Official title: PROSPECTIVE, RANDOMIZED, SINGLE-CENTER STUDY TO COMPARE CLINICAL OUTCOMES BETWEEN CRYOPRESERVED AND LYOPRESERVED STRAVIX AS AN ADJUNCT TO NPWT IN THE TREATMENT OF COMPLEX WOUNDS

NCT number: NCT04405765

IRB Approved date: 04/10/2023

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PROTOCOL NUMBER:
STU-2020-0293

SPONSOR:
OSIRIS THERAPEUTICS (SUBSIDIARY OF SMITH & NEPHEW)
7015 ALBERT EINSTEIN DRIVE
COLUMBIA, MD 21046

ISSUE DATE:
19 July 2021

SPONSOR SIGNATURE PAGE

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Print Name, Title, Company: _____

Signature: _____ Date: _____

INVESTIGATOR SIGNATURE PAGE

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Site Name: _____

Print Investigator Name: _____

Investigator Signature: _____ Date: _____

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Executive Summary

Title of Study	Prospective, Randomized, Single-Center Study to Compare Clinical Outcomes between Cryopreserved and Lyopreserved Stravix as an Adjunct to NPWT in the Treatment of Complex Wounds
Protocol Number	TBD
Study Purpose	The purpose of this study is to evaluate the clinical outcomes using lyopreserved Stravix as compared to cryopreserved Stravix when used as an adjunct to NPWT.
Study Design	Prospective, 2-arm parallel assignment, randomized clinical trial. Stravix will be applied at initial visit with NPWT and will only be reapplied after 4 weeks if wound is not ready for grafting unless debridement is indicated.
Enrollment	40 subjects will complete the study.
Investigational Sites	UT Southwestern and Parkland Hospitals
Study Duration	12 weeks
Efficacy Endpoints	Primary Endpoint: To compare the incidence of complete wound closure of lyopreserved vs cryopreserved Stravix treated groups at 12 weeks post initial application. Secondary Endpoints: To evaluate the outcomes noted below between lyopreserved and cryopreserved Stravix. 1. Time to 100% granulation of deep structures (bone, tendon, etc) 2. Duration of NPWT 3. Time to surgical grafting to cover the wound. 4. Time to complete wound closure (complete epithelization with no drainage) 5. Level of infection 6. Resource utilization 7. Quality of Life – SF36, EQD5 and PROMISE
Safety Endpoints	Ulcer-related adverse events
Study Groups	Subjects will all receive NPWT and be randomized to either lyopreserved or cryopreserved Stravix.

1. Introduction

Background and Rationale

Negative Pressure Wound Therapy (NPWT) has dramatically changed the care of complex foot wounds. Compared to standard wound care, patients with diabetic foot wounds that are treated with NPWT are 5.9 times more likely to heal and 4.4 times less likely to require amputation [1, 2]. NPWT involves the delivery of sub-atmospheric pressure through a vacuum pump connected to a specialized dressing to maintain a closed environment. NPWT increases perfusion to the wound, accelerates granulation tissue formation, reduces edema, and reduces bio-burden [1-3].

Stravix® is a cryopreserved human placental tissue composed of umbilical amnion and Wharton's jelly. Stravix retains the native collagen and hyaluronic acid-rich extracellular matrix (ECM), endogenous growth factors, and endogenous cells including epithelial cells, fibroblasts, and mesenchymal stem cells (MSCs) found in placental tissue. Stravix is a living placental tissue for surgical applications. As a viable wrap for surgical procedures, Stravix conforms to injured tissue, can be sutured, and is arthroscopic and robotic procedure friendly. Stravix is manufactured using a proprietary process allowing the tissue to retain its native components.

Historically, cryopreservation was the only available method that allowed for long-term preservation of living cells and tissues. However, cryopreservation requires ultra-low-temperature freezers and dry ice or liquid nitrogen for storage, which limits the widespread use of cellular therapies. To address this limitation, Osiris has developed Prestige™ Lyotechnology. Unlike all other known lyophilization methods, Prestige™ Lyotechnology enables the preservation of living cells within tissues while stored at ambient temperatures. Moreover, the manufacturing of lyopreserved living tissues with Prestige™ Lyotechnology is scalable and can be applied to many different cell and tissue types.

Benefits and Risks

Potential Benefits to the Subjects

Previous studies of clinical cases demonstrate potential benefit of Stravix use in surgical treatment of complex wounds.

Potential Risk

Participation in this clinical investigation presents low risk to subjects. Stravix is defined by FDA as an HCT/P under Section 361 of the Public Health Service Act and 21 CFR Part 1271. This category of products considered low risk (ref FDA documents). Some general risks associated with any new wound dressing or skin-contact devices are listed in the table below.

Risks	Disorders/Conditions
Skin and Subcutaneous Tissue Reaction/Allergy	• Skin rash, irritation, blistering • Pruritus/itching • Skin excoriation/breakdown • Skin scarring if significant skin irritation were to occur • Skin hyper/hypo-pigmentation at and/or around dressing application area

	• Erythema/redness, edema, inflammation, or swelling at and/or around dressing application area
Mild Pain or Discomfort	• Tenderness/minor ache at and/or around dressing application area • Decreased sleep or sleep quality • Paresthesia (numbness, tingling, prickling, creeping sensation)
Other	• Risks to privacy • Loss of data confidentiality

Potential Risks of a Blood Draw

- Discomfort
- Fainting
- Dizziness
- Bruising at the puncture site (hematoma)
- Nerve injury
- Arterial puncture or laceration

Protection Against Risks

Protected health information (PHI) of subjects in clinical investigations are kept as confidential as possible in accordance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). However, confidentiality cannot be assured. The table below lists the entities granted access to protected health information (PHI).

Entity	Reason for Access
Sponsor's Clinical Representative (Monitor)	Assess data for accuracy and completeness
Institutional Review Board (IRB)	Ensure protection of research subjects
Regulatory Authorities (e.g. Food and Drug Administration	Audit clinical trial for Subject protection and data integrity
Investigator/Site Staff	Collection and assessment of data for accuracy and completeness

Alternatives to Participation:

Subjects are not required to participate in this research study. As an alternative to study participation, subjects will receive wound care treatment per physician discretion.

2. Trial Objectives

The primary objective of this trial is to compare the clinical outcomes between cryopreserved and lyopreserved Stravix adjunct to NPWT in the treatment of diabetes-related lower extremity complex wounds.

3. Selection and Withdrawal of Subjects

Inclusion Criteria:

- Diagnosis of a diabetes mellitus
- Men/women ≥21 years old
- Post-operative foot or ankle wounds sized >4cm^2 that have presented for <1 year
- ABI ≥0.5 or toe pressures >30 mmHg
- Wounds indicated for treatment with NPWT

Exclusion Criteria:

- Active Charcot arthropy
- Unable to use NPWT
- Untreated bone or soft tissue infection
- Is pregnant or plans to become pregnant
- Is nursing or actively lactating
- Developmental disability/significant psychological disorder that in the opinion of the investigator could impair the subject's ability to provide informed consent, participate in the study protocol or record study measures, including untreated schizophrenia, bipolar disorder and psychiatric hospitalization within the last 2 years.
- Active alcohol or substance abuse in the opinion of the investigator that could impair the subject's ability to provide informed consent, participate in the study protocol or record study materials

Withdrawal of Subjects

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the PI for safety, behavioral, or administrative reasons. If a subject does not return for a scheduled visit, every effort should be made to contact the subject, per institutional protocol. In any circumstance, every effort should be made to document subject outcome. The Investigator should inquire about the reason for withdrawal, request that the subject return for a final visit, if applicable, and follow up with the subject regarding any unresolved AE's.

Any related SAE occurring within 14 days following subject discontinuation must be reported to Osiris Therapeutics and be followed up until stabilization or resolution. If the Subject withdraws from the study, and also withdraws consent for disclosure of future information, no further study evaluations will be performed, and no additional data will be collected. The PI may retain and continue to use any data collected before such withdrawal of consent.

The Investigator may choose to withdraw their own participation or discontinue a Subject from study with

or without their consent for any of the following:

- Adverse Events
- Non-compliance
- Safety
- Complications
- Unforeseen events

A patient who experiences the noted changes, relative to previous measures, in the following parameters will be terminated early from study participation

- An increase in wound size by 50% or more prior to debridement.
- An increase in pain rating by 50% or more
- Presence of infection as determined by 2 more bacterial infection indicators that requires treatment outside the study scope
- Need for wound management outside the study scope
- Or for any reason that may, in the opinion of the Investigator, affect negatively the wellbeing of the subject.

If for any reason the subject is withdrawn from the clinical investigation, the Investigator will inform the subject accordingly.

4. Trial Design

Description of Trial Design

This study is a prospective, 2-arm parallel assignment, randomized clinical trial to compare lyopreserved vs cryopreserved Stravix as an adjunct therapy to NPWT.

Trial Endpoints

Primary Endpoint: To compare the incidence of complete wound closure of lyopreserved vs cryopreserved Stravix treated groups at 12 weeks post initial application.

Secondary Endpoints: To evaluate the outcomes noted below between lyopreserved and cryopreserved Stravix.

1. Time to 100% granulation of deep structures (bone, tendon, etc)
2. Duration of NPWT
3. Time to surgical grafting to cover the wound.
4. Time to complete wound closure (complete epithelization with no drainage)
5. Level of infection
6. Quality of Life – SF36, EQ-5D and PROMIS®

Sample Size

For this study, 40 patients from UT Southwestern and Parkland hospitals will complete the study (no cap on screening/enrollment).

Duration of Subject Participation

- Screening: Within 7 days of therapy start
- Treatment period: Up to 12 weeks from therapy start
- Total duration of subject participation: Up to 13 weeks

Screening and Enrollment

Patients who present to the investigator's institution (through clinic admission, direct transfer from another facility, or through the emergency room) may be recruited to participate in the study. No direct marketing for subject recruitment will be done.

Patients approached for study participation will be at least 21 years of age at the time of consent, will undergo wound assessment, and meet all eligibility requirements. Those meeting eligibility criteria for the study will have the study explained to them by the Investigator. An Informed Consent Form will be provided to sign according to Section 11 prior to undergoing any study procedures. Patients will be encouraged to ask questions of the investigators. It will be made clear to the patient that not participating in the study will in no way influence the treatment plan or the relationship with the physician.

There is no cap on the number of subjects that can be screened/enrolled. Additional subjects will continue to be recruited as needed to reach the minimum number of 20 treated per protocol, per arm (40 in total).

Wound Selection

Subjects with only one wound will be included in the study. Subjects with multiple wounds will have each wound measured for volume. The wound with the largest volume meeting all eligibility requirements will be chosen for inclusion in the study as the primary wound. The etiology of the chosen wound, its classification, and if it is a new or recurring, will be documented. All other wounds will be documented as secondary wounds and will be followed throughout the study.

Randomization

Randomization will be performed using www.randomizer.org. Subjects who meet all inclusion and no exclusion criteria will be randomized in a 1:1 ratio to be treated with NPWT and either lyopreserved Stravix or cryopreserved Stravix. Study staff will use the randomization number labels contained in the envelope. The number will become the subject ID. The research staff will note treatment assignment number on the CRF.

Visit Schedule

Study procedures for each phase of study are outlined below. In a later section, details on each study procedure are described, including equipment designation.

Screening

1. Explain purpose and nature of the study and obtain signature on the informed consent document.
2. Screen the subject against protocol inclusion and exclusion criteria, including all pertinent tests

(ex. ABIs), including pregnancy test
3. Complete SF-36, EuroQol 5 dimensions (EQ-5D) and PROMIS®
4. Perform neuropathy assessment
5. Randomize subject if all inclusion and exclusion criteria are met.

Baseline (-within 7 days -after screening)

1. Obtain general medical history and demographic information and social history
2. Complete a physical examination, body weight, height, BMI and vital signs, including measurement of resting heart rate, respiratory rate, and blood pressure while seated.
3. Select target study ulcer
4. Obtain complete history pertinent to DFU disease including duration of the target ulcer, previous and current treatment. Document ulcer classification.
5. Perform debridement and obtain tissue collection (up to 4 tissues and 1 bone if applicable).
6. Collect blood sample at baseline visit (approximately 10mL at each collection) for Ribonucleic Acid (RNA) gene expression.
7. Perform standardized photography and measurement of the primary study wound and, if applicable, secondary study wounds (eKare or available camera with ruler if eKare is not available) pre-debridement. Assess the post-debridement ulcer area (cm^2), perimeter (cm), and greatest depth (cm) using the inSight device (eKare, Fairfax, VA). These values are the baseline measurements for calculating wound closure rate at the two-week run-in visit.
8. Perform hyperspectral imaging of dorsal and plantar aspects of the foot.
9. Collect all relevant concomitant medication (antibiotics, antifungals and other anti-infective therapies)
10. Collect all relevant clinical lab values
11. Place Stravix (lyo or cryo per randomization schedule) and dress wound for NPWT therapy (typically within 24 hours post-operatively for NPWT placement).
12. Document size of Stravix used.
13. Disburse subject stipend

Therapy/Treatment Phase (Weekly visits +/- 4 days)

Study Visit 1-11:

1. Assess target ulcer (if wound has closed, document as such, skip to step 5).
2. Document time on/off NPWT if applicable. NPWT removal/discontinuation will be per Principal Investigator discretion.
3. Perform standardized photography (eKare) of the study primary wound when NPWT is removed.
4. Assess the ulcer(s) area (cm^2), perimeter (cm), and greatest depth (cm) using the inSight device (eKare) and document if the primary wound and, if applicable secondary wound(s), document if subject has investigational product in place or no investigational product- if the wound is deemed closed by the physician, skip to EOS visit.
5. Perform hyperspectral imaging of the dorsal and plantar aspects of the foot if wound is active or closed.
6. Debride wound if indicated. Re-measure primary wound and, if applicable, secondary wounds with the inSight device (eKare) if surgical debridement is performed prior to dressing the wound.
7. Collect tissue and/or bone samples at weeks 4, and 8. If at any visit the subject has an infection, collect tissue and/or bone.
8. Collect blood sample at week 4 (approximately 10mL at each collection) for Ribonucleic Acid

(RNA) gene expression. Will also collect blood if patient develops and recovers from an infection during the study timeframe. Blood will be collected once clinical signs of infection are resolved.
9. Collect all relevant concomitant medication.
10. If wound is still active, redress the wound with assigned Stravix (if removed for debridement) and NPWT. Document all dressings applied including size of Stravix.
11. Disburse subject stipend
12. Note: at week 4, if wound is not ready for grafting, remove existing Stravix and replace with new piece of Stravix (per randomization schedule)
13. Assess for AE/SAEs and/or follow up on previous AE/SAEs.

Study Visit Closed
1. When a subject's study wound has closed, they will perform the EOS evaluation.

Study Visit 12/EOS:
1. At the time of wound closure, subjects will perform EOS visit.
2. A subject whose wound is not deemed closed (epithelialized with no drainage) by physician at week 12 will exit from the study after the week 12 wound evaluation.
3. Assess index ulcer.
4. If the wound is still open and large enough, collect tissue and/or bone samples along with a blood sample.
5. Assess the ulcer(s) area (cm^2), perimeter (cm), and greatest depth (cm) using the inSight device (eKare) and document if the primary wound and, if applicable, secondary wound(s) and if subject has investigational product in place or no investigational product.
6. Perform hyperspectral imaging of the dorsal and plantar aspects of the foot if wound is active or healed.
7. If wound has not closed, redress the wound per physician-directed standard of care.
8. Administer Patient Reported Outcome (PRO) (questionnaires).
9. Collect all relevant concomitant medication
10. Perform EOS visit documentation
11. Follow up on AE/SAEs that have been reported that have not yet been resolved.

Study Visit Follow-up:
1. If the wound heals during the treatment phase or if the wound is not healed after 12 weeks, data from their electronic medical record will be evaluated to identify healing, time to heal, adverse events related to the wound.

Detailed Study Operations

Medical Status/History

- *The New York Heart Association (NYHA) Functional Classification System:* This system will be used to classify stage of heart failure. The New York Heart Association (NYHA) functional classification system is used to classify the stage of heart failure from Class I to IV (as shown in the table below). This system relates symptoms to everyday activities and the patient's quality of life.

Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

- *Body Mass Index (BMI):* BMI is calculated as the ratio of body weight in kilograms (kgs) to height in meters squared (m^2).

- *Medical History:* The following information will be collected from each subject

Co-Morbidities

Cardiac	MI < 6mos		MI > 6mos	None
Coronary Artery Bypass Surgery	Yes/No			
Cardiac Angioplasty	Yes/No			
Stroke	< 6mos		> 6mos	None
Liver Disease	Hepatitis A	Active	Not Active	None
	Hepatitis B	Active	Not Active	None
	Hepatitis C	Active	Not Active	None
	Cirrhosis	Active	Not Active	None
Vision	Retinopathy	proliferative	background	none
	Glaucoma	Yes/No/Not Known		Specify Eye:
	Cataracts	Yes/No/Not Known		Specify Eye:
	Blindness	Yes/No/Not Known		Specify Eye:
Bone Tumors	Yes/No			
Arthritis	Yes/No			
Osteoporosis	Yes/No			
Malignancy	Yes/No			

Clinical Lab Values

- BUN
- Creatinine
- Estimated GFR (glomerular filtration rate)
- Hemoglobin A1c
- Prealbumin (transthyretin)
- Albumin
- CRP (C-reactive protein)
- ESR (Erythrocyte Sed Rate)
- White blood cell count
- RBC
- Hemoglobin
- Hematocrit
- Pregnancy test if subject is child bearing potential

Blood Collection

Whole blood will be collected and the cells lysed. RNA will be isolated and preserved, then analyzed for gene expression. The blood, tissue samples and some clinical data will be sent to Ingham Institute of Applied Clinical Research. These samples and the clinical data will be de-identified and will only contain an assigned study ID number, visit number and date of the visit.

Concomitant Medications

All antibiotics, antifungals and other anti-infective therapies engaged in by the subject will be recorded at screening visits and treatment visits.

Social Factors

The following social factors will be recorded:

- marital status
- years of education
- occupation
- tobacco use history: number of years of smoking, current or previous smoker, use of chewing tobacco, average daily number of cigarettes for current smokers
- alcohol and drug use history: current and/or previous history, types of alcohol/drugs consumed in the past and/or present; frequency and amount of alcohol/drugs consumed in the past and/or present

Demographic Variables

The following demographics will be recorded for each subject:

- Gender: male or female.
- Age
- Language Spoken: English, Spanish, English and Spanish, other(specify)
- Ethnicity: Caucasian, Hispanic, African American, American Indian, Asian/Pacific Islander.

Wound History and Assessment

- *Wound History and Evaluation:* We will use the University of Texas Ulcer Classification to document ulcer severity. Our group developed and validated this classification system. This classification system is a 4 x 4 matrix that includes the depth of the ulcer or the ulcer Grade: (1.) pre-ulcerative site, (2.) full thickness, (3.) extends to tendon or capsule, (4.) extends to bone and the Stage of the ulcer that identifies if there is infection and/or PAD: (A.) No PAD or infection, (B.) infection, (C.) PAD (D.) infection and PAD.

- *Wound Measurements and Debridement:* Debridement will be performed per physician discretion using a scalpel or bone curette to remove all surrounding callus, wound debris, necrotic tissue, fibrin, eschar and non-viable tissue to create a bleeding wound bed. Debridement may also take place in a surgical setting in which case tissue samples will be collected. Tissue and bone samples – the doctor will take 4 small samples of tissue from the subject's wound after debridement

(removal of dead or unhealthy tissue) and 1 sample of bone (if applicable) and these tissue samples will be kept and tested for the amount and type of bacteria that are present (qPCR analysis/laboratory analysis). We will objectively measure ulcer debridement and changes in wound size using changes in wound volume and area from wound measurements with the inSight digital 3D measurement device (eKare, Fairfax, VA). Digital photos will be taken after debridement. We will take post-debridement measurements of wound volume and area. Gardner et al reported that volume measurements with a digital image evaluation system was reliable.

- *Safety Measures:* Each of the following measures will be rated on the following 5-point Likert scale, as well as a descriptive evaluation being recorded, as applicable, for the study wound: 1) absence or 2) presence of the following:
    - Erythema
    - Discharge/drainage
    - Malodor
    - Tissue necrosis

Vascular Assessment

We will assess perfusion with three approaches; Arterial dopplers (bedside may be done for screening if formal ABIs have not yet been performed/resulted), and Hyperspectral Imaging. Hyperspectral imaging (HyperMed, Nashville, TN or Kent Imaging, Calgary, AB, Canada) will be used to evaluate the peri-wound tissue surrounding the ulcer and the angiosomes on the dorsum and plantar aspect of the foot.

Neurological Assessment

Semmes-Weinstein monofilament evaluation will be performed. We will assess light touch and pressure sensation at five sites on the study foot using a 10-gram monofilaments. We will perform Vibration Perception Threshold VPT; a device that uses vibration to test how well your feet can detect gentle pressure.

Infection Evaluation

This system first divides wounds by whether they are clinically infected on the basis of the presence of purulent secretions or local or systemic signs of inflammation or infection. Infected wounds are further divided into those that are considered to be mild, moderate, or severe, on the basis of the size (especially of any cellulitis) and depth (or level of tissue involved) of the infection and presence of systemic manifestations of infection or metabolic instability [4]. Additionally, the subjects will have the following monitored: temperature, heart rate, respirations and WBC.

Description	**International Working Group Infection Severity**
no infection: no pus no inflammation	1
2 or more signs of infection (pus, pain, warmth, swelling. cellulitis < 2cm infection limited to the skin or subcutaneous tissue	2

No systemic signs of infection	
2 or more signs of infection (pus, pain, warmth, induration. cellulitis > 2 cm lymphangistis, abscess infection extends to deep fascia, tendon muscle No systemic signs of infection	3
same as 3 with systemic toxicity meets SIRS criteria fever >38C, tachycardia >90, respiration >12, leukocytosis >12,000	4
osteomyelitis	5

Patient Reported Outcomes

The Medical Outcomes Study (MOS) 36-item Short Form (SF-36) is one of the most commonly utilized instruments in assessing diabetic foot disease and provides information on overall physical and mental quality of life. The SF-36 provides a physical component summary (PCS) score as well as a mental component summary (MCS) score, which are derived from eight different sub- scales. The PCS and MCS are standardized so that a score of 50 represents the normative score for the general population, and a higher score is indicative of better HRQOL (20). Excellent correlation has been found between PCS and MCS scores calculated from the SF-36 in patients with diabetic foot disease [5].

The EuroQol-5-dimension (EQ-5D-5L) questionnaire is a generic instrument which is commonly used for the assessment of QoL in various diseases. The 5-level version, EQ--5D-5L, consists of 2 pages – the EQ-5D-5L descriptive system, and the EQ visual analogue scale (EQ VAS). The descriptive system comprises 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The digits for the 5 dimensions can be combined in a 5-digit number describing the respondent's health state. The questionnaire item results will be converted to an index score using the conversion tool provided by EuroQoL for EQ-5D-5L.

The EQ VAS records the respondent's self-rated health on a 20 cm vertical, visual analogue scale with endpoints labeled 'the best health you can imagine' and "the worst health you can imagine". This information may be used as a quantitative measure of health as judged by the individual respondents.

PROMIS® is a publicly available system of highly reliable, precise measures of patient-reported health status for physical, mental, and social well-being. This web-based resource can be used to measure health symptoms and health-related quality of life domains such as pain, fatigue, depression, and physical function, which are relevant to a variety of chronic diseases.

Offloading

All subjects who can be offloaded will wear the DH OffLoading Walker or a Total Contact Cast. Subjects who have dorsal wounds or who cannot wear a boot/cast will be placed in a diabetic shoe. Offloading method may be dependent on wound location and postural positioning of the subject.

12 Month Retrospective Follow-up:
We will collect 12 month follow-up data to extend the length of follow up to adequately capture the time period with highest incidence of recurrent ulceration and infection.

Schedule of Events Table – Treatment Phase

The table below shows the schedule of planned events for each subject in this study.

Schedule of Events															
	Weekly Visits (±4 days)														
	SC	**BL/0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	***EOS**	**FUP @6mo**
Informed Consent	X														
Inclusion/Exclusion	X														
Randomization	X														
Medical History, Social, Demographics, PE, Vitals		X													
Lab Values		X													
Blood Collection for RNA#		X				X								X	
Pregnancy test if needed	X														
ABI, monofilament, VPT	X														
Wound Assessment, Debridement, Tissue Collection if Applicable+		X				X				X				X	
Stravix Application**		X				**X									
Wound Photography and Measurements, HSI Camera		X	X	X	X	X	X	X	X	X	X	X	X	X	
Offloading		X	X	X	X	X	X	X	X	X	X	X	X		
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	
Concomitant Medication		X	X	X	X	X	X	X	X	X	X	X	X	X	
Administer SF-36, EuroQol 5 dimensions (EQ-5D) and PROMIS®	X													X	
Subject Stipend		X	X	X	X	X	X	X	X	X	X	X	X	X	
EMR Reference															X

*if subject closes prior to week 12, subject will perform EOS visit and enter the FUP phase

**Stravix should only be removed and replaced if the physician deems it necessary for wound outcomes. If Stravix is not changed until week 4 and at week 4 the wound is not ready for surgical closure, it will be replaced. +Only to be collected if surgical debridement is required.
#will also collect blood if subject develops and recovers from an infection during the study timeframe. Blood will be collected once clinical signs of infection are resolved.

Audits and Inspections

Internal audits are done periodically to assure regulations, guidelines and protocols are adhered to appropriately. Patient binders are reviewed for proper informed consent process documentation, case report forms are compared to the source documents and reviewed for accuracy and an independent review of the inclusion and exclusion criteria using a checklist. Additionally, the use of an auditing and monitoring tool which includes sections on consent, HIPAA, on study/treatment, off treatment/off study and a general section. Weekly meetings occur to go over each study. If there are any issues that arise, they are brought up and discussed at the meeting.

5. Assessment of Efficacy

Effectiveness Endpoints

Primary Endpoint: To compare the incidence of complete wound closure of lyopreserved vs cryopreserved Stravix treated groups at 12 weeks post initial application.

Secondary Endpoints: To evaluate the outcomes noted below between lyopreserved and cryopreserved Stravix.

1. Time to 100% granulation of deep structures (bone, tendon, etc)
2. Duration of NPWT
3. Time to surgical grafting to cover the wound.
4. Time to complete wound closure (complete epithelization with no drainage)
5. Level of infection
6. Quality of Life – SF36, EQD5 and PROMISE

6. Assessment of Safety

Primary Safety Endpoint

Number of index wound related adverse events

7. Statistics

Analysis Plan

We will summarize study variables as means and standard deviations (SD) for continuous variables and proportions or percentages for categorical variables. Continuous variables will be presented as median, mean ± standard deviation and dichotomous variables presented as percent. We will use Analysis of Variance (ANOVA) to test for differences in continuous variables. For categorical variables, we will use chi square to compare the proportion of outcomes in each treatment arm with an alpha of 0.05, and we will use Kaplan Meier analysis to compare closure rates of the treatment groups. p-values were reported using the step-up Bonferroni method of Hochberg. We will use an adjusted two-sided analysis with an alpha of 0.05. In the intent to treat analysis, we will use the last observation carried forward to define the clinical outcomes for patients that were lost to follow up.

Tissue samples will be extracted and used for molecular biology analyses. These may include, gene and protein expression of the wound tissue, histological analyses of tissue morphology and pathology, and analyses of bacterial genes to determine levels of infection. All sample analyses will be performed in a de-identified manner and tissue samples WILL NOT be used for genetic sequencing that can be used to deduce the subject's identity.

Sample Size Justification

This is a pilot exploratory study so no sample size justification will be provided.

8. Data Management

Standardized CRFs will be used. Investigators are responsible for the accurate completion and timely submission of the data collected during the trial. All data from the trial will be entered from the CRFs into a central database. Incoming data will be frequently reviewed to identify inconsistent or missing data and any adverse events. Any data issues are to be promptly addressed with the investigator by the Sponsor. Quality assurance procedures will be established to ensure that complete, accurate and timely data are submitted, that protocol requirements are followed and that complications, adverse events and adverse device effects are correctly reported and investigated, as appropriate. Investigators are to maintain all source documents as required by the protocol, including laboratory results, supporting medical records, and signed Informed Consent Forms. The source documents will be used during the regular monitoring visits to verify information from the database against data contained on the completed CRFs.

9. Deviations from the Investigational Plan

Any deviations from the study protocol will be documented as Protocol Deviations in the Source Documentation.

10. Product Accountability

The Sponsor will ship investigational product directly to the site once IRB approval has been obtained. The investigator is responsible for providing a secure storage location for the investigational product, supervising product use, as well as the disposal and/or return of the product as instructed by the Sponsor. In addition, the investigator shall maintain records to document the receipt, use and disposition of all investigational product received by their site.

11. Ethics

Trial Conduct

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 812).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number only. All study records will be kept in a locked research office with limited

access to study personnel and code sheets linking a patient's name to a patient identification number will be stored separately in a secured area within the research office. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

Good clinical practice (GCP) is an international quality standard that is provided by International Conference of Harmonization (ICH), an international body that defines a set of standards, which governments can then transpose into regulations for clinical trials of medications as well as medical devices involving human subjects.

GCP guidelines are important as they provide the necessary enforcement of ethics of a clinical study, protection of human rights for the subjects (such as voluntariness) and assurance of the safety and efficacy of investigational products. High standards are required in terms of comprehensive documentation for the clinical protocol, record keeping (paper and electronic which includes computers and software), training, and facilities. Quality assurance and inspections ensure that these standards are achieved and maintained.

GCP guidelines include standards on how clinical trials should be conducted, define the roles and responsibilities of clinical trial sponsors, clinical research investigators, and monitors. As a research site, we follow the ICH-GCP guidelines. Our GCP includes review of each case to determine whether or not the patient would be a good candidate for a clinical trial and if there is an appropriate clinical trial for the patient. Important aspects include adequate time for the person to review the consent form and have all questions answered, voluntarily give consent and know that they can withdraw at any time. Maintaining the subject's privacy and confidentiality is handled by assigning a subject number for each study participant. Research staff responsibilities include: adherence to the protocol, proper documentation, accuracy of data and appropriate handling of the study product.

Institutional Review Board Review

The protocol and consent form will be reviewed and approved by the IRB/IEC prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to Osiris Therapeutics contact prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently

approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Any amendment to the protocol will be written by the investigator and Osiris Therapeutics contact. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

Informed Consent

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25 [a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

Subjects cannot be asked to sign the Informed Consent form until the study has been fully approved by the investigational site's IRB and the Sponsor has received and reviewed the specific IRB approved Informed Consent form to be used by the site. The potential subject shall be given adequate time to read the consent form, have the study procedures explained, including risks and benefits, as well as alternative procedures, prior to signing the Informed Consent form. An example of the Informed Consent form for this study is provided in Appendix 1: Informed Consent Form. The consent form must be read by the subject, the subject's questions answered, and the form signed by the subject before the treatment can be performed. All subjects are to receive copies of their signed consent form. The date the subject signs the consent form is to be recorded on CRF.

Coverage of Expenses

<u>Subject Compensation</u>

Subjects will be paid 35 dollars for each week of participation in the study at the completion of their participation in the study. Payment will be made after each visit or within 30 days of the ending of their participation in the study. The study sponsor will not pay for transportation to and from the wound care clinic for the follow-up visit requirements.

<u>Cost to Subjects</u>

There will be no cost incurred by the subject for participating in this study. All noted procedures are considered standard of care (SOC) and will be billed to the patient's insurance or whatever program the patient uses.

Confidentiality

Protected health information (PHI) of clinical investigation Subjects are kept as confidential as possible in accordance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Members of the study team who will have access to the PHI information include the following: Research staff at UT Southwestern and Parkland Hospitals, Sponsor (Osiris Therapeutics), and any outside labs/facilities that may handle specimens. When appropriate, specimens and data will only have subject ID. However, confidentiality cannot be assured.

12. Data Handling and Record Keeping

Source Documents

The investigator must maintain detailed source documents on all subjects who are enrolled or who undergo screening in the study. Source documents include subject medical records, hospital charts, clinic charts, investigator subject trial files, as well as the results of diagnostic tests (e.g., laboratory tests, hemodynamic studies).

The following minimum information should be entered into the subject's medical record:

- The date the subject entered the trial and the subject number
- The trial protocol number and the name of the Sponsor
- The date that Informed Consent was obtained
- Evidence that the subject meets the trial eligibility requirements (e.g., medical history, study procedures and/or evaluations)
- The dates of all trial related subject visits
- Evidence that required procedures and/or evaluations were completed
- Use of any concomitant medications
- Documentation of specific device used
- Occurrence and status of any adverse events (AEs)
- The date the subject exited the trial and a notation as to whether the subject completed the trial or was discontinued, including the reason for discontinuation

Data Collection

The investigator must maintain detailed records on all subjects who sign the Informed Consent Form and begin the pre-procedure evaluation. Data for enrolled subjects will be transcribed on to CRFs provided by the Site. All data should be transcribed completely, promptly and legibly. Corrections should be made in a manner that does not obscure or eliminate the original error, by striking through the original data with one line, and initialing and dating the change, along with the reason for the change (if not obvious). The investigator should maintain a copy of all completed CRFs from this trial.

Trial exit forms will be completed for all enrolled subjects, regardless if they did or did not complete the trial (e.g., subject discontinuation, trial termination).

Record Retention

All records relating to the conduct of this trial are to be retained by the investigator for a minimum of 2 years or until notified by the Sponsor or Sponsor's designee that the records may be destroyed, whichever

is longer.

13. Quality Control and Quality Assurance

Site Training

Training records shall be kept for all study-related training.

Investigator Training

Investigator Responsibilities

The investigators are responsible for ensuring that this study is conducted according to this protocol and applicable regulations and that signed Informed Consent is obtained from each subject prior to his inclusion in this study. It is the investigator's responsibility to ensure that all staff assisting with this study have the appropriate qualifications and are fully instructed on the study procedures and respect subject confidentiality, as specified in the Investigator Agreement with the Sponsor

Investigator Records

Standardized Case Report Forms (CRFs) will be used to collect complete and accurate records of the clinical data generated from this study according to Good Clinical Practices (GCP) requirements. The investigators are responsible for collecting and accurately recording the clinical data generated for this study. Investigators are also responsible for maintaining records for at least 7 years per UTSW rules.

Investigator Reports

The investigator will be responsible for providing the following reports, in accordance with CFR§812.150, to the Sponsor for this study:

- Serious Adverse Events (SAEs) and Unanticipated Adverse Device Effects (UADEs): The investigators will report by telephone, email or fax any SAEs or UADEs as soon as possible, within 24 hours of the investigator becoming aware of the event, to the Sponsor and the IRB. The Serious Adverse Event form is to be completed to document the SAE and it is to be faxed or express mailed to the Sponsor and the IRB within ten working days of the event.
- Withdrawal of Approval: If an IRB withdraws the approval to conduct this study for any reason, the investigator will notify the Sponsor as soon as possible, but in no event later than five working days after the withdrawal of the approval
- Progress Reports: The investigator will submit progress reports on the investigation to the Sponsor and the reviewing IRB at regular intervals, but in no event less often than yearly.
- Deviations from the Investigational Plan: The investigator must notify the Sponsor and the reviewing IRB of any deviation from the Investigational Plan to protect the life or physical well-being of a subject in an emergency. This notice must occur as soon as possible, but in no case longer than five working days following the occurrence of the deviation.
- Informed Consent: If the investigator uses a device without obtaining informed consent, the investigator shall report the use to the Sponsor and the IRB within 5 working days after the use occurs.
- Final Report: Within three months after the termination or completion of the study or the

investigator's part in the study, the investigator shall submit a final report to the Sponsor and the IRB.

- Other Reports: Upon request from the IRB or the FDA, the investigator shall provide accurate, complete and current information about any aspect of the study.

The investigator is responsible for ensuring that the study is conducted according to this protocol and that signed Informed Consent is obtained from each subject prior to their inclusion in this study.

14. Adverse Events and Serious Adverse Events

General

All observed or volunteered adverse events regardless of treatment group or suspected causal relationship to the investigational product(s) will be reported as described in the following sections.
For all adverse events, the investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification to the Osiris Therapeutics contact. For all adverse events, sufficient information should be obtained by the investigator to determine the causality of the adverse event. The investigator is required to assess causality. For adverse events with a causal relationship to the device, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and the Osiris Therapeutics contact concurs with that assessment.

Protocol Specific AE Definition/Collection: For the purposes of this study, only AEs that are directly related to the study product, diabetes (including labs & kidney function) or wound healing (including infections related to the wound) will be collected and reported as these events may effect outcomes and analysis of data. AEs not related to the aforementioned product or diagnoses are not relevant to the subject outcomes of this research or data analysis (such as nausea, emesis, pain, constipation, diarrhea, mood disorders, respiratory diagnoses, etc). AEs that do not fall under this protocol specific definition will be assessed and treated as per standard of care. SAEs will be collected as per the usual SAE guidelines (see section on reporting SAEs). In addition, planned procedures related to the study wound for a subject during the same hospitalization (as these subjects will be recruited while in the hospital because they have an infection in their foot wound/ulcer) for incision/debridement/amputation will be considered part of their continuation of SOC and not adverse events unless a complication, new infection or new wound occurs.

Adverse Event Reporting

For serious adverse events, the reporting period to Osiris Therapeutics or its designated representative begins from the time that the subject provides informed consent, which is obtained prior to the subject's participation in the study, i.e., prior to undergoing any study-related procedure and/or receiving investigational product, through and including 28 calendar days after the last administration of the investigational product. Any serious adverse event occurring any time after the reporting period must be promptly reported if a causal relationship to investigational product is suspected. Adverse events (serious and non-serious) should be recorded on the CRF from the time the subject has undergone one treatment through last subject visit.

Definition of an Adverse Event (AE)

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage. Examples of adverse events include but are not limited to:

- Abnormal test findings;
- Clinically significant symptoms and signs or diagnosis;
- Changes in physical examination findings;
- Hypersensitivity;
- Progression/worsening of wound.

Definition of Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is any AE that has any serious unfavorable and unintended sign, symptom, or disease temporally associated with the use of the devices, whether or not considered related, including those that:

- results in death
- is life-threatening
- requires inpatient hospitalization or causes prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- requires intervention to prevent permanent impairment or damage

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, the important medical event should be reported as serious, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes.

Causality Assessment of Adverse Events

The investigator's assessment of causality must be provided for all adverse events (serious and non-serious); the investigator must record the causal relationship in the CRF, as appropriate, and report such an assessment in accordance with the serious adverse reporting requirements if applicable. An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the cleared medical device caused or contributed to an adverse event. If the investigator does not know whether or not medical device caused the event, then the event will be handled as "related to medical device" for reporting purposes. (see Section on Reporting Requirements). If the investigator's causality assessment is "unknown but not related to investigational product", this should be clearly documented on study records.

In addition, if the investigator determines a serious adverse event is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with the serious adverse event reporting requirements, if applicable.

Adverse Event Severity Assessment

The Investigator will provide an assessment of the severity of each adverse reaction by recording a

severity rating on the appropriate SAE reporting page of the subject's file. Severity, which is a description of the intensity of manifestation of the SAE, is distinct from seriousness, which implies a patient outcome or SAE-required treatment measure associated with a threat to life or functionality. Severity will be assessed according to the following scale.

If required on the adverse event case report forms, the investigator will use the adjectives MILD, MODERATE, or SEVERE to describe the maximum intensity of the adverse event. For purposes of consistency, these intensity grades are defined as follows:	
MILD	Does not interfere with subject's usual function.
MODERATE	Interferes to some extent with subject's usual function.
SEVERE	Interferes significantly with subject's usual function.

Note the distinction between the severity and the seriousness of an adverse event. A severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with subject's usual function) but would not be classified as serious unless it met one of the criteria for serious adverse events, listed above.

Withdrawal Due to Adverse Events (See Also Section on Subject Withdrawal)

Withdrawal due to adverse event should be distinguished from withdrawal due to insufficient response, according to the definition of adverse event noted earlier, and recorded on the appropriate adverse event CRF page.

When a subject withdraws due to a serious adverse event, the serious adverse event must be reported in accordance with the reporting requirements defined below.

Eliciting Adverse Event Information

The investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the study subject. In addition, each study subject will be questioned about adverse events by a member of the research staff.

Reporting Requirements

Each adverse event is to be assessed to determine if it meets the criteria for serious adverse events. If a serious adverse event occurs, expedited reporting will follow local and international regulations, as appropriate.

Serious Adverse Event Reporting Requirements

If a serious adverse event occurs, Osiris Therapeutics is to be notified within 24 hours of awareness of the event by the investigator. In particular, if the serious adverse event is fatal or life-threatening, notification to Osiris Therapeutics must be made immediately, irrespective of the extent of available adverse event information. This timeframe also applies to additional new information (follow-up) on previously forwarded serious adverse event reports as well as to the initial and follow-up reporting of Exposure during pregnancy cases.

In the rare event that the investigator or member of the research team does not become aware of the occurrence of a serious adverse event immediately (e.g., if an outpatient study subject initially seeks treatment elsewhere), the investigator or member of the research team is to report the event within 24 hours after learning of it and document the time of his/her first awareness of the adverse event.

For all serious adverse events, the investigator is obligated to pursue and provide information to Osiris Therapeutics in accordance with the timeframes for reporting specified above. In addition, an investigator may be requested by Osiris Therapeutics to obtain specific additional follow-up information in an expedited fashion. This information may be more detailed than that captured on the adverse event case report form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications and illnesses must be provided. In the case of a subject death, a summary of available autopsy findings and/or a copy of the death certificate must be submitted as soon as possible to Osiris Therapeutics or its designated representative.

All AEs/SAEs should be followed until resolution.

Non-Serious Adverse Event Reporting Requirements

All adverse events will be reported on the adverse event page(s) of the CRF. It should be noted that the form for collection of serious adverse event information is not the same as the adverse event CRF. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same adverse event term should be used on both forms. Adverse events should be reported using concise medical terminology on the CRFs as well as on the form for collection of serious adverse event information.

Reporting Requirements to Regulatory Authorities

Adverse events reporting, including suspected serious unexpected adverse reactions, will be carried out in accordance with applicable local regulations.

15. **Publication Policy**

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the investigator and the Osiris Therapeutics contact. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

References

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2. Blume, P.A., et al., *Comparison of negative pressure wound therapy using vacuum-assisted closure with advanced moist wound therapy in the treatment of diabetic foot ulcers: a multicenter randomized controlled trial.* Diabetes Care, 2008. **31**(4): p. 631-6.
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