COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title:

Effect of topical rapamycin ointment on keloid regression: A pilot study in human subjects (RAPA-Keloid)

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Effect of topical rapamycin ointment on keloid regression: A pilot study in human subjects (RAPA-Keloid)

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Study Product: *Rapamycin, 8% topical ointment*

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List of Abbreviations

mTOR – formerly mammalian Target of Rapamycin
RAPA – rapamycin
P-gp – permeability glycoprotein
CLOX – clock drawing task
IL-6 – interleukin 6, a pro-inflammatory cytokine and an anti-inflammatory myokine
VEGF – vascular endothelial growth factor
NGF – nerve growth factor
TGF-B1 – transforming growth factor beta-1

Study Summary

Title	Effect of Topical Rapamycin Ointment on Keloid Regression: A pilot study in human subjects
Protocol Number	HSC20190291H
Phase	Pilot
Methodology	For each participant, one keloid will be treated with topical RAPA ointment while the second keloid will serve as an untreated control. 8% RAPA ointment will be applied to the 'treatment' keloid and the second receives petrolatum-only vehicle, to allow each subject to be his/her own control.
Study Duration	7 months including screening and post treatment follow up
Study Center(s)	Audie L. Murphy VA Hospital
Objective	1. To test whether topical rapamycin regresses established keloids in humans by measuring surface area and height changes in the scar over time (6 months) 2. To test safety of product and feasibility of conduct for future clinical trial
Number of Subjects	Screen up to 20 participants to achieve 5 completers
Inclusion Criteria	1. Age 18-75 years old with at least 2 keloid scars (extant at least 1 year) in similar body locations that are easy to reach to apply ointment 2. Subjects will be in good health with all chronic diseases (hypertension, coronary artery disease, etc.) clinically stable 3. Cognitive functioning (CLOX1 ≥10 and CLOX2 ≥12) sufficient to provide informed consent and be able physically to apply QD ointment to affected area 4. Able to attend monthly clinic visits for 6 months
Exclusion Criteria	1. Diagnosis of diabetes 2. Exclusionary medications: systemic steroid or immunosuppressant therapy within past 6 months or local area steroidal treatment within 3 months 3. History of allergy to rapamycin or petrolatum-based products
Study Product, Dose, Route, Regimen	8% rapamycin in petrolatum ointment vs. vehicle only
Duration of administration	6 months, once daily
Statistical Methodology	Keloid surface area and height data will be analyzed using ANOVA

1. Introduction

This document is a protocol for a human research study. This study shall be conducted according to Good Clinical Practice guidelines as adopted by FDA, applicable government regulations, and Institutional research policies and procedures.

1.1. Background

Keloids are dermal fibroproliferative lesions induced by skin wounding and are most common in darker pigmented persons, particularly Africans, Hispanics, and Asians with up to 16% of these minority populations being at risk (17). Keloids can cause significant physical discomfort including pruritis, pain, and limitation of joint mobility; however, the worst sequelae are significant cosmetic defects and deformities. No truly effective keloid therapy exists, yet one is clearly needed. No laboratory animal models of keloids exist; instead, most work has been done on post-surgical samples with subsequent cell culture. Because keloid recurrence rate is high (up to 54% after excision) obtaining a biopsy solely for research can be considered unethical (17, 19). Based on cell culture studies, increased production of cytokines and growth factors including IL-6, VEGF, NGF, and TGF-B1 that activate of the mechanistic (formerly mammalian) Target of Rapamycin (mTOR) are mechanistically involved in keloid formation (18). As activation of mTOR is likely involved in the keloid formation, extant knowledge suggests that inhibition of mTOR activity with agents such as rapamycin would inhibit keloid development and maintenance.

1.2. Innovation

Because keloid recurrence rate is high (up to 54% after excision) obtaining a biopsy solely for research, though not often done, has revealed some pathophysiological mechanisms in keloid formation and maintenance (17, 19). Based on such cell culture studies with cells derived from human keloid biopsies, increased production of cytokines and growth factors including IL-6, VEGF, NGF, and TGF-B1 that activate of the mechanistic (formerly mammalian) Target of Rapamycin (mTOR) are mechanistically involved in keloid formation (18). Furthermore, and particularly relevant to our proposal, tissue extracts obtained from established keloid scar tissue demonstrated elevated expression of mTOR, p70KDa S6 kinase (p70S6K), and increased levels of their activated forms, suggesting roles in already extant keloid scars (14). Given that many mechanisms implicated in keloid development involve activation of mTOR, mTOR inhibition has been suggested as a potential therapeutic approach to preventing keloids (14, 20, 21). In addition, findings that established keloid scars contain elevated expression of mTOR, p70KDa S6 kinase (p70S6K), and their activated forms, mTOR inhibition may facilitate treatment of already extant keloids (14). Rapamycin (RAPA) is an FDA approved mTOR antagonist. Work in our laboratory established that this agent could be delivered transdermally as an ointment without producing measurable levels in the circulation and with no systemic effects. Subsequent serendipitous work in our laboratory suggested that application of topical RAPA ointment regressed established keloids, again without systemic effects. We will test this innovative topical treatment in persons susceptible to keloids who have established keloid scars that have not received any prior treatment.

The number of diagnosed keloids is increasing as decorative piercing is increasingly practiced, especially in visible anatomical sites and in younger age groups. Patients suffering from visible keloids may be affected by their disfigurement and suffer psychological consequences. Indeed, the quality of life can be severely impaired (40). There is no single effective therapeutic treatment for keloids. Numerous treatments have been described including occlusive dressings, compression therapy, steroid injections, imiquimod cream, laser therapy, surgical excision, cryosurgery, 5-fluorouracil, bleomycin, interferon therapy, and even radiation treatments (31). Unfortunately, these treatments are ineffective long-term and post-treatment recurrence is common (8, 40). A simple, effective, and safe treatment is needed.

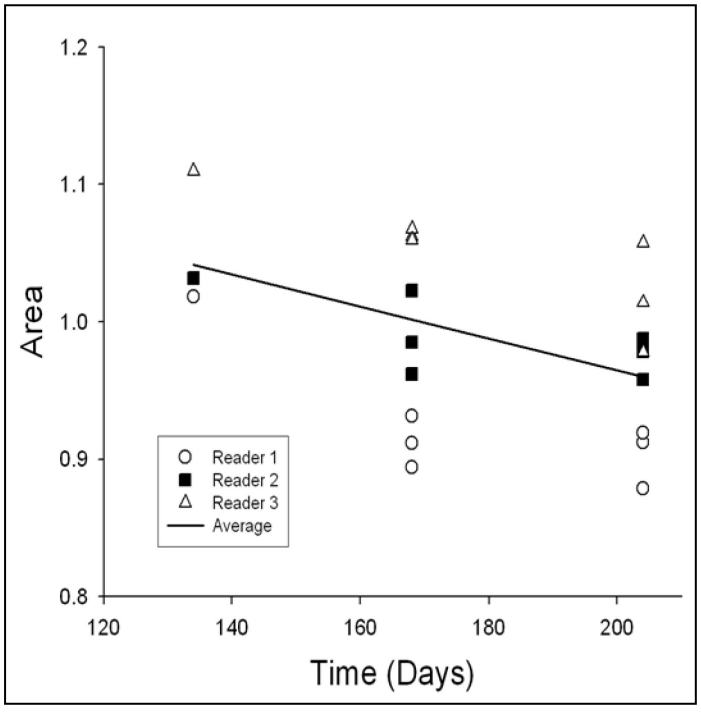
1.3. Preliminary data

Our preliminary data showed that topically applied 8% RAPA ointment produces measurable drug levels in skin, but no systemic levels and was devoid of systemic effects. We also observed that a skin punch biopsy site pretreated with 8% RAPA ointment in a keloid-prone volunteer never underwent keloid formation while the site treated with vehicle alone did, suggesting that topical RAPA can prevent keloid formation. We subsequently examined whether applying 8% RAPA ointment to an established keloid could regress the lesion. Indeed, the lesion did regress with application of 8% RAPA ointment.

Our laboratory completed an NIA funded investigation into the effects of mTOR blockade by topical RAPA on blood flow in vivo in middle-aged human volunteers. In preparation for blood flow experiments, we compared the ability of topical 1% versus 8% RAPA ointments to deliver RAPA into skin. The 1% and 8% RAPA ointments were applied over different intradermal microdialysis probes to examine which, if either, RAPA concentration would deliver the agent into skin. Microdialysis perfusates from the cutaneous interstitial space were collected and measured by HPLC-mass spec. These experiments showed that topical application of 1% RAPA ointment produced inconsistent interstitial RAPA levels, but 8% RAPA ointment universally produced measurable skin interstitial levels. Simultaneous blood sampling showed that topical 8% RAPA ointment produced no measurable systemic level. Based on these results with application time of a few hours, 8% RAPA ointment was chosen for subsequent experiments of a week's duration. In our week-long studies, 8% RAPA ointment produced an average level of 11.47±4.03ng/ml in skin after 7 days of application, again with no detectable RAPA level in blood.

As part of the original RAPA study, 3mm skin punch biopsies are performed, one at a site that received 8% RAPA for a week and a second biopsy done at a nearby site that was treated with the ointment vehicle only. During a post-biopsy visit with one keloid-prone subject, we noted that the biopsy site that received vehicle only healed with a small keloid while the site that received 8% RAPA ointment healed without any lesion whatsoever. This serendipitous observation suggested that 8% RAPA ointment could prevent keloid formation.

To explore this possibility a keloid-prone volunteer applied 8% RAPA ointment to an established keloid once per day. Digital photographs with metric rulers adjacent to the keloid were taken to objectively monitor changes in lesion size. Surface area calculations from the digital photos were calculated by image analysis with Image J. This program can measure surface areas based on pixel analyses when calibrated against a standard; in our case the standard was a millimeter ruler in the photograph, adjacent to the keloid.



After 2 months of treatment the volunteer stated that he believed the lesion was getting smaller, although this was not apparent from lesion surface area calculations. (In retrospect, we speculate in the first months of treatment the surface area did not decrease but the height of the lesion above the surrounding skin decreased). After 4 months, the keloid surface area calculated from the photographs showed a tendency toward reduction. After 7 months, surface area calculations made by 3 different blinded observers with NIH Image J from digital photographs taken under identical lighting and distance conditions showed a clear reduction in surface area (see figure, prior page-calculated

surface area in cm^2 with linear regression lines for months 4-7). The plot shows the lesion area measured by three readers from multiple photographs vs. follow-up time. The data were analyzed using analysis of variance (ANOVA) for repeated measurements (22). The statistical model included the effect of follow-up time, a random effect of photograph within follow-up time, a random effect of reader, an interaction of time and reader, an interaction of reader and photograph, and a residual error. The largest variance component was due to reader (variance component estimate 0.0036) with the residual error being the second largest (0.0011); estimates of the other variance components were substantially smaller (photograph within time 0.0002, reader by time 0.0002, and reader by photograph 0.0001). There was a significant linear trend (slope = -0.0013, P=0.0163); the quadratic trend was not statistically significant (P=0.2728). The negative linear trend was observed for each reader (Reader 1 -0.0016, P=0.0066; Reader 2 -0.0008, P=0.1118; Reader 3 -0.0013, P=0.0459). This exciting observation led us to propose this pilot study to test topical application of 8% RAPA ointment to regress keloids.

1.4. Investigational Agent

Rapamycin (RAPA) concentration of 8% RAPA in a petrolatum vehicle will be compounded and supplied by Doyle's Pharmacy (Houston, TX). We will have subjects self-administer study product or placebo to be applied directly to each treatment keloid. This schedule replicates that which generated our preliminary keloid-regressing results. Our placebo control ointment will be the petrolatum vehicle only. Both ointments will have the same consistency and smell.

1.5. Dose Rationale and Risk/Benefits

Our pilot trial will use 0.5-1.0ml applications of 8% RAPA in a petrolatum vehicle to established keloid lesions in 5 persons with at least 2 chronic keloids greater than 1-year duration in similar body regions. In preliminary studies, we found that applying this 8% RAPA ointment reliably produced local transdermal delivery of RAPA without producing any measurable RAPA levels in systemic blood (6). Treatment will be applied once daily for 6 months. Surface areas of keloids will be monitored monthly and compared over time to evaluate responses.

Topical RAPA Ointment: RAPA from LC Labs was used in our preliminary studies (7, 11). Initially, we used a formulation of 1% RAPA in a petrolatum vehicle (6). This topical preparation had been demonstrated to regress skin angiofibromas in tuberous sclerosis patients (6). No systemic RAPA blood levels, alterations in complete blood counts, changes in complete metabolic profiles, or skin irritation occurred in the tuberous sclerosis patients in that study (6). An alternative topical formulation of RAPA in a vehicle of either 50:40:10 capric acid/isopropyl myristate/benzoyl alcohol or 50:50 capric acid/benzoyl alcohol has also been tested in humans (15). The latter formulations, in up to 8% RAPA concentrations, improved psoriasis, was undetectable in blood, and produced no clinical blood test changes; however, a few participants had adverse reactions to the vehicle. Because topical 1% RAPA in petrolatum was documented to exert local effects without any systemic effects or adverse reactions to the vehicle, we chose this formulation and strength for our initial studies. We assessed transdermal delivery by applying the 1% RAPA ointment over an intradermal μD probe and sampled RAPA delivery into the cutaneous interstitial space. We found 1% RAPA produced measurable interstitial RAPA concentrations in some, but not all cases. We therefore increased the RAPA concentration in petrolatum to 8% and repeated our μD measurements. We found that we could reliably achieve an interstitial level of 11.47±4.03ng/ml RAPA with this 8% RAPA ointment that was chosen for further studies. This 8% RAPA formulation subsequently prevented keloid formation at a skin biopsy site and regressed an established keloid in a keloid-prone subject leading to the present pilot proposal.

One keloid will be treated with topical RAPA while the second keloid will serve as an untreated control by receiving petrolatum vehicle only. 8% RAPA ointment will be applied to the 'treatment' keloid but not the second, to allow each subject to be his/her own control.

Potential Risks: In a randomized, double blind, vehicle-controlled multicenter study of 179 patients with facial angiofibromas secondary to tuberous sclerosis complex, 59 enrolled and randomized to 1% RAPA, 63 to 0.1% RAPA, and 57 to the vehicle-only group (23). Topical rapamycin was generally well-tolerated, with no measurable systemic absorption. Nearly all AEs were mild, with no drug-related moderate, severe, or serious events.

Apparent drug-related adverse effects at the application site were limited to 10% or less incidence for:

- Discomfort or pain
- Pruritis
- Acne
- Erythema
- Irritation

In this same trial, generalized symptoms occurred in a few subjects, including nasopharyngitis, nasal congestion, sinusitis, application site papules and applications site paresthesia. There were no reports of generalized symptoms such as pruritis, nausea, mouth ulceration or cough.

2. Study Objective

We will test the primary hypothesis that treatment of extant keloids with 8% topical RAPA ointment will regress keloid surface area and height.

Finally, to assess safety, we will gather data on anticipated common side effects of the medication, including any adverse drug reactions other than those listed as potential risks in Section 1.5 above.

Objectives:

1. To compare the effect of topical RAPA 8% ointment to treat established keloid versus untreated keloid
2. To test whether regression of surface area occurs after 6 months of treatment
3. To test whether regression of height of treated keloid occurs after 6 months

3. Study Design

3.1. Subjects

We will screen up to 20 persons ages 18-75 without diabetes or unstable, chronic diagnoses. We intend to complete 5 subjects achieving 6 months of treatment with topical RAPA. Refer to Section 4, Inclusion/Exclusion Criteria.

3.2. General Design

Our pilot trial will use daily 0.5-1.0ml applications of 8% RAPA in a petrolatum vehicle to treat established keloid lesions in 5 persons with at least 2 chronic keloids in similar body regions (greater than 1-year duration). Treatment will be applied once daily for 6 months. One keloid will receive 8% RAPA ointment and the other keloid will receive petrolatum-only ointment as a control. Surface areas and height of keloids will be monitored pre- and post-treatment to evaluate responses.

We will measure keloid surface area and height changes pre-treatment and thereafter on a monthly basis using standardized digital photography with a tripod-mounted 8MP Canon S5is camera and calipers to measure keloid height (37).

Surface areas of all photographed lesions will be measured with Image J (http://rsbweb.nih.gov/ij/index.html), a public image-processing program that we used to analyze our preliminary data and will use the same approach in this study.

3.3. Study Endpoints

Primary outcome:

- Change in keloid surface area and keloid height

3.4. Potential Risks to Subject Safety

3.4.1. Drug Administration.

The most common side effects of rapamycin include:

TOPICAL:	SYSTEMIC: (≥ 20%)		
• Discomfort or pain	• Stomatitis	• Acne	• Dizziness
• Pruritis	• Diarrhea	• Chest pain	• Myalgia
• Erythema	• Abdominal pain	• Peripheral	• Hypercholesterolemia
• Irritation	• Nausea	• URI	
• Rash	• Nasopharyngitis	• Headache	

3.4.2. Blood withdrawal. Subjects will undergo venipuncture to obtain blood specimen for complete blood count (CBC) and comprehensive chemistry (CMP) for general safety purposes. Although our research staff are experienced in venipuncture and aseptic technique, the most common risks to phlebotomy include mild pain from the needlestick or venous catheter, bleeding, bruising, and rarely infection.

3.4.3. Psychosocial. Subjects may experience psychological distress from assessments of cognitive function (CLOX), which will be minimized with use of privacy barriers.

4. Subject Selection and Withdrawal

4.1. Inclusion Criteria

- Both sexes of ages 18-75 years in good general health
- All ethnic groups regardless of Veteran status
- Must have at least two (2) keloids present for at least 1 year, located in a similar body area that can be easily reached, and be willing to self-administer investigational ointment and placebo control
- Cognitive functioning (CLOX1 ≥10 and CLOX2 ≥12) sufficient to provide informed consent and be able physically to apply QD ointment to affected area
- Negative pregnancy test

4.2. Exclusion Criteria

- Diagnosis of active or chronic skin disorders
- History of, or active treatment for local or systemic infections, especially of skin

- Current treatment with systemic steroid or immunosuppressant therapy within the past 6 months or local steroidal treatment within 3 months. Potential subjects may be re-screened if local treatment is discontinued for the 3-month period.
- Active/unstable conditions: inflammatory, thyroid, autoimmune, gastrointestinal (GI), hematologic, or neoplastic disorders. Subjects with clinical lab values outside the normal range and determined to be clinically significant by investigator will be excluded.
- Subject is considered unsuitable for the study in the opinion of the investigator for any other reason

4.3. Subject Recruitment and Screening

Subjects will be recruited and by referral from any Audie L. Murphy VA Hospital outpatient clinic or research unit, through medical record review, and by referral from dermatology and other outpatient clinics at UT Health San Antonio. Fliers and other means of advertisement may be distributed per applicable institutional policy.

Screening will be performed following consent at Visit 1, prior to baseline data collection and assessment of eligibility criteria listed above.

4.4. Early Withdrawal of Subjects

4.4.1. When and How to Withdraw Subjects

Subjects have the right to withdraw fully or partially from the study at any time and for any reason without prejudice to his or her future medical care by the physician or at the institution.

Withdrawal of full consent for a study means that the subject does not wish to receive further investigational treatment and does not wish to or is unable to continue further study participation. This may include any follow-up in person, by phone, through third parties including relatives or friends, via discussion with other treating physicians, and by use of medical records. Subject data up to withdrawal of full consent will be included in the analysis of the study. Any subject may withdraw full consent to participate in the study at any time during the study. The Investigator will discuss with the subject appropriate procedures for withdrawal from the study.

4.4.2. Data Collection and Follow-up for Withdrawn Subjects

If a subject withdraws consent to participate in the study, attempts will be made to obtain permission and capture final photographic images of the keloids and measurement before being disenrolled.

If subjects are withdrawn prematurely from the study, Principal Investigator will consult with Study Statistician about handling any incomplete data set as compared to the full data set that fully supports the analysis. The Principal Investigator will determine whether to replace the withdrawn subject to achieve the goal of 5 completers.

Lost to follow up (LTFU) will be defined as a subject not answering or responding to 2 or more consecutive phone follow up calls after starting study medication, or after returned-receipt of 1 certified letter concerning discontinuation of participation in the study.

5. Study Drug

5.1. Description

5.1.1. Rapamycin powder (from a supplier to Doyle's pharmacy)

Synonym: 23,27-Epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclohentriacontine, AY 22989, Sirolimus;
PubChem Substance ID 57654583
CAS No. 53123-88-9

An FDA-approved macrocyclic triene antibiotic forms a complex with FKBP12 that binds to and inhibits the molecular target of rapamycin (mTOR). Rapamycin (RAPA) is a potent immunosuppressant and has anticancer activity.

Mechanism of Action
The RAPA oral formulation developed by Pfizer (RAPAMUNE, sirolimus) inhibits T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (Interleukin [IL]-2, IL-4, and IL-15) stimulation by a mechanism that is distinct from that of other immuno-suppressants. Sirolimus also inhibits antibody production. In cells, sirolimus binds to the immunophilin, FK Binding Protein-12 (FKBP-12), to generate an immunosuppressive complex. The sirolimus: FKBP-12 complex has no effect on calcineurin activity. This complex binds to and inhibits the activation of the mammalian Target Of Rapamycin (mTOR), a key regulatory kinase. This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle. Dosage forms and strengths include: oral solution (60mg/60mL in amber glass bottle) and oral tablets (0.5, 1, and 2mg).

Drug interactions with systemic use include: Inducers of CYP3A4 and P-gp may decrease sirolimus concentrations whereas inhibitors of CYP3A4 and P-gp may increase sirolimus concentrations. Avoid concomitant use of sirolimus with strong inducers (e.g., rifampin, rifabutin) and strong inhibitors (e.g., ketoconazole, voriconazole, itraconazole, erythromycin, telithromycin, clarithromycin) of CYP3A4 and P-gp. Avoiding consumption of grapefruit juice is also cited.

Safety
From oral administration, serious allergic reactions identified by the manufacturer include:

- swelling of face, eyes, or mouth
- trouble breathing or wheezing
- throat tightness
- chest pain or tightness
- feeling dizzy or faint
- rash or peeling of skin

In previous human studies cited earlier in this protocol, topical administration did not produce systemic or serious reactions. See Section 3.4 for Potential Risks due to topical RAPA. Participants will be instructed to notify the research team and get help right away if any of the above symptoms of an allergic reaction occur.

Storage
The powder is packaged 1 mg in a glass bottle with instructions to keep container tightly closed in a dry and well-ventilated place.

5.1.2. Petrolatum (Vaseline, vehicle only)

Mechanism of Action

Petrolatum is a pale yellow to yellow-colored, translucent, soft unctuous mass. It is odorless, tasteless, and not more than slightly fluorescent by daylight, even when melted. Petrolatum is an inert material with few incompatibilities.

Safety

Petrolatum is mainly used in topical pharmaceutical formulations and is generally considered to be a nonirritant and nontoxic material. Animal studies, in mice, have shown petrolatum to be nontoxic and noncarcinogenic. Although petrolatum is generally nonirritant in humans following topical application, rare instances of allergic hypersensitivity reactions have been reported, as have cases of acne, in susceptible individuals following repeated use on facial skin. However, given the widespread use of petrolatum in topical products, there are few reports of irritant reactions. The allergic components of petrolatum appear to be polycyclic aromatic hydrocarbons present as impurities. The quantities of these materials found in petrolatum vary depending upon the source and degree of refining. Hypersensitivity appears to occur less with white petrolatum and it is often the preferred material for use in cosmetics and pharmaceuticals.

Storage

Petrolatum is an inherently stable material owing to the unreactive nature of its hydrocarbon components; most stability problems occur because of the presence of small quantities of impurities. On exposure to light, these impurities may be oxidized to discolor the petrolatum and produce an undesirable odor. Petrolatum should not be heated for extended periods above the temperature necessary to achieve complete fluidity (approximately 70°C/158°F). When heated to decomposition it emits acrid smoke and irritating fumes.

Petrolatum may be sterilized by dry heat. Petrolatum should be stored in a well-closed container, protected from light, in a cool, dry place.

Regulatory Status

GRAS listed. Accepted for use in certain food applications in many countries worldwide. Included in the FDA Inactive Ingredients Database (ophthalmic preparations; oral capsules and tablets; otic, topical, and transdermal preparations).

5.1.3. Compounded investigational product

The investigational product and the placebo will be compounded and supplied by Doyle's Pharmacy in Houston, TX. A certificate of analysis from the source for the powder form of RAPA used in compounding can be provided. The literature states the compounded product will remain stable for 3 months (6) and extended stability testing will not be performed since new product can be ordered every 2 months through duration of the trial.

5.2. *Treatment Regimen*

Each ointment (8% RAPA and placebo) will be applied to the appropriately designated keloid (RAPA experimental or placebo control) once daily for 6 months.

To avoid treatment confusion, the chosen keloids will be marked with a color-code by permanent marker; one color will be red, and a second will be green. Metered dispensers will be similarly color coded. The dispenser coded in red will be applied to the keloid labeled "right" and the lime green coded dispenser will be applied to the keloid labeled "left." Participants will be given color-coded photographs of the 2

keloids with identified landmarks to show which keloid receives which color-coded ointment.

5.3. Administration of Study Drug

The initial application will be done in the laboratory for subject education. Color-coded permanent markers will be given to participants to re-mark sites as needed.

Participants will be responsible thereafter for applying the different ointments to the color designated keloids once daily for the study's duration. Two medication dispensers (8% RAPA and vehicle control) will be given to each subject on a monthly basis.

Subjects will be instructed to turn the Topi-CLICK of each container once per application and apply all the expressed ointment to the matching, color-coded keloid. Subjects will be instructed to apply ointments after bathing or showering, avoid washing the treated areas for at least 4 hours after application, avoid cross-contamination between sites by washing hands between applications, and use new disposable gloves (provided by study) for each ointment application.

By managing with color-coded dispensers to match lesions, the participants will be "blind" to which lesion is receiving placebo and which keloid gets active treatment.

5.4. Subject Compliance Monitoring

Subjects will be called weekly between Visits 1 and 2 to verify compliance and at midpoints between Visits 2 and 7. Subjects also receive a drug diary to help record administrations and any notable effects, which supports reporting the participant's experiences at phone follow-ups and clinic visits.

Each dispenser will be weighed before being given out and at monthly intervals to quantify compliance.

5.5. Prior and Concomitant Therapy

5.5.1. Exclusionary medications:

- Systemic steroid or immunosuppressant therapy
- Body lotions, astringents, or other skin applications including cosmetics and especially sunscreen to the keloid area(s)
- Other antifungal, antibiotic, or topical preparations

5.5.2. Possible drug interactions:

- See Section 5.1 above

5.6. Packaging

Drug and placebo will be supplied by the compounding pharmacy in a metered dispenser called the Topi-CLICK® container. The opaque Topi-CLICK® dispenses 0.5-1mL of ointment, protects the product from light exposure, and serves to conceal any subtle differences between placebo and investigational product.

5.7. Management of Study Drug

5.7.1. Storage

The compounded preparation of 8% RAPA in petrolatum will be stored in a locked cabinet at room

temperature in a cool, dry place.

5.7.2. Dispensing of Study Drug

Designated study staff maintain the drug purchase documentation and administration logs to track how, when and to whom the investigational drug was assigned. Inventory is managed by the research pharmacy. Study clinical staff keep administration records regarding dosing compliance, returned/unused drug, and any damaged or wasted study drug product. Routine Study Drug reconciliation will be performed based on clinical site policy and standard operating procedures.

5.7.3. Return or Destruction of Study Drug

The procedures for final reconciliation of the site's drug supply at the end of the study will be in accordance with research pharmacy standard operating procedures.

Unused study product will be disposed in accordance within the standard medication destruction policies of the hospital.

6. Study Procedures

Study visits. All study visits will be performed at the Audie L. Murphy VA Hospital or by telephone.

6.1. Visit 1 – Consent, Screening and Baseline

6.1.1. Informed Consent

Eligible subjects will be asked to come to the research area to review and sign the consent form. Staff conducting the consent process adhere to local standard operating procedure (SOP) for consent administration. When scheduling consent appointments, staff will ask participants to arrive fasting.

6.1.2. Screening and Baseline

Following consent and signature, participants will undergo minimal cognitive screen (CLOX1 ≥10 and CLOX2 ≥12) to ensure consent is valid. Study staff will:

- Obtain vital signs, height and weight, lab work (fasting) to ensure safety and rule out systemic infection (complete blood count [CBC], comprehensive metabolic panel [CMP], lipid panel, and urine pregnancy test)
- Document medical history, concomitant medication review, physical examination, and
- Perform evaluation of eligibility criteria listed in Sections 4.1 – 4.2 above.

Additional baseline activities and data collection to include:

- Current and recent medications within past 3 months
- Marking of keloid lesions selected for study
- Photography and caliper measurements
    - Standard calipers will be used for measuring height of keloid above normal skin surface
    - Photography and use of Image J software – Study staff will upload digitized photographs and transfer directly from camera to computer software; images of the keloid are visualized, and outlined with an electronic drawing tool contained in the software. The software calculates the surface area within the outlined region and stores the data in Image J for analysis (see Analysis section next page).
- 1st medication administration for education
- Investigational medication prescribed/dispensed for 1 month, and participant is given written instructions for self-administering medication and care of the study lesion

- Schedule Visit 2

Study staff will contact participants weekly by phone for follow up (3 calls) during the first month of treatment. If the participant has a SmartPhone, a scheduled video visit (such as FaceTime, Imo or Skype) is optional to conduct weekly interim assessments but not required. Visits 2-7 must be performed in the research area and cannot be conducted virtually due to photography standardization.

Standardized digital photography and caliper measurements - Analysis

We will measure keloid surface area and height changes pre-treatment and thereafter on a monthly basis using standardized digital photography with a tripod-mounted 8MP Canon S5is camera and calipers to measure keloid height (37). The key to successful use of photography is to maintain consistent image quality and thus ensure that images can be compared fairly. Factors that influence image quality will be standardized including lighting, 10cm distance from keloid surface, super macro camera setting, a proper center of focus perpendicular to the keloid surface, and inclusion of a standard millimeter ruler as a reference for calculation of surface areas by Image J. Photos thus taken will be suitable for sequential measurements over the 6 months of the study because they will be taken by the same camera at the same angle under identical conditions and using the same standardized ruler by the same person. Image J (http://rsbweb.nih.gov/ij/index.html), is a public domain image-processing program that can calculate area and pixel value statistics of user-defined selections, measure distances and angles, create density histograms, and line profile plots from digital images. Spatial calibration is available to provide real dimensional measurements in standard units such as mm^2. We used Image J to analyze our preliminary data and will use the same approach in this study. Surface areas of all photographed lesions will be measured with Image J by three trained reviewers blinded to all aspects of the photograph (date taken, treatment, subject, etc.). In addition, keloid height measurements (the maximum vertical elevation above normal skin) in millimeters will be made with calipers (37). Prior to measurements, we conduct training sessions for the readers to improve agreement among readers. Reviewers will be trained to standardized definitions (color, texture, etc.) of lesions thus minimizing inter-reviewer variability. We also will assess agreement within readers by repeating the measurements at a later time. The percentage of keloid flattening will be calculated as the percentage reduction normalized to pre-treatment height. Finally, we will record clinical observations of keloid changes to extend and subjectively verify our objective measurements.

6.2. Visit 2 (approximately 4 weeks after Visit 1) – Month 1 of treatment

- Adverse event review and urine pregnancy test
- Keloid observation and clinical assessment
- Photography and caliper measurements – (same as baseline)
- Dispense medication

6.3. Visit 3 (approximately 4 weeks after Visit 2) – Month 2

- Adverse event review and urine pregnancy test
- Keloid observation and clinical assessment
- Photography and caliper measurements – (same as baseline)
- Dispense medication

6.4. Visit 4 (approximately 4 weeks after Visit 3) – Month 3

- Same as above, plus repeat CBC, CMP, lipid panel

6.5. Visit 5 (approximately 4 weeks after Visit 4) – Month 4

- Same as above and urine pregnancy test

6.6. Visit 6 (approximately 4 weeks after Visit 5) – Month 5

- Same as above and urine pregnancy test

6.7. Visit 7 (approximately 4 weeks after Visit 6) – Month 6 – Final Study Visit

We will ask participants to return to the hospital to undergo post-treatment procedures, conclude the study data collection and disenrollment processing.

- Vital signs, weight, and lab work to ensure safety or rule out systemic infection or effects (CBC, CMP, lipid panel)
- Update medical history, concomitant medications, focused physical examination, and
- Evaluation of medication compliance
- Final adverse event review
- Keloid observation and clinical assessment
- Photography and caliper measurements – (same as baseline)

6.8. Summary Visit Schedule

Event	Screening & Baseline Visit 1		Visit 2 (V1 +1m)	Visit 3 (V2 +1m)	Visit 4 (V3 +1m)	Visit 5 (V4 +1m)	Visit 6 (V5 +1m)	Visit 7 (V6 +1m)
Consent	X							
Vital signs	X		X	X	X	X	X	X
Height (V1 only) and weight	X							X
CLOX exam	X							
Urine HCG, monthly	X		X	X	X	X	X	
Labs: CBC, CMP, and lipid panel	X				X			X
History	X							X
Physical Exam	X							X
ConMed Review	X		X	X	X	X	X	X
Keloid Observed		X	X	X	X	X	X	X
Photo and Caliper		X	X	X	X	X	X	X
Dispensing Rx		X	X	X	X	X	X	
Medication Instructions and phone calls weekly between V1-V2 and at midpoint between V2-7 for compliance monitoring		X	X	X	X	X	X	
AE Review			X	X	X	X	X	X
Questionnaires		X						X
Disenrollment								X

7. Statistical Plan

7.1. Statistical Methods

For all analyses, the statistical threshold for significance will be set at $p<0.05$.

Keloid surface area and height data will be analyzed using ANOVA for repeated measurements using a

model similar to that described for the analysis of the preliminary data (see INNOVATION, above) with the addition of a random effect for subject (47). This analysis can be modified to include between-subject factors if such factors (sex, race, etc.) are identified for investigation.

8. Safety and Adverse Events

8.1. Definitions

8.1.1. Adverse Event (AE)

In general, AE is used very broadly and encompasses physical and psychological harms and includes:

> Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not it is considered related to the subject's participation in the research.

Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

8.1.2. Serious Adverse Event (SAE)

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

8.1.3. Unanticipated Problems Involving Risk to Subjects or Others (UPIRSO)

Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- <u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- <u>Suggests that the research places subjects or others at greater risk of harm</u> (including physical, psychological, economic, or social harm).

8.1.4. Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

8.1.5. Pre-existing Condition

A preexisting condition is one that is present at the start of the study. A pre-existing condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

At screening, any clinically significant abnormality should be 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 must also be recorded and documented as an adverse event.

8.1.6. Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a clinically significant degree requiring active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

8.1.7. Hospitalization, Prolonged Hospitalization or Surgery

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

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition (e.g., colonoscopy, SCI annual evaluation, respite care).
- Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2. Recording of Adverse Events

At each contact with the subject, the investigator or study staff will seek information about adverse events by specific questioning and, if appropriate, by examination. Information on all AEs will be recorded immediately in the source documentation. Site staff will record AEs using the appropriate data collection form in REDCap, which will be exported as an AE Log (See Section 9.3) for periodic review, at least monthly and ad hoc, depending on severity and expected/unexpected nature of the event.

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported per Institutional policy and according to FDA requirements.

8.3. Reporting of Serious Adverse Events and Unanticipated Problems

SAEs and UPIRSOs will be reported per local IRB policy and procedure.

Each subject is evaluated for any adverse events (AE). Any event that is reported to either the principal investigator or designated research staff by either the subject or medical staff caring for the subject and which meets the criteria will be documented. Any AE reported as serious will necessitate an adverse event report, which will be submitted to the IRB, R&D Committee, and to the Pepper Center DSMB.

Unanticipated risks to subjects or others (UPIRSO) that are a result of study participation are promptly reported to the IRB, the VA R&D Committee, and, if deemed appropriate, to the Pepper Center DSMB. The report will include a description of the event, when and how it was reported, as well as any official chart records or documentation to corroborate the event or the reporting of the event. All adverse events will be graded as mild, moderate, or severe. All adverse events will be summarized annually and submitted to the IRB and R&D Committees. Any action resulting in a temporary or permanent suspension of this study (e.g. local site IRB actions) will be reported to FDA or drug manufacturer per IRB stipulations.

8.3.1. Investigator reporting: notifying the Pepper Center DSMB

Any study-related SAE-UPIRSO, must be reported to the Principal Investigator by telephone within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed and submitted within 24 hours. The investigator will keep a copy of this SAE form on file at the study site.

Report SAEs by email and facsimile to:

Dean L. Kellogg, Jr, MD, PhD	or	Sara E. Espinoza, MD
Professor, Medicine-Geriatrics		Associate Professor, Medicine-Geriatrics
Email: kelloggd@uthscsa.edu		Email: espinozas2@uthscsa.edu
210-617-5132 fax 210-235-3681 pager		210-310-5859

Within the following 48 hours, the Principal Investigator provides further information on the SAE or the UPIRSO in the form of a written narrative. This should include a copy of the completed SAE form, and any other diagnostic information that will assist the understanding of the event.

8.3.2. Investigator reporting: notifying the UTHSCSA IRB

Notifying the IRB and or FDA if SAE or UPIRSO

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening

-or-

– a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Any finding from tests in laboratory animals that:

– suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements

Post marketing surveillance and adverse events may be submitted on FDA Form 3500A or in a narrative format. The contact information for submitting safety reports is noted below:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Dermatology and Dental Products (DDDP)
5901-B Ammendale Road
Beltsville, MD 20705-1266
Phone: (301) 796-2290
Fax: (301) 796-9712

8.4. Medical Monitoring

The Investigator and or Co-PI will review the safety and progress of this study on a monthly basis or when needed if SAE or SAE-UPIRSO occurs.

8.5. Investigator Reporting of Protocol Deviations/Violations

Departures during the conduct of a research study constitute a protocol deviation, violation or exception and as such must be reported to the UTHSCSA IRB.

Tracking and reporting of protocol deviations and violations to the IRB is the responsibility of the PI. To determine whether deviations or violations require prompt reporting or other action, refer to the IRB document entitled “Decision Tree – Evaluating Departures” on the IRB website. Failure to report departures from the protocol according to IRB policy may constitute possible non-compliance, which will require a Prompt Report Form and possible FDA reporting by IRB.

8.5.1. Deviations and violations may be identified in a number of ways including:

- A report by an individual can be made directly to the IRB Office.
- The IRB may learn of event through its continuing review of ongoing research.
- Compliance reviews (audits) conducted by the Office of Regulatory Affairs and Compliance or one of the HSC affiliated institutional compliance offices.
- A report by an individual can be made directly to the Office of Regulatory Affairs and Compliance (Hotline) or one of the HSC affiliated institutional compliance offices.
- A report by another committee, department, institution, or official.
- An audit or report from the study sponsor or sponsor’s monitoring entity.

8.5.2. Definitions of Protocol Deviations/Violations

- Protocol deviations – such as out of window visit, missed lab, usually recognized after the fact, etc.
- Protocol violations – enrolling an ineligible participant, using wrong consent version, willful act of

not following protocol
- Emergency violations Refer to UTHSCSA IRB Policy website: https://research.uthscsa.edu/irb/policy/deviations for more information

8.6. Stopping Rules

Should a subject develop a rash during study intervention, RAPA medication will be stopped and their participation will cease.

Should a female participant of childbearing age become pregnant during study intervention, RAPA medication will be stopped and participation will be withdrawn.

In the unlikely event that a study-related death or SAE occurs, the decision to stop the trial, either temporarily or permanently, will be the collaborative responsibility of the Pepper Center DSMB and the Principal Investigator.

9. Data Handling and Record Keeping

9.1. Confidentiality

Information learned about all subjects will be kept confidential. All data and protected health information in paper form will be kept confidential by assigned anonymous identifier and kept secured (password protected and/or double locked). Subjects will not be identified in any way in any publication.

9.2. Source Documents

Source data are contained in source documents found in paper subject files at the research site and in VA CPRS medical records. Print all entries legibly in blue or black ink. Erasures and white-out material are prohibited. If any entry error has been made on paper, 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. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.3. Case Report Forms

The study case report forms (CRF) are the primary data collection instruments for the study. Data requested on the CRF will be collected from the subject encounter and from the subject diaries, pharmacy logs, and medical records (VA CPRS), then entered into the REDCap database. All missing data will be routinely queried, corrected, and or 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”.

9.4. Data Management

Database Management Software: All data management for this project will be maintained using the UT Health San Antonio REDCap platform which is managed by the Department of Epidemiology and Biostatistics.

Data System: REDCap is a computing environment developed by Vanderbilt University consisting of a collection of instruments, under the management of UT Health San Antonio’s systems, policies, and procedures that govern its informatics operations. Data projects are designed to be end-user oriented and constructed to optimize workflow and minimize errors.

All data will be input using a web front-end interface. All users are individually assigned authorization for access to specific components of the database application. Information that is input is checked for logical and range consistency and mandatory data fields must be entered in order to input a record.

9.5. Records Retention

The regulatory binder is maintained by the assigned OAIC Pepper Center Regulatory Coordinator and stored in the PI Research Folder at Barshop Institute if conducted at UTHSA site and on designated VHASTX server if conducted at VA.

The Principal Investigator is responsible for maintaining study essential documents for at least 3 years after the funding grant period ends or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product, whichever is longer.

These documents should be retained for a longer period if required by a funding agency, the FDA or other institutional retention policy. In such an instance, it is the responsibility of the VA Audie Murphy Hospital or Principal Investigator to inform the institution as to when these documents no longer need to be retained.

10. Study Monitoring, Auditing, and Inspecting

10.1. Study Monitoring Plan

The Principal Investigator will ensure that the designated regulatory coordinator or other quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct study monitoring visits as assigned.

10.2. Auditing and Inspecting

The Principal Investigator will permit study-related monitoring, audits, and inspections by the IRB, the funding sponsor, the OAIC Pepper Center Data Safety and Monitoring Board (DSMB), government regulatory bodies, and University compliance groups 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 University compliance and quality assurance offices.

11. Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312) applicable government regulations and Institutional research policies and procedures.

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

12. Study Finances

12.1. Funding Source

This pilot study is financed through a grant from the Department of Medicine

12.2. Conflict of Interest

None reported. All VA investigators submit Conflict of Interest Disclosure forms as part of the review and approval process.

12.3. Subject Stipends or Payments

There are no funds available to reimburse participants for time and transportation. The participants who screen pass and move on to study intervention will receive a box of surgical gloves, a surgical pen in two colors, and study medication and placebo at no cost.

13. Publication Plan

The study will be registered and published on www.clinicaltrials.gov in accordance with FDAAA and institutional policy.

The Institution and its respective designees may present or publish the results of a scientific investigation involving this study in accordance with ICJME standards and guidelines.

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15. Attachments

A. Sample Consent Form
B. Grant award
C. SOP for drug management (pending)
D. Imaging instructions for photography (pending)

16. SUMMARY OF PROTOCOL CHANGES

Date of Change	Version	Section Modified	Before Change	After Change
04-16-19	1.0 initial to IRB	all	NA	NA
04-30-19	1.1	IRB number, version date 04-30-19	Pending	Updated to 20190291H
05-14-19	1.2	Section 4.1 Inclusion	Change criterion	Added "negative pregnancy test" and made Urine HCG "prn" in Summary Visit Table
		Section 5.7	Drug inventory managed by clinical study staff	Drug storage and management by Research Pharmacy
		Stopping Rules	¶ 1, regarding AE due to drug	Replaced ¶ 1 to state stopping drug and withdrawal from study if participant develops a rash
		General formatting update		Corrected formatting of margins Sect 8.3 to end
06-05-19	1.3	Section 5	Sigma Aldrich	Changed to "supplier to Doyle's pharmacy"; removed Sigma Aldrich as needed
07-15-19	1.4	Visit Schedule Table and Visit narratives	CBC, BMP	Modified Safety Labs: Replaced BMP with CMP and added lipids; plus repeat labs at Visit 4 and urine pregnancy testing monthly (per FDA recommendation)
02-13-20	1.5	Study Drug and Visits	Topi-pump	Changed dispenser to Topi-click
04-20-20	1.6	Cover page	NCT# pending	NCT# updated