Double Blind, Single-Center, Randomized, Within-Subject Placebo, Phase I Study Evaluating the Effects of Novel Topical Gel in Prevention of Hypertrophic Scar Formation

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List of Abbreviations

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
ECM	Extracellular Matrix
ECG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IDS	Investigational Drug Services
IND	Investigational New Drug Application
IRB	Institutional Review Board
LSR	Local Skin Reaction
PCCA PP	Professional Compounding Centers of America Pracasil™-Plus
PHI	Protected Health Information
PI	Principal Investigator
RCT	Randomized Clinical Trial
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
SPP1	Osteopontin
SSE	Surgical Scar Excision
TP	Topical Pentamidine + PCCA Pracasil™-Plus
US	Ultrasound
VAS	Visual Analog Scale
VSS	Vancouver Scar Scale
POSAS	Patient and Observer Scar Assessment Scale

Study Summary

Title	Double Blind, Single-Center, Randomized, Within-Subject Placebo, Phase I Study Evaluating the Effects of Topical Pentamidine in Prevention of Hypertrophic Scar Formation
Running Title	Novel Topical Gel for Hypertrophic Scar Prevention
IRB Protocol Number	17-005230
Phase	Pilot Study
Methodology	Double Blind, Single-Center, Randomized, Within-Subject Placebo Phase I Study
Overall Study Duration	3 months
Subject Participation Duration	3 months
Single or Multi-Site	Single site
Objectives	Evaluate safety of topical pentamidine (TP) as an adjuvant to surgical scar excision (SSE) in the treatment of hypertrophic scar formation. Safety will be measured by serial monitoring for adverse events following the procedure and during treatment duration. Secondarily, study will evaluate drug quality and standardizations as well as investigate initial signs of efficacy.
Number of Subjects	10
Diagnosis and Main Inclusion Criteria	Hypertrophic scar diagnosed in Mayo Clinic Department of Dermatology or Division of Plastic Surgery, Rochester, Minnesota.
Study Product, Dose, Route, Regimen	Pentamidine, delivered as topical formulation containing 2% TP in silicone-containing base, PCCA Pracasil™-Plus (PP), versus placebo base alone. Approximately 1.8 mL of product in allotted single dose containers will be applied every 48 hours for 4 weeks following surgical scar excision.
Duration of Administration	Once every 48 hours for 4 weeks.
Reference therapy	SSE with silicone compounding base only (PCCA PP).
Statistical Methodology	The primary analysis will use descriptive statistics to document adverse events between treated and untreated lesions. The secondary analysis will use paired t-test or Wilcoxon signed rank test to compare the change in lesion height and characteristics between the treated and placebo groups following SSE (TP + PCCA PP vs. PCCA PP alone). In addition, two-factor analysis of variance (ANOVA) with repeated measures on both factors will be used to evaluate the effect of treatment and follow-up time for other continuous measures such as Vancouver Scar Scale (VSS) and Patient and Observer Scar Assessment Scale (POSAS).

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Hypertrophic scars are formed by an excessive tissue response to dermal injury characterized by local fibroblast proliferation and overproduction of collagen [1, 2]. With an incidence of hypertrophic scarring occurring at rates of 40-70% post-surgery and up to 91% following burn injury [3, 4], an effective topical treatment would offer immense benefits to a patient's quality of life. Current treatments for hypertrophic scars rely on management via surgical intervention [5], compression therapy [6], silicone gel sheeting [7], laser [8, 9] or corticosteroid injection [10, 11]. While these may offer temporary relief, interventions such as corticosteroid therapy have recurrence rate of up to 50% after 5 years [12, 13] and various skin complications including dermal atrophy, skin ulceration and hypo/hyperpigmentation [14].

The use of silicone gel sheeting to prevent and treat hypertrophic scarring is still relatively new, and started in 1981 with treatment of burn scars [15]. Topical silicone gel sheet and silicone cream have been evaluated as efficacious in treating keloids and hypertrophic scars due to their hydrating effect on the stratum corneum [16-18]. Silicone-based therapies are currently being investigated in several clinical trials for hypertrophic abdominal scar (NCT01078428), hypertrophic and keloid scar (NCT00754247), and post-surgical hypertrophic scarring (NCT01861119; NCT00849004) (http://clinicaltrials.gov/) [19]. Preliminary results from the Investigation of a Novel Silicone Dressing to Maximize the Outcomes of Scar Revision Procedures (IMPROVE) trial (NCT01430130) showed improvement in the visual analog scale (VAS) in the silicone dressing cohort. This proof-of-concept demonstration suggests that silicone-based therapy may provide a solution for hypertrophic scar prevention. Despite these findings, treatment of hypertrophic scarring varies and management targeting the root cause of abnormal wound healing is warranted.

Early inflammation following dermal injury is essential in the progression of normal wound healing; in contrast, chronic or excessive inflammation leads to pathological scarring and fibrosis [20, 21]. Our recent preliminary findings show that osteopontin (SPP1), a secreted chemokine-like protein [22] involved in intracellular signaling [23], modulates extracellular matrix (ECM) remodeling [24]; this interaction plays a role in scar formation as SPP1 is known to drive tissue fibrosis and aging [25]. Previous studies have shown that SPP1 local knockdown leads to accelerated repair and decreased granulation tissue and scar formation [26]. Thus, SPP1 expressed by wound fibroblasts as a consequence of signals from inflammatory cells may hinder the rate of wound repair and regeneration. Screening pharmaceutically active compounds that inhibit the SPP1 promoter led to the discovery of pentamidine isethionate, which inhibited SPP1 promoter activity by ≥80% with minimal in vitro cell toxicity.

Here we investigate a novel topical agent, SPP1 inhibitor, pentamidine isethionate, compounded in silicone-containing base, as an adjuvant therapy to surgical scar excision (SSE) to prevent adverse scarring and enhance skin rejuvenation by upregulating anti-fibrotic and anti-aging processes. Initial studies in rabbit ear hypertrophic scar formation model (n=18 rabbits) showed significantly reduced scar elevation index following 4 weeks of topical pentamidine (TP) treatment

compared to silicone base, PCCA Pracasil™-Plus (PP), versus no treatment. As such, TP serves as a primed agent for trial as a clinical-grade product for human use.

1.2 Investigational Agent

SPP1 is a pro-inflammatory inducer of skin fibrosis and aging. Screening pharmaceutically active compounds that inhibit the SPP1 promoter led to the discovery of pentamidine isethionate, which inhibited SPP1 promoter activity by ≥80% with minimal in vitro cell toxicity. Mayo Clinic Compounding Pharmacy was utilized to produce pentamidine isethionate, delivered as topical cream formulation containing 2% TP in PCCA PP base.

1.3 Preclinical Data

Pre-clinical testing (animal and biocompatibility) of TP in silicone base (PCCA PP) demonstrated safety and biocompatibility across numerous rabbit models of hypertrophic scar formation. Pre-clinical animal and biocompatibility data are provided below.

We developed a hypertrophic scar formation rabbit ear model following IACUC approval (A00001970-16). Surgical ligation of leporine auricular vessels induced an ischemic landscape for hypertrophic scar production. Specifically, cranial artery and vein were ligated; caudal artery and vein were preserved; and central artery was ligated but central vein was preserved in the rabbit ear (Figure 1A). Following vascular ligation, one linear full-thickness skin wound was created on the ventral side of each ear with a surgical blade. The skin and perichondrium were removed and wound edges were closed with 4-0 Nylon sutures, generating tension for hypertrophic scarring.

Following ischemic wounding, we assessed the relative ischemia created in the ear at time of surgery and time of sacrifice with a fluorescent light assisted angiography (Spy Elite, LifeCell) (Figure 1B). Ischemic perfusion coupled with mechanical suture tension yielded hypertrophic scar formation.

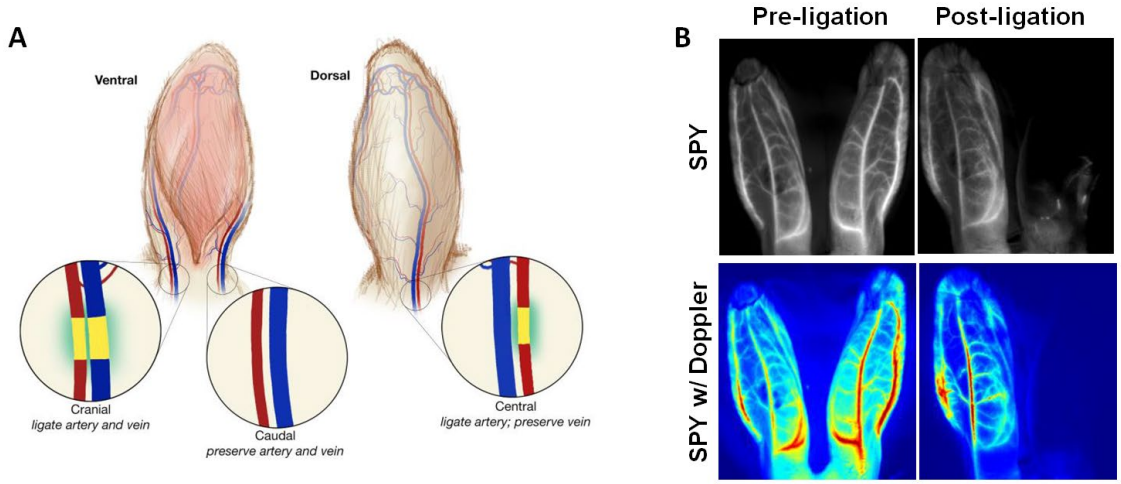


Figure 1. Ischemic Rabbit Ear for Hypertrophic Scar Model. Surgical Ligation of Leporine Auricular Blood Vessels, A. Fluorescent light-assisted angiography (Spy Elite) with and without Doppler, B.

Following vascular ligation and linear wounding, rabbits were assigned to treatment groups: (1) control group with synthetic bandage (no treatment), (2) PCCA PP (base only), or (3) TP in PCCA PP. Treatment with 2% TP in PCCA PP demonstrated scar reduction following 4 weeks of topical gel application (Figure 2). These results provide proof-of-concept for the proposed trial using 2% TP in PCCA PP for hypertrophic scar reduction.

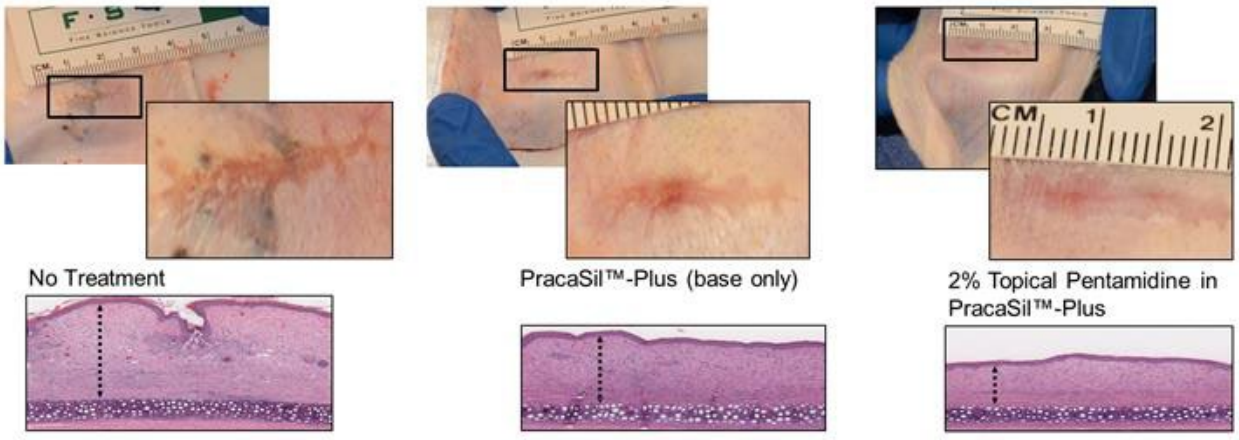


Figure 2. Hypertrophic Scar Reduction.

1.4 Clinical Data to Date

There is no available clinical research data to date on topical application of pentamidine isethionate to treat hypertrophic scarring.

1.5 Dose Rationale

Drug titration testing with 1%, 2% and 5% pentamidine isethionate identified 2% as the optimal active dose for wound healing.

1.6 Study Risks and Benefits

1.6.1 Anticipated Risks

Although the risk is extremely small, subjects are theoretically at increased risk of infection through participation in this study. In addition to standard of care with surgical scar excision (SSE), patients in this study will have skin biopsied from their scar site. There also exists a potential risk for adverse skin reactions following topical drug application and/or concomitant drug interactions. The type of infections and adverse skin reactions that are theoretically possible and how we will monitor and address are listed below:

- Acute soft tissue infection:

Risk of infection from scar excision alone is extremely low. In Dr. Moran's practice, there has been very limited instance of infection. However, we will contact patients by phone at 24 hours post-discharge as well as at 2 months and 3 months to address any of the following symptoms: increasing pain at the wound site, redness around the wound or fever [defined as body temperature above 100.4°F (38.0°C)], as deemed appropriate by the primary investigator. In addition, the study subjects will be asked to self-monitor for symptoms of pain or fever and contact the study investigators immediately if they have any of above symptoms.

- Adverse skin reactions:

In its use as an antifungal treatment, topical pentamidine (TP) has been proven to have minimal to no adverse skin reactions (Patent Publication# US5230897-A). We will be monitoring patients weekly during the first month. Follow-up visits at week 2and week 4 will assess change in physical exam or pattern of pain. Patients will also be encouraged to self-monitor for symptoms of pain or fever. Patients will seek immediate medical attention for fever above 103˚F (39.4°C).

With the proposed protocol, acute risks exist and are as follows:

- Pain
- Itching
- Urticaria
- Infection
- Inflammation
- Bruising
- Crusting/Scabbing
- Hyper/hypo/depigmentation
- Thickening of the skin
- Change in vascularization

1.6.2 Overall Study Risk Management

The following measures implemented by Mayo Clinic will minimize the risks associated with patients' participation in the clinical study:

- Mayo Clinic selects personnel with extensive experience in conducting clinical studies and in performing procedures involved in the protocol including skin biopsies and surgical procedures.
- All examinations, treatment procedures and interpretation of clinical data generated during the study is directed, overseen and analyzed by an appropriately licensed and credentialed physician who has been trained to the clinical study protocol.
- Mayo Clinic developed the clinical protocol and training programs to ensure that the study personnel at Mayo Clinic have a strong knowledge and understanding of the clinical protocol, including patient selection criteria and procedure requirements.
- Mayo Clinic carefully developed patient eligibility criteria for the investigation including clearly defined inclusion and exclusion criteria to ensure that only properly selected patients will be enrolled in the clinical study.
- The protocol is designed so that patient treatment and follow-up procedures will be consistent with those of the clinically established standard of care.

1.6.3 Scar Excision and Skin Biopsy Risk Management

Investigators and all study personnel will be trained in the details of all aspects of the study procedures. To minimize risks and increase the chances of a favorable clinical outcome the following measures are required:

- Enrollment of subjects who qualify per the inclusion/exclusion criteria in the protocol
- Strict adherence to the study protocol
- Use sterile technique throughout the procedure
- Use standard of care for the monitoring of and treatment of possible adverse events following scar excision and biopsy procedure
- Depending on the site of the scar, the surgeon will explain the types of activities to avoid
- Close surgical incision and biopsy site with appropriate suture

1.6.4 Adverse Reaction Risk Management

Investigators and all study personnel will be trained in the details of all aspects of the study procedures. Standardization of topical drug and placebo application techniques is critical to a good clinical outcome. To minimize risks and increase the chances of a favorable clinical outcome the following measures are required:

- Subjects will be educated on treatment application during pre-operative visit
- Potential risks will be listed in the patient consent form and quantified during the course of treatment via patient questionnaires (VSS and POSAS)
- Follow-up will continue for 3 months following surgery: in-person appointments with study staff will occur during treatment period at 2 weeks and4 weeks after surgery. A final visit will be encouraged at 3 months after surgery. A follow-up phone call will be made by study staff 24 hours after surgery and postoperative weeks 1 and 3, and month 2 (and 3 if the patient is unable to return for in-clinic follow-up)2.

1.6.5 Potential Benefits

Each patient will receive both treatment and within subject placebo following SSE. Either the distal or proximal portion of the wound will receive the investigative treatment (TP) versus placebo (PCCA PP) with adjuvant SSE. Our preclinical data suggests that topical treatment with pentamidine may provide an improved environment for lesion healing in the setting of hypertrophic scar. However, this has not been formally tested for safety or efficacy in a RCT within the United States. As far as cosmetic benefits, patients will have the potential for the reduction of visible scarring. Hypertrophic scarring can cause patients to experience physical pain, as well as psychological and social costs that can be emotionally and financially difficult. The proposed topical treatment aims to reduce the thickness, redness and altered pigmentation of scar tissue by targeting the root cause of pathological scarring and fibrosis. Thus, the focus of this pilot study will be procedural safety and preliminary efficacy.

2 Study Objectives

The primary goal of this randomized clinical trial will be to directly evaluate safety of topical scar prevention product and placebo base control in patients with hypertrophic scarring. Patients with known diagnosis of hypertrophic scar will be enrolled. Secondarily, we will evaluate initial signs of treatment efficacy using topical product.

3 Study Design

3.1 General Description

This study is a double-blind pilot study of scar excision procedure followed by treatment with either TP in PCCA PP versus PCCA PP silicone base only. Current management for patients with hypertrophic scar includes silicone-based treatment. At our institution we treat patients with scar excision surgery without topical scar cream following the procedure. However, a significant proportion of patients continue to fail this treatment modality leading to scar recurrence. Indeed, individual patient response to therapy varies based on patient characteristics including ethnicity. As such, we plan to treat the same patient with both TP in PCCA PP and PCCA PP alone at different ends of their scar. In this way, we can better determine efficacy of these topical agents in biologically controlled setting with reduced response variability. We plan to enroll patients with minimum hypertrophic scar length ≥ 3 cm.

Those patients who successfully qualify based on inclusion and exclusion criteria will be invited to participate in the trial. Following informed consent, eligible subjects will undergo pre-operative tests to include blood work and EKG. Preoperative photographs of the scar site will be taken. Scar quality questionnaires (VSS and POSAS) will be completed and a scar ultrasound performed.. A 5-mm skin punch biopsy will be obtained from the lateral edge of the scar at both the proximal and distal ends of the scars, under local anesthesia. Alternatively, the first punch biopsy may occur prior to the surgical procedure while the patient is under anesthesia. Part of the sample will be stored in formalin and prepared for histological staining. The other portion will be frozen and prepared for molecular analysis. All participants will receive both treatments to be applied either at the proximal end or the distal end of the scar. Treatment application will be randomized. For example, topical agent A will be applied to the proximal end of the scar and will contain either TP

in PCCA PP or PCCA PP. Topical agent B will treat the distal portion of the scar and contain either TP in PCCA PP or PCCA PP alone. 1-2cm in the middle of the scar will remain untreated as the border zone. It is known that blood perfusion influences scar formation; thus to reduce potential bias from distal scar site receiving reduced blood flow compared to proximal scar site, treatment application will be randomized to scar site. Prior to treatment initiation, patients will undergo scar excision procedure with a 24-hour recovery period. Treatments will be provided in single dose containers for application every 48 hours following surgical recovery (total of 14-16 applications; total treatment duration of 4 weeks).

Patient Treatment Schema

Visit 0: (Preoperative screening)

- Informed consent

Visit 1: (Preoperative period)

- Pre-operative evaluation including history, clinical exam with baseline neuropsychiatric assessment, and laboratory studies to include hepatic and renal function, complete blood count with differential, blood glucose level and calcium. The specific laboratory tests for hepatic and renal function will include ALT, AST, Bilirubin, BUN and Creatinine. Any abnormal value twice the upper limit of normal will meet exclusion criteria: AST (Normal: 8-48 U/L); ALT (Normal: 7-55 U/L); Bilirubin, Total (Normal <= 1.2 mg/dL); BUN (Normal 8-24 mg/dL); Creatinine (0.8-1.3 mg/dL).
- Baseline cardiac monitoring (ECG)
- Scar photography
- Quality questionnaires (VSS and POSAS)
- Pre-operative US and/or 3D imaging
- Treatment application patient education
- 5-mm skin punch biopsy under local anesthesia from distal and proximal sites (Alternatively, the first punch biopsy may occur prior to the surgical procedure while under anesthesia.)

Visit 2: (Day of procedure)

- Surgical scar excision under local or general anesthesia depending on size of scaring and surgeon preference for excision
- Provide single dose containers with de-identified treatment agents (TP in PCCA PP and PCCA PP alone) and treatment recording log for two weeks

Phone Interview #1: (Week 0, Day 1 postop)

- Assessment of adverse events

Phone Interview #2 (Week 1 postop)

- Assessment of adverse events

Visit3: (Week 2 postop)

- Scar photography
- Quality questionnaires (VSS and POSAS)

- Post-operative evaluation including clinical exam with local safety evaluation to assess tolerability (e.g. erythema, edema, vesicle formation, necrosis, ulceration etc.) and neuropsychiatric adverse event assessment
- Laboratory studies (including hepatic and renal function, complete blood count with differential, blood glucose level and calcium)
- Post-operative cardiac monitoring (ECG)
- Post-operative US and/or 3D imaging
- Provide single dose containers with de-identified treatment agents (TP in PCCA PP and PCCA PP alone) and treatment recording log for two weeks

Phone Interview #3 (Week 3 postop)

- Assessment of adverse events

Visit4: (Week 4 postop)

- Scar photography
- Quality questionnaires (VSS and POSAS)
- Post-operative evaluation including clinical exam with local skin reaction evaluation and neuropsychiatric adverse event assessment
- Laboratory studies will include hepatic and renal function, complete blood count with differential, blood glucose level and calcium. Post-operative US and/or 3D imaging
- 5-mm skin punch biopsy under local anesthesia from distal and proximal sites

Phone Interview #4: (Month 2 postop)

- Assessment of adverse events

Visit #5: (Month 3 postop)

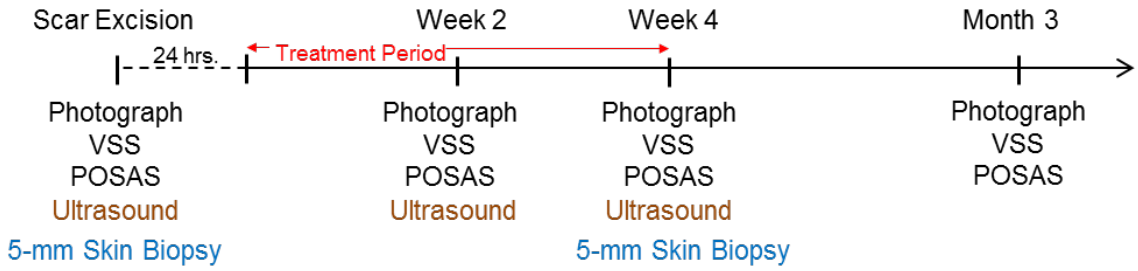
- Scar photography
- Quality questionnaires (VSS and POSAS)
- Assessment of adverse events
- Note: if the patient is unable to return for an in-clinic appointment, a phone interview will be conducted

3.2 Number of Subjects

Ten subjects will be enrolled in this trial.

3.3 Duration of Participation

Length of study treatment will be 4 weeks. Per treatment schema, patients will have 5 total visits including initial pre-operative evaluation (visit 1); surgical excision procedure (visit 2) and follow-up visits at postoperative week 2 and 4, and month 3. Patients will receive a phone call 24 hours following their surgical excision to assess adverse events. Another phone call will be made at postoperative weeks 1 and 3, and postoperative month 2.



3.4 Primary Study Endpoints

Aim #1: Identify the type and number of serious adverse events related to TP in PCCA PP compared to PCCA PP base alone following surgical scar excision. While we do not anticipate any severe adverse events, we will monitor for the following:

- Skin infection
- Skin irritation
- Wound dehiscence
- Clinically significant abnormalities in physical examination and vital signs.

3.5 Secondary Study Endpoints

Aim #2: Evaluate initial signals of efficacy as measured by US dimensions (length, width and height) and volume quantification, skin biopsy histological features (semi-quantitative assessment of skin fibrosis) and patient-reported outcome metrics (per VSS and POSAS). Preoperative US as well as 2 week and 4 week postoperative US will be compared based on formal quantification of the hypertrophic scar lesion volume size (unit of measure: cm^3). Preoperative 5-mm skin biopsy and 4 week postoperative skin biopsy will be used for histological evaluation (including epidermal hyperplasia/hyperkeratoses, hair follicles, apocrine glands, smooth muscles, fibroplasia, vascular proliferation and collagen orientation) [27] and epidermal scar thickness calculation. Quality of life and scar elevation scores will also be assessed at serial follow-up as measured by VSS and POSAS.

3.6 Identification of Source Data

The following source data will be directly recorded on the Case Report Form (CRF):

- Patient demographic data:
  - Age
  - Sex
  - Ethnicity
  - Indication for scar excision
  - Concomitant medications
- Inclusion criteria
- Exclusion criteria
- Physical examination
- Trial medication administration
  - Date of dosing
  - Time of dosing
- Assessment

  - POSAS
  - VSS
- Adverse events
  - Pain
  - Rash
  - Skin Ulcer
  - Erythema
  - Pruritus
  - Urticaria
  - Erosions
  - Scabbing
  - Infection
  - Depigmentation
  - Hypopigmentation
  - Hyperpigmentation

The following source data will not be directly collected in the Case Report Form (CRF), but will be captured in supportive documentation (EMR):

- Photographs of lesion area
- Ultrasound 3D radiographic images
- Histology report from 5-mm skin biopsy

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- Males and females 18 years of age or older.
- Diagnosis of hypertrophic scar by a Mayo Clinic plastic surgeon or dermatologist
- Target disease or condition: Hypertrophic scar
- Subject with a hypertrophic scar that meet all of the following criteria:
  - Linear scar ≥3 cm to ≤40 cm in length
  - Present for minimum 6 months
  - Located anywhere in the body except on the face or front of neck
  - Resulting from surgical or traumatic injury, or other scar considered appropriate for surgical excision
- Ability to safely undergo scar excision surgery
- Capacity to provide informed consent
- Ability to comply with protocol
- Subject is judged, by the clinical investigator, to be healthy as evidenced by lack of clinically significant abnormal findings on medical history, physical examination, electrocardiogram, vital signs, and clinical laboratory tests.

4.2 Exclusion Criteria

- Subjects identified as having a keloid or a scar not appropriate for surgical excision

- Subjects who are positive for hepatitis B surface antigen (HbsAg), hepatitis C antibody and HIV as determined in screening the subject's electronic medical record.
- Concurrent use of systemic corticosteroids (excludes inhaled or topical steroids), COX-2 inhibitors and/or drugs that are strong inhibitors and inducers of CYP enzymes
- Are immuno-compromised (HIV infected, cancer and other disease affecting the basal immune response)
- Clinically significant cardiovascular, pulmonary, renal, endocrine, hepatic, neurological, psychiatric, immunological, gastrointestinal, hematological, or metabolic disease that is, in the opinion of the investigator, not stabilized or may otherwise impact the results of the study.
- Subjects with renal and hepatic impairment.
- Known allergy or hypersensitivity to the study drug(s) or one of the ingredients of the formulation.
- Any infection or wound in the area to treat including photosensitive dermatosis or inflammatory acne.
- Existence of any surgical, medical or laboratory condition that, in the judgment of the clinical investigator, might interfere with the safety, distribution, metabolism or excretion of the drug
- Participation in another clinical study in the past 30 days or concurrent participation in another clinical trial.
- Patients with poorly controlled diabetes mellitus ($HbA1_C \geq 8\%$), peripheral neuropathy, or known concomitant vascular problems.
- Pregnant or lactating female patients.
- Prisoners.
- Subjects who smoke cigarettes and/or use other tobacco products.

4.3 Subject Recruitment, Enrollment and Screening

Prior to discussing study enrollment with the patient, principal investigator, Alexander Meves, MD and/or Steven Moran, MD, will discuss with study coordinator to ensure that that target enrollment has not yet been met.. Subjects will be recruited at Mayo Clinic Department of Dermatology and Division of Plastic Surgery in Rochester, Minnesota.

At the time of patient enrollment in the trial the following must be verified:

1. IRB approval of the trial and open enrollment status with both Mayo Clinic and clinicaltrials.gov.
2. Open place on the enrollment log (confirmation from the PI and study coordinator).
3. Patient is willing and able to travel to the Mayo Clinic, Rochester for all tests, treatment and follow up required in the protocol.

At the time of patient enrollment the following will be confirmed and documented:

1. A signed consent form.

2. The existence of a signed authorization for use and disclosure of protected health information.
3. A completed inclusion/exclusion enrollment checklist.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects may withdraw from the study for any of the following reasons: safety issues, inability or unwillingness to adhere to protocol requirements, or if the subject desires to withdraw from the study. A single subject withdrawn from the study will not be replaced. If two or more patients have withdrawn (with the exception of safety reasons) one of the subjects will be replaced with a new enrollee. The decision to withdraw subjects and the responses below will occur under the following circumstances.

- Patients wishing to withdraw from the study: Patients wishing to withdraw from the study should provide written indication of these wishes.
- Patients unable or uncooperative to continue in the study: If possible, patients should be contacted in an attempt to determine the reasons for the inability to complete the study. After at least three attempts and failure to complete required testing the patient will be considered out of the study.
- Withdrawn from study due to safety reasons: See section below for the response to drug related adverse events and patient withdrawal from the study. Generally, even patients with a drug related adverse event will continue with the test schedule as the test schedule includes minimal risk tests and is used to monitor and gauge continued long term effects of the treatment.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

The sponsor of the study is responsible for following up on patients who develop adverse reactions to treatment. All study participants will undergo risk-appropriate follow-up until study-related adverse events have been fully treated and resolved.

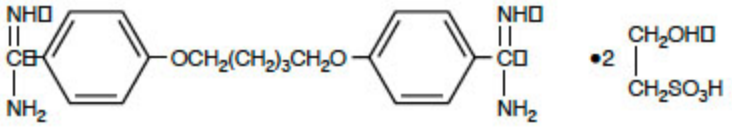
5 Study Drug

5.1 Description

Pentam 300 (pentamidine isethionate), an anti-protozoal agent, is a sterile, nonpyrogenic, lyophilized product. Pentamidine isethionate is a white crystalline powder soluble in water and glycerin, slightly soluble in alcohol and insoluble in ether, acetone, and chloroform. Currently used as an oral inhalation/intramuscular/intravenous antifungal agent.

Pentamidine isethionate, an aromatic diamidine, is known to have activity against *Pneumocystis carinii*. The mode of action of pentamidine is not fully understood. *In vitro* studies indicate that the drug interferes with protozoal nuclear metabolism by inhibition of DNA, RNA, phospholipid and protein synthesis.

It is chemically designated as 4,4-[1,5-pentanediylbis(oxy)]bis-benzenecarboximidamid with the following structural formula:



$C_{19}H_{24}N_4O_2 \cdot 2C_2H_6O_4S$ 592.68

Each vial contains:
Pentamidine isethionate . . . . . 300 mg

Pharmacokinetics
Biotransformation of pentamidine in humans is unknown. At least six metabolites have been identified in rats. Elimination of systemic drug is primarily renal, and half-life is increased in patients with renal dysfunction. Half-lives vary, depending on the route of administration. The terminal half-life is 2—4 weeks. Urinary excretion may persist for up to 8 weeks after discontinuation. Multiple dosing may result in drug accumulation, even in patients with normal renal function. No information on fecal excretion in humans is available.

Affected cytochrome P450 isoenzymes and drug transporters: CYP3A5, CYP2D6, CYP2C19, CYP1A1, CYP4A11. Previous data suggest that pentamidine was metabolized by the CYP2C19 isoenzyme; however, this has not been confirmed. In vitro data suggest that the CYP1A1, 3A5, and 4A11 isoenzymes may contribute to the metabolism of pentamidine, but data from healthy volunteers show that the CYP1A1 and 2D6 are the mediating enzymes in humans. Drug interactions with pentamidine are not well studied. Given these interactions, clinicians should be cautious using concurrent drugs that have similar toxicities.

Base
The vehicle used in this study is Pracasil™-Plus (PCCA #30-4655; PCCA PP). This non-aqueous silicone-based compounding agent contains the following ingredients:

- Cyclopentasiloxane
- Polysilicone-11
- PEG-16 Macadamia Glycerides
- Dimethicone
- C30-45 Alkyl Cetearyl Dimethicone Crosspolymer
- Pentaclethra Macroloba Seed Oil

- Oenocarpus Bataua Pulp Oil
- Phosphatidylcholine
- Tocopheryl Acetate
- BHT

5.2 Treatment Regimen

First dose will be applied 24 hours post-surgical scar excision, then scheduled every 48 hours. Approximately 1.8 mL (0.06 oz) of topical pentamidine (TP) or PCCA PP control will be topically applied via single dose containers provided the day of surgery and at their 2-week postoperative follow-up visit. Participants will receive two sets of single dose containers (set A and set B). One set will be randomized to contain TP with de-identified label; the other set will be randomized to contain PCCA PP with de-identified label. Each set (A and B) will contain 8 single dose containers, all with a de-identified label. The study drug will be applied every 48 hours. Specifically, topical agent (either TP or PCCA PP) will be applied to either the distal or the proximal end of the wound. Study participants will receive treatment application education during their pre-operative visit; further details provided in Section 6.1. Treatment duration is 4 weeks.

5.3 Preparation and Administration of Study Drug

Standard USP 40-NF 35, Chapter 795 compounding techniques will be followed. The compounder is responsible for compounding preparations of acceptable strength, quality, and purity and in accordance with the prescription or medication order. The compounder is also responsible for dispensing the finished preparation, with appropriate packaging and labeling, and in compliance with the requirements established by the applicable state agencies, state boards of pharmacy, federal law, and other regulatory agencies where appropriate.

Professional Compounding Centers of America Pracasil™-Plus (PCCA PP) is a topical silicone-based compounding base agent. Mayo Clinic Compounding Laboratory produced topical gel pentamidine (Pentam-300) in PCCA PP in USP (U.S. Pharmacopeial Convention)-grade facility. This gel will be sent to the Research Facility and stored at controlled room temperature (20-25˚C) and stable until the end of the study period (4 weeks). Contact Melissa Wendling, Pharm.D., R.Ph. 507-293-1157 in the Research Pharmacy for more information.

Treatments will be provided in single dose containers for application every 48 hours following surgical recovery window (total 14 applications); total duration will be 4 weeks. Approximately 1.8 mL (0.06 oz) of pentamidine and within-patient placebo control will be topically applied to the designated scar region. Single dose containers will ensure each patient receives the same dosage.

5.4 Subject Compliance Monitoring

Subjects will be seen in the outpatient clinic as part of their follow-up (total of three follow-up visits in addition to four follow-up phone calls) during study duration. To ensure that study subjects are qualified in treatment application, each subject will complete an education session with the RN study coordinator. Subject compliance will be monitored during the follow-up visits and phone calls. Subjects will also receive a treatment log to record the date and time of treatment application as well as record any adverse events.

5.4.1 Prior and Concomitant Therapy

No topical therapies other than either TP or placebo PCCA PP are permitted for the duration of the study.

5.5 Packaging

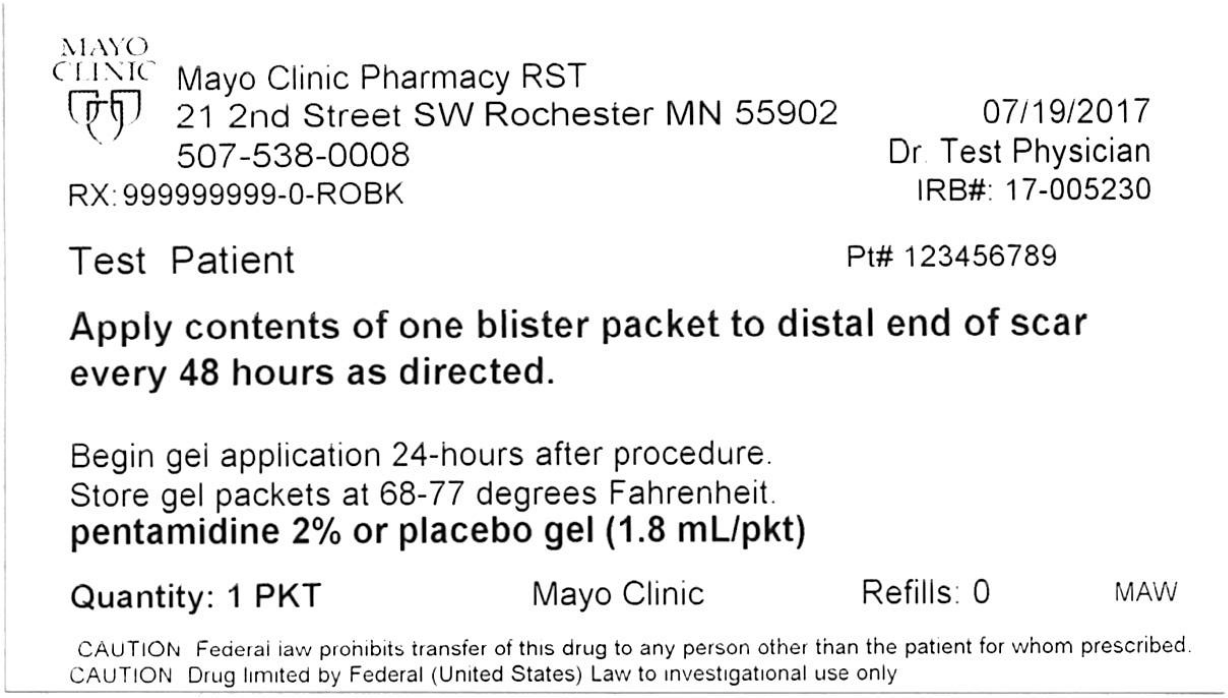
The research pharmacy will order 300 mg Pentam vials via Rochester Methodist Campus (RMC) pharmacy purchasing specialists from APP Pharmaceuticals. Pentam vials will be stored within the research pharmacy (20-25˚C) and sent to the compounding pharmacy when the study drug order is placed. The study drug order will be initiated by the study coordinator following patient consent, and as needed based on the date of surgery and 2 week postoperative visit. The Principle Investigator will sign the order which is then forwarded to the research pharmacy to be filled. For production, 300 mg Pentam (pentamidine isethionate) product will be compounded into non-aqueous silicone base vehicle (Pracasil™-Plus (PCCA #30-4655; PCCA PP) for 2% topical gel.

The compounding pharmacy will prepare the single containers for storage and dispensation by the research pharmacy. The research pharmacy will dispense a specified quantity of containers according to patient-specific prescription signed by IRB-authorized prescriber. The study coordinator will pick up the prescription from the research pharmacy and provide to the patient on visit 2 (day of surgery) and visit 3 (2 week postop clinic visit). Each dispensation will contain 8 active and 8 placebo containers to be applied every 48 hours; all containers (used and unused) will be returned by the patient at their next follow-up visits.

The research pharmacy will perform randomization schema to determine treatment to dispense for proximal versus distal scar areas. The research pharmacy maintains electronic records of investigational medication inventory and dispensation activities for the study; thus randomization will be performed for enrolled patients electronically via Medidata and Scientific Data Management System (SDMS). All Mayo Clinic Research Pharmacy staff members are required by policy to complete the Mayo Clinic Human Subject Protection and CITI Good Clinical Practice Training once every 3 years. The designated Research Pharmacy staff member assigned primary responsibilities for the study reviews all sponsor documents and prepares instruction sheets outlining storage, handling, electronic order entry and preparation of all investigational agents as indicated in the study protocol and pharmacy manual.

Following randomization, the distal location randomized treatment group (TP or PCCA PP) will be placed into a light-resistant bag with the below label on the exterior. Similar label will be provided for the proximal treatment group. Label will include caution statement: “Caution: New Drug--Limited by Federal (or United States) law to investigational use."

Example of label:


MAYO CLINIC
Mayo Clinic Pharmacy RST
21 2nd Street SW Rochester MN 55902
507-538-0008
07/19/2017
Dr. Test Physician
IRB#: 17-005230
RX: 999999999-0-ROBK
Test Patient
Pt# 123456789
Apply contents of one blister packet to distal end of scar every 48 hours as directed.
Begin gel application 24-hours after procedure.
Store gel packets at 68-77 degrees Fahrenheit.
pentamidine 2% or placebo gel (1.8 mL/pkt)
Quantity: 1 PKT
Mayo Clinic
Refills: 0
MAW
CAUTION Federal law prohibits transfer of this drug to any person other than the patient for whom prescribed.
CAUTION Drug limited by Federal (United States) Law to investigational use only

5.6 Masking/Blinding of Study

This is a double-blinded study; subjects and clinical investigators (physicians, nurse practitioners and study delegates) will be blinded to the treatment. Study treatments (either TP or control PCCA PP base) will be packaged at the Mayo Clinic Compounding Pharmacy as described above. Compounding pharmacists will be aware of the study drug and will provide an unidentified label (please see example in Section 5.5) with lot number only for each treatment application. At the completion of the study, investigators will be unmasked following data interpretation and statistical analysis.

5.7 Receiving, Storage, Dispensing and Return

Per Mayo Clinic Research Pharmacy policy, investigational product(s) are received by study personnel acting on behalf of the investigator. Research pharmacy staff verifies information on the packing slip matches the contents of the shipment. The following are verified by receiving personnel upon receipt of shipment: product identification, sponsor product label, quantity of items received, package size(s), lot numbers, expiration dates if available, product condition, and maintenance of storage conditions in transit. Any discrepancies in shipment are communicated immediately to study sponsor. Investigational products that do not contain an expiration date are deemed in date and acceptable to use until notice of expiration is received from sponsor or supplier. Research staff contacts sponsors and/or suppliers quarterly to determine if retest date or expiration is available. After shipment has been checked and verified, IP is immediately placed in appropriate storage conditions as indicated in study documentation. For product delivered with a temperature recording device which may be electronically interrogated, data will be downloaded and saved in PDF format in the Investigational Drug Services (IDS) electronic management application if the device supports such function.

Invoices are dated, signed, scanned, and uploaded electronically into the IDS electronic management application at time of receipt. Signed/dated hard copies of invoices are double checked per page against scanned uploaded images to ensure scanned image represents a true copy of the original document. Once accuracy of the scanned image has been verified, hard copies of invoices/shipping documents are discarded.

Product No	NDC No.	
11310	63323-113-10	PENTAM® 300 (pentamidine isethiionate for injection) 300 mg, lyophilized product in single-dose vials, packages of 10.

Store dry product at 20° to 25°C (68° to 77°F) [see USP Controlled Room Temperature]. Protect from light. Preservative Free. Discard unused portion.

Mayo Clinic compounding pharmacy will produce the topical gel pentamidine (Pentam-300) in PCCA PP in USP (U.S. Pharmacopeial Covention)-grade facility. This gel is then sent to the research pharmacy in single dose containers and stored at controlled room temperature (20-25˚C) and stable until the end of the study period (4 weeks).

Study coordinators will pick up study drug containers from the research pharmacy for dispensation to the patient. Any unused doses will be returned to the research pharmacy to be discarded properly.

5.7.1 Receipt of Drug Supplies

All drug formulation and compounding will be performed in the Mayo Clinic Compounding Laboratory. Pentam-300 and PCCA PP will be purchased from their respective manufacturers and shipped to the Research Pharmacy. Purchase will occur via Mayo Clinic Rochester Methodist Campus (RMC) pharmacy purchasing specialists from APP Pharmaceuticals. Further production details in Section 5.5. This is a single-site study conducted in Mayo Clinic Rochester, MN.

5.7.2 Storage

The compounded drug will be stored at controlled room temperature (20-25˚C) in light resistant containers placed in amber Ziploc bags. These will be kept secure in the Research Pharmacy until receipt of compounding request from the clinical trial coordinator. Special access is required to access this location to prevent unintended or unauthorized use of the drug. No special handling is required. Since the drug is formulated from a lyophilized, stable product and compounded with a non-aqueous base, the topical formulation will be stable for up to 180 days.

Per Mayo Clinic Research Pharmacy policy, the Investigational Pharmacy is a secure, badge access area accessible to authorized pharmacy personnel only. Investigational products are stored as per study documentation. Temperature monitoring is recorded via a continuous electronic monitoring system. All storage areas and devices that contain investigational products are monitored by this system. Temperature sensor devices are calibrated per local policy and calibration certificates are available upon request. Sponsor specific temperature monitoring devices are not utilized. Temperature data from the continuous electronic temperature monitoring system is stored

electronically in a dedicated server and is readily retrievable upon request. Local procedures are followed in the event of an out of range condition reported by the continuous electronic monitoring system.

5.7.3 Dispensing of Study Drug

Per Mayo Clinic Research Pharmacy policy, pharmacists and/or pharmacy technicians under the supervision of a pharmacist will prepare and dispense investigational products per study protocol. Lot numbers and expiration dates of commercial supplies utilized in the preparation of investigational products such as syringes, needles, IV bags, tubing, diluents, and standard of care products are not tracked.

Single dose containers will help to ensure accurate and consistent dosing across all patients participating in the trial. Approximately 1.8 mL (0.06 oz) of TP and placebo PCCA PP will be topically applied to the designated scar region. The randomized treatment group (TP versus PCCA PP) will be assigned to subjects in a double-blinded manner by the research pharmacist; details in Section 5.5. Patients will be educated on topical drug application during the pre-operative clinic visit. The patient will be instructed to apply enough study drug to thoroughly cover the area to be treated. It is not necessary to use the entire dose for each treatment application, but a minimum of at least half should be used. The patient will be asked to administer a sample product in the presence of a study coordinator to verify their application technique and amount. Once the patient demonstrates understanding and skill, the patient may personally administer the remaining treatments during the trial.

Single dose containers will be distributed to patients at visit 2 and visit 3. Each treatment set will contain 8 active and 8 placebo containers to be applied every 48 hours. Any unused containers will be returned by the patient at their next follow-up visit (return bag for used containers will be provided). Randomized treatment label will contain instructions for site-specific application. Patients are held accountable for keeping track of dispensed drug at all times. If a dose of the drug is lost due to unforeseen circumstances, a single replacement may be available.

Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

5.7.4 Return or Destruction of Study Drug

Per Mayo Clinic Research Pharmacy policy, investigational product and associated wastes generated on site as a consequence of participation in a sponsor's study are disposed of via placement in a designated, appropriate waste container per local policy and regulation. Empty containers, vials, or empty injectable syringes of investigational products generated from preparation and subsequent administration of an investigational product to a patient are not retained for monitor inspection following dose preparation; instead these items are disposed of by placement in an appropriate waste container based on the hazard risk of the investigational product (e.g. hazardous, chemotherapeutic, biohazardous). Disposition is documented in the IDS electronic management application. Unless pharmacy is the only unblinded party in a study, IDS pharmacy staff will not document investigational product destruction or drug returns within sponsor electronic systems. In addition, all investigational products (i.e. tablets, capsules, injections, and/or

containers) that are returned by subjects, as well as any returned empty boxes and cartons are disposed of in the manner described above immediately after appropriate documentation of such return has occurred in the IDS electronic management application.

At the completion of the study, there will be a final reconciliation of amounts of cream manufactured, dispensed, returned (if applicable), destroyed and any drug remaining. This reconciliation will be documented, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Visit 0 (Preoperative screening)

Patients will provide written consent if they are interested in participating in this trial and meet appropriate inclusion and exclusion criteria.

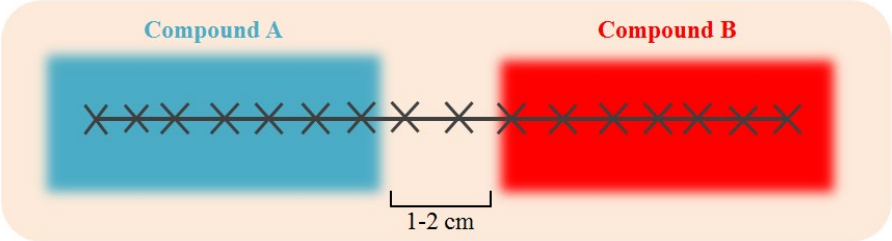
6.2 Visit 1 (Preoperative period)

Patients will receive a comprehensive pre-operative evaluation. This evaluation will gather information relating to medical history as well as include a standard clinical exam and necessary laboratory studies. Once consent is provided and patient meets inclusion criteria as a qualified participant for the study, photographs will be taken of the lesion area with a digital camera and/or photography service. Pre-operative ultrasound and/or 3D imaging will also be performed to determine initial scar height and morphology. The scar will be assessed using the Vancouver Scar Scale (VSS) [28] and the Patient and Observer Scar Assessment Scale (POSAS) [29]. These scales will determine the initial level of hypertrophy and scar attributes before treatment initiation. Safety evaluation will include assessment of systemic and local safety and thus baseline clinical exam with neuropsychiatric evaluation, laboratory studies (including hepatic and renal function, complete blood count with differential, blood glucose level and calcium) and baseline cardiac monitoring with electrocardiogram (ECG) will be performed.

This visit will include two 5-mm skin punch biopsy (one from distal site and one from proximal scar site) performed under local anesthesia in a dedicated procedure room. Alternatively, the first punch biopsy may occur prior to the surgical procedure while under anesthesia. This sample will be processed by the study staff for histological/molecular evaluation to analyze scar characteristics.

Patients will also be educated on proper treatment application. Treatment regimen and duration will be summarized. For example, following their surgical scar excision procedure, they will be provided single dose containers with topical study agents. There will be two different formulations given to patients; one is the placebo control (PCCA PP) and the other is the active treatment (TP). Patients and providers blinded to treatment regions but their treatment label will have appropriate instructions for application either at the distal or proximal region.

Based on the distal or proximal region assigned to each formulation by the pharmacist and appropriately labeled single dose containers, patients will apply the unit dose. Compound A (either TP or PCCA PP)) will be applied to proximal site and Compound B (either TP or PCCA PP) will be applied to the distal site;1-2 cm border zone in the middle of the scar will remain untreated.



The study coordinator will provide a demonstration of application using a sample container containing petroleum gel or similar non-therapeutic agent. Prior to treatment application, site preparation and wound care techniques will be reviewed (patient education hand-out provided). Patients will be advised to carefully clean the skin around the wound using either normal saline solution or mild soapy water. Using medical gloves, topical gel will be applied to either the proximal or distal wound. Gloves will be changed between each treatment application. Patients will then practice topical gel application and wound dressing to verify their understanding. Regarding total surface area to be treated, the following guidelines are recommended:

- Scar length 3 cm to 8 cm: Treat 2 cm proximal (site A) and 2 cm distal (site B)
- Scar length 8 cm to 10 cm: Treat 3 cm proximal (site A) and 3 cm distal (site B)
- Scar length 10 cm to 15 cm: Treat 4 cm proximal (site A) and 4 cm distal (site B)
- Scar length >15cm: Treat 5 cm proximal (site A) and 5 cm distal (site B)

After each treatment application, the treated area will be covered with a non-adherent dressing (which will be provided to the patient). There will be a 1-2 cm untreated gap between the two scar treatment sites (proximal and distal to prevent cross contaminations.

At this visit, a detailed schedule will be provided including date of surgery and follow-up visits/phone calls. All procedures, surveys, imaging and testing will be explained.

6.3 Visit 2 (Day of Procedure)

Patients will be undergoing surgical scar excision using local or general anesthesia depending on the size of scarring and surgeon preference for excision. Dr. Steven Moran and/or his surgical staff will perform the surgical scar excision. Patients will receive their first 2 weeks of study drug with de-identified treatment agents to be applied either distal or proximal region of surgical site.

6.4 Phone Interview #1 (Week 0, Day 1 postop)

Patients will be contacted at 24 hours following scar excision. Any adverse effects present will be assessed and noted. Measures to address these effects will be taken on a per-patient basis.

6.5 Phone Interview #2 (Week 1 postop)

Patients will be contacted 1 week following scar excision. Any adverse effects present will be assessed and noted. Measures to address these effects will be taken on a per-patient basis.

6.6 Visit 3 (Week 2 postop)

The scar will be photographed and quality questionnaires (VSS and POSAS) will be filled out. Patients will receive their third and fourth week of study drug with de-identified treatment agents to be applied either distal or proximal region of surgical site. Post-operative ultrasound and/or 3D imaging will be performed to assess scar height. Post-operative safety testing with laboratory studies (including hepatic and renal function, complete blood count with differential, blood glucose level and calcium) and cardiac monitoring (ECG) will be performed. Additional safety evaluation including clinical exam with local safety evaluation to assess tolerability (e.g. erythema, edema, vesicle formation, necrosis, ulceration etc.) and neuropsychiatric adverse event assessment will be completed.

6.7 Phone Interview #3 (Week 3 postop)

Patients will be contacted 3 weeks following scar excision. Any adverse effects present will be assessed and noted. Measures to address these effects will be taken on a per-patient basis.

6.8 Visit 4(Week 4 postop)

The scar will be photographed and quality questionnaires (VSS and POSAS) will be filled out. Post-operative ultrasound and/or 3D imaging will be performed to assess scar height. This visit will include two 5-mm skin punch biopsy (one from distal site and one from proximal scar site) performed under local anesthesia in a dedicated procedure room. This sample will be processed by the study staff for histological/molecular evaluation to analyze scar characteristics. Post-treatment safety testing with laboratory studies (including hepatic and renal function, complete blood count with differential, blood glucose level and calcium) and cardiac monitoring (ECG) will be performed. Additional safety evaluation including clinical exam with local safety evaluation to assess tolerability (e.g. erythema, edema, vesicle formation, necrosis, ulceration etc.) and neuropsychiatric adverse event assessment will be completed.

Follow-up visits may be scheduled per patient request.

6.9 Phone Interview #4: (Month 2 post op)

Patients will be contacted 2 months following scar excision. Any adverse effects present will be assessed and noted. Measures to address these effects will be taken on a per-patient basis.

6.10 Visit #5: (Month 3 post op)

The scar will be photographed and quality questionnaires (VSS and POSAS) will be completed. Patients will be assessed for any adverse events.

Range for phone interviews for call 2 and 3: +/- 1 day
Range for phone interview for call 4: +/- 1 week
Range for study visits 3 and 4: +/- 1-3 days
Range for study visit 5: +/- 2 weeks

6.11 *Schedule of Events*

Schedule of Events

Study Activity	Visit 0 Pre-op	Visit 1 Pre-op	Visit 2, Week 0, Day 0 Surgery	Phone 1, Week 0, Day 1	Phone 2 Week 1	Visit 3, Week 2	Phone 3 Week 3	Visit 4, Week 4	Phone 4, Month 2	Visit 5 Month 3
Informed consent	X									
Randomization		X								
General evaluation, physical exam and vital signs[a]		X								
Scar photography		X	X			X		X		X
Neuropsychiatric exam and local safety evaluation (baseline and adverse event assessment)[b]		X				X		X		
Laboratory studies[c]		X				X		X		
Quality questionnaires (VSS and POSAS)[d]		X				X		X		X
US and/or 3D imaging[e]		X				X		X		
Cardiac monitoring (ECG)[f]		X				X		X		
Treatment application patient education		X								
Dispense study drug [g]			X			X				
Surgical scar excision			X							
5-mm skin punch biopsy		X						X		
Phone interview				X	X		X		X	
Adverse event evaluation				X	X	X	X	X	X	X

a: Vital signs include blood pressure, pulse, temperature and respiratory rate. Physical exam includes skin check.
b: Clinical exam for neuropsychiatric exam will include evaluation for confusion and hallucination.
c: Hepatic and renal function, complete blood count with differential, blood glucose level and calcium
d: Quality questionnaires: Vancouver Scar Scale (VSS) and Patient Observer Scar Assessment Scale (POSAS).
e: Ultrasound (US) and/or 3D-scanner imaging used to evaluate scar height.
f: Safety evaluation will include cardiac monitoring with electrocardiogram (ECG).
g: Single dose containers with de-identified treatment agents (active topical drug and silicone-base only).

7 Statistical Plan

7.1 Sample Size Determination

This is a first in-human exploratory study to determine the efficacy of topical pentamidine. There will be 80% power to detect an effect size of 1.3 standard deviations for the comparison of MED between any two treatment groups. This calculation is based a two-sided unpaired t-test with a type I error of 0.05.

7.2 Statistical Methods

Primary Hypothesis
This primary hypothesis will be evaluated by paired t-test.

7.3 Subject Population(s) for Analysis

This study will include only subjects who completed ALL study-related procedures and follow up.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Drug Interactions

No drug interaction studies with Pentam 300 have been conducted.

Because the nephrotoxic effects may be additive, the concomitant or sequential use of pentamidine isethionate and other nephrotoxic drugs such as aminoglycosides, amphotericin B, cisplatin, foscarnet, or vancomycin should be closely monitored and avoided, if possible.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

The safety evaluation of this topical drug product during the conduct of a clinical trial includes an assessment of systemic safety as well as evaluation of local safety. Evaluation of systemic safety addresses warnings, precautions, and adverse events from approved product labeling for pentamidine isethionate (e.g., laboratory studies including renal and hepatic function, hematology, blood glucose, and calcium; periodic cardiac monitoring with electrocardiogram [ECG], and assessment for neuropsychiatric adverse events [including confusion and hallucinations]. The evaluation of systemic safety will be conducted at baseline (Visit 1, pre-operative), during treatment (Visit 3, Week 2), and after treatment (Visit 4, Week 4) and extended follow-up (Visit 5, Month 3). The local safety evaluation includes an active assessment of local tolerability (e.g. erythema, edema, vesiculation, necrosis, ulceration etc.) using an acceptable scale called the local skin reaction (LSR) grading scale [30]. The LSR grading scale uses a 0-4 numerical rating, with clinical descriptor and representative photographic images for each rating. The scale provides the ability to rate each LSR category (erythema, flaking/scaling, crusting, swelling, vesiculation/postulation and erosion/ulceration) and generate a composite objective LSR score [30].

Serious Adverse Events
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly
- tissue necrosis

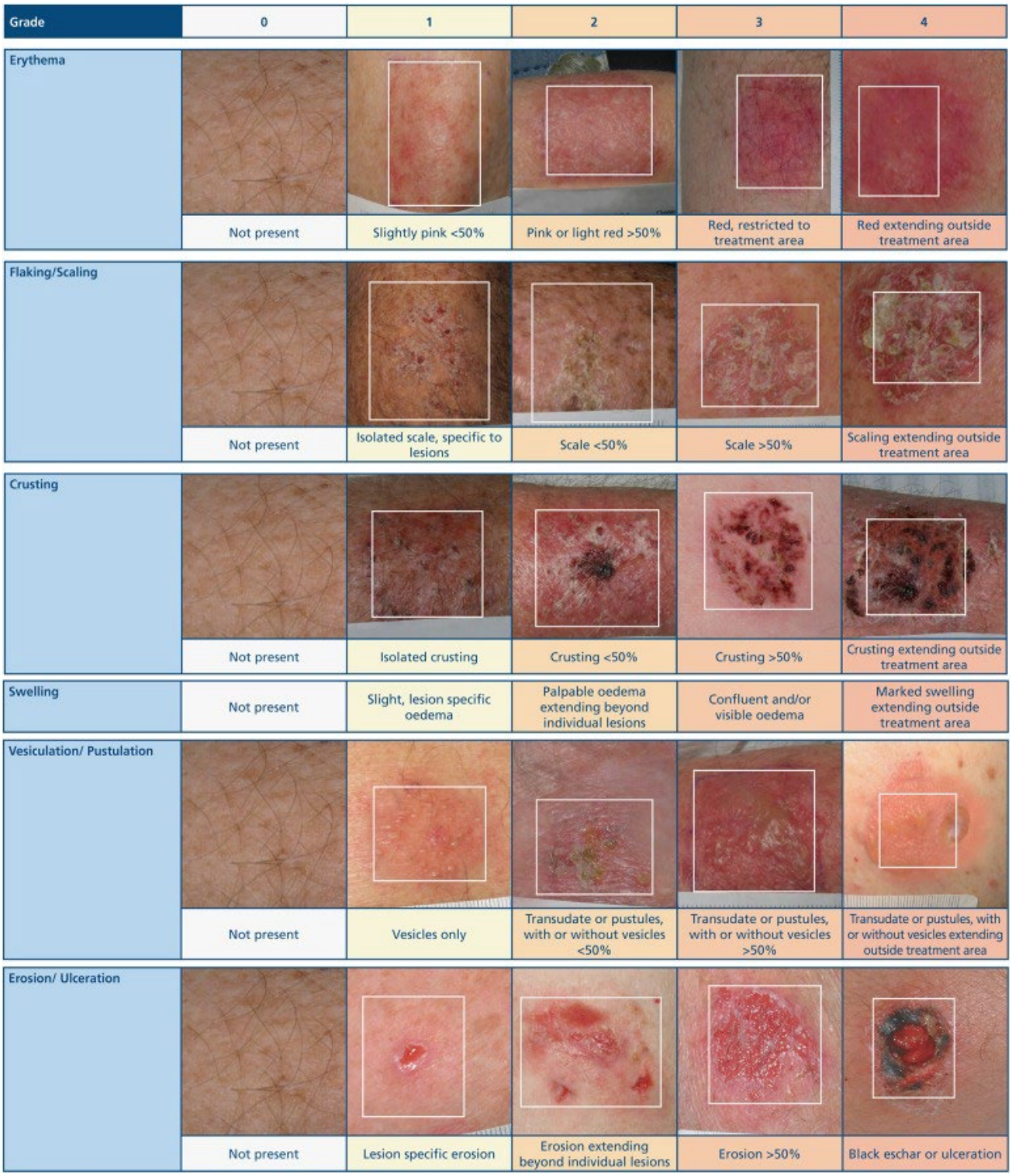
and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Non-serious Adverse Events

- skin ulcers
- erythema
- reaction at site of administration
- rash

Routine monitoring of safety evaluation includes an active assessment of local tolerability (e.g. erythema, edema, vesiculation, necrosis, ulceration etc.) using an acceptable scale called the local skin reaction (LSR) grading scale as outlined below [30].



Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as 3 months following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition is recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality is recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event is recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events are followed by the sponsor-investigator until the events have resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event(s) and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

According to Mayo IRB Policy any serious adverse event (SAE) which the Principal Investigator has determined to be a UPIRTSO must be reported to the Mayo IRB as soon as possible but no later than 5 working days after the investigator first learns of the problem/event.

Information collected on the adverse event worksheet (and entered in the research database):

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention*):
- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5**)
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

All adverse events will be recorded in the Case Report Form. The sponsor-investigator will review all adverse events to determine if reports need to be made to the IRB and FDA. The sponsor-investigator will sign off on any adverse events that are reported in the Case Report Form within 24 hours. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

We will use of a table to define Relationship of an Event to the Research and to define the severity of each event.

*** Relationship Index**

The relationship of an AE to the Investigational Drug is a clinical decision by the sponsor-investigator (PI) based on all available information at the time of the completion of the CRF and is graded as follows:

1. Not related: a reaction for which sufficient information exists to indicate that the etiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is c1early related to other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy.

2. Unlikely: a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals, or underlying disease provide plausible explanations.

3. Possible: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals; information on drug withdrawals may be lacking are unclear.

4. Probable: a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfi1 this definition.

5. Definite: a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).

** Severity Index
The maximum intensity of an AE during a day should be graded according to the definitions below and recorded in details as indicated on the CRF. If the intensity of an AE changes over a number of days, then separate entries should be made having distinct onset dates.

1. Mild: AEs are usually transient, requiring no special treatment, and do not interfere with patient's daily activities.

2. Moderate: AEs typically introduce a low level of inconvenience or concern to the patient and may interfere with daily activities, but are usually ameliorated by simple therapeutic measures.

3. Severe: AEs interrupt a patient's usual daily activity and traditionally require systemic drug therapy or other treatment.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all serious, unexpected and suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction.

This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 Unmasking/Unblinding Procedures

The study statistician will be responsible for revealing the treatment assignments (i.e. unblinding) when necessary. Since no interim analysis is planned for this study, unblinding will occur only when 1) a serious adverse event is observed and the treatment assignment must be made known and/or 2) at the conclusion of the study at the time of data analysis to evaluate treatment safety and preliminary efficacy.

8.5 Stopping Rules

The stopping rules specified below are based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment under investigation. The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (i.e. an adverse event with attribute specified as "possible", "probable", or "definite") that satisfy either of the following:

- If the first two patients or 3 consecutive at any point experience a SAE deemed related to study drug as defined in section 8.3.
- If a patient death considered related to the agent occurs.

The protocol will be reviewed by the study team. It will not be re-opened without proper protocol modification. If no modifications are possible then the study will close and be permanently stopped.

If a subject experiences an adverse event assessed as ≥ Grade 2 in the System Organ Classes (SOCs) of Cardiac Disorders and Blood and Lymphatic System disorders, or ≥ Grade 3 for all other SOCs, according to Common Terminology Criteria for Adverse Events (CTCAE v4), the subject will not receive additional doses. Subjects should remain in the study and be followed until the adverse event resolves or stabilizes.

We note that we will review grade 4, and 5 adverse events deemed "unrelated" or "unlikely to be related" to verify their attribution and to monitor the emergence of a previously unrecognized treatment-related adverse event.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction.

Data Management

Validation of source data will be performed by the investigator or his delegates.

Data Processing

Raw data will be recorded in case report form and then transferred into a password-secured electronic database.

Data Security and Confidentiality

Data will be stored on the secure Mayo Clinic network, which is backed up nightly.

Data Quality Assurance

Data will be audited internally by the study investigator and co-investigators to rule out errors in data entry and data collection. MED data will be cross-validated by images of skin obtained during the course of the study.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study for at least 2 years. These will include subject case histories and regulatory documents.

The sponsor-investigator will retain the specified records and reports for the longer of these periods;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored on a routine basis during the conduct of the trial. The Mayo Clinic Office of Research Regulatory Support will provide clinical monitoring for the trial as a service for the sponsor-investigator. Clinical trial monitoring requires review of the study data generated throughout the duration of the study to ensure the validity and integrity of the data along with the protection of human research subjects. This will assist sponsor-investigators in complying with Food and Drug Administration regulations.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

Data and safety monitoring provides a clinical investigation with a system for appropriate oversight and attention to the protection of human subjects by the investigator, research team, or an independent reviewer. A Data and Safety Monitoring Plan is a quality assurance plan for a research study. A written Data and Safety Monitoring Plan (DSMP) prospectively identifies and documents monitoring activities intended to protect the safety of the subjects, the validity of the data and the integrity of the research study. The DSMP may also identify when to terminate a subject's participation (i.e. individual stopping rules) and/or the appropriate termination of a study (i.e. study stopping rules).

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study is financed through a grant from the Mayo Clinic.

13 Publication Plan

The sponsor-investigator has the primary responsibility for publication of the results of the study. The trial will be registered on clinicaltrials.gov prior to enrollment and results published, as applicable.

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