotics for an index ulcer infection) and the peri-ulcer area (such as treatment for erythema, irritation, itching), together with the indication for the medication
  - Concomitant medications used to treat all SAE, and all AE considered "RELATED" to the product will be recorded in the appropriate eCRF.
- Assess subject compliance regarding off-loading since the last visit
- Assess the foot and lower extremity, then assess the index ulcer after removal of the dressing (but do not remove residual TTAX01 in the treatment group).
  - Determine whether skin and soft tissue infection (SSTI) is present. If not, follow the scheme shown in ***Figure 12.1-1*** (Appendix)
  - If SSTI is present, without evidence of osteomyelitis, follow the scheme shown in ***Figure 12.1-2*** (Appendix); withhold TTAX01 application, obtain a deep tissue biopsy for histopathology, culture and sensitivity, and adjust the antibiotic regimen

(start a 2 week course of antibiotics if none are being taken, or revise antibiotics if still being taken for a previous infection)

- If new, recurrent or persistent osteomyelitis is suspected, follow the scheme shown in ***Figure 12.1-3*** (Appendix); repeat procedures to diagnose osteomyelitis (PTB plus imaging or scanning). If osteomyelitis is confirmed, follow the procedures for the Initial Procedure Visit, including application of TTAX01 (start a 6 week course of antibiotics if none are being taken, or revise antibiotics if still being taken for a previous osteomyelitis).

- Clean the wound using sterile saline, non-ionic cleanser or a hypochlorous solution. Antiseptic agents including hydrogen peroxide, acetic acid, chlorhexidine, povidone/iodine, and cetrimide should not be used.
- The wound should be sharply debrided to remove callus, macerated, necrotic/devitalized/non-viable tissue and purulence observed on the wound edge and wound bed. Debride until the wound edge is clean and sharply defined, with at least punctate bleeding at the edge and wound bed.
- Obtain photograph and measure the area and volume of the wound using the provided electronic wound imaging and measuring device (after debridement, if performed).
- Determine if additional TTAX01 is necessary **only for subjects in the treatment group** who have not achieved closure.
    - Remove any visible sutures or staples from prior treatment.
    - Remnant TTAX01, if present in the ulcer bed following debridement, should be left in place beneath reapplication.
    - TTAX01 may be reapplied following sharp debridement, for the index ulcer displaying the following criteria:
        - When there is no TTAX01 remnant detected one week after each application, suggesting dislodgement.
        - At least four (4) weeks from previous application of TTAX01 under the following conditions:

- Neither SSTI or osteomyelitis are observed, and
- Wound healing has stalled, defined as no change of the wound surface area compared to the wound surface area at last application, or
- Wound base does not exhibit hyperemic granulation tissue, or
- Wound edge is edematous and elevated.

- TTAX01 should be secured in place by sutures or staples.
- TTAX01 must be fenestrated.
- Obtain post-application photograph of TTAX01 using the electronic wound imaging and measuring device

▪ TTAX01 will be withheld for 3 weeks following each application, and withheld if the wound shows any of the following favorable signs of healing:

- Presence of TTAX01 tissue remnant
- Wound base covered by a black or yellow cap/eschar
- Wound base showing hyperemic granulation tissue
- Wound showing epithelialization.

- Apply wound dressing including a non-adherent, standard foam pad or alginate dressing (e.g., hydrogels, foam dressings, and alginates) that is appropriate for the amount of drainage, and a secondary bandage to ensure a moist wound environment, which adequately controls exudate and avoids maceration of the surrounding skin.
- Continue with the off-loading device appropriate to the location of the ulcer (preferably with full length boot or total contact cast, unless not appropriate per the physician, in which case a substitute off-loading device can be made with sponsor approval)
- Adjust infection management including antibiotics as needed, as indicated by the results from the microbiology and histopathology bone biopsy.

Schedule the next Treatment Period visit in one week (7 ± 2 days) or, if the subject is at Visit 16, schedule the first Weekly Observation Period Visit 17 in one week (7 ± 2 days).

8.1.5 Weekly Observation Period (Visits 17 – 20; 7 ± 2 days)

[Purpose]: To monitor safety and wound healing and adjust antibiotic regimens

No further applications of TTAX01 are permitted beyond Visit 16. If the wound deteriorates to ≥ 50% compared to the Initial Procedure post debridement wound area measurement, the Investigator may begin to utilize adjunctive therapies as explained in Section 6.5.

If at any Weekly Observation Period Visit the index ulcer is observed to be closed, perform the following procedures:

- Assess adverse events
- Review concomitant medications
- Assess subject compliance regarding off-loading since the last visit
- Obtain photographs of the closed ulcer using the electronic wound imaging and measuring device
- Observe the wound – if it is fully epithelialized with no drainage or need for a dressing, record it as closed.

The next visit scheduled will serve as the first consecutive confirmation visit in 14 ± 2 days **and will begin the confirmation of closure procedures** (refer to Section 8.1.9).

If the wound remains open, conduct the following procedures:

- Assess adverse events
- Review concomitant medications. These will be recorded in the subject's source document, but should only be recorded in the eCRF for the following:
  - All current and past treatments for the index ulcer only
  - Concomitant medications used to treat the index ulcer (e.g., systemic antibiotics for an index ulcer infection) and the peri-ulcer area (such as treatment for erythema, irritation, itching), together with the indication for the medication
  - Concomitant medications used to treat all SAE, and all AE considered "RELATED" to the product will be recorded in the appropriate eCRF.

- Assess subject compliance regarding off-loading since the last visit
- Assess the foot and lower extremity, then assess the index ulcer after removal of the dressing (but do not remove residual TTAX01 in the treatment group).
- Clean the wound using sterile saline, non-ionic cleanser or a hypochlorous solution. Antiseptic agents including hydrogen peroxide, acetic acid, chlorhexidine, povidone/iodine, and cetrimide should not be used.
- The wound should be sharply debrided to remove callus, macerated, necrotic/devitalized/non-viable tissue and purulence observed on the wound edge and wound bed. Debride until the wound edge is clean and sharply defined, with at least punctate bleeding at the edge and wound bed. **Leave in place** any TTAX01, regardless of appearance.
- Obtain photograph and measure the area and volume of the wound using the provided electronic wound imaging and measuring device (after debridement, if performed).
- Apply wound dressing including a non-adherent, standard foam pad or alginate dressing (e.g., hydrogels, foam dressings, and alginates) that is appropriate for the amount of drainage, and a secondary bandage to ensure a moist wound environment, which adequately controls exudate and avoids maceration of the surrounding skin.
- Continue with the off-loading device appropriate to the location of the ulcer (preferably with full length boot or total contact cast, unless not appropriate per the physician, in which case a substitute off-loading device can be made with sponsor approval)
- Adjust infection management including antibiotics as needed, as indicated by the results from the microbiology and histopathology bone biopsy.

Schedule the next Treatment Period visit in one week (7 ± 2 days) or, if the subject is at Visit 20, schedule the first Bi-Weekly Observation Period Visit in two weeks (14 ± 2 days).

8.1.6 Bi-Weekly Observation Period (Visits 21 – 23; 14 ± 2 days)

[Purpose]: To monitor safety and wound healing, adjust antibiotic regimens

No further applications of TTAX01 are permitted beyond Visit 16. If the wound deteriorates to ≥ 50% compared to the Initial Procedure post debridement wound area measurement, the Investigator may begin to utilize adjunctive therapies as explained in Section 6.5.

If at any Bi-Weekly Observation Visit the index ulcer is observed to be closed, perform the following procedures:

- Assess adverse events
- Review concomitant medications
- Assess subject compliance regarding off-loading since the last visit
- Obtain photographs of the closed ulcer using the electronic wound imaging and measuring device
- Observe the wound – if it is fully epithelialized with no drainage or need for a dressing, record it as closed.

The next visit scheduled will serve as the first consecutive confirmation visit in 14 ± 2 days **and will begin the confirmation of closure procedures** (refer to Section 8.1.9).

If the wound remains open, conduct the following procedures:

- Assess adverse events
- Review concomitant medications.
- Assess subject compliance regarding off-loading since the last visit, if applicable
- Assess the foot and lower extremity, then assess the index ulcer after removal of the dressing (but do not remove residual TTAX01 in the treatment group).
  - Determine whether skin and soft tissue infection (SSTI) is present. If SSTI is present, without evidence of osteomyelitis, obtain a deep tissue biopsy for histopathology, culture and sensitivity, and adjust the antibiotic regimen (start a 2 week course of antibiotics if none are being taken, or revise antibiotics if still being taken for a previous infection)
  - If new, recurrent or persistent osteomyelitis is suspected, determine whether the wound probes to bone, and obtain imaging or scanning. If osteomyelitis is

confirmed, start a 6 week course of antibiotics if none are being taken, or revise antibiotics if still being taken for a previous osteomyelitis.

- Clean the wound using sterile saline, non-ionic cleanser or a hypochlorous solution. Antiseptic agents including hydrogen peroxide, acetic acid, chlorhexidine, povidone/iodine, and cetrimide should not be used.
- The wound should be sharply debrided to remove callus, macerated, necrotic/devitalized/non-viable tissue and purulence observed on the wound edge and wound bed. Debride until the wound edge is clean and sharply defined, with at least punctate bleeding at the edge and wound bed. **Leave in place** any TTAX01 regardless of appearance.
- Obtain photograph and measure the area and volume of the wound using the provided electronic wound imaging and measuring device (after debridement, if performed).
- Apply wound dressing including a non-adherent, standard foam pad or alginate dressing (e.g., hydrogels, foam dressings, and alginates) that is appropriate for the amount of drainage, and a secondary bandage to ensure a moist wound environment, which adequately controls exudate and avoids maceration of the surrounding skin. Alternative therapies are allowed during this period, per Section 6.5.
- Continue with the off-loading device appropriate to the location of the ulcer (preferably with full length boot or total contact cast, unless not appropriate per the physician, in which case a substitute off-loading device can be made with sponsor approval).
- Adjust infection management including antibiotics as needed, as indicated by the results from the microbiology and histopathology bone biopsy.

Schedule the next Observation Period visit for 14 ± 2 days or, if the subject is at Visit 23, schedule the first Long Term Observation period visit in 28 ± 3 days.

8.1.7 Long Term Observation Period (Visits 24 – 28; 28 ± 4 days)

[Purpose] To evaluate safety and observe wound status

No further applications of TTAX01 are permitted beyond Visit 16. Subjects enter this Long Term Observation Period whether they have not completely healed their wound, or, they have achieved complete closure. If they have achieved complete wound closure, where healing has been confirmed at two consecutive visits each two weeks apart, but their wound re-opens during this period, it will be regarded as a failure of durable healing.

If at any Long Term Observation Visit a previously unhealed index ulcer is observed to be closed, perform the following procedures:

- Assess adverse events
- Review concomitant medications
- Assess subject compliance regarding off-loading since the last visit
- Obtain photographs of the closed ulcer using the electronic wound imaging and measuring device
- Observe the wound – if it is fully epithelialized with no drainage or need for a dressing, record it as closed.

The next visit scheduled will serve as the first consecutive confirmation visit in 14 ± 2 days **and will begin the confirmation of closure procedures** (refer to Section 8.1.9).

Otherwise,

- Assess adverse events
- Review concomitant medications. These will be recorded in the subject's source document, but should only be recorded in the eCRF for the following:
  - All current and past treatments for the index ulcer only
  - Concomitant medications used to treat the index ulcer (e.g., systemic antibiotics for an index ulcer infection) and the peri-ulcer area (such as treatment for erythema, irritation, itching), together with the indication for the medication

  - Concomitant medications used to treat all SAE, and all AE considered "RELATED" to the product will be recorded in the appropriate eCRF.
- Assess the foot and lower extremity, then assess the index ulcer after removal of the dressing.
  - Determine whether skin and soft tissue infection (SSTI) is present. If SSTI is present, without evidence of osteomyelitis, obtain a deep tissue biopsy for histopathology, culture and sensitivity, and adjust the antibiotic regimen (start a 2 week course of antibiotics if none are being taken, or revise antibiotics if still being taken for a previous infection)
  - If new, recurrent or persistent osteomyelitis is suspected, determine whether the wound probes to bone, and obtain imaging or scanning. If osteomyelitis is confirmed, start a 6 week course of antibiotics if none are being taken, or revise antibiotics if still being taken for a previous osteomyelitis.
- Clean the wound using sterile saline, non-ionic cleanser or a hypochlorous solution. Antiseptic agents including hydrogen peroxide, acetic acid, chlorhexidine, povidone/iodine, and cetrimide should not be used.
- The wound should be sharply debrided to remove callus, macerated, necrotic/devitalized/non-viable tissue and purulence observed on the wound edge and wound bed. Debride until the wound edge is clean and sharply defined, with at least punctate bleeding at the edge and wound bed.
- Obtain photograph and measure the area and volume of the wound using the provided electronic wound imaging and measuring device (after debridement, if performed).
- Apply wound dressing including a non-adherent, standard foam pad or alginate dressing (e.g., hydrogels, foam dressings, and alginates) that is appropriate for the amount of drainage, and a secondary bandage to ensure a moist wound environment, which adequately controls exudate and avoids maceration of the surrounding skin. Alternative therapies are allowed during this period, per Section 6.5.

- Continue with the off-loading device appropriate to the location of the ulcer (preferably with full length boot or total contact cast, unless not appropriate per the physician, in which case a substitute off-loading device can be made with sponsor approval).
- Adjust infection management including antibiotics as needed, as indicated by the results from the microbiology and histopathology bone biopsy.

Schedule the next Long Term Observation Period visit for 28 ± 4 days or, if the subject is at Visit 28, schedule the End of Study visit for 28 ± 7 days.

8.1.8 End of Study Visit (Visit 29; Day 344 ± 7)

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

Begin with the following procedures:

- Assess adverse events
- Review any changes in concomitant medications
- Obtain photograph of the index ulcer using the electronic wound imaging and measuring device
- Assess the index ulcer

If at this visit a previously unhealed index ulcer is observed to be closed,

perform the following procedures:

- Assess adverse events
- Review concomitant medications
- Assess subject compliance regarding off-loading since the last visit
- Obtain photographs of the closed ulcer using the electronic wound imaging and measuring device

- Observe the wound – if it is fully epithelialized with no drainage or need for a dressing, record it as closed.

The next visit scheduled will serve as the first consecutive confirmation visit in 14 ± 2 days **and will begin the confirmation of closure procedures** (refer to Section 8.1.9).

Otherwise,

- Perform physical examination, including vital signs (i.e., heart and respiratory rates, blood pressure, and temperature while patient is seated)
- Perform Laboratory Assessments as listed in Table 8.1.1-1 including the pregnancy test for females of childbearing potential
- Instruct subject to complete the PROMIS Lower Extremity Function questionnaire and the CWIS.

Complete subject disposition forms and exit the subject from the study.

8.1.9 Confirmation of Closure ("C" Visits)

[Purpose:] These visits serve to confirm that a wound has achieved complete closure

Confirmation of Closure visits are used to record the initial observation of closure, and persistent closure. Ideally, a wound that shows initial closure will still be closed at a confirmation visit two weeks later, and at a second confirmation visit four weeks later. At each of the confirmatory visits, use the Confirmation of Closure visit form(s) to record observations. Continue with the off-loading device appropriate to the location of the ulcer during the period of confirmatory visits.

Perform the following procedures:

- Assess adverse events
- Review concomitant medications
- Assess subject compliance regarding off-loading since the last visit

- Obtain photographs of the ulcer using the electronic wound imaging and measuring device
- Observe the wound – if it is fully epithelialized with no drainage or need for a dressing, record it as closed.

If the wound is found to be open at either confirmation visit or if the subject misses a scheduled confirmation visit, record it as such and then move directly to the original schedule of visits. This may become a treatment visit for example if the subject has not passed Visit 16, or an observation visit if the subject has passed Visit 16.

If the index ulcer is closed at the first confirmation visit, schedule the subject to return for a second consecutive confirmation visit in 14 ± 2 days. If the index ulcer remains closed at the second confirmation visit, schedule the subject to begin the Long Term Observation Period at the next study day that will put the subject onto the planned 28 day schedule of visits. For example, if a subject completes the second confirmation visit on Day 50, they should return on Day 64 and continue thereafter with visits every 28 ± 3 days. Days 92, 120, 148, 176, 204, or 232 are the other days on which these subjects can enter into Long Term Observation. Note that Day 176 has a unique window of plus or minus 2 days, otherwise these visits have windows of plus or minus 3 days.

If the initial closure is observed at Visit 28 or 29, the subject should move immediately to the End of Study procedures once both confirmation visits are completed, or if the wound is found to be open at either of the two confirmatory visits.

8.1.10 Unscheduled Visits

Unscheduled exams may be conducted at the discretion of the Investigator. All obtained information should be recorded in the source documents and on the Unscheduled Visit CRF.

8.1.11 Early Termination Visit

If a subject is withdrawn from their randomized treatment for any reason, they should be encouraged to continue with the subsequent scheduled visits for observation. The Observational Period visits are particularly valuable in monitoring long-term adverse events, (e.g., unresolved ongoing adverse events, and new treatment related adverse events including limb amputation for ulcers that do not heal).

If a subject is being withdrawn from the trial, attempt to schedule an early termination visit at which the End of Study procedures are carried out (refer to Section 8.1.6). Complete procedures described therein and document the reason for the subject's withdrawal.

8.2 Efficacy Assessments

The efficacy assessments rely primarily on observation of the wound, in order to determine if it has healed. While complete epithelialization is the standard definition of healing, the definition used in this protocol conforms to the 2006 FDA Guidance on wound healing, which requires two additional observations of healing at two week intervals. Photographic images of healed wounds will be reviewed by an independent expert in wound healing. Wounds that re-open (or re-ulcerate) after confirmed closure are also of interest, with the assessments in this case consisting of single observations. Patient reported outcomes are also recorded in order to evaluate the impact of a healed, or unhealed wound, on the subject's quality of life.

8.3 Safety and Other Assessments

Safety is evaluated at each visit by examination of the lower extremity 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 and severity of adverse events.

All adverse events must be reported in the subject's source documents; AE associated with the index ulcer or related to the test article must be recorded on an Adverse Event Form 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, 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.

8.4.2.1 Overdose

Not applicable.

8.4.3 Classification of AE

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 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 AE or 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:

Herbert B. Slade, MD Cell Phone: (817) 226-8760 E-mail: hslade@tissuetechinc.com

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.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

- Primary Efficacy Endpoint(s):

The primary efficacy endpoint is time in weeks from baseline to the initial observation closure of a complete wound healing, over 26 weeks, that is confirmed at two consecutive visits each two weeks apart. The primary efficacy endpoint will be analyzed using the Cox regression analysis (i.e., proportional hazards model) to assess the effect of TTAX01 treatment on time to complete wound healing compared to SC in terms of hazard ratio and difference in the curve of the proportion of subjects reaching the complete wound healing over the 26-week treatment period.

The null hypothesis is that the hazard ratio of TTAX01 arm vs, SC arm is not statistically different from 1.0. The alternative hypothesis is that the hazard ratio is lower than 1.0 at the significance level of <0.05.

- Secondary Efficacy Endpoint(s):

Secondary efficacy endpoints in this study include proportions of index ulcers healed by Week 50, proportions of wound re-ulcerations by Week 50, proportions of minor and major amputations by Week 50, and total score of PROMIS Lower Extremity Function Questionnaire at end of study.

For index ulcer healing by Week 50, the null hypothesis is that the proportion of subjects with wound healing will not be statistically different between the two treatment arms of this study at Week 50. The alternative hypothesis is that a greater proportion of subjects in the SC plus TTAX01 arm will have index ulcers healed at Week 50, compared to the SC alone arm.

For index ulcer re-ulceration by Week 50, the null hypothesis is that, among those subjects who have had the index ulcer healed before Week 50, the proportion of subjects with wound re-ulcerated will not be statistically different between the two treatment arms of this study at

Week 50. The alternative hypothesis is that a smaller proportion of subjects in the SC plus TTAX01 arm will have index ulcers re-ulcerated at Week 50, compared to the SC alone arm.

For minor and major amputations by Week 50 following initial procedure, the null hypothesis is that the proportion of subjects receiving minor and/or major amputations will not be statistically different between the two treatment arms of this study by Week 50. The alternative hypothesis is that a smaller proportion of subjects in the SC plus TTAX01 arm will have minor and/or major amputations by Week 50, compared to the SC alone arm.

For total score of CWIS and PROMIS Questionnaires, the null hypothesis is that a total score (or better quality-of-life) of this measure will not be statistically different between the two treatment arms of this study at the end of study. The alternative hypothesis is that a greater total score (or better quality-of-life) of one or both of these measures will be seen for the SC plus TTAX01 arm at the end of study, compared to the SC alone arm.

9.2 Sample Size Determination

The sample size for this trial was estimated using CreoStat HB StudySize software, v3.0.2. Using a log rank, two-sided, non-parametric Proportional Hazards (Freedman) approach, with accrual time assumed to be zero, α set to 0.025, 1-β set to 0.90, wound "survival" in the control arm set to 60% (thus 40% healed), and wound "survival" in the TTAX01 arm set to 35% (thus 65% healed), assuming 1:1 randomization and a 10% dropout, it is estimated that 94 subjects per group will be needed. As an added margin, 110 will be enrolled per group in the US portion of the trial (increases power to 94%). Three additional sites in Taiwan are expected to participate in this multicenter trial, but consistency of data between the US and TW sites is unknown. Therefore, the TW sites will be allowed to enroll 10 subjects per site, for an additional 30 subjects overall (15 per arm). This raises the power to 97%, assuming all data are acceptable for analysis.

9.3 Populations for Analyses

The Intent-to-Treat (ITT) population will consist of all enrolled and randomized 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 26-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 analyses of safety endpoints.

The Long-Term Observation (LO) Population includes all subjects who entered the long-term observation period of Visits 22 thru 26. The Long-Term Observation Population will be used for the analyses of those efficacy and safety endpoints that are assessed over the long-term observation period.

9.4 Statistical Analyses

9.4.1 General Approach

All data will be presented using summary statistics or frequency tables, as appropriate, and will be analyzed for superiority comparisons between SC plus TTAX01 arm and SC alone arm for both the treatment period and long-term observation period. The description of the sample will be done using summary statistics (n, mean, standard deviation, median, and maximum/minimum) for continuous data and using frequency statistics (counts and percentages) for categorical data. Hypothesis testing, unless otherwise indicated, will be performed at the 5% significance level (2-sided). All *P*-values will be rounded to four decimal

places; *P*-values less than 0.0001 will be presented as '<0.0001' in all tables. Unless specifically stated, all confidence intervals will be two-sided with 95% coverage.

The Sponsor, or their designee, will analyze the data using SAS® Statistical Analysis System Version 9.1.3 or higher. Detailed descriptions of the statistical analysis methods, data conventions, and sensitivity analysis as well as handling of missing data for both efficacy and safety measures will be described in detail in the Statistical Analysis Plan (SAP).

9.4.2 Analysis of the Primary Efficacy Endpoint

The primary efficacy endpoint of time in weeks from baseline to the initial observation closure of a complete wound healing, over 26 weeks, confirmed at two consecutive visits each two weeks apart will be analyzed using the Cox regression analysis (i.e., proportional hazards model) to assess the effect of TTAX01 treatment on time to complete wound healing compared to SC in terms of hazard ratio and difference in the curve of the proportion of subjects reaching the complete wound healing over the 26-week treatment period. For those subjects who discontinue from the treatment period prematurely without wound closure data collected over the confirmation period, those subjects who have missing data of wound closure over the confirmatory period and those subjects whose index ulcers are not deemed to be completely closed at the end of the 26-week treatment period, the time to the closure of a complete wound healing will be considered being censored at the corresponding time point.

The Cox regression model will include the treatment as the main predictor, whereas the subject's baseline index ulcer size and duration will be included in the model as covariates to adjust potential effects on time to wound closure. SAS PROC PHREG will be used to conduct the Cox regression analysis. The estimated hazard ratio of SC plus TTAX01 arm vs, SC alone arm and its 95% confidence intervals (CI) for wound healing will be reported.

For summary statistics and graphic illustrations of the primary efficacy endpoint, the obtained proportion of subjects with complete wound healing, the estimated median of time in weeks to the complete wound healing and the 95% CI as well as the estimated probabilities at each of

the 26 weeks of index ulcer non-closure will be analyzed and reported for each of the two treatment arms, using the method of Kaplan-Meier survival analysis with SAS PROC LIFETEST.

The analysis of the primary efficacy endpoint will be performed for the ITT population and PP population.

9.4.3 Analysis of the Secondary Endpoints

Secondary efficacy endpoints in this study include proportions of index ulcer healed by Week 50, proportions of wound re-ulcerations by Week 50, proportions of minor and major amputations by Week 50, and total score of PROMIS Lower Extremity Function Questionnaire at end of study.

The non-parametric Cochran-Mantel-Haenszel (CMH) test with adjustment for baseline wound size cohort will be used to examine treatment effects for the SC plus TTAX01 arm vs. SC alone arm on proportion of subjects with index ulcer achieving and remaining healed at Week 50, proportion of subjects (among those with complete wound healing before Week 50) with wound re-ulceration at Week 50, and proportion of subjects with minor and/or major amputations by Week 50. Subjects' missing values at Week 50 in these endpoints, due to either no assessment or early termination from the study, will be imputed using the last-observation carry forward (LOCF).

An analysis of covariance model (ANCOVA) with treatment and baseline wound size as effect will be used to analyze total score of PROMIS Lower Extremity Function Questionnaire at end of study. Subjects' missing values in this endpoint, due to either no assessment or early termination from the study, will utilize the assessment collected at the early termination visit.

The analyses of the secondary efficacy endpoints will be performed for the ITT population and PP population as well as the LO population.

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 as well as total score of Cardiff Wound Impact Schedule (CWIS).

Where applicable, analyses of safety measures will be conducted, respectively, for the 26-week treatment period of the safety population and for the long-term observation period of the LO population. Missing values in safety measures will remain as missing in analyses.

9.4.5 Baseline Descriptive Statistics

The following subject demographic and baseline data will be collected and reported:

Demographic variables will include but not limited to the following:

- Age
- Gender
- Ethnicity
- Race
- Smoking status

- Medical history
- Disease diagnosis, index ulcer measurement, severity, and prior treatment
- Physical examinations
- Laboratory tests
- PROMIS and CWIS QoL measures

9.4.6 Planned Interim Analyses

No interim analysis is planned for this study.

9.4.7 Subgroup Analyses

Primary and secondary efficacy endpoints will be reported by subgroup as follows:

- male vs. female
- by race based on combined categories of American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, and White
- by age group
- confirmed osteomyelitis vs. non-confirmed
- by baseline wound size cohort
- by use of additional therapies that were excluded prior to V9
- Use of antibiotics within first 6 weeks of treatment
- Ulcer debridement performed within first 4 and 8 weeks of treatment post initial procedure

9.4.8 Tabulation of Individual Participant Data

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

9.4.9 Exploratory Analyses

None.

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 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 are 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 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

Sponsor's Contact	**Medical Monitor**
Tommy Lee, VP Clinical Operations	Herbert B. Slade MD Chief Medical Officer
TissueTech, Inc.	*TissueTech, Inc.*
7300 Corporate Center Drive, Suite 700 *Miami, FL 33126*	*7300 Corporate Center Drive, Suite 700* *Miami, FL 33126*
786-815-7103	*817-226-8760*
tlee@tissuetechinc.com	*hslade@tissuetechinc.com*

10.1.6 Safety Oversight

Safety oversight will be under the direction of an internal Data Safety Monitoring Board (DSMB), 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 DSMB 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.

The members of the DSMB will include the Medical Monitor, Executive VP of Clinical & Regulatory Affairs, Trial Biostatistician, and the lead Principal Investigator. Notification of any halt will be sent to all Investigators and IRB pending a final determination.

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, 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 RAVE EDC, a 21 CFR Part 11-compliant data capture system provided by Medidata. 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.

10.1.10 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.

10.1.11 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.

10.1.12 Conflict of Interest Policy

Under the applicable regulations the trial sponsor may be required to submit a list of clinical investigators who conducted covered clinical studies and certify and/or disclose certain financial arrangements as follows:

a. Certification that no financial arrangements with an investigator have been made where trial outcome could affect compensation; that the Investigator has

no proprietary interest in the tested product; that the Investigator does not have a significant equity interest in the sponsor of the covered trial; and that the Investigator has not received significant payments of other sorts; and/or

b. Disclosure of specified financial arrangements and any steps taken to minimize the potential for bias.

Disclosable Financial Arrangements:

c. Compensation made to the Investigator in which the value of compensation could be affected by trial outcome.
d. A proprietary interest in the tested product, including, but not limited to, a patent, trademark, copyright or licensing agreement.
e. Any equity interest in the sponsor of a covered trial, i.e., any ownership interest, stock options, or other financial interest whose value cannot be readily determined through reference to public prices. This requirement applies to all covered studies, whether ongoing or completed;
f. Any equity interest in a publicly held company that exceeds $50,000 in value. The requirement applies to interests held during the time the clinical investigator is carrying out the trial and for 1 year following completion of the trial; and
g. Significant payments of other sorts, which are payments that have a cumulative monetary value of $25,000 or more made by the sponsor of a covered trial to the Investigator or the Investigators' institution to support activities of the Investigator exclusive of the costs of conducting the clinical trial or other clinical studies (e.g., a grant to fund ongoing research, compensation in the form of equipment or retainers for ongoing consultation or honoraria) during the time the clinical Investigator is carrying out the trial and for 1 year following completion of the trial.

10.2 Additional Considerations

Not Applicable.

10.3 List of Abbreviations

ABI	Ankle to Brachial Index
AE	Adverse Event
AM	Amnion
BP	Blood Pressure
cGMP	current Good Manufacturing Practices
cGTP	current Good Tissue Practices
CLI	Critical Limb Ischemia
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CWIS	Cardiff Wound Impact Schedule
DFU	Diabetic foot ulcer
DSMB	Data and Safety Monitoring Board
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HA	Hyaluronic acid
HbA1c	glycated hemoglobin A1c
HC	Heavy chain
HCT/P	Human cell or tissue based product
ICF	Informed Consent Form
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IDSA	Infectious Diseases Society of America
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intent to Treat
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Affairs
MRI	Magnetic Resonance Imaging
mg	Milligram
mmHg	Millimeters Mercury
NIH	National Institutes of Health
OR	Operating Room
PP	Per Protocol
PTB	Probe to bone
PTX3	Pentraxin-3
SAE	Serious Adverse Event
SC	Standard Care
SOA	Schedule of Activities
SSTI	Skin and Soft Tissue Infection
TcPO2	Transcutaneous Pressure of Oxygen
TTI	TissueTech Inc.
TW	Taiwan
UC	Umbilical Cord
UDI	Unique Device Identification

10.4 Protocol Amendments

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by TissueTech Inc. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRB/IEC is notified within 5 days.

Any permanent change to the protocol must be handled as a protocol amendment. The written amendment must be submitted to the IRB/IEC and the Investigator must await approval before implementing the changes. TissueTech Inc. will submit protocol amendments to the appropriate regulatory authorities for approval.

If in the judgment of the IRB/IEC, the Investigator, and/or TissueTech Inc., the amendment to the protocol substantially changes the study design and/or increases the potential risk to the subject and/or has an impact on the subject's involvement as a study participant, the currently approved written informed consent form will require similar modification. In such cases, informed consent will be renewed for subjects enrolled in the study before continued participation.

Summary of Changes from Previous Version:

Version	Affected Section(s)	Summary of Revisions Made	Rationale
1.0	N/A	Original Issue	
1.1	§ 2.2	Updated reference to TTAX01-CR001	TTAX01-CR001 was not completed at the time of original issue, it was completed at the time of this amendment
	§ 8	Clarified procedures on first observation of closure, removed statements that no dose or frequency would be recorded for ConMed and other treatments	Previous wording implied that nothing needed to be done at the visit where closure was first seen; corrected an error regarding data collection
	§ 9.2	Removed reference to single trial, added accrual assumption	Following request by FDA

	§ 10.1.5	Corrected e-mail address	Corrects an error
	§ various	Spelling and grammatical errors corrected, updated wording in Cardiff Wound Impact Schedule	Opportunistic

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12 APPENDIX

12.1 Decision Flow Diagrams for Placement of TTAX01

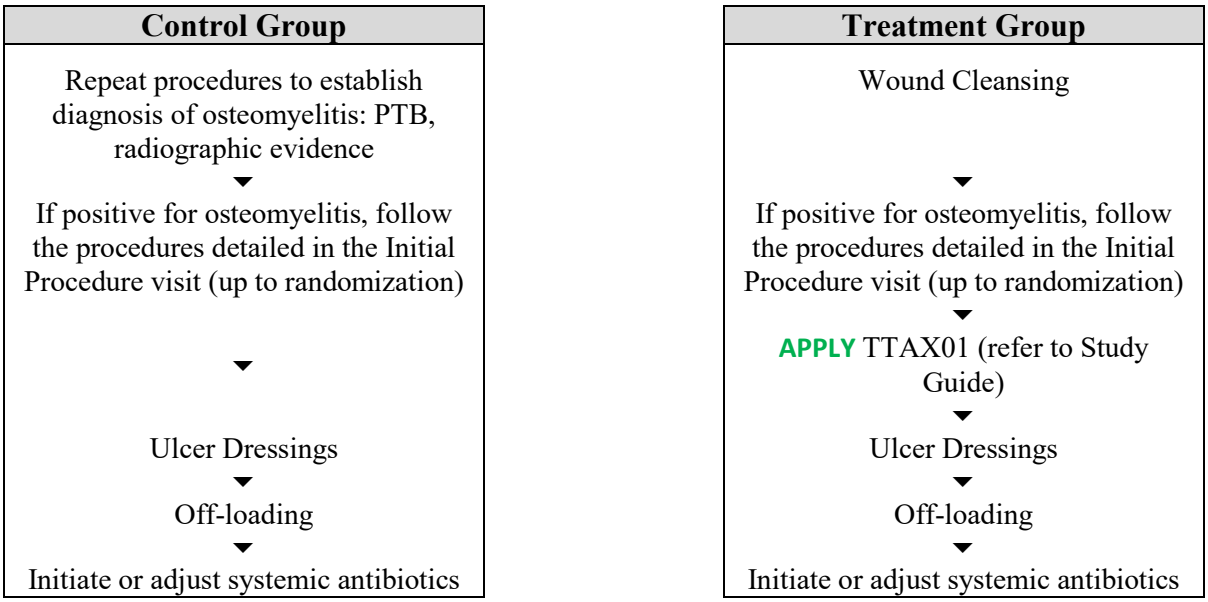
Figure 12.1-1. Treatment if there is no SSTI observed

Control Group	Treatment Group
Wound Cleansing ▼ Debridement ▼ Ulcer Dressings ▼ Off-loading	Wound Cleansing ▼ Debridement ▼ Assess reapplication / withhold criteria of TTAX01 (refer to Study Guide) ▼ Ulcer Dressings ▼ Off-loading

Figure 12.1-2. Treatment if SSTI is observed <u>without</u> recurrence of clinical suspicion of osteomyelitis

Control Group	Treatment Group
Wound Cleansing ▼ Debridement ▼ Obtain & send deep tissue biopsy for microbiology testing, initiate or adjust antibiotics ▼ Ulcer Dressings ▼ Off-loading	Wound Cleansing ▼ Debridement ▼ Obtain & send deep tissue biopsy for microbiology testing, initiate or adjust antibiotics ▼ **WITHHOLD** TTAX01 until clinical signs/symptoms resolve (refer to Study Guide) ▼ Ulcer Dressings ▼ Off-loading

Figure 12.1-3. Treatment if SSTI is observed <u>with</u> clinical suspicion of osteomyelitis



12.2 PROMIS Lower Extremity Function questionnaire

The subject will be asked to "Please respond to each question or statement by marking one box per row."

	Without any difficulty	With a little difficulty	With some difficulty	With much difficulty	Unable to do
Are you able to get on and off the toilet?...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Are you able to step up and down curbs?...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Are you able to get in and out of a car?...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Are you able to get out of bed into a chair?...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Are you able to push open a heavy door?...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Are you able to run errands and shop?...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Are you able to get up off the floor from lying on your back without help?...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Are you able to go for a walk of at least 15 minutes?...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

	No difficulty	A little difficulty	Some difficulty	A lot of difficulty	Can't do
How much DIFFICULTY do you currently have standing up from an armless straight chair (e.g., dining room chair)?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have sitting down on and standing up from a chair with arms?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have moving from sitting at the side of the bed to lying down on your back?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have standing up from a low, soft couch?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

How much DIFFICULTY do you currently have going up and down a flight of stairs inside, using a handrail?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have walking on uneven surfaces (e.g., grass, dirt road or sidewalk)?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have walking around one floor of your home?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have taking a 20-minute brisk walk, without stopping to rest?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have walking on a slippery surface, outdoors?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have climbing stairs step over step without a handrail? (alternating feet)?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
How much DIFFICULTY do you currently have walking in a dark room without falling?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

12.3 Cardiff Wound Impact Schedule

The subject will be provided with, and asked to complete the following:

How often do you see your family and friends?

Daily ☐ Once a week ☐ Once a month ☐ Less than once a month ☐

How <u>stressful</u> has this experience been for you during the past 7 days?

	Not at all/ not applicable	Slightly	Moderately	Quite a bit	Very
Difficulty getting out and about	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Relying more on others	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Your family/friends being overly protective	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Unable to enjoy your usual social life (e.g. hobbies)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Limited contact with family/friends	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Not going out for fear of bumping your wound site	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Wanting to withdraw from people	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

Have you <u>experienced</u> any of the following during the past 7 days?

	Not at all/ not applicable	Seldom	Sometimes	Frequently	Always
Difficulty getting out and about	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Relying more on others	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Your family/friends being overly protective	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Unable to enjoy your usual social life (e.g. hobbies)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

Limited contact with family/friends	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Not going out for fear of bumping your wound site	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Wanting to withdraw from people	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

To what extent do you agree/disagree with the following statements?

	Strongly disagree	Disagree	Not Sure	Agree	Strongly Agree
I feel anxious about my wound(s)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
I feel frustrated with the time it is taking for the wound(s) to heal	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
I am confident that the wound(s) I have will heal	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
I worry that I may get another wound in the future	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
The appearance of the wound site is upsetting	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
I worry about bumping the wound site	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
I worry about the impact of the wound(s) on my family/friends	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

Have you experienced any of the following during the past 7 days?

	Not at all/ not applicable	Seldom	Sometimes	Frequently	Always
Disturbed sleep	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Difficulty in bathing	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

Immobility around the home	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Immobility outside the home	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Leakage from the wound(s)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pain from the wound site	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Discomfort from the bandaging / dressing	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Unpleasant odor or smell from the wound(s)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Problems with everyday tasks (e.g. shopping)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Difficulty in finding appropriate footwear	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Problems with the amount of time needed to care for the wound site	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Financial difficulties as a result of the wound(s)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

How stressful has this experience been for you during the past 7 days?

	Not at all/ not applicable	Slightly	Moderately	Quite a bit	Very
Disturbed sleep	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Difficulty in bathing	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Immobility around the home	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Immobility outside the home	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Leakage from the wound(s)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pain from the wound site	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Discomfort from the bandaging / dressing	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

Unpleasant odor or smell from the wound(s)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Problems with everyday tasks (e.g. shopping)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Difficulty in finding appropriate footwear	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Problems with the amount of time needed to care for the wound site	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Financial difficulties as a result of the wound(s)	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

13 SPONSOR SIGNATURES

CLINICAL PROTOCOL APPROVAL FORM

Protocol Title: A Multicenter Phase 3 Trial of Biotherapy using Cryopreserved Human Umbilical Cord (TTAX01) for Late Stage, Complex Non-healing Diabetic Foot Ulcers (AMBULATE DFU)

Study No: TTAX01-CR003

Original Protocol Date: 08 Aug 2019

Protocol Version No: v1.1

Protocol Version Date: 30 Oct 2019

This study protocol was subject to critical review and has been approved by the appropriate protocol review committee of the sponsor. The information contained in this protocol is consistent with the current risk-benefit evaluation of the investigational product.

The Investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

Name and Title	Approval	Signature	Date
Author: **Herbert B. Slade MD** Chief Medical Officer	(Yes) No (circle one)	Herbert B. Slade MD	11/1/19
Clinical Operations: **Tommy D. Lee MSHS** VP Clinical Operations	(Yes) No (circle one)		01Nov2019
Biometrics: **Yuxin Zhang PhD** Xtiers Consulting (biostatistics)	(Yes) No (circle one)		01Nov2019
Regulatory Affairs: **Frank E. Young MD PhD** Exec VP Clinical & Regulatory Affairs	(Yes) No (circle one)		01Nov2019

14 INVESTIGATOR SIGNATURE

Study Title:	A Multicenter Phase 3 Trial of Biotherapy using Cryopreserved Human Umbilical Cord (TTAX01) for Late Stage, Complex Non-healing Diabetic Foot Ulcers (AMBULATE DFU)
Study Number:	TTAX01-CR003
Version Date:	30 Oct 2019

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: _____