

Developing strategies for effective debridement in patients for venous leg ulcers

IRB Number: 20180256

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Clinical Trial Protocol Statement of Compliance

This clinical trial shall be conducted in compliance with the protocol, as referenced herein, and all applicable local, national, and international regulatory requirements to include, but not be limited to:

- **International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (GCP)**
- **Ethical principles that have their origins in the Declaration of Helsinki**
- **Food and Drug Administration (FDA) Code of Federal Regulation (CFR):**
 - **Title 21CFR Part 50 and 45 CFR Part 46, Protection of Human Patients**
 - **Title 21CFR Part 54, Financial Disclosure by Clinical Investigators**
 - **Title 21CFR Part 56, Institutional Review Boards**
 - **Title 21CFR Part 312, Investigational New Drug Application**
 - **Title 45 CFR Parts 160, 162, and 164, Health Insurance Portability and Accountability Act (HIPAA)**

As the Principal Investigator, I understand that my signature on the protocol constitutes my agreement and understanding of PI responsibilities to conduct the clinical trial in accordance to the protocol and applicable regulations. Furthermore, it constitutes my understanding and agreement that any changes initiated by myself, without prior agreement in writing from the Funding Agency, shall be defined as a deviation from the protocol, and shall be formally documented as such.

I understand that my signature constitutes agreement and understanding of acceptance of the defined responsibilities of an Investigator as defined by the protocol, applicable FDA Regulations, and/or business contracts, but does not in any capacity relieve me of my responsibilities as the Investigator. Additionally, my signature constitutes my understanding and agreement that any changes to the protocol shall be implemented timely with my review and approval prior to implementation.

INVESTIGATOR'S STATEMENT

I have reviewed the protocol and agree to conduct this study as outlined in the protocol and in compliance with ICH/GCP Guidelines.

I have read and agree to follow the Study procedures as outlined.

Print Name of PI

PI's Signature

Date

Print Name of PI

PI's Signature

Date

CLINICAL PROTOCOL SYNOPSIS

Title of Trial:	Developing strategies for effective debridement in patients for venous leg ulcers	
UM IRB Number:	20180256	
Principle-Investigator:	Marjana Tomic-Canic, PhD	
Trial Duration:	5 years	Phase of Trial: N/A
Trial Population:	Venous leg ulcers	
Trial Centers:	University of Miami	
Objectives:	<p><u>Primary:</u> Change in the genetic profile after debridement in the intervention group and how that correlates to wound healing.</p> <p><u>Secondary:</u> Percent rate of healing after 4 weeks between standard of care control group and debridement intervention group.</p>	
Trial Design:	Randomized, longitudinal	
Number of Patients:	360 patients	
Trial Interventions:	Debridement and standard of care	
Inclusion Criteria:	<ol style="list-style-type: none"> 1. >18 years of age 2. Confirmation of venous disease by non-invasive venous studies with either Doppler-confirmed venous reflux, or having ≥ 2 clinical characteristics of venous insufficiency (varicose veins, lipodermatosclerosis, venous dermatitis, atrophie blanche, edema) 3. have a venous ulcer between the knee and ankle, at or above the malleolus 4. wound size would be greater than or equal to 5cm² in area without exposed tendon, muscle or bone 5. wound duration of at least 6 months 	

	<ol style="list-style-type: none"> 6. VLU containing yellow/white slough with or without fibrous/scar tissue and/or non-viable tissue 7. ability of subject to tolerate limb compression bandage
Exclusion Criteria:	<ol style="list-style-type: none"> 1. history of diabetes mellitus and a HbA1c > 12% (obtained within past 6 months) 2. Ankle brachial index(ABI) less than 0.80 3. any active cancer other than a nonmelanoma skin cancer; any previous cancer must be in remission for at least 5 years 4. suspicion of malignancy within VLU 5. life expectancy <6 months 6. history of kidney disease and creatinine greater than 2.0 (obtained within past 6 months) 7. history of liver disease and liver function test (ALT, AST, ALK PHOS, and bilirubin) >2x upper limit of normal (obtained within past 6 months) 8. requirement for long-term systemic corticosteroids or immunosuppressive therapy, or history of corticosteroid or immunosuppressive use in the 4 weeks prior to study entry 9. history of immunodeficiency 10. ulcers due to none venous etiology and leg ulcers associated with mixed etiology 11. Untreated osteomyelitis 12. Hepatitis 13. acute deep venous thrombosis 14. allergy to lidocaine and/or epinephrine 15. Subject's inability to successfully tolerate compression therapy 16. Skin ulcer previously treated within the last 4 weeks with biologic therapies (e.g. cell therapy or growth factors) 17. if currently incarcerated 18. known pregnancy
Statistical Methodology:	<p>With a 2:1 ratio of sample size 360, significance level of 0.05, two-sided, to achieve 80% power, minimum 28% overall healing for the intervention group is needed, which requires a minimum 21% intervention group will show changed gene profile.</p>

ABBREVIATIONS

- Adverse Event – AE
- Alanine Aminotransferase – ATL
- Alkaline Phosphate – ALK
- Ankle Brachial Index – ABI
- Aspartate Aminotransferase – AST
- Case Report Form – CRF
- Code of Federal Regulation – CFR
- Data Safety Monitoring Plan – DSMP
- Diabetic Foot Ulcer – DFU
- Echocardiogram – ECG
- End of Therapy –EOT
- False Detection Rate – FDR
- Food and Drug Administration – FDA
- Good Clinical Practice – GCP
- Health Insurance Portability and Accountability Act – HIPAA
- Hemoglobin A1c – HbA1c
- Informed Consent Form – ICF
- Institutional Review Board – IRB
- Internal Conference on Harmonization – IHC
- National Institute of Arthritis and Musculoskeletal and Skin Diseases – NIAMS
- National Institutes of Health – NIH
- Over-the-counter – OTC
- Polymerase Chain Reaction – PCR
- Principle Investigator – PI
- Protected Health Information – PHI
- Protocol Deviation – PD
- Quality Assurance - QA
- Rosenstiel Medical Science Building – RMSB
- Safety Officer – SO
- Serious Adverse Event – SAE
- Trial Master File – TMF
- Unexpected Problem – UP
- University of Miami – UM
- University of Miami Hospital – UMH
- Venous Leg Ulcer – VLU

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1. INTRODUCTION

1.1. Background and Rationale for the Trial

Chronic non-healing venous leg ulcers (VLUs) have an intrinsic healing impairment that is associated with dysfunctional gene expression patterns. We have shown that tissue from the non-healing edge of chronic VLUs exhibits characteristic histopathology including epidermal hyperproliferation, dermal fibrosis, accumulation of intracellular pro-collagen and dysregulation of genes involved in epidermal differentiation, migration and proliferation. We have demonstrated that biopsies taken from the non-healing edges of VLUs before and after debridement have distinct morphologies and distinguishable gene expression patterns, providing the biological basis and justification of debridement. These observations are supported by the findings that primary cells grown from tissue biopsies before and after debridement also have distinct and typical genomic patterns, with cells from pre-debridement edge biopsies exhibiting a non-healing phenotype as evidenced by loss of migration and loss of ability to respond to growth factor stimuli. Moreover, we used a genomic approach in a clinical trial to determine mechanism of action of cell based therapy in patients with VLU and identified a specific set of genes responsible for therapeutic reprogramming that shifts non-healing ulcer into acute wound-like healing VLU.

Taken together, preliminary data suggests a loss of healing potential, prior to debridement which is distinct from cells taken from a post-debridement biopsy, which show an ability to migrate and respond to growth factors. Limited clinical data suggests a positive response in wound size reduction in VLUs following wound debridement, but debridement is not performed, routinely, consistently and systematically and thus is often not considered standard of care for VLUs. Furthermore, in contrast to the edge of diabetic foot ulcers (DFUs) where a possible margin of debridement maybe visually delineated by callus at the wound edge, VLUs lack callus and therefore it is much more difficult to assess to what extent debridement should be performed, thereby questioning

its overall benefit and efficacy. Therefore, we hypothesize that genes (their transcription level or protein patterns) can be utilized to guide wound edge debridement in patients with VLUs and further that such guided debridement will improve healing outcome in patients suffering from chronic non-healing VLUs. Moreover, we propose to use genomic profiles to further advance and guide development of PCR-based approach or immunostains that can serve as a simplified diagnostic test to identify debridement margin and non-healing VLUs tissue.

2. TRIAL OBJECTIVES

2.1. Trial Objectives

Primary: The primary objective is the change in the genetic profile after debridement in the intervention group and how that correlates to wound healing.

Secondary: The secondary objective the percent rate of healing after 4 weeks between standard of care control group and debridement intervention group.

3. TRIAL POPULATION

3.1. Inclusion Criteria

1. >18 years of age
2. Confirmation of venous disease by non-invasive venous studies with either Doppler-confirmed venous reflux, or having ≥ 2 clinical characteristics of venous insufficiency (varicose veins, lipodermatosclerosis, venous dermatitis, atrophie blanche, edema)
3. have a venous ulcer between the knee and ankle, at or above the malleolus
4. wound size would be greater than or equal to 5cm^2 in area without exposed tendon, muscle or bone
5. wound duration of at least 6 months
6. VLU containing yellow/white slough with or without fibrous/scar tissue and/or non-viable tissue
7. ability of subject to tolerate limb compression bandage

3.2. Exclusion Criteria

1. history of diabetes mellitus and a HbA1c $> 12\%$ (obtained within past 6 months)
2. Ankle brachial index(ABI) less than 0.80
3. any active cancer other than a nonmelanoma skin cancer; any previous cancer must be in remission for at least 5 years
4. suspicion of malignancy within VLU
5. life expectancy < 6 months
6. history of kidney disease and creatinine greater than 2.0 (obtained within past 6 months)
7. history of liver disease and liver function test (ALT, AST, ALK PHOS, and bilirubin) $> 2x$ upper limit of normal (obtained within past 6 months)
8. requirement for long-term systemic corticosteroids or immunosuppressive therapy, or history of corticosteroid or immunosuppressive use in the 4 weeks prior to study entry
9. history of immunodeficiency
10. ulcers due to none venous etiology and leg ulcers associated with mixed etiology

11. Untreated osteomyelitis
12. Hepatitis
13. acute deep venous thrombosis
14. allergy to lidocaine and/or epinephrine
15. Subject's inability to successfully tolerate compression therapy that is changed weekly
16. Skin ulcer previously treated within the last 4 weeks with biologic therapies (e.g. cell therapy or growth factors)
17. if currently incarcerated
18. known pregnancy

3.3. Discontinuation from Trial Treatment

Patients will be discontinued from trial treatment for any of the following reasons:

- Any patient with an adverse event of grade 3 or higher, determined to be at least possibly related to study drug, will be discontinued from treatment
- Patient requests to withdraw from the trial and discontinue treatment
- Patient requests to discontinue treatment
- Inability of the patient to comply with trial requirements
- Conditions requiring therapeutic intervention not permitted by the protocol
- Inter-current illness (this will be at the investigator's discretion)
- Non-compliance/lost to follow-up

4. TRIAL REGISTRATION

All patients and/or their legal guardians shall receive written and verbal information concerning the clinical trial. This information will emphasize that participation in the clinical trial is voluntary and that the patient may withdraw from the clinical trial at any time and for any reason. All patients will be given opportunity to ask questions and will be given sufficient time to consider before consenting. This trial will be registered on ClinicalTrials.gov.

The signed and dated informed consent to participate in the clinical trial must be obtained prior to any clinical trial related procedure being carried out.

5. TRIAL DESIGN

This is a single center, open label trial that will last up to 4 weeks. A total of 360 patients will be enrolled.

Once eligibility is confirmed, at baseline patients will be randomized in a 2:1 ratio to the interventional arm or the control arm. Patients randomized to the control arm will receive standard of care treatment of their VLU. Standard of care treatment will consist of a four-layer compression therapy (e.g. Profore) which will be changed weekly by the study team. Patients randomized to the intervention arm will receive wound edge debridement in addition to the standard of care. These subjects will also under go specimen collection at the wound edge (2 3mm punch biopsies before and after wound edge debridement) and at control location (1 3mm punch biopsy of the upper thigh; optional). Starting at baseline and continuing weekly for 4 weeks, wound area measurements and photographs will be collected before debridement or standard of care is applied.

A Data Safety Monitoring Plan (DSMP) including a Safety Officer (SO) will be used to monitor safety on an ongoing basis. If adverse events and other safety parameters are acceptable, the study will continue as planned. If a significant safety concerns exists, no further enrollment will occur until a full evaluation has taken place.

6. STUDY RECRUITMENT METHODS

Patients with VLU seen for their routine care at the Dermatology Outpatient Clinics at the University of Miami Hospital or South Miami satellite site will be identified for possible study eligibility by any of the dermatologists in our practice. If the treating dermatologist is a study team member they will ask the patient if they would be interested in being contacted by the study team to learn more about a research study on VLUs. A partial HIPAA waiver is needed, so that if the patient gives permission to be contacted, they will then be approached by a member of the study team while they are in clinic or if that is not feasible then the treating dermatologist will ask if the patient would like to be contacted by telephone. If the treating dermatologist is not a study team member, they will provide the potential subject with the study team's contact information or study flyer so that the patient may contact the study team for more information. If it is then determined that they are interested in participating, they will be scheduled for a visit at our dermatology clinical research site where informed consent and all other study procedures will take place. The risks/benefits of the study will be presented to the subjects, as well as the disclaimer that refusal to participate in the study will in no way, shape, or form alter the type or quality of their care.

A partial HIPAA waiver is also needed as patients will also be recruited from a chart review using research IT and URIDE University of Miami Hospital. Once eligibility is determined, the treating physician of the potential subject will be contacted by the study team and asked to introduce the study during the potential subject's next clinic visit. The treating dermatologist will ask the patient if they would be interested in being contacted by the study team to learn more about a research study on VLUs. If the patient gives permission to be contacted, they will then be approached by a member of the study team while they are in clinic or if that is not feasible then the treating dermatologist will ask if the patient would like to be contacted by telephone. If it is then determined that they are interested in participating, they will be scheduled for a visit at our dermatology clinical research site where informed consent and all other study procedures will take place. The risks/benefits of the study will be presented to the subjects, as well as the disclaimer that refusal to participate in the study will in no way, shape, or form alter the type or quality of their care.

Furthermore, we will announce this study on the Facebook page of the University of Miami Department of Dermatology and Cutaneous Surgery to recruit patients with VLUs. Emails will also be sent to the Miami Society for Dermatology and Cutaneous Surgery list serve and Palm Beach County Society for Dermatology list serve to inform community clinicians about this study. We will also post fliers at designated locations throughout University of Miami buildings, and in our dermatology clinics.

7. DATA AND SPECIMEN BANKING

Study records will be in a locked cabinet in the Dermatology clinical research site (UMH west building, room 504-508, 1321 NW 14 St, Miami, FL 33125).

Collected samples and data will be allotted numeric identifiers that will not be associated with any identifying personal information. These samples and data will be

sent to Dr. Tomic's laboratory (RMSB 2029A, RMSB 6056 1600 NW 10th Ave, Miami, FL 33136) for analyses.

8. DATA MANAGEMENT

The samples will be given a numeric code that will be kept in the password protected file that lists which numeric code corresponds to which sample donor. The subjects' identities as participants in this research study will be kept confidential in any publication or presentation of the results of this study. Additionally, no identifiable patient information will be attributed to the samples, thereby keeping them anonymous. Sample anonymity extends to all areas of this research; therefore, patient identity will not be revealed to collaborators or co-investigators involved in any aspect of the research.

9. SETTING

Study procedures will take place at one of these 4 locations:

- Department of Dermatology's Clinical and Translational Research Unit
University of Miami Hospital
West Building, Suites 504, 505, 506, 508
1321 NW 14th Street
Miami, Florida 33136
- Dermatology Outpatient Clinics
Wound Care Clinic
1295 NW 14th St
South Building, 1st floor
Miami, FL 33136
- Dermatology Outpatient Clinics
University of Miami Hospital
South Building, Suites K, L, M
1295 NW 14th Street
Miami, Florida 33125
- Dermatology Outpatient Clinics
South Miami Satellite
7000 SW 62nd Ave
Penthouse A
Miami, Florida 33143

10. ADMINISTRATION OF TRIAL INTERVENTIONS

10.1. Trial Interventions

Standard of care will include foam dressing (e.g. Mepilex) and a four-layer compression system (e.g. Profore), which will be changed weekly by the study team. This dressing and compression system will be used in all participants.

The intervention arm will include tissue collection and wound debridement. Wound debridement will be performed using a sharp circular disposable dermal 5mm curette (Integra Miltex, New Jersey) with the patient in a reclining or supine position to remove the slough, nonviable tissue, and any fibrous tissue down to the vascular base. Local anesthetics will be applied to the wound 30-45 minutes prior to debridement. Tissue

collection (2 3mm punch biopsies) will be performed at the wound edge before debridement and after debridement, using standard sterile punch biopsy technique. In addition, an optional 3mm punch biopsy of normal, healthy skin will be obtained from the upper inner thigh. After tissue sample collection, patients in the intervention arm will then receive standard of care bandaging and compression mentioned above.

10.2. Duration of Treatment

Once eligibility is determined, patients will be randomized into the control or intervention arms at the baseline visit. Then they will be followed up weekly for 4 weeks.

10.3. End of Trial

The end of the trial is defined as the last visit of the last patient, unless it is necessary to terminate the study for safety concerns before then.

10.4. Prior and Concomitant Medications

No other treatments or bandaging systems should be applied to the treatment area during the course of the study. All medications, including over the counter drugs (OTCs) and nutritional supplements, taken during the preceding 4 weeks will be record at Baseline. Thereafter, a record of all medications, including OTCs and supplements, taken during the course of the study will be made. Information regarding the total daily dose, route of administration, start and discontinuation dates, and indication will be recorded on the patient's paper CRF.

11. TRIAL ASSESSMENTS AND TREATMENT

Procedure	Baseline	Follow Up Visit 1 (±2 Days)	Follow Up Visit 2 (±2 Days)	Follow Up Visit 3 (±2 Days)	Follow Up Visit 4 (±2 Days)
Informed Consent/ HIPAA form	X				
Inclusion/ Exclusion	X				
Demographics	X				
Medical History	X				
Concomitant Meds	X	X	X	X	X
Randomization	X				
Ulcer History	X				
Ulcer Photographs	X	X	X	X	X
Ulcer Measurement	X	X	X	X	X

Pre-Debridement Sample Collection ^a	X				
Debridement ^a	X				
Post-Debridement Sample Collection ^a	X				
Control Sample Collection ^{a,b}	X				
Ulcer Standard of Care Dressing Applied	X	X	X	X	X
Ulcer Standard of Care Compression Applied	X	X	X	X	X
AE/SAE Collection	X	X	X	X	X

^a Intervention arm only; ^b optional

11.1. Overview

All patients should visit the trial center on the days specified within this protocol. The complete Schedule of Assessments for this trial is shown in Section 8.

11.2. Baseline/Randomization

Following signed informed consent, obtained in the presence of the investigator or designee, the eligibility criteria will be reviewed.

At the baseline visit, patients will undergo assessments (demographics, medical history, ulcer history, ulcer measurements, and concomitant medications) to confirm eligibility prior to randomization. Once eligibility is confirmed the subject will be randomized to the control or interventional treatment arm.

All subjects will undergo wound photography and measurements (Silhouette system) before receiving debridement and/or standard of care dressing and compression.

If randomized to the interventional arm, subjects will undergo tissue collection and wound debridement. Tissue collection will be 2 3mm punch biopsies from the wound edge pre- and post-debridement. An optional 3mm punch biopsy of normal, healthy skin can be collected from the upper thigh. Wound photography and measurement will also be collected post-debridement. Patients in the interventional arm will then receive the same standard of care dressing and compression as the control arm.

11.2.1. Randomization

A 2:1 ratio of intervention to control treatment randomization scheme will be used, with one third (n=120) randomized into the control group that will receive only standard of care wound dressing and compression and the other two thirds (n=240) into the intervention group that will receive wound debridement and tissue sample collection

along with standard of care wound dressing and compression.

A predetermined computer-generated randomization scheme will be contained in a sealed envelope. The randomization envelopes will be sequentially numbered and will be opened in sequential order. Prior to treatment the ulcer (e.g., debridement and biopsy collection) the correct randomization envelope shall be opened to reveal the treatment assignment, control or intervention.

11.2.2. Skin Sample Collection

Under sterile conditions, wound bed will be anesthetized using topical local anesthetic Emla cream, and the wound edge skin using lidocaine 1% with epinephrine (1:100,000) and buffered with sodium bicarbonate (9:1). Using a 3mm punch biopsy, two tissue samples per location will be taken from the edge of the wound at beginning and end of debridement at the initial visit. One 3mm punch biopsy will also be taken from the thigh.

Based on the PI and study team's judgment, hemostasis of the wound edge biopsies will be done with suture, gel foam, or standard of care wound compression/dressing. The thigh biopsy will be closed with sutures or gel foam. The research team will provide the subject with proper aftercare and suture removal instructions.

Tissue biopsies will be immediately stored in the RNAlater solution (Ambion) to avoid RNA degradation until histological evaluation of frozen OCT embedded biopsies. Once biopsies are evaluated by histology for presence/absence of marked hyper-proliferation, hyper- and para-keratosis, and presence of dermal fibrosis we will proceed with RNA isolation.

11.3. Post-Treatment Follow-up Visit

All patients will be seen weekly for 4 weeks after the Baseline visit to assess wound healing rate. During follow up visits, wound photographs, wound measurements, and adverse events will be collected.

11.4. Wound infection

If a wound infection is suspected during the trial, a clinic visit will be scheduled at the earliest time convenient for the patient. At the visit, clinical assessment, photograph, and wound measurements will be taken, and appropriate topical and systemic antimicrobial treatment will be implemented by the principal investigator. Weekly follow-up visits will be made to monitor for resolution of wound infection.

11.5. Healing of Wound Prior to End of Study

If the wound heals completely prior to the end of study, the patient will be instructed to return for an earlier visit to document healing.

11.6. Patient Withdrawal

The following medical and other reasons justify a premature termination (by patient or investigator) of the investigational treatments:

- Withdrawal of informed consent,
- SAEs,
- Patient's request,
- Patient is lost to follow-up, and/or
- Investigator's judgment.

If a patient withdraws from the study, all efforts will be made to complete a final evaluation, if possible, including the reason for withdrawal. The final evaluation will include all end of treatment (EOT) procedures.

Withdrawals due to non-attendance or non-compliance must be followed up to obtain the reason for non-attendance or non-compliance. Withdrawals due to intercurrent illness or adverse events (AEs) are to be fully documented with supplementary information where available and appropriate. Patients discontinued due to an AE will be followed until the AE is resolved, a reasonable explanation is provided for the event, or the patient is referred to his/her own primary medical doctor. The specific AE in question will be recorded on the appropriate CRF.

12. INVESTIGATIONAL INTERVENTION

The investigation intervention will be standard wound debridement, along with tissue collection.

The intervention arm will include tissue collection and wound debridement. Wound debridement will be performed using a sharp circular disposable dermal 5mm curette (Integra Miltek, New Jersey) with the patient in a reclining or supine position to remove the slough, nonviable tissue, and any fibrous tissue down to the vascular base. Local anesthetics will be applied to the wound 30-45 minutes prior to debridement. Tissue collection (2 3mm punch biopsies) will be performed at the wound edge before debridement and after debridement, using standard sterile punch biopsy technique. In addition, an optional 3mm punch biopsy of normal, healthy skin will be obtained from the upper inner thigh. After tissue sample collection, patients in the intervention arm will then receive standard of care bandaging and compression mentioned above.

12.1. Precautions and Risks Associated with Investigational Intervention

Collection of a 3mm punch biopsy from either the upper thigh or the edge of a VLU is minimally invasive procedure. The associated risks include pain, infection, scarring, wound dehiscence and pigmentary alteration and these will be clearly discussed with the patients prior to the biopsy. Prior to the procedure, subjects will be assessed for any known allergies or reaction to any medications/materials (i.e. latex, local anesthesia) as well as any contraindications for the use of anesthetics or other materials used in the procedure. Subsequent to the procedure, subjects will be thoroughly educated about the signs and symptoms of wound infection at the biopsy site (tenderness, purulence, redness, pain) that necessitate immediate medical attention, and study investigators will be made available to participants should any questions or problems arise.

As with any drug, it is possible that subjects could experience an allergic reaction to **Emla** and/or **lidocaine/epinephrine**. Such allergic reactions include: itching, skin rash,

sudden drop in blood pressure, loss of consciousness and/or associated with seizures, including the possibility of death.

Another risk includes the remote possibility of breach of confidentiality. Every attempt will be made to ensure that protected health information is kept secure. All study data will be kept in a locked office and kept under password protection on a computer that is only accessible by study personnel.

12.2. Potential Benefits to Subjects

The potential benefit to the subject in the interventional arm is improved wound healing.

12.3. Vulnerable Populations

No vulnerable subjects will be enrolled.

12.4. Sharing Results with Subjects

Individual results will not be shared with subjects. Results will only be shared through scientific publication.

13. RESPONSE EVALUATIONS AND MEASUREMENTS

The investigator(s) will assess the wound size on each patient during each study visit.

13.1. Safety Evaluation

The incidence of AEs and SAEs will be recorded and analyzed throughout the study.

14. STATISTICAL CONSIDERATIONS

A total of 360 patients who meet the inclusion criteria will be enrolled in the proposed study. Since the intervention group will be subdivided by whether or not their gene profile changes before and after wound edge debridement, a 2:1 ratio of intervention to control randomization scheme will be used, with one third (n=120) randomized into control group that doesn't get wound edge debridement and the other two thirds (n=240) into intervention group who receive wound edge debridement. Based on wound literature for VLU, a refractory subset of wounds exists which only 15% of those will heal with standard care, therefore in our population of refractory wounds, we expect that only 15% will meet the healing criteria in controls as well as in the subgroup of non-changed gene profile (incomplete debridement) in the intervention group. However, in patients who are less refractory over 75% heal with standard care and thus we expect 75% will meet the healing criteria for the remaining patients with changed gene profile (successful debridement) in the intervention group. With a 2:1 ratio of sample size 360, significance level of 0.05, two-sided, to achieve 80% power, minimum 28% overall healing for the intervention group is needed, which requires a minimum 21% intervention group will show changed gene profile.

Primary: The change in genetic profile after debridement in the intervention group will be examined by comparing the gene expression pattern in samples collected pre-debridement to samples collected post-debridement. The post-debridement gene expression patterns will then be correlated to healing outcomes.

This objective has ample statistical power to evaluate using genomic profiles to select and develop immunostain-based complementary assessment method for guiding surgical debridement. Using logistic regression model and assuming at least 40% those healed after 4 weeks possess healing genomic profiles, with sample size 360, significance level 0.05, two-sided, an odds ratio of 1.4 will result in study power of 0.86. Based on preliminary data, we are confident that the patients with healing genomic profiles will have greater than 40% healing rate than those without.

Data analysis: RNA-seq data analyses will be performed in collaboration with the UM Center for Computational Science. After filtering, the raw read files are analyzed for quality control. Quality control of the reads via FastQC will include per base quality, GC content, and sequence read lengths and distribution. Differential expression will be performed by CuffDiff or edgeR. These two methods offer substantial literature coverage and established distributional properties, which may better support a more straightforward interpretation of results. In case their outcomes overlap, to some extent, this evidence might lead us to consideration of the union of their delivered outcomes. Just as for traditional methods of assessing gene expression, the high degree of accuracy is needed to reduce the false discovery rate (FDR). The union model will be used to assign uniquely aligned reads to Ensembl (v85, with controls done on other releases) homo sapiens mRNA/miRNA annotation. Analyses will also include annotations for lncRNA using specialized repositories like GENCODE. The fold change will be determined across all groups (healed, non-healed,) based on the number of reads. Only pairs which have $FDR < 0.05$ and $\log\text{-ratio} > 2$ in paired t-tests between, upon correction for by the false discovery rate (FDR) method of Benjamini and Hochber will be retained as significant. We will be paying close attention to the fact that lower expression levels are expected for non-coding RNAs, compared to other biotypes, and this may require further sensitivity analysis to establish the most appropriate cutoff range for lncRNA. Fastq files will be submitted to the NCBI GEO database.

Secondary: To evaluate the percent healing rate, the area of each patient's wound will be measured at every visit and divided by 4 to calculate the average wound healing rate in cm^2/week and the average percent reduction for each study arm.

15. SAFETY REPORTING AND ANALYSIS

15.1. Safety Analyses

Safety and tolerability assessments will consist of ongoing monitoring and recording of all adverse event (AEs) and serious adverse event (SAE) reports, and the regular monitoring of signs and symptoms of cutaneous irritation.

15.2. Adverse Events

The PI is responsible for identifying, documenting and reporting adverse events to the IRB and FDA as applicable.

15.2.1. Definitions of Adverse Events

An adverse event is the development of an undesirable medical condition, or the deterioration of a pre-existing medical condition following or during exposure to a medicinal product, whether or not considered causally related to the product.

An undesirable medical condition can be symptoms (e.g., chest pain), signs (e.g., tachycardia), or the abnormal results of an investigation (e.g., laboratory findings).

15.2.2. Recording of Adverse Events

All adverse events of any patient during the course of the trial will be reported in the case report form, and the investigator will give his or her opinion as to the relationship of the adverse event to trial drug treatment (i.e., whether the event is related or unrelated to trial drug administration). If the adverse event is serious, it should be reported within 24 hours to the IRB as per UM policy. Other untoward events occurring in the framework of a clinical trial are also to be recorded as AEs (i.e., AEs that occur prior to assignment of trial treatment that are related to a protocol-mandated intervention, including invasive procedures such as biopsies, medication washout, or no treatment run-in).

All AEs, regardless of seriousness or relationship to trial treatment, spanning from the start of trial treatment until the 4th week follow up visit are to be recorded in the patients CRF.

15.2.3. Handling of Adverse Events

All adverse events resulting in discontinuation from the trial should be followed until resolution or stabilization. Patients must be followed for AEs for 30 calendar days after treatment (e.g., debridement and/or tissue collection). All new AEs occurring during the follow up period must be reported and followed until resolution unless, in the opinion of the investigator, these values are not likely to improve because of the underlying disease. In this case, the investigators must record their reasoning for this decision in the patient's medical record and as a comment on the CRF. After 30 days, only AEs, SAEs, or deaths assessed by the investigator as treatment related are to be reported.

15.3. Serious Adverse Events

15.3.1. Definitions of Serious Adverse Events

The definitions of serious adverse events (SAEs) are given below. The principal investigator is responsible for ensuring that all staff involved in the trial are familiar with the content of this section.

An SAE or reaction is defined as any untoward medical occurrence that: results in death, is immediately life-threatening, requires at least a 24-hour inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.

The definition of SAE also includes any important medical event. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-

threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the previous definition. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Hospitalization during the trial for a pre-planned surgical or medical procedure (one which was planned prior to entry in the trial), does not require reporting as a serious adverse event.

15.3.2. Serious Adverse Event Reporting

It is important to distinguish between “serious” and “severe” adverse events, as the terms are not synonymous. Severity is a measure of intensity; however, an AE of severe intensity need not necessarily be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but may not be considered an SAE. On the other hand, a stroke which results in only a limited degree of disability may be considered only a mild stroke, but would be considered an SAE. Severity and seriousness should be independently assessed when recording AEs on the CRF and SAEs on the SAE Report Form.

Adverse events classified by the treating investigator as serious require documentation and reporting to the IRB according to UM policies and procedures in order to comply with regulatory requirements. Serious adverse events may occur at any time from the signing of the informed consent form start of trial treatment through the 30-day follow-up period after the last trial treatment.

To report a SAE, the SAE Report Form should be completed with the necessary information. The CRF pages required for SAE reporting include:

- Adverse Event and Safety Complementary pages
- Concomitant Medications
- Demographics
- Medical History

All SAEs and medically confirmed deaths (regardless of causality assessment) occurring on trial treatment or within 28 days of last trial treatment must be reported on the SAE Report Form and followed until resolution (with autopsy report if applicable).

Deaths occurring within 30 days after last trial treatment that are deemed ‘possibly’ or ‘probably’ related to study drug must be reported as SAEs on the SAE Report Form (with an autopsy report if available). Deaths occurring within 30 days after last trial treatment and not attributed to trial treatment (e.g., disease progression) need not be reported as SAEs, but simply captured on the appropriate CRF.

Investigators must report SAEs and follow-up information to their responsible Institutional Review Board (IRBs)/Ethics Committee according to the policies of the responsible IRB/IEC. If the Investigator believes the AE impacts the risk to study subjects, the AE will be reported to the IRB within 10 business days of knowledge of the

event. If the Investigator believes there is no impact, then the AE will be reported to the IRB in summary format at the time of continuing review. SAEs will be reported to the IRB within 24 hours of knowledge of the event.

Any event that is reported to the IRB by the Investigator will also be reported to the Funding Agency (NIAMS) and the SO via KAI within the designated timeline.

15.3.3. SAE Reporting Requirements

UM is responsible for reporting relevant SAEs to the competent authority, other applicable regulatory authorities, and participating investigators, in accordance with ICH guidelines, FDA regulations, and/or local regulatory requirements.

UM is responsible for reporting unexpected fatal or life-threatening events associated with the use of the trial drugs to the regulatory agencies and competent authorities via telephone or fax within 7 calendar days after being notified of the event. UM will report all related but unexpected SAEs including non-death/non-life-threatening related but unexpected SAEs associated with the use of the trial medication to the appropriate competent authorities (according to local guidelines), investigators, and central IRBs/IECs by a written safety report within 15 calendar days of notification.

15.4. Unanticipated Problems

15.4.1. Definitions of Unanticipated Problems

The definition of Unanticipated Problems (UPs) are any information that is (1) unexpected and (2) related or probably related to the research, and (3) indicates that the subjects or others are at increased risk of harm.

15.4.2. Unanticipated Problems Reporting

Investigators must report UPs to the IRB and 24 hours of the investigator becoming aware of the event. Any UP event that is reported to the IRB by the Investigator will also be reported to the Funding Agency (NIAMS) and the SO via KAI within the designated timeline.

15.5. Protocol Deviation Recording and Reporting

Non-compliance or Protocol Deviations (PDs) during the course of the trial will be reported in the case report form, and the investigator will give his or her opinion as to the PDs impact on subject safety, data integrity, and risk. If the Investigator believes the PD impacts the study in these categories, the PD will be reported to the IRB within 10 business days of knowledge of the event. If the Investigator believes there is no impact, then the PD will be reported to the IRB in summary format at the time of continuing review.

Any event that is reported to the IRB by the Investigator will also be reported to the Funding Agency (NIAMS) and the SO via KAI within the designated timeline.

15.6. Recording of Adverse Events, Unanticipated Problems, and Serious Adverse Events

Investigators should use correct medical terminology/concepts when recording UPs, AEs or SAEs on the SAE Report Forms and AE CRF. Avoid colloquialisms and abbreviations.

All UPs and AEs, including those that meet SAE reporting criteria, should be recorded on the AE CRF; AEs and UPs that meet the definition of an SAE should additionally be reported following the procedures noted above.

15.6.1. Diagnosis vs. signs and symptoms

All AEs should be recorded individually in the patient's or caregiver's own words (verbatim) unless, in the opinion of the Coordinating Investigator or designated physician, the AEs constitute components of a recognized condition, disease, or syndrome. In the latter case, the condition, disease, or syndrome should be named rather than each individual sign or symptom. If a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE as appropriate on the relevant form(s) (SAE Report Form and/or AE CRF). If a diagnosis is subsequently established, it should be reported as follow-up information is available. If a diagnosis is determined subsequent to the reporting of the constellation of symptoms, the signs/symptoms should be updated to reflect the diagnosis.

15.6.2. Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation time points. Such events should only be recorded once on the SAE Report Form and/or the AE CRF. If a persistent AE becomes more severe or lessens in severity, it should be recorded on a separate SAE Report Form and/or AE CRF.

A recurrent AE is one that occurs and resolves between patient evaluation time points, and subsequently recurs. All recurrent AEs should be recorded on an SAE Report Form and/or AE CRF.

15.6.3. Abnormal Laboratory Values

Any grade 3 or 4 laboratory abnormalities or any clinically significant grade 1 or 2 hematology or biochemistry laboratory value(s) should be recorded as an AE. If an abnormal laboratory value or vital sign is associated with clinical signs and/or symptoms, the sign or symptom should be reported as an AE, and the associated laboratory value or vital sign should be considered additional information that must be collected on the relevant CRF or EDC. If the laboratory abnormality is a sign of a disease or syndrome, only the diagnosis needs to be recorded on the SAE Report Form or AE CRF.

15.6.4. Deaths

Deaths that occur during the protocol-specified AE reporting period that are attributed by the investigator solely to progression of disease will be recorded on the CRF. All other on-trial deaths, regardless of attribution, will be recorded on an SAE Report.

When recording a serious adverse event with an outcome of death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the SAE Report Form and Adverse Event page of the CRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Death NOS" on the CRF.

15.6.5. Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization of >24 hours or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol. There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE.

15.6.6. Pre-Existing Medical Conditions

A pre-existing medical condition is one that is present at the start of the trial. Such conditions should be recorded on the General Medical History CRF. A pre-existing medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the trial. When recording such events on an SAE Report Form and/or AE CRF, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors.

15.6.7. Expected and Unexpected Adverse Events

Expected AEs include infection of the wound, cellulitis, worsening of the wound (increased wound size), and deep vein thrombosis.

Unexpected AEs is an AE considered unexpected if it is not consistent with the risk information described in the protocol or other study documents. Unexpected AEs include pulmonary embolism, renal failure, liver failure, myocardial infarction, and death.

15.7. Analysis of Safety Data

Events that are definitely or probably related to the study intervention will be considered for analysis. Analyses of these events will examine the magnitude, timing, and duration of the excess risks. The analyses will include all participants who were randomly assigned to the different study treatment groups.

16. ETHICAL, FINANCIAL, AND REGULATORY CONSIDERATIONS

16.1. IRB Approval

The trial protocol, ICF, patient documents, patient recruitment procedures (e.g., advertisements), information about payments (i.e., PI payments) and compensation available to the patients and documentation evidencing the PI's qualifications should be submitted to the IRB for ethical review and approval if required by local regulations, prior to the trial start.

The PI will follow all necessary regulations to ensure appropriate, initial, and ongoing, IRB trial review. The PI must submit and, where necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent document.

16.2. Regulatory Approval

As required by local regulations, the PI will ensure all legal aspects are covered, and approval of the appropriate regulatory bodies obtained, prior to trial initiation. If required, the PI will also ensure that the implementation of substantial amendment to the protocol and other relevant trial documents happen only after approval by the relevant regulatory authorities.

16.3. Informed Consent

Informed consent is a process by which a patient voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the patient's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.

The research team will follow the "HRP-090 SOP: Informed Consent Process for Research" to obtain informed consent and the "HRP-091 SOP: Written Documentation of Informed Consent" to document informed consent in writing.

The informed consent form will be submitted for approval to the IRB that is responsible for review and approval of the trial. Each consent form must include all of the relevant elements currently required by the FDA, as well as local county authority or state regulations.

Before recruitment and enrollment into the trial, each prospective candidate will be given a full explanation of the trial. Once the essential information has been provided to the prospective candidate, and the investigator is sure that the individual candidate understands the implications of participating in this trial, the candidate will be asked to give consent to participate in the trial by signing an informed consent form. A notation that written informed consent has been obtained will be made in the patient's medical record. A copy of the informed consent form, to include the patient's signature, will be provided by the investigator to the patient.

If an amendment to the protocol substantially alters the trial design or the potential risks to the patients, the patient's consent to continue participation in the trial should be obtained.

16.4. Confidentiality

Confidentiality of patient's personal data will be protected in accordance with the Health Insurance Portability and Accountability Act of 1996 (HIPPA). HIPPA regulations require that, in order to participate in the trial, a patient must sign an authorization from the trial that he or she has been informed of following:

- What protected health information (PHI) will be collected from patients in this trial;
- Who will have access to that information and why;
- Who will use or disclose that information;
- That health information may be further disclosed by the recipients of the information, and that if the information is disclosed the information may no longer be protected by federal or state privacy laws;
- The information collected about the research trial will be kept separate from the patient's medical records, but the patient will be able to obtain the research records after the conclusion of the trial;
- Whether the authorization contains an expiration date; and
- The rights of a research patient to revoke his or her authorization.

In the event that a patient revokes authorization to collect or use his or her PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of patient authorization. For patients that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the patient is alive) at the end of their scheduled trial period.

In compliance with ICH GCP guidelines and applicable parts of 21 CFR, it is a requirement that the investigator and institution permit authorised representatives, the regulatory authorities and the IRB direct access to review the patient's original medical records at the site for verification of trial-related procedures and data.

Measures to protect confidentiality include: only a unique trial number and initials will identify patients on the CRF or other documents. Patients will be informed of their rights within the ICF.

16.5. Compensation for Research-Related Injury

If a patient is hurt or gets sick as a result of being in this study, treatment will in most cases be available. If the patient has insurance, his / her insurance company will be billed. The patient will be informed that their insurance may or may not pay for these costs. If the patient does not have insurance, or if his / her insurance company refuses to pay, the patient will be expected to pay. Funds to compensate for pain, expenses, lost wages, and other damages caused by injury are not available.

16.6. Economic Burden to Subjects

All study related procedures will not be an economic burden to the subjects for participating in this study.

16.7. Financial Information

NIH NIAMS will provide funding for the study. The trial procedures for all trial participants will be provided free of charge for the duration of the trial, up to a limit agreed upon in the contract.

17. RECORD RETENTION AND DOCUMENTATION OF THE TRIAL

17.1. Amendments to the Protocol

Amendments to the protocol shall be planned, documented and signature authorized prior to implementation.

All amendments require review and approval of the Principal Investigator supporting the trial.

Amendments specifically involving change to trial design, risk to patient, increase to dosing or exposure, patient number increase, addition or removal of new tests or procedures, shall be reviewed and approved by the IRB at the Investigator's facility.

The amendment will be submitted formally to the FDA or other regulatory authorities as applicable, after IRB approval and specifically when an increase to dosing or patient exposure and/or patient number has been proposed; or, when the addition or removal of an Investigator is necessitated.

17.2. Trial Documentation and Storage

The PI must maintain a list of appropriately qualified persons to whom he/she has delegated trial duties and should ensure that all persons assisting in the conduct of the trial are informed of their obligations. All persons authorized to make entries and/or corrections on the CRFs are to be included on this document. All entries in the patient's CRF are to be supported by source documentation where appropriate.

Source documents are the original documents, data, records and certified copies of original records of clinical findings, observations and activities from which the patient's CRF data are obtained. These can include, but are not limited to, hospital records, clinical and office charts, laboratory, medico-technical department and pharmacy records, diaries, microfiches, ECG traces, copies or transcriptions certified after verification as being accurate and complete, photographic negatives, microfilm or magnetic media, X-rays, and correspondence.

The PI and trial staff are responsible for maintaining a comprehensive and centralized filing system (Trial Master File (TMF)) of all trial-related (essential) documentation, suitable for inspection at any time by applicable regulatory authorities. The TMF must consist of those documents that individually or collectively permit evaluation of the conduct of the trial and the quality of the data produced. The TMF should contain as a minimum all relevant documents and correspondence as outlined in ICH GCP Section 8 and 21 CFR Part 312.57, including key documents such as the IB and any amendments, protocol and any amendments, signed ICFs, copies of completed CRFs, IRB approval documents, Financial Disclosure forms, patient identification lists, enrolment logs, delegation of authority log, staff qualification documents, laboratory normal ranges, records relating to the trial drug including accountability records. Drug accountability records should, at a minimum, contain information regarding receipt, shipment, and disposition. Each form of drug accountability record, at a minimum, should contain PI name, date drug shipped/received, date, quantity and batch/code, or lot number for identity of each shipment. In addition, all original source documents supporting entries in the CRF must be maintained and be readily available.

The Investigator shall maintain adequate records of case histories and any other trial-related records as per 21 CFR Part 312.62 for no less than 6 years after study closure.

To enable evaluations and/or audits from regulatory authorities the investigator additionally agrees to keep records, including the identity of all participating patients (sufficient information to link records e.g., CRFs and medical records), all original, signed informed consent forms, and copies of all CRFs, SAE Reporting forms, source documents, detailed records of treatment disposition, and related essential regulatory documents. The documents listed above must be retained by the investigator for as long as instructed to comply with national and international regulations (generally 6 years after study closure).

17.3. Data Collection

The trial CRF is the primary data collection instrument for the trial. CRFs will be completed using the English language except for patient/patient questionnaires in non-English speaking countries and should be kept current to enable the monitor to review the patients' status throughout the course of the trial.

In order to maintain confidentiality, only trial number, patient number, and initials will identify the patient in the CRF. The investigator will maintain a personal patient identification list (patient numbers with corresponding patient identifiers) to enable records to be identified and verified as authentic. Patient data/information will be kept confidential, and will be managed according to applicable local, state, and federal regulations.

All missing data must be explained. When a required laboratory test, assessment, or evaluation has not been done, a note on the CRF should be created verifying that the field was "Not Done" or "Unknown".

17.4. Trial Monitoring, Auditing, and Inspecting

The investigator will permit trial-related monitoring, quality audits, and inspections by, government regulatory authorities, of all trial-related documents (e.g., source documents, regulatory documents, data collection instruments, case report forms). The investigator will ensure the capability for inspections of applicable trial-related facilities. The investigator will ensure that the trial monitor or any other compliance or QA reviewer is given access to all trial-related documents and trial-related facilities. Participation as an investigator in this trial implies the acceptance of potential inspection by government regulatory authorities.

17.5. Quality Assurance and Quality Control

In addition to the Clinical Monitoring component of this protocol, Quality Assurance (QA) to assess compliance with GCP and applicable regulatory requirements. Data or documentation audited shall be assessed for compliance to the protocol, accuracy in relation to source documents and compliance to applicable regulations.

17.6. Disclosure and Publication Policy

All information provided regarding the trial, as well as all information collected/documented during the course of the trial, will be regarded as confidential.

The Principle-Investigator will register the trial on www.clinicaltrials.gov. In addition, Principle-Investigator will publish the results of the trial.