PROTOCOL SUMMARY

TITLE OF THE STUDY: MERIT: Beta Adrenergic Antagonist For The Healing of Chronic Diabetic Foot Ulcers

Start Date: 04/01/2017
Ending Date 12/31/2024

PHASE: 2

METHODOLOGY: It is a prospective, randomized, double blinded, controlled, and parallel-group study with 2 arms. Follow up cohort will include a real-world group.

STUDY DURATION: 31 weeks

TREATMENT PHASE: 12 weeks

FOLLOW-UP PHASE: 16 weeks

REAL-WORLD GROUP: 12 weeks

STUDY CENTERS: One centralized study location Multispecialty Wound Healing Clinic at Sacramento VA Medical Center.
Note: Primary Care, Vascular, Podiatry and Dermatology clinics from the at the Veteran Affairs Northern California Health Care System (VANCHS) satellite clinics (Redding, Chico, Martinez, Oakland, McClellan, and Fairfield) will refer patients to the study location.

NUMBER OF SUBJECTS: 2 treatment arms, 24 subjects per arm for a total of 48; Screen-failed patients to be followed passively on the status of their diabetic foot ulcer via CPRS. Additional patients will be screened and enrolled for exploratory follow up to study inflammatory markers in non-healing patients.

STUDY OBJECTIVES: The primary objective of this study is to assess the effectiveness of the topically applied Timoptic-XE® or timolol (a non-selective beta adrenergic receptor antagonist (βAR)) versus standard of care for the treatment of non-healing diabetic foot ulcers. The primary endpoint will be the measurement of the complete ulcer closure (as defined by “skin re-epithelialization without drainage or dressing requirements) at 12 weeks. The secondary outcomes will be the following: comparison of percent difference change in size from the randomization visit to the endpoint visit (post 12 weeks) between the treatment and control group, the time to wound closure between the two groups, and wound healing rates with impact of wound size between the treatment groups,change in trans-epidermal water loss measurements at sites of healed wounds, measurement of quality of life using Veterans Rand (VR-36) Health Survey, measurement of the Lower Extremity Functional Scale Outcome and the Charlson Comorbidity Index.

The secondary objective of study is to profile the safety of topically applied Timoptic- XE® in the treatment of DFU. We will monitor serum levels of timolol post-application to determine the plasma concentration of timolol in relation to its systemic bioavailability. Timolol levels will be determined using reversed-phase ultraperformance liquid chromatography and UV detection. The primary safety outcome will be the measurement of timolol serum during the treatment phase.

The secondary safety outcomes will be the following: the mean changes from baseline in systolic and diastolic blood pressure in mmHg, the mean changes from baseline in heart rate in beats per minute, and the measurement of all the adverse events associated with the use of Timoptic-XE®.

INCLUSION CRITERIA:

1. Male or female subject of any race 18 years old or older
2. Lower extremity ulcer located anywhere on the foot up to the ankle:
    a. Of more than 30 days duration and less than 2 years duration (medically documented)
    b. Surface area between 0.5cm^2 and 20cm^2 (as measured with the Silhouette imaging system at randomization). The ulcer with largest surface area meeting inclusion criteria will be selected as index ulcer
    c. If two ulcers present with the same surface area, the ulcer of the longest duration will be selected as index ulcer
3. Documented Ankle Brachial Index (ABI) between 0.8 and 1.2 on the study limb or toe pressure over 65mmHg within 3 months of screening phase
4. Documented biopsy report to rule out malignancy of ulcer of > 6 months duration

EXCLUSION CRITERIA*:

A subject will be excluded from the study if any of the following criteria are met:

1. Ulcer of non-diabetic etiology, such as venous, arterial and burn wounds
2. Index ulcer is less than 3 cm in distance from any other ulcer on the same extremity
3. There are greater than 3 ulcers on the study foot
4. Index ulcer presents with any of the following: cellulitis, osteomyelitis, exposed bone, tendon or fascia, purulent exudate or gangrene
5. Index ulcer shows evidence of infection (defined as a moderate or severe rating of all of the following clinical signs/symptoms: 1) increased warmth, 2) increased pain, 3) erythema, and 4) malodorous exudate at Screening or at Randomization (Visit 1), OR total organism count > 1 x 10^5 colony forming units (CFU) from the screening visit study ulcer culture sample)
6. Index ulcer surface area has decreased or increased > 40% between Screening and at Randomization (Visit 1) as assessed by the Silhouette imaging system
7. Has medically documented history of Human Immunodeficiency Virus (HIV)
8. Has active malignancy on the study limb
9. Has uncontrolled diabetes mellitus as defined by glycosylated hemoglobin A1C > 12% within 3 months of screening
10. Has immunodeficiency as defined by serum IgG, IgA, and IgM less than one-half the lower limit of normal
11. Has severe protein malnutrition as defined by serum albumin < 2.5 g/dL

12. Has serum aspartate aminotransferase (AST, SGOT, GOT) or serum alanine aminotransferase (ALT, SGPT, GPT) levels greater than twice the upper limit of normal
13. Has fatigue, palpitations, dyspnea, and/or angina at rest
14. Has a medically documented or self-reported history, within the previous 12 months from date of Screening Visit, of alcohol or drug abuse, particularly methadone or heroin
15. Has received previous treatment with the following during the 60 days prior to Screening: Immunosuppressive agents, radiation, chemotherapy, growth factors (epidermal growth factor, tumor necrosis factor, transforming growth factor, platelet derived growth factor, etc.)
16. Has received previous treatment with the following during the 30 days prior to screening: the site of the study ulcer, split- or full-thickness skin graft at the site of the study ulcer, biologically-active (or engineered) cellular or acellular product(s) at the site of the study ulcer, investigational drug or device
17. Has been hospitalized for treatment of a diabetic foot ulcer within the previous 30 days from Screening
18. Has history of bradycardia (heart rate less than 60)
19. Has ESR>70mm/hr and CRP>100 mg/L at time of screening
20. Has medically documented history of hypotension/orthostatic hypotension and/or symptomatic hypotension (systolic blood pressure below 90 and diastolic blood pressure less than 60). (Note: There is no standard testing regimen protocol for orthostatic hypotension, even for patients starting on oral timolol, per the consultant Cardiologist, Dr. Schaefer)
21. Currently taking asthma or COPD medications (as documented in chart)
22. Has a medically documented diagnosis of myasthenia gravis, untreated hyperthyroidism, , Type 2, Type 3 heart block, cardiogenic shock, overt cardiac failure
23. Female who is pregnant or refuses to use adequate contraceptive methods and is of childbearing age during the trial
24. Prisoners, institutionalized individuals or vulnerable population

(The real-world group will not have any exclusion criteria related to timolol (criteria 18-22)

(*)Note: The exclusion criteria were modified compared to the original submission based on the recommendation from the DMC, which suggested that these criteria would significantly impair the enrollment. Those were also revised upon evaluation by the consultant Cardiologist, Dr. Schaefer.

TREATMENT: Total duration of study per subject: Up to 31 weeks (Includes to 2 weeks of screening and up to 16 weeks until the end of the follow-up period). 12 weeks for the real-world group.

DURATION OF ADMININSTRATION: Daily application for 12 weeks (starting at Visit 3/Week 3).

STUDY DESIGN: This is a prospective, randomized, double blinded, controlled, and parallel-group study with 2 arms: βAR antagonist (SOC plus topically applied Timoptic-XE®) and control (standard of care (SOC) plus non-biologically active gel (hydrogel, Kendall®, placebo medication).

EFFICACY ENDPOINTS:
PRIMARY EFFICACY OUTCOME
1. Complete closure of ulcer by Visit 14/Week 14

SECONDARY EFFICACY OUTCOME
1. Complete closure of ulcer by Week 31
2. Percent difference change in size from the randomization visit to the endpoint visit (post 12 weeks)
3. Time to wound closure
4. Rate of ulcer healing
5. Change in transepidermal water loss of re-epithelialized wound after wound closure

SAFETY ENDPOINT:
PRIMARY SAFETY OUTCOME
1. Timolol serum levels during the treatment phase

SECONDARY SAFETY OUTCOME
1. Mean changes from baseline in systolic and diastolic blood pressure in mmHg
2. Mean changes from baseline in heart rate in beats per minute
3. Adverse events associated with the use of timolol.

QUALITY OF LIFE ASSESSMENT: We will use ANCOVA to analyze the quality of life from the VR-36 Health Survey and the Lower Extremity Functional Scale Outcome Questionnaire.

DATA ANALYSIS:
The primary outcome is complete ulcer closure by Week 12 (end of the Active Phase) of the study and the rate of ulcer healing by the 12th week of care between the two arms.

Hypothesis to be tested: The null hypothesis is that there is no difference in the proportion of subjects with complete wound closure of DFU between the timolol treatment group and the control group. Complete wound closure is defined as 100% closure, or skin re-epithelialization without any drainage present or dressing requirements. Two-sided Fisher's exact test will be used to compare the proportion of complete ulcer closure by Week 12 (end of the Active Phase) of the study and the rate of ulcer healing by the 12th week of care between the two arms. The Kaplan-Meier method will be used to estimate the survival function of time to achieve complete ulcer closure (if less than 12 weeks) for each arm, and a two-sided log-rank test will be used to compare the survival curves between the two arms.

The area of the target ulcer will be summarized by treatment group and visit. Both actual value and change from the previous visits ulcer area will be presented. A second summary of target ulcer area will also be given which will present change from baseline. The weekly change and the percentage weekly change in the target ulcer area from visit to visit will be summarized by treatment group and visit. The two-sided t-test or Wilcoxon rank-sum test as appropriate will be used to compare relative ulcer area from baseline to Week 12 between the two arms.

Chi-square test or Fisher's exact test as appropriate will be used to compare wound healing rates in relationship to wound size, as well as wound healing rates between the treatment groups at the 4-week follow-up and at the 12-week follow-up period and recidivism rate.

Additional secondary analyses to evaluate the association of specific wound characteristics (such as wound size, wound location) and subject characteristics (body mass index, chronicity of the wound, history of DVT, length of diabetes, HbA1c, and ABI/toe pressure will be conducted using logistic regression. Further logistic regression will be used to investigate the relationship between the occurrence of each type of adverse event and treatment in order to adjust for each of the potential confounding factors previously listed.

A two-way ANOVA with a post hoc test will be used to determine the relationship between timolol serum levels and wound healing.

Further statistical analysis will be used to determine the relationship between serum inflammatory markers and wound healing.

For demographic and clinical characteristic data at baseline, continuous variables will be summarized (when appropriate) by using mean, median, standard deviation, co-efficient of variation, minimum value and maximum value. Categorical variables will be summarized with frequency tables. Baseline comparability between the two treatment groups will be assessed by using the independent two-sample t-test or Wilcoxon rank-sum test and the Chi-square test or Fisher's exact test.

Following wound closure, comparison of changes in the epidermal barrier function of healed ulcers treated with timolol vs those treated with saline control will be non-invasively measured quantitating the transepidermal water loss (TEWL). Categorical variables will be summarized with frequency tables. Baseline comparability between the two treatment groups will be assessed by using the independent two-sample t-test or Wilcoxon rank-sum test and the Chi-square test or Fisher's exact test.

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1. BACKGROUND AND RATIONALE

Diabetes has become a worldwide pandemic affecting over 387 million individuals, including 29 million people in the United States alone. It is estimated that 15 to 25 percent of diabetic patients are expected to develop a diabetic foot ulcer (DFU) during their lifetime. As the DFU becomes intractable, the patient's quality of life and productivity are considerably affected.

The longer that a wound remains non-healed, there is an increased risk for infection, leading to amputation or even possible death. It is estimated that over 245 billion dollars are spent in total medical cost and lost wages related to the disease. The cost per episode of care can exceed $38,000, and associated with hospitalization, ulcer recurrence, amputations, home health care and dressing changes, decrease work productivity and premature disability, feelings of social isolation and depression. In a study of Veterans with diabetes and foot ulcer, there was a 2.39 increased relative risk of death compared to those without a foot ulcer. With the estimated cost per episode of care of $38,000, this remains a costly and important problem of particular relevance to the VA. In fact, in the VA health system alone, diabetic care takes 4% of the overall budget and 28% of the pharmacy budget with one and a half billion spent annually on diabetic Veterans.

Despite adhering to the standard wound care regimens, a major challenge faced by wound care specialists is to achieve successful treatment of these wounds. Research has shown that after 20 weeks of good wound care, only 31% of DFU heal. More recent therapeutic approaches to wound healing, such as bioengineered tissues and biologically active growth factors are very costly and have only shown modest clinical efficacy. It is important to recognize that the VA health system may not be able to justify additional financial burden of these costly new therapies. Therefore, the drive to heal DFU to prevent further complications and high costs associated with treatment is critical; as such, we are investigating, using a safe, inexpensive, well-characterized, easy to use drug that could be implemented system wide to enhance DFU repair and that may have far-reaching consequences.

Of the several identified classes of adrenergic receptors (α and β, and their subtypes), it is of particular interest to note that the major cell types of the skin--human keratinocytes, melanocytes and dermal fibroblasts express primarily β-2 adrenergic receptors (βARs) . Keratinocytes also have the capacity to synthesize the catecholamines epinephrine and norepinephrine, in essence creating a self-contained, catecholamine signaling network. The functional role of this network has been elusive. Our work suggests that it contributes to the control of cell migration, and thus to skin wound healing. When skin is wounded, repair mechanisms are activated to restore skin integrity as quickly as possible. The repair process involves the orchestration of interactions between cellular components, growth factors, chemokines, extracellular matrix proteins, which

regulate the migration and proliferation of the keratinocytes into the wound. This directional migration of keratinocyte into the wound is critical for repair and reestablishment of epithelial coherence. Our work and that of others has established that stress catecholamines such as epinephrine and norepinephrine, the natural ligands for the βAR that are present within the wound environment, impair pro-reparative functions of keratinocytes, fibroblasts, mesenchymal stem cells, and endothelial cells.

Importantly, and specifically relevant to this proposal, we have shown that blockade of the βAR with antagonists improves healing in vitro, and in animal models. Work by other investigators also supports the hypothesis that βAR antagonists can improve DFU healing. Collagen synthesis (in a pulmonary injury model) is increased by βAR antagonists. More specifically, studies have demonstrated that the topical application of βAR antagonists to wounds in diabetic rats improves not only rate of healing, but also wound vascularity. Since impairment of angiogenesis is a critical obstacle in DFU healing, the data that demonstrate that βAR antagonists improve endothelial cell migration, new vessel outgrowth from rat aortic rings, and in the classic angiogenesis assay of the chicken chorioallantoic membrane, as well as in increasing vascularity in murine skin wounds, lend particular significance to the proposed study. Indeed, a patent application for use of βAR antagonist for healing of DFU has already been filed by other investigators, albeit with no human clinical data. Additionally, there are published reports of timolol improving the epidermal barrier function in skin. Since a deficient barrier inevitably compromises the functional property of the repaired skin, one of our secondary hypotheses is that timolol restores the barrier and thus may result in a more durable healed ulcer. We will monitor transepidermal water loss (TEWL), an indicator of epidermal barrier function, using a non-invasive handheld pen-like device (Vapometer), and a secondary outcome will be the change in TEWL in timolol treated vs control treated wounds.

Our goal with the currently proposed clinical trial is to generate these unequivocal data, and to demonstrate the efficacy of this approach to improve healing in human chronic DFU.

2. **OBJECTIVES**

The primary objective of this study is to assess the effectiveness of the topically applied Timoptic-XE® or timolol (a non-selective beta adrenergic receptor antagonist) versus standard of care for the treatment of non-healing diabetic foot ulcers. The primary endpoint will be the measurement of the complete ulcer closure (as defined by “skin re-epithelialization without drainage or dressing requirements) at 12 weeks. The secondary outcomes will be the following: comparison of percent difference change in size from the randomization visit to the endpoint visit (post 12 weeks) between the treatment and control group, the time to wound closure between the two groups, and wound healing rates with impact of wound size between the treatment groups and measure quality of life using VR-36 Health Survey and the Lower Extremity Functional Scale Outcome Questionnaire.

The secondary objective of study is to profile the safety of topically applied Timoptic-XE® in the treatment of DFU. We will monitor serum levels of timolol post-application to determine the plasma concentration of timolol in relation to its systemic bioavailability. Timolol levels will be determined using reversed-phase ultraperformance liquid chromatography and UV detection.

Safety endpoint will be the measurement of all the adverse events associated with the use of Timoptic-XE®.

A tertiary/ancilliary objective of the study is to compare efficacy of both arms of this study, Group A (SOC + Timoptic XE) and Group B (SOC + non-biologically active gel), to a **"Real-world cohort"** – patients who are not randomized and continue to receive care for their diabetic foot ulcer(s) not within the context of this research study. Passive follow-up these screen-failed patients via CPRS will be limited to charts related to the care of their foot ulcer(s).

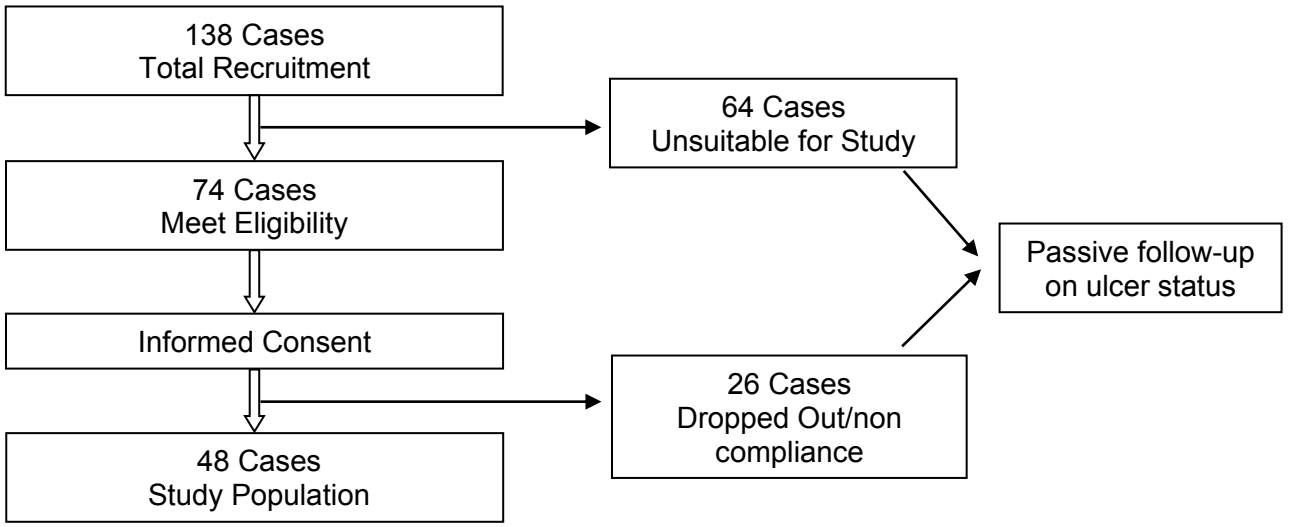
3. STUDY POPULATION

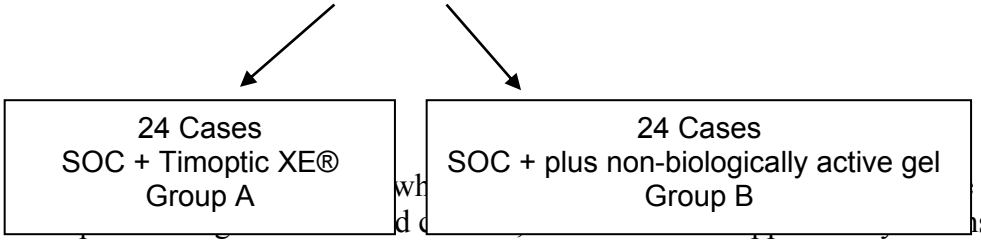
The study population includes all Veterans' age 18 or older, with a documented diagnosis of diabetes and a foot ulceration that has existed for at least 4 weeks at the time of enrollment into the study. Diabetic patients with foot ulcers will be selected from a random sample of patients from the Multispecialty Wound Healing Clinic at Sacramento VA Medical Center, and the six clinical sites at the VANCHCS. In fact, there were over 15,146 patients with diabetes mellitus receiving care at the VANCHCS, which includes Sacramento VA Medical Center and 6 other satellite clinics, and approximately 1002 patients with DFU were receiving care at the VANCHCS, within the last 2 fiscal years.

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 138 subjects and assuming approximately 35% dropout rate, the goal is to enroll 48 subjects in total, 24 subjects in each arm.

Selection of Cases and Controls:
The investigators will query the CPRS/VISTA database at the 7 respective sites of the VANCHCS. Eligible subjects will be called by the research coordinator and invited to participate in the study.
Those who agree will be given an appointment for screening and enrollment. A subject must meet all the following criteria to be eligible for study inclusion.

Figure 2: Summary of Selection Process





wh to join the study will
d c stitutional Review Board (IRB). We will perform simple allocation of the subjects using an electronic randomization for treatment assignment-since our target enrollment population is fairly homogenous.

Run-in phase (screening 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. 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. These sealed envelopes will be provided to the pharmacist will dispense the Timoptic-XE® or non-biologically active gel hydrogel for the patients according to the arm to which they have been randomized. Subject demographics will not be provided until this assignment is complete. Participants will be randomly placed into Group A (SOC plus Timoptic-XE®) or Group B (SOC plus non-biologically active gel). There will be an estimated of 24 patients in each group. The patients will not be informed of treatment administered.

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, or the patient displays non-compliance with study schedule. 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 prospective, randomized, double blinded, controlled, and parallel-group study with 2 arms to assess the effectiveness of the topically applied Timoptic-XE® or timolol (a non-selective beta-adrenergic receptor antagonist) versus standard of care for the treatment of non-healing DFU in the VA system.

4.2 DESCRIPTION OF STUDY GROUPS

Standard of care will consist of the typical foot evaluation and care provided by the Department of Veteran Affairs facilities, which will include: weekly ulcer assessment/progress and area measurement by digital photography before and after sharp debridement of ulcer, maintenance of a clean, moist ulcer environment with a non-biologically active gel (hydrogel) the use of an offloading device that protects the wound from pressure or trauma related to ambulation and other acts of daily living (such as orthotics, contact casts, crutches, wheelchairs), and infection management, if indicated.
The investigator will document the standard of care provided during clinic visits including weekly follow-up evaluations. Given the nature and complexities associated with DFU, the offloading device provided will be dependent on the subjects' ability to tolerate the specific offloading device. Adherence to offloading device will be evaluated by the investigator at each visit by observing plantar wear patterns and inquiring from the patient if offloading was used consistently as instructed. Alternative to total contact cast or instant total contact cast (camwalker), offloading devices will be offered including, but not limited to the use of felt/foam adhesive/post op shoe, custom offloading insole, and customized healing shoe in combination with gait assistive device such as a roll-a-bout scooter, walker, wheelchair or motorized scooter.

Group A: SOC plus Timoptic-XE®
Patients in the timolol group will undergo the same wound care and offloading modality as the SOC group, with weekly wound evaluation dressing changes, moist sterile gauze, dry gauze dressings.
The dosage used in this study will be 3 drops/3cm2/day that is equivalent to 0.75mg/3cm2/day.

Group B: SOC plus non-biologically active gel (hydrogel)
The placebo medication will be applied to the wound daily with the same dosage as above.

"Group" C: Passive follow up of screen-failed patients ***Does not change total # of patient enrollment
Patients that are not randomized into group A or B (i.e. meet exclusion criteria, do not meet all inclusion criteria, non-compliance, withdrawal) will be passively followed via CPRS on the status of their diabetic foot ulcers.

Drug management and record keeping
The research pharmacist will receive and manage both the non-biologically active gel (placebo) and the Timoptic-XE®. The pharmacist will compound and dispense the Timoptic-XE® or non-biologically active gel hydrogel for the patients according to the arm to which they have been randomized. She will repackage the medications into identical dispensers with a coded label and will dispense a 12-week supply to each patient according to their individual randomized unique identifier to ensure accurate storage and dispensing records. The un-blinded research pharmacist will keep record of the drug and patient treatment group assignment. Please see the VA Research Pharmacist's letter of agreement to participate as described. For Timoptic-XE® the recommended ocular dosage is 0.25mg/day (1 drop in each eye once a day). The average exposed ocular surface is about 3 cm^2. Based on the studies using timolol topically for ulcerative hemangiomas and the numerous case studies of topical timolol on chronic wounds, the dosage used in this study will be 3 drops/3cm^2/day that is equivalent to 0.75mg/3cm^2/day. Depending on

the size of the wound, the research pharmacist will dispense one or two bottles (with either Timoptic-XE® or placebo) for the 12 weeks supply as described in the table below.

Wound size (cm^2)	Number of drops	Timolol dosage (mg/day)	Timolol dosage (mL/day) Note: 1 drop = 0.05 ml	Dosage for 12-week supply (ml)	Number of bottles (timolol or placebo) dispensed to patient for 12-week supply (1 bottle = 5ml)
< 0.5	1	0.25	0.05	4.2	1
> 0.5 - 0.9	1	0.25	0.05	4.2	1
> 1.0 - 1.9	1	0.25	0.05	4.2	1
> 2.0 - 2.9	2	0.5	0.1	8.4	2
> 3.0	3	0.75	0.15	12.6	3

1.1. STUDY FLOW CHART

Study Plan Schematic

Procedure	**Screening Phase** Visits 1-2 (Week 1-2)		**Active Phase** Visits 3-14 (Week 3-14) *Including a 1st confirmatory visit if healed before 12 weeks of treatment*		**2nd Confirmatory Visit** **Prior to Follow-up Phase** Visit 15 (Week 15)	**Follow-up Phase** Visits 16, 17, 18 & 19 (Week 19, 23, 27 & 31)			
	Visit 1 (Week 1)	Visit 2 (Week 2)	Visit 3 (Week 3) **Randomization**	Visits 4-14 (Week 4-14) **Study Endpoint at Week 14***	Visit 15 (Week 15)	Visit 16 (Week 19)	Visit 17 (Week 23)	Visit 18 (Week 27)	Visit 19 (Week 31)
Inclusion/Exclusion Criteria	X	X	X						
Informed Consent/HIPAA document	X								

VR-36 Health Survey Questionnaire	X			X*					
Lower Extremity Functional Scale Outcome Questionnaire	X	X		X					X
Charlson-Comorbidity Index	X								
Physical Exam	X			X*					
EKG	X			X*					
Vital Signs	X	X	X	X	X	X	X	X	X
Ankle-Brachial Index/Duplex Scan	X	X							
Debrided Tissue for genetic analysis (Weeks,1-9)	X	X	X	X					
Bacterial, Fungal Infection and Malignancy Evaluation	X								
Laboratory evaluation (hematology, renal, and liver function)	X								
Vapometer Readings@ (@If healed, readings recorded from point of closure + 4 months)				X@	X	X	X	X	X
Blood Sample for Timolol assay/Inflammatory markers(weeks 4,8,12)	X			X†					
Ulcer Assessment	X	X	X	X	X	X	X	X	X
Debridement († if needed)	X	X	X	X	X†	X†	X†	X†	X†
Ulcer Photography and Area Measurement	X	X	X	X	X	X	X	X	X
Offloading device	X	X	X	X	X	X	X	X	X
Randomization			X						
SOC + Timoptic-XE® or SOC + non biologically active gel (hydrogel as placebo medication)			X	X					

SOC			X	X	X	X	X	X	X
Concomitant Medications and Adverse Events	X	X	X	X	X	X	X	X	X

4.3.1 Screening/Baseline Period (Visit 1, 2)

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: A comprehensive history and physical exam along with a battery of tests) will be performed on each study applicant to assess eligibility during the screening phase. Those subjects with known drug allergy to Timoptic-XE® and those not meeting the exclusion criteria will not be enrolled.

1) Demographic information: gender, age, race.
2) Medical history: medical problems, surgeries, trauma, history of previous ulcers, amputations, characteristic and duration.
3) Comprehensive history and physical exam: vital signs, height, weight, Body Mass index
4) General health and lifestyle: smoking history, alcohol, drugs abuse.
5) Lower extremity exam: vascular – pedal pulses, color of skin, temperature, edema; dermatological – clinical description of the ulcer, 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: hematology, chemistry, EKG, microbiology and pathology HbA1c, 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, VR-36 Health Survey and the Lower Extremity Functional Scale Outcome Questionnaire.
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®) 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/Treatment (Visit 3/Week 3)

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 SOC+Timoptic-XE or SOC + non biologically active gel (hydrogel as placebo medication) or the control (standard therapy).

Patients that are not randomized into group A or B due to ineligibility (i.e. meet exclusion criteria, do not meet all inclusion criteria, non-compliance, withdrawal) will be passively followed via CPRS on the status of their diabetic foot ulcers for the duration of the study.

4.3.3 Treatment Phase (Visits 4 to 14/Weeks 4 to 14)

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.

- All subjects will have weekly visit including:
- Vital signs
- Ulcer assessment and measurement
- Lab tests per protocol
- Sharp debridement
    - Weeks 1-9: debrided tissue will be collected and analyzed for molecular markers of inflammatory characterization of wound microbiome.
- Wound cleansing and moist wound healing dressing
- Infection assessment if indicated with confirmatory bacterial culture (deep swab curette, tissue specimen/biopsy), radiographical X-rays and blood work (CBC, ESR, CRP, and chemistry) if deemed necessary by investigator clinician.
- Use of an offloading device that protects the wound from pressure or trauma related to ambulation and other acts of daily living. The total contact cast or instant total contact cast would be ideal offloading devices. However, given the nature and complexities associated with DFU, it is unrealistic to expect that all patients to tolerate such offloading devices. Thus, the offloading device provided will be dependent on the subjects' ability to tolerate the specific offloading device. Adherence to offloading device will be evaluated by the investigator at each visit by observing plantar wear patterns and inquiring from the patient if offloading was used consistently as instructed. Alternative to total contact cast or instant total contact cast (cam walker), offloading devices will be offered including, but not limited to the use of felt/foam adhesive/post op shoe, custom offloading insole, and customized healing shoe in combination with gait assistive device such as a roll-a-bout scooter, walker, wheelchair or motorized scooter.
- Application of treatments:
    - Standard of Care: Application of non biologically active gel (hydrogel as placebo medication), non-adhesive dressing (Adaptic® or Mepitel®), over wound bed, covered by dry gauze dressings
    - Timoptic-XE®: Application of Timoptic-XE® , non-adhesive dressing (Adaptic® or Mepitel®), over wound bed, covered by dry gauze dressings. Up to 12 weeks of daily applications will be done to wound until healed.
- Offloading devices (see above)

- Changes in concomitant medications, adverse events, compliance to offloading, will be recorded.
- If ulcers heal sooner than 12 weeks, the subject will be assessed the following week for a confirmatory visit, followed another week later by a 2nd confirmatory visit. Ulcer healing is defined by 100% epithelialization as determined by the clinician. The subject with healed ulcer will also return for follow-up visits Weeks 19, 23, 27 & 31 (visits 16, 17, 18 and 19).
    - Vapometer recordings will be collected on visits corresponding to weeks 19, 23, 27, and 31 on the newly healed ulcer site, 5 cm away from healed ulcer site. Readings on the contralateral side will also be marked and taken and recorded. **5 recordings for each site will be taken and the average will be documented.

NOTE: if ulceration remains open after the 12th week of application – then will switch back to standard of care and the subject will be followed monthly until completion of the study.

Blood samples will be taken on each subject on weeks 4, 8 and 12 to determine serum Timolol level. We do not anticipate blood levels in our patients to be higher than those seen in patients who receive Timoptic XE® gel for ocular indication (normal range: 0.3-0.5 ng/ml). We will terminate study on patients in whom Timolol level is 0.7 ng/ml or higher.

4.3.4 Study Endpoint (Visit 14/Week 14)

- Subjects to complete the health-related quality of life survey or the VR-36 Health Survey and the Lower Extremity Functional Scale Outcome Questionnaire.
- Vital signs will be obtained and recorded.
- The ulcer will be assessed – visual inspection – complete ulcer characteristics form (see appendix). Determine if reaches 100% epithelialization determined by clinician.
- 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.
- Vapometer recordings will be collected on visits corresponding to weeks 19, 23, 27, and 31 on the newly healed ulcer site, 5 cm away from healed ulcer site. Readings on the contralateral side will also be marked and taken and recorded. **5 recordings for each site will be taken and the average will be documented
- Changes in concomitant medications, adverse events, compliance to offloading, will be recorded.

4.3.5 2ND CONFIRMATORY VISIT (VISIT 15/WEEK 15)

4.3.6 Follow-Up Phase (Visits 16 to 19/Weeks 19, 23, 27 & 31)

- The ulcer will be assessed – visual inspection – complete ulcer characteristics form (see appendix). Determine if reaches 100% epithelialization 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.
- Vapometer recordings will be collected on visits corresponding to weeks 19, 23, 27, and 31 ,on the ipsilateral healed ulcer site, 5 cm away from healed ulcer site. Readings on the contralateral side will also be marked and taken and recorded. **5 recordings for each site will be taken and the average will be documented

4.3.7 Real-World Group Phases

Screening/Baseline Period (Visit 1/Week1)

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 1st visit.

Screening assessments and pre-treatment: A comprehensive history and physical exam along with a battery of tests) will be performed on each study applicant to assess eligibility during the screening phase. Those who do not meet the inclusion criteria and meet any exclusion criteria for the real-world group phase will be excluded.

1) Demographic information: gender, age, race.
2) Medical history: medical problems, surgeries, trauma, history of previous ulcers, amputations, characteristic and duration.
3) Comprehensive history and physical exam: vital signs, height, weight, Body Mass index
4) General health and lifestyle: smoking history, alcohol, drugs abuse.
5) Lower extremity exam: vascular – pedal pulses, color of skin, temperature, edema; dermatological – clinical description of the ulcer, 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: hematology, chemistry, EKG, microbiology and pathology HbA1c, 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, VR-36 Health Survey and the Lower Extremity Functional Scale Outcome Questionnaire.
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®) 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.
15) Blood will be collected for inflammatory marker analysis.

Follow-Up Phase (Visit 2/Week 6)
Subjects will be evaluated and receive standard of care. Appointments will be made as determined by investigator at each VA facility.

All subjects during this visit will have;

- Vital signs
- Ulcer assessment and measurement
- Lab tests per protocol
- Sharp debridement
    - debrided tissue will be collected and analyzed for molecular markers of inflammatory characterization of wound microbiome.
- Wound cleansing and moist wound healing dressing
- Infection assessment if indicated with confirmatory bacterial culture (deep swab curette, tissue specimen/biopsy), radiographical X-rays and blood work (CBC, ESR, CRP, and chemistry) if deemed necessary by investigator clinician.
- Use of an offloading device that protects the wound from pressure or trauma related to ambulation and other acts of daily living. The total contact cast or instant total contact cast would be ideal offloading devices. However, given the nature and complexities associated with DFU, it is unrealistic to expect that all patients to tolerate such offloading devices. Thus, the offloading device provided will be dependent on the subjects' ability to tolerate the specific offloading device. Adherence to offloading device will be evaluated by the investigator at each visit by observing plantar wear patterns and inquiring from the patient if offloading was used consistently as instructed. Alternative to total contact cast or instant total contact cast (cam walker), offloading devices will be offered including, but not limited to the use of felt/foam adhesive/post op shoe, custom offloading insole, and customized healing shoe in combination with gait assistive device such as a roll-a-bout scooter, walker, wheelchair or motorized scooter.
- Application of treatments:
    - Standard of Care: Application of non biologically active gel (hydrogel as placebo medication), non-adhesive dressing (Adaptic® or Mepitel®), over wound bed, covered by dry gauze dressings
- Offloading devices (see above)
- Changes in concomitant medications, adverse events, compliance to offloading, will be recorded.
- Blood will be collected for inflammatory marker analysis

Study Endpoint (Visit 3/Week 12)

- Subjects to complete the health-related quality of life survey or the VR-36 Health Survey and the Lower Extremity Functional Scale Outcome Questionnaire.
- Vital signs will be obtained and recorded.
- The ulcer will be assessed – visual inspection – complete ulcer characteristics form (see appendix). Determine if reaches 100% epithelialization determined by clinician.
- 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.
- Blood will be collected for inflammatory marker analysis.

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 Monitory Committee:
A Central Data Monitoring Committees board will be established with Hines VA Hospital, Hines, IL 60141.

Consultant Services
Dr. Hien Nguyen (Internal Medicine, Section Chief of Infectious Diseases, Assistant Professor of Medicine at UC Davis Medical Center) will assist in the management of subjects and subject safety.
Dr. Arthur Swislocki (Endocrinology, Section Chief of Endocrinology) will assist in the management of patient regarding issues related to diabetic glucose control.
Dr. Saul Schaefer (Cardiology, Section Chief of Cardiology) will assist in the management of patients regarding issues pertaining to blood pressure and pulse monitoring.

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 Visit 14/Week 14, and final secondary endpoint measurement at Visit 17/Week 31. The ulcer will be assessed weekly and will be treated as described above. A designated inter-observer (blinded to the treatment of the subjects) will regularly review images and physical exam findings to determine primary and secondary endpoints. 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 Visit 14/Week 14 will have follow-up assessment one week after healing, a 2nd assessment a week after, then 4 monthly visits at Weeks19, 23, 27 & 31. If the ulceration healed after 12 weeks, then the subjects will be similarly assessed. The subject with healed ulcer will also return for follow-up visits Weeks19, 23, 27 & 31 (visits 16, 17, 18 and 19).

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, 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 Visit 14/Week 14 and Visit 17/Week 31. Complete healing will be defined as full re-epithelialization 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 using Silhouette Mobile™ ulcer tracing and surface area calculation.
- 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 epithelialization, 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 incidences of adverse effects will be reported from all sites and will be withdrawn from the study.
- VR-36 Health Survey, and the Lower Extremity Functional Scale Outcome Questionnaire (self-administered questionnaires) will be used to measure quality of life and provided at beginning of the study and at the study endpoint.
- Rate of transepidermal water loss will be measured weekly subsequent to wound closure and compared in the two groups.

Sample Size Estimation:
From previous studies, we expect that about 20% of the study subjects will heal in the control arm of our study by the 12th week of care. We estimate that the experimental treatment will improve this outcome with 63% of the study subjects healing by the 12th week of care (a healing rate close to that reported in the most recent case series of topical use of timolol on chronic wounds.

We will perform simple allocation of the subjects using an electronic randomization for treatment assignment-since our target enrollment population is fairly homogenous.

The sample size calculation will require the enrollment of 48 subjects (24 subjects in each arm) to provide a power of 80% to detect a difference of 43% (= 63% - 20%) in the rate of ulcer healing between the two arms by using two-sided Fisher's exact test at a significance level of 5%. It is estimated that the overall attrition will be 35%. Specifically, 10% of the subjects who are enrolled will be exited from the study prior to randomization, 10% will not meet the primary endpoint analysis inclusion requirement and 15% will not complete the study due to other factors such as treatment failure, loss to follow-up, adverse events, clinical/safety issues, and/or non-compliance. We will track the various reasons for study exit. Therefore, 138 subjects (69 subjects in each arm) will be recruited into the study after taking into account the overall attrition of 35%.

The consulting statistician will review implementation and compare major baseline demographic and prognostic characteristics to ascertain that randomization was successful. The power analysis was formulated using the STPLAN Version 4.5 (2010) with the input of the study consultant biostatistician.

Analyses:

The primary outcome is complete ulcer closure by Week 14 (end of the Active Phase) of the study and the rate of ulcer healing by the 12th week of care between the two arms.

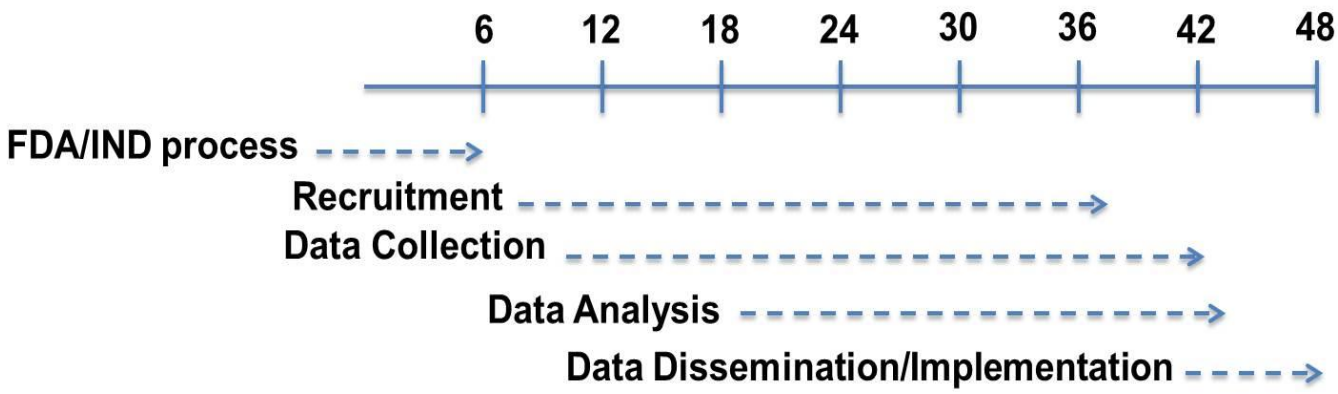
Hypothesis to be tested: The null hypothesis is that there is no difference in the proportion of subjects with complete wound closure of DFU between the timolol treatment group and the control group. Complete wound closure is defined as 100% closure, or skin re-epithelialization without any drainage present or dressing requirements. Two-sided Fisher's exact test will be used to compare the proportion of complete ulcer closure by Week 12 (end of the Active Phase) of the study and the rate of ulcer healing by the 12th week of care between the two arms. The Kaplan-Meier method will be used to estimate the survival function of time to achieve complete ulcer closure (if less than 12 weeks) for each arm, and a two-sided log-rank test will be used to compare the survival curves between the two arms.

The area of the target ulcer will be summarized by treatment group and visit. Both actual value and change from the previous visits ulcer area will be presented. A second summary of target ulcer area will also be given which will present change from baseline. The weekly change and the percentage weekly change in the target ulcer area from visit to visit will be summarized by treatment group and visit. The two-sided t-test or Wilcoxon rank-sum test as appropriate will be used to compare relative ulcer area from baseline to Week 12 between the two arms.

Chi-square test or Fisher's exact test as appropriate will be used to compare wound healing rates in relationship to wound size, as well as wound healing rates between the treatment groups at the 4-week follow-up and at the 12-week follow-up period and recidivism rate.

Additional secondary analyses to evaluate the association of specific wound characteristics (such as wound size, wound location) and subject characteristics (body mass index, chronicity of the wound, history of DVT, length of diabetes, HbA1c, and ABI/toe pressure will be conducted using logistic regression. Further logistic regression will be used to investigate the relationship between the occurrence of each type of adverse event and treatment in order to adjust for each of the potential confounding factors previously listed. A two-way ANOVA with a post hoc test will be used to determine the relationship between timolol serum levels and wound healing. For demographic and clinical characteristic data at baseline, continuous variables will be summarized (when appropriate) by using mean, median, standard deviation, co-efficient of variation, minimum value and maximum value. Categorical variables will be summarized with frequency tables. Baseline comparability between the two treatment groups will be assessed by using the independent two-sample t-test or Wilcoxon rank-sum test and the Chi-square test or Fisher's exact test. Finally, we will use ANCOVA to analyze the quality of life (QOL) using the VR-36 Health Survey and the Lower Extremity Functional Scale Outcome Questionnaire, and the Charlson Comorbidity Index.

Timeline and Milestones:


6
12
18
24
30
36
42
48
FDA/IND process
Recruitment
Data Collection
Data Analysis
Data Dissemination/Implementation

7. APPENDIX 1 – Wound Scoring System

Ulcer Characteristic Form

Size: Dimensions _____x_____x_____cm

Number of leg ulcers: Right Leg _____ Left Leg _____

If multiple ulcers, write distance from the study ulcer to other ulcers: _____cm
Where is the study ulcer located? □ Right Leg □ Left Leg

Describe the study ulcer location: ☐ foot ☐ ankle ☐ low calf ☐ high calf ☐ other: _____

Describe the study ulcer position: ☐ dorsal ☐ ventral ☐ medial ☐ anterior ☐ lateral ☐ posterior

How old is the current target diabetic foot ulcer (in weeks): _____ weeks

Event that precipitated target ulcer: ☐ Ill-fitting shoes ☐ Poor foot care ☐ Acute accidental event (trauma) ☐ Not determined ☐ Other, please specify: _____

Has the study ulcer ever healed and recurred? ☐ Yes ☐ No

Has the subject ever suffered a DVT in the study limb? ☐ Yes ☐ No

Does the Study Limb/Leg Ulcer present with any of the following? *(Check all that apply)*
☐ Hyperpigmentation ☐ Varicosities ☐ Dermatitis ☐ Swelling/Edema
☐ Lipodermatosclerosis ☐ Other: _____

	Parameter	Assessment	Score	Total
1	Erythema	Absent	0	
		Mild	1	
		Moderate	2	
		Severe	3	
2	Induration	Absent	0	
		Mild	1	
		Moderate	2	
		Severe	3	
3	Tenderness	Absent	0	
		Mild	1	
		Moderate	2	
		Severe	3	
4	Pain	Absent	0	
		Mild	1	

		Moderate	2	
		Severe	3	
5	Local warmth	Absent	0	
		Mild	1	
		Moderate	2	
		Severe	3	
6	Size (cm^2)	<1	0	
		1–2	1	
		> 2–5	3	
		> 5–10	6	
		> 10–30	8	
		> 30	10	
7	Depth (mm)	<5	0	
		5–9	3	
		10–20	7	
		> 20	10	
8	Undermining (mm)	< 2	3	
		2–5	5	
		>5	8	
			TOTAL SCORE	

Wound Assessment Tool Interpretation

1. **Erythema**, congestive or exudative redness surrounding the wound caused by engorgement of the capillaries in the lower layers of the skin. Grade: none: absent; mild: pink, barely perceptible; moderate: pale red with defined edges; severe/extreme: red to dark red.
2. **Induration**, inflammatory hardening or thickening of tissues. Grade: none: absent; mild: localized to the site of infection; moderate: limited extension from the site of infection; severe: extending from the site of infection to involve a substantial portion of the affected lower extremity.
3. **Tenderness** (sign), palpation of the site elicits a report by the patient of tenderness; measured on a 0 (no tenderness) to 10 (the worst imaginable tenderness) scale. Grade: none: absent; mild: score of ≤ 5; moderate: score of 6–8; severe: score of ≥ 9.
4. **Pain** (symptom), subjective reporting of discomfort or the perception of pain at the site of the wound as reported by the patient; measured on a 0 (no pain) to 10 (the worst imaginable pain) scale. Grade: Grade: none: absent; mild: score of ≤ 5; moderate: score of 6–8; severe: score of ≥ 9.
5. **Local warmth** (sign), Increase in skin temperature relative to the uninfected contralateral foot. Grade: none: temperature of the two sides the same; mild: temperature slightly, but perceptibly warmer; moderate: temperature clearly warmer; severe: marked difference in temperature.
6. **Size** (cm^2) - the ulcer area.

7. **Depth** (mm) - measured in the deepest apparent part of the wound using a sterile cotton-tipped wooden swab held 90^0 to the wound and marked with a pen held parallel to the surface of the intact skin.
8. **Undermining** (mm), measurement of any tunneling, subepithelial tissue loss, or shearing, as measured using a sterile cotton-tipped wooden swab.

Lipsky *et al*. The value of a wound score for diabetic foot infections in predicting treatment outcome: a prospective analysis from the SIDESTEP trial. Wound Repair Regen. 2009 Sep-Oct;17(5):671-7. Epub 2009 Aug 11.

1: Denda M, Fuziwara S, Inoue K. Beta2-adrenergic receptor antagonist accelerates skin barrier recovery and reduces epidermal hyperplasia induced by barrier disruption. J Invest Dermatol. 2003 Jul;121(1):142-8. PubMed PMID: 12839574.

2: Berardesca E, Loden M, Serup J, Masson P, Rodrigues LM. The revised EEMCO guidance for the in vivo measurement of water in the skin. Skin Res Technol. 2018 Aug;24(3):351-358. doi: 10.1111/srt.12599. Epub 2018 Jun 20. PubMed PMID: 29923639.

3: Jansen van Rensburg S, Franken A, Du Plessis JL. Measurement of transepidermal water loss, stratum corneum hydration and skin surface pH in occupational settings: A review. Skin Res Technol. 2019 Sep;25(5):595-605. doi: 10.1111/srt.12711. Epub 2019 May 20. Review. PubMed PMID: 31111588.

8. APPENDIX 2 – Hemostasis Procedure:

The goal of debridement is to achieve "pinpoint" bleeding at the wound base that is easily controlled with minimal pressure. In case of perfuse bleeding study staff will attempt to control bleeding with prolonged firm pressure. If bleeding is not controlled after 5 minutes of continuous pressure, study staff will attempt to achieve hemostasis using sterile electrodesiccation. If the subject bleeding cannot be controlled or the subject develops signs and symptoms of early shock, study staff will urgently escort the subject to the ED. Serious adverse event should then be reported.

9. APPENDIX 3 – Infection Exclusion Procedure

The following should be considered positive infection criteria and should exclude a subject from participating in the study: clinical signs and symptoms of infection resulting in clinical assessment of infection, quantitative tissue biopsy demonstrating pathological species exceeding 10^6 CFU/gram of tissue, positive fungal culture, positive x-ray, or being treated for infection within the last 30 days.

In equivocal cases such as isolated elevation of ESR, CRP or WBC (without other clinically obvious source) or an equivocal X-ray reading, a definitive MRI study should be ordered to exclude osteomyelitis. If the MRI is equivocal, a bone biopsy should be performed.

Exclusion of infection should be exhaustively pursued in order to avoid enrolling a subject with "brewing" infection as detailed in inclusion/exclusion criteria.

10. APPENDIX 4 – Home Care Instructions

Handout to be handed out on to patients

Timolol Trial - Home Wound Care Instructions (to be distributed as handouts to the subjects)

Dear patient,
Please read and follow carefully ALL the instructions at home DAILY:

1. Keep your dressing clean and dry at all times. When bathing, wrap the entire dressing with two large garbage bags and seal with surgical tape provided. DO NOT tighten the tape so it cuts blood flow. Do your best to avoid direct water contact with the wrapped foot.
2. Change your dressing once a day using specific materials provided to you by your doctor.
3. Apply the exact number of drops of the medication, as instructed by your doctor.
4. Cover the wound with Adaptic/Mepitel, sterile gauze and secure with paper tape (or kerlix wrap and tape).

Thank you for your cooperation.

Provider instructions:

1. Please review the instructions with the patient. Please ensure the patient understands the instructions and is capable of providing home care.
2. Do not forget to order relevant supplies.
3. Secondary dressings include use of Adaptic/Mepitel, gauze (or kerlix wrap), and tape placed by the clinician. Remember: Additional absorptive dressings may be used if there is excessive drainage.
4. Dressings should be changed daily per protocol instructions above.

11. APPENDIX 5 –Veterans Rand-36 (VR-36) Health Survey

Please see attachment in IRBNet, titled, "VR-36 Health Questionnaire"

12. APPENDIX 6 –Lower Extremity Functional Scale

The Lower Extremity Functional Scale

We are interested in knowing whether you are having any difficulty at all with the activities listed below **because of your lower limb problem** for which you are currently seeking attention. Please provide an answer for **each** activity.

Today, *do you* or *would you* have any difficulty at all with:

	Activities	Extreme Difficulty or Unable to Perform Activity	Quite a Bit of Difficulty	Moderate Difficulty	A Little Bit of Difficulty	No Difficulty
1	Any of your usual work, housework, or school activities.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
2	Your usual hobbies, re creational or sporting activities.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
3	Getting into or out of the bath.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
4	Walking between rooms.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
5	Putting on your shoes or socks.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
6	Squatting.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
7	Lifting an object, like a bag of groceries from the floor.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
8	Performing light activities around your home.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
9	Performing heavy activities around your home.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
10	Getting into or out of a car.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
11	Walking 2 blocks.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
12	Walking a mile.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
13	Going up or down 10 stairs (about 1 flight of stairs).	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
14	Standing for 1 hour.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐
15	Sitting for 1 hour.	0 ☐	1 ☐	2 ☐	3 ☐	4
16	Running on even ground.	0	1	2	3	4
17	Running on uneven ground.	0	1	2	3	4
18	Making sharp turns while running fast.	0	1	2	3	4
19	Hopping.	0	1	2	3	4
20	Rolling over in bed.	0	1	2	3	4
	Column Totals:					

Minimum Level of Detectable Change (90% Confidence): 9 points **SCORE: ____/ 80 (fill in the blank with the sum of your responses)**

13. APPENDIX 7 – Charlson Comorbidity Index

https://www.mdcalc.com/charlson-comorbidity-index-cci

14. APPENDIX 8 – Timolol IND 122399 - FDA "Safe to proceed"

Please see attachment "Timolol IND 122399"