TITLE OF THE STUDY: A comparative efficacy study: bioengineered living dermal replacement tissue vs. non-viable extracellular matrix for the treatment of non-healing diabetic foot ulcers.

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PROTOCOL SUMMARY

TITLE OF THE STUDY: A comparative efficacy study: bioengineered living dermal replacement tissue vs. non-viable extracellular matrix for the treatment of non-healing diabetic foot ulcers.

PHASE: Equivalence Study

METHODOLOGY: Randomized, controlled, stratified, prospective

STUDY DURATION: 8 weeks

FOLLOW-UP: 16 weeks

STUDY CENTERS: 2 centralized study locations (Mather and Martinez Wound Clinics)
Note: Satellite clinics (Redding, Chico, Oakland and Fairfield) will refer patients to the study locations

NUMBER OF SUBJECTS: 3 treatment arms, 57 subjects per arm for a total of 171

STUDY OBJECTIVES: The primary objective of this study is to assess the effectiveness of cellular dermal replacement tissue vs. non-viable extracellular matrix (ECM) for the treatment of non-healing diabetic foot ulcers. The primary endpoint will be the measurement of wound closure at 12 weeks, secondary outcomes will be measured at 20 weeks and the rate of healing in each treatment arm.

Secondary objective of study is to determine cost effectiveness of the bioengineered cellular vs. acellular ECM products. Cellular ECMs (Dermagraft®) are costly devices, $1800 per application, difficult to use and time sensitive (weeks). The acellular ECM, (Oasis®) is easier to use and at a cost $80 per application and has a longer shelflife (2 years). These devices typically require multiple applications, on a weekly basis. If the findings show that these devices are equivalent, then the use of acellular ECM would lead to significant savings for the VA. Genetic markers of non-healing vs. healing diabetic ulcers will also be investigated, which will be useful information in predicting healing potential.

INCLUSION CRITERIA:
Subjects must meet all of the following inclusion criteria to enroll in the study:

1. Patients with ICD-9 codes of diabetic foot ulcer or other foot ulcer present for at least 4 weeks (any location on the foot, no more than 1 ulcer on the target limb).
2. Diagnosis of diabetes with either Type 1 or 2, and HbA1c less than 10%.
3. Over the age of 18 and younger than 85 years of age.
4. Based on the Curative Health Services Classification, subjects with a Grade 2 foot ulcer, full thickness and subcutaneous tissue that extend through the dermis but without tendon, muscle, capsule or bone exposure.
5. Area greater than or equal to 1.0 cm^2 and less than or equal to 25.0 cm^2 post debridement.
6. Foot ulceration with no clinical signs or symptoms of infection, as determined with clinical assessment of 2 or more signs of inflammation, and quantitative analysis of bacterial load of the wound. No systemic signs of infection, such as fever/chills, nausea or vomiting, and normal CBC.

a) A sample of the wound after debridement with curettage technique will be taken for quantitative analysis of bacterial load. If findings of 1 x 105 CFU per gram of tissue, the wound will be considered subclinical infected and excluded from study.
b) Lab test taken at start of study, CBC—white blood count within normal range.

7. Patients will be required to have an assessment for arterial insufficiency, which includes a formal ankle-arm index. If the ankle-arm index is equal to or greater than 0.8 and less than 1.4 or a toe-arm index is equal to or greater than 0.6 will be eligible. Note: Patients that have inconclusive physical examination or indirect physiologic testing (ankle-arm index) will require an arterial duplex studies that can image directly the arterial flow velocities and perfusion to the distal extremity or computed tomography angiography and conventional angiography, which may be considered as a subset group if findings show no arterial insufficiency per vascular surgeon consultant. In addition, patients that undergo revascularization procedure and do meet ABI requirement post-intervention will be included in the study as a subset group.
8. Females of childbearing age potential, with negative serum pregnancy test and not lactating during length of the study.
9. Willingness to comply to wound care assigned and attend follow-up visits.

EXCLUSION CRITERIA:
Those subjects will be excluded from the study with any of the following reasons:

1. Diagnosis of cancer, treatment with immunosuppressive or chemotherapeutic agents, radiotherapy or systemic corticosteroids less than 30 days before enrollment.
2. Immunocompromised diseases (HIV/AIDS).
3. Bleeding disorders.
4. Connective tissue diseases.
5. Pregnant women.
6. History of drug or alcohol abuse within one year of the study.
7. Infected wounds or osteomyelitis (confirmed by bone biopsy, MRI or bone scan).
8. Active Charcot as described by Saunder's classification system.
9. Poor nutritional status, Albumin level < 2.9.
10. Ulceration size less than 1.0 cm^2 or greater than 20 cm^2.
11. Porcine allergy.
12. Chemistry tests serum creatinine, 2 times above the upper limits and LFTs 3 times above the upper limits.

TREATMENT: Total duration of study per subject: Up to 22 weeks (Includes to 2 weeks of screening and up to 12 weeks until the end of the follow-up period).

DURATION OF ADMININSTRATION: 8 weekly applications (starting at Visit 4/Week 1).

STUDY DESIGN: This is a randomized, single-blinded, four-armed controlled clinical equivalence trial comparing the effectiveness of three intervention arms (acellular ECM, Oasis® and a cellular ECM, fibroblast-only Dermagraft®) and a standard treatment arm.

EFFICACY ENDPOINTS:
PRIMARY EFFICACY OUTCOME

1. Complete closure of ulcer by Week 12

SECONDARY EFFICACY OUTCOME

1. Complete closure of ulcer by Week 20
2. Rate of ulcer healing

3. Cost effectiveness comparing Oasis® and Dermagraft®

QUALITY OF LIFE ASSESSMENT: We will also evaluate the cost-effectiveness of the Oasis® compared to Dermagraft® and the standard of care by measuring QALYs as the measure of effectiveness, based on results obtained from SF-36v2™ questionnaire. Multiple regression will be used to allow to evaluate continuous dependent variable outcomes, including change scores between baseline and end of study physical (PCS) and mental (MCS) component summaries from the SF-36v2TM questionnaire.

DATA ANALYSIS: The primary outcome is wound closure by Week 12, will use Chi-square test to compare the percentages of subjects with complete closure in each group. Exploratory analysis with logistic regression, using the complete closure at 12 weeks as the dependent variable and the independent variables would include, treatment, size of ulcer, location on foot, how long ulcer has been present, previous revascularization, offloading compliancy, concomitant medications, foot deformity, history of amputation, diabetes complications index, CRP level, and glucose control (HbA1c). Selection of covariates will be performed by stepwise modeling procedures.

Secondary outcomes, will use ANOVA or Kruskal-Wallis test to analyze cost effectiveness. depending if data is normally distributed for comparison of the three treatment arms. If significant, we will further analyze with pair-wise comparisons by using t-test or Wilcoxon rank-sum test with the Bonferroni correction to control for type I experiment-wise error rate of 0.05.

Rates of healing among the four groups will be analyzed using log-rank test to compare time of healing within 20 weeks.

Descriptive statistics will include demographics, smoking history, and characteristics of the wound. For comparison nominal categorical secondary outcome variables, we will use Chi-square test. For comparison of ordinal categorical variables, we will use the Wilcoxon rank-sum test (for two independent group comparison) and Kruskal-Wallis test (for three independent group comparison).

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1 BACKGROUND AND RATIONALE

Diabetic foot ulcers (DFU), chronic, non-healing wounds, pose a serious problem to our healthcare system. Those with DFU are at higher risk for developing infections, which lead to limb amputations. The long-term effects are detrimental on quality of life and productivity loss. Within the VA system, diabetics with foot ulcers account for significant amount of sums of money to treat wounds and to improve healing in DFU. Thus far, studies have shown that the current standard of care to heal wounds is about 10-20% slower after 12 weeks of treatment compared to advanced therapy regimens. This unfavorable cure rate has prompted increased research for therapeutic alternatives, and novel approaches including topical application of growth factors, extracellular matrix scaffold materials and bioengineered living tissue replacements have been developed. This study plans to address the question of the comparative effectiveness of wound care modalities that could potentially translate into millions of dollars of savings for our health care system, as well as simplification of the treatment regimens for our patents—all without sacrificing clinical outcome. We propose to compare the clinical efficacy of two of the most expensive novel treatments for DFU, bioengineered cell-based bioengineered extracellular matrix tissues containing living dermal fibroblasts (Dermagraft®) to a device that is 15-fold less expensive but has related biologic rationale—an extracellular matrix scaffold devoid of living cellular elements (Oasis®). We hypothesize that these three treatments will yield equivalent clinical outcomes, supporting the adoption of the less expensive, cell-free, matrix device. The importance of this study would then be to establish a protocol approach to wound care that would be implemented system wide.

2 OBJECTIVES

The primary objective of this study is to assess the effectiveness of cellular dermal replacement tissue vs. non-viable extracellular matrix (ECM) for the treatment of non-healing diabetic foot ulcers. The primary endpoint will be the measurement of wound closure at 12 weeks, secondary outcomes will be measured at 20 weeks and the rate of healing in each treatment arm.

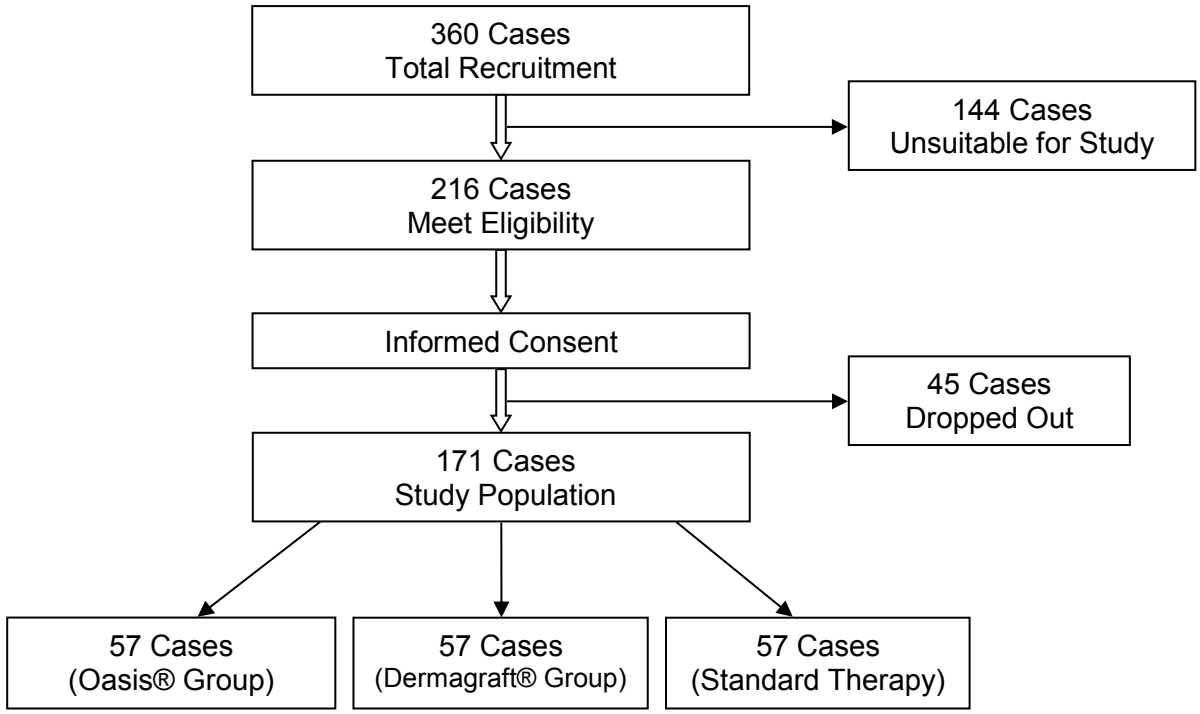
The secondary objective of study is to determine cost effectiveness of the bioengineered cellular vs. acellular ECM products. Cellular ECMs (Dermagraft®) are costly devices, $1800 per application, difficult to use and time sensitive (weeks). The acellular ECM (Oasis®) is easier to use and at a cost $80 per application and has a longer shelf life (2 years). These devices typically require multiple applications, on a weekly basis. If the findings show that these devices are equivalent, then the use of acellular ECM would lead to significant savings for the VA. Genetic markers of non-healing vs. healing diabetic ulcers will also be investigated, which will be useful information in predicting healing potential.

3 STUDY POPULTATION

The study population includes all Veterans' between the ages of 18-85, with a documented diagnosis of diabetes and a foot ulceration that has existed for at least 4 weeks at the time of enrollment into the study. Diabetic patients with foot ulcers will be selected from a random sample of patients from six clinical sites at the Veteran Affairs Northern California Health Care System (VA NCHCS): Mather, McClellan, Fairfield, Martinez, Chico and Redding. There were approximately 25,815 patients with diabetes mellitus receiving care at VA NCHCS within the last 2 fiscal years.[1] Of these, 893 patients were treated with diabetic foot ulcerations.[2] Figure 2 illustrates the selection process with the anticipated number of participants in the study. With a minimum of 60% participation, there should be adequate recruitment for this study. The sites will accrue a total target sample of approximately 360 subjects and assuming approximately 20% dropout rate, the goal is to enroll 171 subjects in total from all sites involved in study.

Selection of Cases and Controls:
The investigators will query the CPRS/VISTA database at the 6 respective sites for eligible participants. Patients will be screened at the various VA clinics as potential study subjects which will be called by the research nurse and invited to participate in the study. Those who agree will be given an appointment for screening and enrollment.

FIGURE 2: Summary of Selection Process



Enrollment/Study Groups:
Participants that are recruited who have met the inclusion criteria and agree to join the study will be required to sign an informed consent, which has been approved by the Institutional Review Board (IRB). The selected cases will be randomly stratified matched by age, gender, race, and date of diagnosis with diabetes and size of ulceration.

Run-in phase in which all potential subjects are given the standard of therapy for 2 weeks, will be performed to determine compliance with return visits and performing standard of care dressings.

Each subject will be given a unique identifier that will have no personal information linked after assignment of a randomly computer generated list of three repeated numbers which will be available for each site. The request for assignment will be emailed to the Data Coordinator with a completed eligibility form, and no personal identifiers except for initials and a last 4 digits of social security. The Data Manager will assign a number and the next sequential group assignment with each subsequent request will be placed in sealed envelopes. Subject demographics will not be provided until this assignment is complete. Participants will be randomly placed into the standard therapy, Oasis® or Dermagraft® groups. There will be an estimated of 57 patients in each group.

The patients will not be informed of treatment administered. However, due to the handling of the Dermagraft® (packaging and thawing), whereas Oasis® is readily available at room

temperature, the Investigator/clinician will be able to identify the two treatments. Therefore, to avoid assessment bias, we will have an Inter-observer that will be blinded to treatment to perform all follow-up wound evaluations and record measurements. Following measurement/wound assessment, the treatment will be performed by another assigned clinician, which will not the involvement of the Inter-observer.

Withdrawal:
The subjects may withdraw from the study at any time during the study, upon request. The investigator may also discontinue a subject if it is determined that the subject has not responded to therapy or adverse event occurred, which requires alternative therapy. Those subjects that have undergone treatment will be followed and documented until end of the study. If there is more than 10 percent loss to follow-up, then investigators will attempt to recruit additional subjects.

4 STUDY PROCEDURES

4.1 DESIGN AND METHODOLOGY

We propose a randomized, single blinded, three-armed controlled clinical equivalence trial comparing the effectiveness of two intervention arms (acellular ECM, Oasis® and cellular ECM, Dermagraft®) and a standard treatment arm to address the problem of efficacy and cost effectiveness among synthetic skin grafts in treating DFUs in the VA system

4.2 DESCRIPTION OF STUDY GROUPS

Standard (Conventional) Therapy:
Standard therapy will consist of the typical foot evaluation and care provided by the Department of Veteran Affairs facilities, which will include weekly sharp debridement, cleansing of wound with normal saline, and application of non-adhesive dressing (Adaptic® or Mepitel®), Iodosorb® gel over wound bed, covered by dry dressings. The investigator will document the standard care provided during clinic visits including weekly follow-up evaluations. Offloading will be standardized, which will involve placement in modified offloading insert (trilaminar plastazote) and removable Cam Walker boot.

Oasis®:
Patients in the Oasis® group will undergo the same wound care and offloading modality as the Standard Therapy group, with weekly wound evaluation dressing changes. The Oasis® matrix will be applied on the wound (cut to size), moistened with normal saline to assure adherence to wound bed, then apply non-adhesive dressing (Adaptic® or Mepitel®), moist sterile gauze, dry and gauze dressings. Up to 8 weekly applications will be applied to wound until healed.

Dermagraft®:
Patients in the Dermagraft® group will receive the same Standard Therapy, with weekly wound evaluation and dressing changes. The Dermagraft® will be thawed as directed by the manufacturer, then under sterile technique Dermagraft® will be cut to size of wound, covered with non-adhesive coverage (Adaptic® or Mepitel®), moistened sterile gauze and dry dressings. Up to 8 weekly applications will be applied to wound until healed.

4.3 STUDY FLOW CHART

Study Plan Schematic

Procedure	Visit 1 (Week -2)	Visit 2 (Week -1)	Visit 3 (Week 0) Randomization	Visits 4-10 (Week 1-7) Treatment	Visits 11-18 (Week 8-15) Treatment	Visit 19 (Week 16) Study Endpoint	Visits 20-23 (Week 17-28) Follow-Up
Informed Consent	X						
Inclusion/Exclusion Criteria	X	X	X				
Physical Exam	X					X	
Vital Signs	X	X	X	X	X	X	
Ankle-Brachial Index	X						
Fungal Infection Culture and Malignancy Evaluation	X						
Blood Sample Drawn for Labs	X						
SF-36v2™, EQ-5D, QALYs Questionnaires	X						Visit 20 only
Ulcer Assessment	X	X	X	X	X	X	X
Debridement	X	X	X	X	X	X	X
Ulcer Photography and Area Measurement	X	X	X	X	X	X	X
Randomization			X				
Dermagraft® Tx + SOC			X	X			
Oasis® Tx + SOC			X	X			
SOC	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X

4.3.1 Screening/Baseline Period (Visit 1, 2/Week -2, -1)

Subjects that meet all criteria of the study will be given an informed consent form to review and sign. Subjects will be required to complete all screening assessments within the 2 weeks prior to randomization and start of treatments.

Screening assessments and pre-treatment:

1) Demographic information: gender, age, race, socio-economic status.
2) Medical history: medical problems, surgeries, trauma, history of previous ulcers, amputations, characteristic and duration.
3) Physical examination: vital signs, height, weight, Body Mass index
4) General health and lifestyle: smoking history, alcohol, drugs abuse, regular physical activity.
5) Lower extremity exam: vascular - pedal pulses, color of skin, temperature, edema; dermatological – clinical description of the ulcer (staging per UT wound classification), fungal infection of skin and/or nails, skin integrity (calluses, dryness); musculoskeletal – foot deformities such as bunion, hammertoe, bony prominence, fat pad atrophy, altered gait; neurological – absence or presence of sensation with 5.07/10 Semmes-Weinstein monofilament, reflexes.
6) Non-invasive vascular study: ankle-brachial systolic pressure (ABI) and toe-brachial systolic pressure (TBI). In order to meet criteria, ankle-arm index must be equal to or greater than 0.8 and less than 1.4 or a toe-arm index is equal to or greater than 0.6.
7) Foot ulcer history (location, length of time, treatments used, pain, etiology of ulcer).
8) Laboratory: Hga1c, chemistry, CBC, pregnancy test (for women of childbearing ages), LFT, ESR, CRP and albumin.

9) Radiological imaging – plain foot and/or ankle films for baseline.
10) Subjects will be given the health-related quality of life survey, the Medical Outcomes Study 36 item Short Form (SF-36v2™) health survey.
11) Sharp debridement of ulcer will be performed per standard method. A small sample will be collected for microbiology (gram stain, cultures/sensitivities, and fungal) and pathology.
12) Photographs of ulcer will be obtained before and after debridement, using Silhouette Mobile™ ulcer tracing, surface area calculation.
13) Dressings applied will include non-adhesive dressing (Adaptic® or Mepitel®), Iodosorb® gel over wound bed, covered by dry dressings.
14) Off-loading shoes will be given, modified offloading insert (trilaminar plastazote) as determined appropriate per discretion of clinician.

4.3.2 Randomization (Visit 3/Week 0)

Those subjects that meet all eligibility criteria will be randomly assigned to one of the three groups. A randomized number will be assigned to each subject participating in the study, where one will receive either Dermagraft®, Oasis® or the control (standard therapy).

4.3.3 Treatment Phase (Visits 4 to 18/Weeks 1 to 15)

Subjects will be evaluated and receive treatments on weekly basis (7 days +/- 2 days). Appointments will be made as determined by investigator at each VA facility.

- At each visit, vital signs will be obtained and recorded.
- The ulcer will be assessed – visual inspection – complete ulcer characteristics form (see appendix).
- If there is concern for infection after evaluation of ulcer, then a confirmatory bacterial culture will be obtained (deep swab culturette or tissue specimen/biopsy). Radiographical xrays and blood work (CBC, ESR, CRP, and Chem) may also be performed if deemed necessary by investigator clinician.
- Next, a digital image will be taken one image before debridement and one image immediately after debridement (camera will capture dimensional size of ulcer). The images will be stored on a central server, with unique identifier as well as loaded on CPRS Vista imaging.
- Surgical debridement of ulcer will be performed using curette or blade. Clinician should debride wound to promote mild bleeding from ulcer site and removal of necrotic or fibrinous tissue and excess hyperkeratotic margins if possible. Clinician may use lidocaine topical jelly/ointment if ulcer is too tender to debride.
- All wound beds will be cleaned gently with normal saline 0.9%.
- Application of treatments:
    - Standard (Conventional) Therapy:
      Application of non-adhesive dressing (Adaptic® or Mepitel®), Iodosorb® gel over wound bed, covered by dry gauze dressings.
      Oasis®:
      The Oasis® matrix will be applied on the wound (cut to size using scissors), moistened with normal saline to assure adherence to wound bed, then apply non-adhesive dressing (Adaptic® or Mepitel®), moist sterile gauze, dry and gauze dressings. Up to 8 weekly applications will be applied to wound until healed.
      Dermagraft®:
      The Dermagraft® will be thawed as directed by the manufacturer, then under sterile technique Dermagraft® will be cut to size of wound using scissors,

covered with non-adhesive coverage (Adaptic® or Mepitel®), moistened sterile gauze and dry dressings. Up to 8 weekly applications will be applied to wound until healed.

- Offloading devices:
    - Plantar foot ulcers – offload with post operative style shoe (with plastazote cut out (aperature or extravasation) as necessary to offload ulcer pressure area.
    - Heel ulcers – use multi-podus splint or Rooke boot.
- Changes in concomitant medications, adverse events, compliance to offloading, will be recorded.
- If ulcers heal sooner than 8 weeks, the subject will be assessed the following week. Ulcer healing is defined by 100% epithelization as determined by the clinician. The subject with healed ulcer will also return for follow-up visits Weeks 9, 16, 20.

  NOTE: if ulceration remains open after 8th application – then will switch back to standard of care therapy (if budget allows, may consider extending graft course).

4.3.4 Study Endpoint (Visit 19/Week 16)

- Subjects to complete the health-related quality of life survey, the Medical Outcomes Study 36 item Short Form (SF-36v2™) health survey.
- Vital signs will be obtained and recorded.
- The ulcer will be assessed – visual inspection – complete ulcer characteristics form (see appendix). Determine if reaches 100% epithelization determined by clinician.
- Labs drawn from each subject, hematological and chemistry panel. Pregnancy test of childbearing age.
- If concern for infection, confirmatory bacterial culture will be obtained (deep swab culturette or tissue specimen/biopsy). Radiographical x-rays and blood work (CBC, ESR, CRP, and Chem) may also be performed if deemed necessary by investigator clinician.
- Digital imaging of ulcer location, if remains non-healing then 2 images to be obtained prior to and after surgical debridement.
- Surgical debridement of ulcer if not healed, will be performed using curette or blade and standard therapy.
- Changes in concomitant medications, adverse events, compliance to offloading, will be recorded.

4.3.5 Follow-Up Phase (Visits 20 to 23/Weeks 17 to 28)

- The ulcer will be assessed – visual inspection – complete ulcer characteristics form (see appendix). Determine if reaches 100% epithelization by clinician.
- Digital imaging of ulcer location, if remains non-healing then 2 images to be obtained prior to and after surgical debridement.
- Surgical debridement of ulcer if not healed, will be performed using curette or blade and standard therapy.
- Changes in concomitant medications, adverse events, compliance to offloading, will be recorded.

5 ADVERSE EVENTS

All adverse events reported spontaneously by the subject/or in response to questioning or observation by the investigator will be recorded.

Reporting of Adverse Events:
The Investigator will be responsible for assessing the relationship of the adverse event to the investigational product, and the seriousness and expectedness of the adverse event at the time of occurrence. All adverse events that occur during the trial will be documented.

AE's/SAE's reported during the study, or SAE's reported within 30 days of the end of the study, should be followed to resolution of the AE/SAE or, within thirty days from the end of the study, a further and final assessment of the outcome should be made. Each AE will be categorized as "serious" or "not serious" based on the definition of an SAE. An SAE is defined as an AE resulting in at least one of the outcomes described in the sections below.

The severity of AEs will be classified as "mild", "moderate", or "severe", based on the following definitions:

- Mild: Awareness of sign, symptom, or event, but easily tolerated; does not interfere with usual daily activities or tasks.
- Moderate: Discomfort enough to cause interference with usual daily activity; may warrant therapeutic intervention.
- Severe: Incapacitating; inability to perform usual activities and daily tasks; significantly affects clinical status; requires therapeutic intervention.

Adverse events will be assigned a relationship (causality) to the study products. The Investigator will be responsible for determining the relationship between an AE and the study product. The type of event, organ system affected, and timing of onset of the event will be factors in assessing the likelihood that an AE is related to the treatment. Relationship of AEs to study products will be classified as follows:

- Not Related: No relationship exists between the AE and the treatment. The event is attributed to a pre-existing medical condition or an intercurrent event unrelated to the study product.
- Possibly Related: Follows the treatment, but may have developed as a result of an underlying clinical condition or treatments/interventions unrelated to the study product.
- Probably Related: Follows the treatment, but is unlikely to have developed as a result of the subject's underlying clinical condition or other treatment or other interventions.
- Definitely Related: Follows the treatment and physical evidence shows a convincing relationship to the treatment.
- Unknown: Follows the treatment, but unable to determine the relationship to the treatment.

Subject Follow-up:
Subjects who experience an AE will be followed until the AE has resolved, if possible.

Serious Adverse Events:
A serious AE (SAE) will be defined as any untoward medical occurrence that occur after signing the informed consent until the Final Evaluation that:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,

- Is a congenital anomaly/birth defect,
- Other (event not covered by SAE categories but in the investigator's opinion, should be considered serious).

Important medical events that may not result in death, be immediately life threatening, or require hospitalization may be considered an SAE when, based upon medical judgment, they may jeopardize the subject and require intervention to prevent one of the outcomes listed above.

Reporting Serious Adverse Events:
The Investigator must report all SAEs (including any subject deaths) occurring during the study (from informed consent until the Final Evaluation). Once the Investigator becomes aware of an SAE, he/she must e-mail (preferred method of communication) or telephone the IRB within 24 hours.

A written report must follow within 48 hours of the time the Investigator learned of the event. This written report must include a full description of the event and all supporting documentation available at that time (e.g., lab reports, electrocardiogram [ECG] reports, etc.). Additional follow-up information must be reported to the Sponsor on the appropriate form as it becomes available and/or upon Sponsor request.

Data Safety Monitoring Board:
Since this is an open-label trial, no data safety monitoring board will be established.

Stopping Criteria:
The emerging clinical and safety data will be reviewed during the trial, and as a result of this review it may be necessary to terminate the study before all patients have completed the protocol. In such circumstances subjects will be followed up for safety assessment.

6 STATISTICAL DESIGN

Measures:
Measures will be collected at baseline, weekly thereafter, with primary endpoint of complete healing at Week 12, and final secondary endpoint measurement at Week 20. The ulcer will be assessed weekly and will be treated as described above. A designated inter-observer will be at each of the testing sites, (blinded to the treatment of the subjects) which will perform all measurements. The following measurements will be taken: size (surface area calculation), photos (digital includes ruler) and complete foot ulcer assessment (description of ulcer/characteristics), before and after debridement. Subjects where ulceration healed earlier than Week 12 will have follow-up assessment one week after healing, then at Week 12 and 20. If ulcerations healed after 12 weeks, then the subjects will be assessed at one week after healing and at Week 20. There will be 4 follow-up visits (Weeks 17, 20, 24, 28), assessed every 4 weeks for a total of 16 weeks.

During the study, if ulceration appears to be infected as determined by clinical presentation characterized by the presence of two or more signs of inflammation,[3] the clinician will follow the protocol of obtaining wound culture for microbiology, radiographs, blood work (CBC, chemistry panel, sedimentation rate, and C-reactive protein), and treatment immediately with appropriate antibiotics. Subjects with infected ulcers will be withdrawn from study and investigators will be notified. In addition, adverse events will be well documented and treated per protocol, and subjects will be withdrawn from study.

Clinical Outcomes:

- The percentage of subjects that have complete closure of ulceration by Week 12 and Week 20. Complete healing will be defined as full re-epithelization with no drainage, or callus formation and remains closed after 1 week of wound closure.
- Rate of wound healing to achieve complete closure. Weekly measurements of surface area will be recorded on a grid marked acetate sheet, using a metric ruler to determine length and width of the wound.
- A diabetic foot ulcer assessment form will be filled out at weekly follow-up visits, that describes characteristics of ulceration including peri-ulcer erythema, wound margins, fibrin, granular tissue, peri-ulcer pruritus, edema and location, pain, amount of drainage and type, amount of epithelization, pain level, depth, and bioburden.
- Number of incident subjects with infection, osteomyelitis and acute Charcot during the study, will be withdrawn from the study.
- Number of incidence of adverse effects will be reported from all sites, and will be withdrawn from the study.
- Veterans' Short Form Health Survey (SF-36v2^{TM}), a self-administered questionnaire will be used to measure quality of life, given at beginning and at the end of the study.
- EuroQol Questionnaire (EQ-5D) to evaluate overall quality of life and to facilitate the calculation of health utilities and quality adjusted life years (QALYs). The EuroQol questionnaire (EQ-5D) will be administered at beginning and at the end of the study.

Sample Size Estimation:

The sample size was estimated based on 80 percent power, significance level 0.05, to detect a difference in the incidence of ulcer closure of the two study groups and the standard of care group (Stplan4.3).[4] It is expected that 50% of the Dermagraft® and Oasis® groups will reach complete ulcer closure by Week 12 and approximately 25% closure with those subjects that received the standard of care (numbers based on previous studies as described previously). We would expect the difference to be 25% between the standard and the treatment group. By using a Chi-square test without continuity correction test would require 57 subjects in each group. If we estimate the difference to be 20% and lower of the cure rates to be 30%, then we would require 88 patients in each group.

Analyses:

The primary end point is wound closure by Week 12, which will be analyzed using Chi-square test to compare the percentages of subjects with complete closure in each group. We will also perform exploratory analysis with logistic regression, using the complete closure at 12 weeks as the dependent variable and the independent variables would include, treatment, size of ulcer, location on foot, how long ulcer has been present, previous revascularization (will analyze as subset group), offloading compliancy, concomitant medications, foot deformity, history of amputation, diabetes complications index, and glucose control (HbA1c). Selection of covariates will be performed by stepwise modeling procedures.

We will perform a cost-effectiveness analysis comparing the treatment groups Dermagraft®, Oasis® and the standard care group at the NCHCS using ANOVA or Kruskal-Wallis test depending if data is normally distributed for comparison of the three treatment arms. If significant, we will further analyze with pair-wise comparisons by using t-test or Wilcoxon rank-sum test with the Bonferroni correction to control for type I experiment-wise error rate of 0.05. A p-value less than 0.05/3 =0.0167 will be considered statistically significant.

We will evaluate the cost-effectiveness of the Oasis® compared to Dermagraft® and the standard of care by measuring QALYs as the measure of effectiveness, based on results obtained from SF-36v2™ questionnaire. Multiple regression will be used to allow to evaluate continuous dependent variable outcomes, including change scores between baseline and end of study physical (PCS) and mental (MCS) component summaries from the SF-36v2™ questionnaire.

Rates of healing among the three groups will be analyzed using log-rank test to compare time of healing within 20 weeks among the 3 treatment groups.

Secondary endpoint (complete healing at 20 weeks), recurrence ulceration rate at 20 weeks will be analyzed using Chi-square tests. In addition, demographics, smoking history, and characteristics of the wound will also be summarized in table. Data that are continuous variables will be summarized by using mean, median, standard deviation, coefficient of variation, minimum and maximal values. Frequency tables will summarize categorical variables.

Timeline and Milestones:

	Year 1		Year 2	
Months	1-6	7-12	1-6	7-12
Aim 1: Recruitment				
Step 1: Screening (2-week run-in phase)	✓	✓	✓	
Step 2: Randomization/Assignment	✓	✓	✓	
Aim 2: Data Collection				
Step 1: Healed by Week 12	✓	✓	✓	✓
Step 2: Wound not healed by Week 12, continue in study until Week 20	✓	✓	✓	✓
Step 3: If wound has not reached closure beyond the 8 weeks of Dermagraft® – will switch to standard of care (expensive). Oasis® – continue to the end of study	✓	✓	✓	✓
Aim 3: Data Analysis				
Step 1: Outcome effectiveness If outcome show significant difference will cross over for intent-to-treat	✓	✓	✓	✓

7 References

1. Vista/CPRS data record system from 2006-2008, retrieved on 4/7/2009.
2. Vista/CPRS data record system from 2006-2008, retrieved on 4/7/2009.
3. Lipsky BA, Berendt AR, Deery HG Diagnosis and treatment of diabetic foot infections. Clinical Infectious Diseases: An official publication of the infectious diseases society of America. 2004; 203:885-910.
4. Stplan4.3 Statistical Software, Department of Biostatistics and Applied Mathematics, University of Texas M.D. Anderson Cancer Center, 2006.

8 APPENDIX

Ulcer Characteristic Form

Size: Dimensions _____x_____x_____cm

Location of Foot Ulcer:

A. Dorsal foot
B. Plantar digits (1, 2, 3, 4 or 5)
B. Plantar forefoot (excludes digits)
C. Plantar midfoot (cuneiform, navicular level)
D. Plantar heel

Wound Bed/Base:

Percentage of Granulation (Red) Tissue

A. Bright, beefy red; 75% to 100% of wound bed
B. Bright, beefy red; Greater than 50% to less than 75% of wound bed
C. Pink, dull red; Greater than 25% to less than 50% of wound bed
D. No granulation tissue present and/or less than 25% of wound bed

Percentage of Fibrinous (Yellow) Tissue

A. None
B. Less than 25% of wound bed
C. 25% to less than 50% of wound bed
D. 50% to less than 75% of wound bed
E. 75% to 100% of wound bed

Percentage of Necrotic (Black/Eschar) Tissue

A. None
B. Less than 25% of wound bed
C. 25% to less than 50% of wound bed
D. 50% to less than 75% of wound bed
E. 75% to 100% of wound bed

Epithelialization (appearance of skin – may appear pink or red skin):

A. Less than 25% of wound bed
B. 25% to less than 50% of wound bed
C. 50% to less than 75% of wound bed
D. 75% to less than 100% of wound bed
E. 100% all intact skin

Wound Edges/Margins:

A. Healed
B. Greater than 50% advancing epithelialization of wound edges
C. Less than 50% advancing epithelialization of wound edges
D. No advancing border of epithelium

Depth:

A. None – intact skin
B. Partial thickness involving skin loss of epidermis and/or dermis
C. Full thickness involving skin loss of epidermis/dermis and subcutaneous layer

D. Tendon/joint capsule exposed
E. Probes to bone

Peri-wound Erythema:
A. Normal, no appearance of redness/pinkness
B. Mild pinkness surrounding wound
C. Bright red and/or blanches to touch
D. Pallor, pale
E. Violaceous, dusky red and or non-blanchable
F. Black, necrosis

Peri-wound Edema:
A. None
B. Minimal edema
C. Non-pitting edema within 4 cm of wound
D. Non-pitting edema extends beyond 4 cm of wound
E. Pitting edema within 4 cm of wound
R. Pitting edema extends beyond 4 cm of wound

Drainage/Exudate:
A. None
B. Serosanguinous
C. Serous
D. Seropurulent
E. Purulent

Amount of Drainage/Exudate:
A. None
B. Scant (less than 1 ml)
C. Mild (Less than 25% of dressing saturation)
D. Moderate (50% to less than 75% of dressing saturation)
E. Copious (75% or more of dressings saturated)

Tenderness/Pain at Wound Site (pain scale 0-10):
A. None
B. 1 to 2
C. Greater than 3 to 4
D. Greater than 4 to 7
E. 8 to 10

Statistical Analysis Plan

Analyses: The primary outcome is wound closure by Week 12, which will be analyzed using Chi-square test to compare the percentages of subjects with complete closure in each group. We will also perform exploratory analysis with logistic regression, using the complete closure at 12 weeks as the dependent variable and the independent variables would include, treatment, size of ulcer, location on foot, how long ulcer has been present, previous revascularization (will analyze as subset group), offloading compliancy, concomitant medications, foot deformity, history of amputation, diabetes complications index, CRP level, and glucose control (HbA1c). Selection of covariates will be performed by stepwise modeling procedures, and overseen by biostatistician Dr. Li.

Cost effectiveness analysis: We will perform a cost-effectiveness analysis comparing the treatment groups Dermagraft®, Apligraf®, Oasis® and the standard care group at the NCHCS using ANOVA or Kruskal-Wallis test depending if data is normally distributed for comparison of the three treatment arms. If significant, we will further analyze with pair-wise comparisons by using t-test or Wilcoxon rank-sum test with the Bonferroni correction to control for type I experiment-wise error rate of 0.05. A p-value less than 0.05/3 =0.0167 will be considered statistically significant.

We will evaluate the cost-effectiveness of the Oasis® compared to Dermagraft® or Apligraf® and the standard of care by measuring QALYs as the measure of effectiveness, based on results obtained from SF-36v2™ questionnaire. Multiple regression will be used to allow to evaluate continuous dependent variable outcomes, including change scores between baseline and end of study physical (PCS) and mental (MCS) component summaries from the SF36v2™ questionnaire.

Rates of healing among the four groups will be analyzed using log-rank test to compare time of healing within 20 weeks.

Secondary outcomes such as complete healing at 20 weeks, recurrence ulceration rate at 20 weeks will be analyzed using Chi-square tests. In addition, demographics, smoking history, and characteristics of the wound will also be summarized in table. For comparison nominal categorical secondary outcome variables, we will use Chi-square test. For comparison of ordinal categorical variables, we will use the Wilcoxon rank-sum test (for two independent group comparison) and Kruskal-Wallis test (for three independent group comparison).