OPEN-LABEL, SINGLE-ARM PILOT STUDY OF THE EFFECTS OF TOPICAL 5% IMIQUIMOD CREAM ON PREVENTING KELOID RECURRENCE AFTER SURGICAL KELOIDECTOMY

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CONFIDENTIAL

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List of Abbreviations

actinic keratosis (AK)
human papilloma virus (HPV)
Toll-like-receptor-7 (TLR7)

Study Summary

Title	Open-label, single-arm pilot study of the effects of topical 5% Imiquimod cream on preventing keloid recurrence after surgical keloidectomy
Short Title	Imiquimod for preventing keloid recurrence
IRB Protocol Number	
Phase	Pilot study
Methodology	Open-label, single-arm
Study Duration	Enrollment of patient will take place over the next year. The project will begin as soon as the IRB is approved.
Study Center(s)	Single-center
Objectives	Primary objective: - To assess the efficacy of topical 5% Imiquimod cream on decreasing keloid recurrence after excision. Secondary objectives: - To assess the tolerability of topical 5% Imiquimod cream
Number of Subjects	10
Diagnosis and Main Inclusion Criteria	**Key inclusion criteria** Patients age ≥ 18 with keloids < 5cm on trunk (below neck and excluding groin) and extremities (excluding hands and feet) requesting excision Able and willing to provide informed consent **Key exclusion criteria** Pregnant or breastfeeding Taking systemic immunosuppressive medication, life threatening disease, or inherent sun-sensitivity Hypersensitivity to study drug Age < 18 Keloids located in sections as noted above Unable to follow-up for 10 weeks Involvement in a trial of another experimental intervention within 30 days Unable or unwilling to provide informed consent
Study Product, Dose, Route, Regimen	5% Imiquimod cream, topical application to cover skin lesion once daily x 5 days per week for 6 weeks
Duration of administration	6 weeks
Reference therapy	None
Statistical Methodology	Response rate will be presented as a percentage, with 95% confidence intervals. Other endpoints will be presented descriptively, with an appropriate measure of variation (e.g. standard deviation) if necessary.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This is an open-label, single-arm, pilot study on the effects of topical imiquimod treatment in preventing keloid recurrence after surgical excision. Keloids are abnormal scars that form in certain genetically predisposed individuals following trauma to the skin. They can be physically disabling and cause social impairment. Many therapies have been proposed and trialed for the permanent removal of keloids, but they all have limited efficacy. (1)

Topical imiquimod therapy has been reported to decrease keloid recurrence following keloidectomy in human patients (8-13). In our lab, we observed that topical imiquimod pre-treatment (imiquimod initiated prior to onset of trauma) improves wound closure and decreases scar formation in mouse ear-punch models of wound healing. Given all previous reports of adjuvant imiquimod therapy to keloidectomy initiated imiquimod therapy after keloidectomy, we would like to test the efficacy of topical imiquimod pre-treatment in preventing keloid recurrence after surgical excision.

1.1 Background

Keloids are benign dermal fibroproliferative tumors that represent a form of abnormal wound healing following any insult to the deep dermis, including lacerations, abrasions, surgery, piercings, vaccinations, and burns in predisposed individuals (1). They are distinguished from hypertrophic scars, another type of abnormal scar formation, by their extension beyond the borders of the original wound, failure to regress spontaneously, and tendency to recur following excision (1).

Keloids tend to occur more frequently in dark-skinned individuals, with an incidence of up to 16% in those of black and Hispanic backgrounds (2). These lesions appear to affect male and female subjects equally and most commonly occur in persons aged 10-30 years (2). They typically form on the ears, face, chest, and shoulders and can cause pain, burning, itching, and restriction of motion. Furthermore, the scars can be extremely disfiguring and potentially cause significant social and psychological distress in affected individuals.

The process by which keloids develop is poorly understood, though most theories of keloid etiology relate to fibroblast dysfunction (2). These lesions are difficult to treat, as they frequently recur following excisional removal. Current treatment options include surgical excision, laser, intralesional injections, pressure and silicone dressings, topical preparations, oral agents, radiation, and cryotherapy (1). Oftentimes, surgical excision is combined with adjuvant therapies such as corticosteroids and immunomodulators at the time of skin closure because surgical excision alone has recurrence rates ranging between 45% and 100% (3). Additionally, because surgical excision requires complete removal of all abnormal tissue, post-excision keloid recurrence may lead to a larger keloid than prior to excision. Although many adjuvant therapies to surgical excision and modalities for keloid removal have been proposed, there has been no consensus regarding how to best treat these lesions, and there is currently no medication approved for the indication of reducing keloid recurrence after excision.

Imiquimod is an immunomodulating agent that binds to Toll-like-receptor-7 (TLR7) and acts as a potent inducer of interferon and cytokine release at the site of skin application. It has been shown to decrease excessive collagen production and upregulate certain apoptosis-related genes in keloid fibroblasts (4). When formulated as a 5% cream, Imiquimod is a safe and well-tolerated drug. Side effects include skin erosion, excoriation, flaking, and edema at site of application (5).

1.2 Investigational Agent

Imiquimod is an immune response modifier that binds to TLR7 and, when applied topically to the skin, induces the production of a variety of cytokines (6). It is manufactured as a cream at concentrations of 2.5%, 3.75%, and 5%. 5% Imiquimod is currently approved for the treatment of actinic keratosis (AK), superficial basal cell carcinoma, and external genital and perianal warts (condylomata acuminata) from human papilloma virus (HPV) infection (7).

Imiquimod is applied topically, and percutaneous absorption of Imiquimod is minimal. 5% Imiquimod cream will be used in this study. Each gram of 5% Imiquimod cream contains 50 mg of Imiquimod in an off-white oil-in-water vanishing cream base consisting of isostearic acid, cetyl alcohol, stearyl alcohol, white petrolatum, polysorbate 60, sorbitan monostearate, glycerin, xanthan gum, purified water, benzyl alcohol, methylparaben, and propylparaben (7).

A mean peak serum drug concentration of 0.4 ng/ml was observed in a small study of 12 patients with genital/perianal warts following an average applied dose of 4.6 mg. In patients with actinic keratosis who applied 5% Imiquimod cream topically 3-times weekly for 16 weeks, the mean peak drug concentrations at the end of week 16 were approximately 0.1, 0.2, and 3.5 ng/mL for applications to face (12.5 mg Imiquimod), scalp (25 mg Imiquimod), and hands/arms (75 mg Imiquimod), respectively. After up to 3 weeks of dosing with the 3.75% cream (18.75 mg/day applied to the face and scalp) in 17 patients with actinic keratosis, the mean peak serum Imiquimod concentration was 0.323 ng/mL with a median t_{max} of 9 hours after dosing. It appears that systemic exposure may be more dependent upon surface area of application than amount of applied dose. The half-life of the 3.75% cream was 29.3 +/- 17 hours at the end of the study; therefore, steady-state concentrations are anticipated to occur by day 7 with once daily dosing. The apparent half-life of the 5% cream was about 10-times greater with topical dosing than the 2-hour apparent half-life seen following subcutaneous dosing, suggesting prolonged retention in the skin. (7) (16)

1.3 Preclinical Data

Multiple case reports have suggested that Imiquimod application to the sites of surgical wounds and to hypertrophic scars may reduce scar occurrence/recurrence. In our lab, we have shown that topical application of 5% Imiquimod increases the rate of wound closure and leads to increased tissue regeneration with decreases in scarring in mouse ear hole punch models of tissue regeneration. In our preclinical study, we applied 5% Imiquimod cream for 5 days one week prior to ear hole punch **(Figure 1A).** In the presence of Imiquimod, there is a marked increase in the rate of closure when compared to control **(Figure 1C)** often leading to complete closure of the wound **(Figure 1B, D).** The drug treated group also demonstrates more complete regeneration of skin appendages such as sebaceous glands (arrowhead) and ear cartilage (arrow) in the new tissue **(Figure 1D)**, a feature consistent with tissue regeneration and not scar formation.

To eliminate the possibility that this Imiquimod phenotype is simply due to nonspecific skin irritation, other skin irritants such as calcipitriol and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were also tested. Wound closure rates were not significantly reduced compared to vehicle control in each case (data not shown).

These preliminary results suggest that Imiquimod application enhances wound closure and biases the healing process towards tissue regeneration and away from scarring.

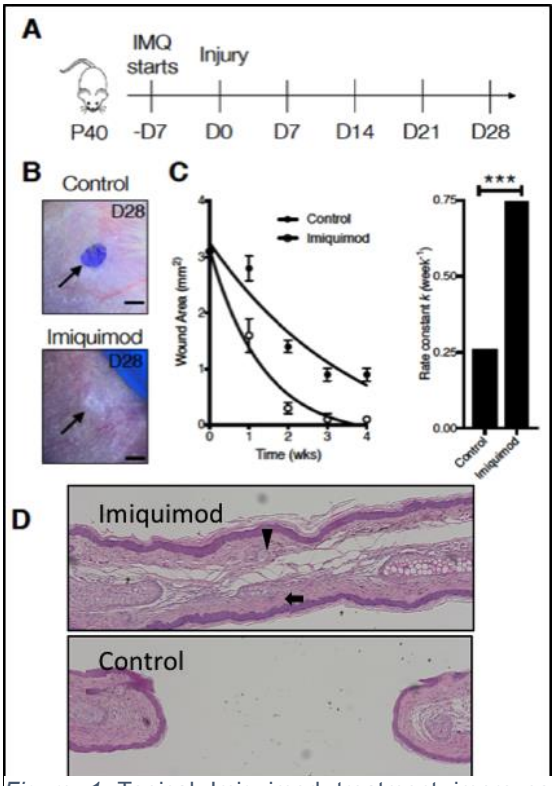


Figure 1 Topical Imiquimod treatment improves mouse ear wound healing

1.4 Clinical Data to Date

Case series and prospective trials of 5% Imiquimod after surgical keloidectomy have been reported. However, the results have been variable. As summarized in **Table 1**, post-excision treatment with Imiquimod appears to be most effective in preventing recurrence of keloids on the ears but has limited efficacy in other locations. Additionally, some studies were limited by poor patient follow-up compliance.

All of the reported studies were designed such that Imiquimod was started after surgical excision of keloids. There are no reports of initiating Imiquimod prior to surgical excision, and our preclinical data suggests that there may be a benefit to pre-excision Imiquimod therapy.

Table 1 Summary of published clinical data for Imiquimod therapy post-keloidectomy

Reference study	Number of keloids	Sites treated	Recurrence (%)	Follow-up duration
Berman (2002) (8)	11 (10 patients)	Earlobe (10) and back (1)	0 (0%)	24 weeks
Martin-Garcia (2005) (9)	8 (6 patients)	Earlobe	2 (25%)	24 weeks
Stashower (2006) (10)	8 (4 patients)	Earlobe	0 (0%)	12 months
Maholtra (2007) (11)	3 (2 patients)	Presternal	3 (100%)	12 weeks
Cacao (2009) (12)	9 (9 patients)	Trunk	8 (89%)	12 weeks
Frias (2018) (13)	41 (25 patients)	Trunk (9), suprapubic (2), extremity (4), auricular (20)	9 trunk (100%), 0 suprapubic (0%), 4 extremity (100%), 3 auricular (15%)	6 months

1.5 Dose Rationale and Risk/Benefits

Once daily application of 5% Imiquimod cream is currently approved by the FDA for the treatment of many skin disorders. Although the exact mechanism of action has not been elucidated, when used to treat pre-cancerous lesions and skin cancers, Imiquimod results in inflammation, which destroys the lesion and can result in complete regression. Additionally, as mentioned above, Imiquimod has been shown to be effective in preventing recurrence after surgical keloidectomy, though to a varying degree depending on the site of the lesion.

The proposed dosage regimen for this study is in accordance with the current recommended dosing for superficial basal cell carcinoma – apply once daily before bedtime 5-times per week for a total of 6 weeks. This regimen has been shown to be safe, tolerable as well as effective in inducing molecular and clinical responses in patients.

Areas treated with imiquimod may become inflamed. The symptoms may include itching, burning, redness, ulceration (sores), scabbing, flaking, and pain. There could be increased sensitivity to the sun in areas of drug application. This could be avoided by proper sun protection such as wearing protective clothing and/or applying sunscreen of SPF 30 or greater 15 minutes prior to sun exposure with reapplication every 2 hours. Systemic side effects may include “flu-like” symptoms such as fever, fatigue, headache, nausea, diarrhea, and muscle pain. These symptoms are less common and are generally mild.

Unlike previously reported studies, this study aims to initiate treatment with Imiquimod one week prior to keloidectomy. Our preclinical data suggests that pre-treatment with Imiquimod prior to injury increases wound healing and decreases scar formation. Additionally, it has been shown that when using

corticosteroids as an adjunct to surgical excision, it is best to initiate treatment in the preoperative period and continue treatment for up to 3 months post-excision (14).

Because the effects of the drug are not well studied in pregnancy and during breastfeeding, it is possible that there are harmful side effects that are not yet known to both the mother and unborn or breast-feeding child. Therefore, pregnant and breastfeeding subjects are excluded from the study. Additionally, the risks of this study includes risks of breach of confidentiality. While every effort will be made to protect the confidentiality of study subjects, there is a potential risk of an inadvertent or unintended breach of confidentiality.

As demonstrated in patients who have used 5% Imiquimod for other FDA approved indications, risks of topical application of 5% imiquimod are minimal. Additionally, previous studies as well as our preclinical data indicates that 5% imiquimod could modulate wound healing and prevent keloid recurrence in patients when applied prior to keloid-excision and continued after the procedure. Therefore, we believe that the benefit of possible decrease in keloid recurrence outweighs the minimal risks posed by topical 5% imiquimod therapy.

2 Study Objectives

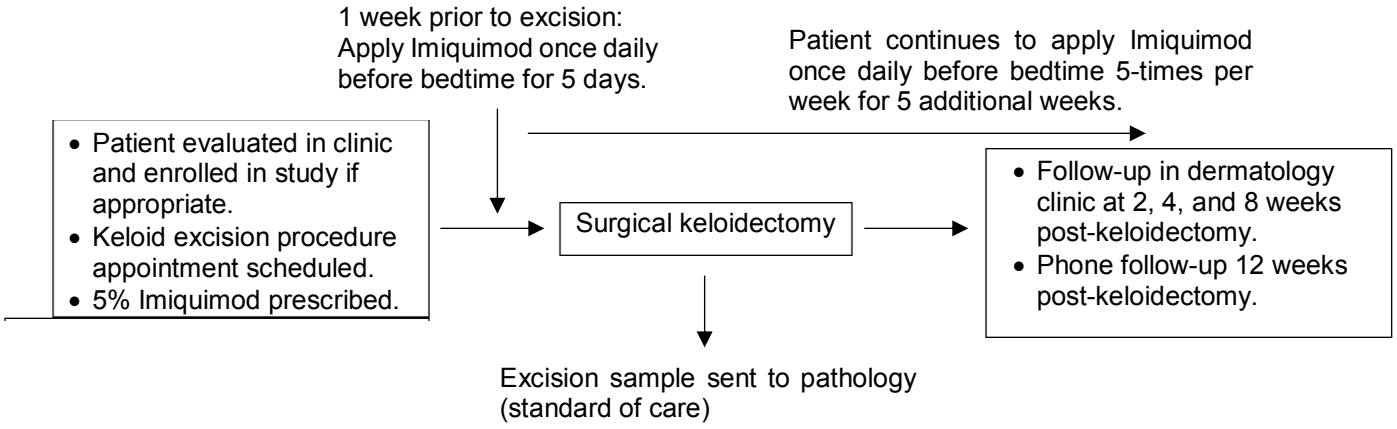
Primary Objective
To assess the efficacy of 5% Imiquimod cream on preventing keloid recurrence after excision. Subjects will be evaluated by a consultant dermatologist at 2, 4, and 8 weeks following excision and by phone 12 weeks after excision to assess for clinical evidence of lesion recurrence.

Secondary Objectives
To assess the safety and tolerability of 5% Imiquimod in subjects undergoing keloid excision. Subjects will be evaluated by a consultant dermatologist at 2, 4, and 8 weeks following excision to assess for local site reaction such as redness, discomfort, swelling, and ulceration.

3 Study Design

3.1 General Design

- Prospective, open-label, non-randomized, single-arm pilot study
- Subjects are expected to participate for a total of approximately 14 weeks from initial dermatology consultation visit



3.2 Primary Study Endpoints

To assess the efficacy of 5% Imiquimod cream on decreasing keloid recurrence after excision when initiated prior to excision

3.3 Secondary Study Endpoints

To investigate the tolerability of 5% Imiquimod cream

3.4 Primary Safety Endpoints

To assess the safety and tolerability of 5% Imiquimod in subjects undergoing keloid excision.

At each clinic follow-up visit (2, 4, 8 weeks after excision), the investigator will rate the local site reaction on the following scale:

- None
- Mild reaction (slight redness, discomfort, no swelling, no ulceration)
- Moderate reaction (redness, pain and swelling, no ulceration)
- Severe reaction (redness, pain, swelling, and ulceration)

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Age ≥ 18
2. Men and women who present clinically with keloids requesting excision
3. Any number of keloids
4. Keloid no larger than 5cm in diameter at the base
5. Clinical findings consistent with keloid formation, including: a scar that does not spontaneously regress, extends beyond borders of normal tissue, may be pruritic, may be painful.
6. Location of keloid in low-risk areas – areas other than above the neck, hands, feet, or groin
7. Able and willing to give informed consent

4.2 Exclusion Criteria

1. Age < 18
2. Hypersensitivity to Imiquimod or to any of the excipients (methylhydroxybenzoate, propylhydroxybenzoate, cetyl alcohol, and stearyl alcohol)
3. Involvement in a trial of another experimental intervention within 30 days
4. Life threatening disease
5. Use of immunosuppressive medications such as oral corticosteroids
6. Bleeding disorders
7. Not available for follow-up for 10 weeks
8. Pregnant, intention to become pregnant during treatment phase of trial, or breastfeeding

4.3 Subject Recruitment and Screening

Patients will be recruited through the routine work of the Dermatology departments at the Hospital of the University of Pennsylvania. Recruitment and consent will be obtained by the principle investigator or another Dermatology physician as delegated by the investigator seeing the patient in the outpatient clinic. Patients seen in the outpatient clinic with clinical representation of keloids will be approached by the

Dermatology attending as part of clinical evaluation, and written, informed consent will be obtained from the patient prior to any research related procedures are conducted.

It will be explained to the potential participant that entry into the trial is entirely voluntary and that their treatment and care will not be affected by their decision. Should any Penn affiliates be approached, that their decision of whether to participate will not impact their standing with the institution. It will also be explained that they can withdraw at any time. In the event of their withdrawal it will be explained that their data collected so far will be retained and used as part of the study analysis.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

The participant will stop Imiquimod treatment early for the following reasons:

- New clinical/diagnostic information (e.g. pathology report) suggests a diagnosis other than keloid
- Intolerable local or systemic toxicity from Imiquimod
- Clinical evidence of keloid recurrence. These participants will progress to intralesional steroid injections or excision as per standard of care.
- Any participant who becomes pregnant during the study
- Withdrawal of consent

Subjects who withdraw consent will be withdrawn from the trial. The subjects will be made aware that this will not affect their standard of care. Subjects will be made aware (via the information sheet and consent form) that should they withdraw the data collected will be retained and used as part of the study analysis.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Protected Health Information to be collected include names and medical record numbers. Additional information that is to be collected from patient's electronic medical record are:

- Race/ethnicity/gender
- And all elements of dates for dates that are directly related to the patient
- Current medications
- Symptoms related to treatment
- Physical examination
- Treatment and response
- Unidentifiable photodocumentation of lesions

Patients who choose to withdraw from the trial will be asked for permission for phone follow-up 12 weeks after excision date.

Patients who are withdrawn from the study for any reason but have initiated at least 1 imiquimod treatment will be seen in the outpatient clinic for one additional follow-up visit after their withdrawal date to observe the application site.

This information will be associated with a study ID. A master list with PHI and study IDs will be maintained by personal investigators in a secure location. Access will be given to only study personnel as needed. Data sets will only include assigned study IDs. Data will be backed up as a password protected file on a Penn Medicine secure server. Links to identifiers will be destroyed after the study is completed.

5 Study Drug

5.1 Description

Imiquimod is a topical immunomodulator approved for the treatment of actinic keratosis, superficial basal cell carcinoma, and external genital and perianal warts (condylomata acuminata) from human papilloma virus (HPV) infection. Each gram of 5% Imiquimod cream contains 50 mg of Imiquimod in an off-white oil-in-water vanishing cream base consisting of isostearic acid, cetyl alcohol, stearyl alcohol, white petrolatum, polysorbate 60, sorbitan monostearate, glycerin, xanthan gum, purified water, benzyl alcohol, methylparaben, and propylparaben (7).

5% Imiquimod is supplied in single-use packets each of which contains 250mg of the cream, equivalent to 12.5mg of Imiquimod. It is supplied in a box of 24 packets each (7).

5.2 Treatment Regimen

Topical dosage (5% cream)
Apply a thin coat of Imiquimod cream to cover the entire lesion once daily 5-times per week prior to normal sleeping hours, for 6 weeks, starting 1 week prior to keloid excision. Cream should be left in place for approximately 8 hours. The target keloid should have a maximum diameter of no more than 5cm and be located on the trunk (excluding anogenital skin), below the neck, or extremities (excluding hands or feet). The treatment area should include a 1 cm margin of skin around the tumor. The diameter of cream droplet applied should range from 5 mm to 10 mm for keloid areas of 0.5 cm to 5 cm, respectively. Subjects should wash hands thoroughly prior to and after application of medication. Maximum to be prescribed: 36 packets during the 6-week treatment period.

A rest period of several days during treatment may be required due to local skin reactions. Follow-up visits will be scheduled at 2, 4, and 8 weeks after excision and as needed for local skin reactions.

The following dosing algorithm will be used at the 2 and 4 visits and unscheduled visits due to local toxicity to adjust the frequency of application accordingly.

- If intolerable at 5 days per week; stop completely for 1 week then restart but at a reduced dose of 3 days per week. Increase to 5 days per week as soon as tolerable.
- If intolerable at 3 days per week, a further week off, start again at 3 days per week. Stop if intolerable.

Subjects will be requested to contact the local investigator for advice before stopping or changing treatment.

5.3 Method for Assigning Subjects to Treatment Groups

This is a single-arm study. All patients will be receiving open-label active drug.

5.4 Preparation and Administration of Study Drug

The principle investigator or dermatology physician designated by the PI will prescribe the 5% Imiquimod cream for the patient. Study medication will consist of the number of packets as ordered by the PI necessary to treat the Keloid. The study drug will be purchased with start-up funds from the PI, and it will be provided to the patient free of charge. Study medication will be open label (see section for study drug details).

5.5 Subject Compliance Monitoring

Compliance with study procedures will be monitored throughout the trial. It is expected that the Investigator will comply with the protocol requirements and encourage subjects to follow all procedures carefully.
Subject compliance with treatment administration will be calculated as the percentage of the reported number of doses administered compared to the number that should have been administered while the subject is on study. The total number of packets utilized will be compared to the total number prescribed. Additionally, application site will be evaluated for erythema and local site reaction.

5.6 Prior and Concomitant Therapy

Concomitant medications/treatment for the keloid lesions should be kept to a minimum during the study. However, if considered necessary for the subject's welfare they may be given at the discretion of the investigator according to the local standard of care. The drug, dose and duration should be recorded.

Immunosuppressive medications including oral corticosteroids are specifically excluded for the duration of this study. A reconciliation of recorded medications in the electronic medical system will be performed at the screening visit.

5.7 Packaging

5% Imiquimod cream is supplied in single-use packets each of which contains 250mg of the cream, equivalent to 12.5mg of Imiquimod. 5% Imiquimod cream is supplied in a box of 24 packets each (7).

As there is no blinding in this study, the imiquimod will be prescribed in its standard packaging, with additional labelling to comply with regulatory requirements.

5.8 Blinding of Study Drug

The study is not blinded.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The investigator will make a notation of any damaged or unusable study treatments that were supplied to the investigator's site.

5.9.2 Storage

The drug will be stored and handled per the specifications on the package insert. The drug will be stored at 4-25ºC (39-77ºF) with the temperature being monitored in a secure location contained in an appropriately designated secure area. Avoid freezing. Keep out of reach of children.

5.9.3 Dispensing of Study Drug

The study physician or designee in the dermatology clinic will dispense the medication and keep detailed dispensing records. The physician will write a prescription for the amount of medication to be dispensed.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, subjects will be asked to return all unused medication at the final follow-up visit. There will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

Patients will be recruited through the routine work of the Dermatology departments at the Hospital of the University of Pennsylvania. Recruitment and consent will be obtained by the principle investigator or another Dermatology physician as delegated by the investigator seeing the patient in the outpatient clinic. Patients seen in the outpatient clinic with clinical representation of keloids will be approached by the Dermatology attending as part of clinical evaluation, and written, informed consent will be obtained from the patient prior to any research related procedures are conducted.

It will be explained to the potential participant that entry into the trial is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time. In the event of their withdrawal it will be explained that their data collected so far will be retained and used as part of the study analysis.

	Visit 1 (Week 1)	Visit 2 (Week 2)	Visit 3 (Week 4)	Visit 4 (Week 6)	Visit 5 (Week 10)	Phone encounter (Week 14)
Informed consent	X					
Urine pregnancy test	X					
Imiquimod application	X (start after visit)	X	X	X		
Keloid excision		X				
Excision specimen sent to pathology per SOC		X				
Photograph of lesion	X (optional)	X (optional)	X (optional)	X (optional)	X (optional)	
Evaluation of local site reaction		X	X	X	X	
Evaluation for recurrence			X	X	X	X

6.1 Visit 1

Diagnosis of keloid will be made clinically by a Dermatology attending in the University of Pennsylvania Dermatology outpatient clinic. If the patient meets all inclusion and exclusion criteria set by the study and wishes for the keloid to be surgically removed, the patient will be approached by the Dermatology team for recruitment into the study. The patient will be given the opportunity to have a further discussion about the study and receive answers to any questions they may have. If the patient is agreeable to participating in the study, written and informed consent will be obtained from the patient or the patient's legally authorized representative. A medical history will be taken, demographic data will be collected, and photo-documentation of the lesions will be recorded.

A urine pregnancy test will be conducted prior to receiving study drug, for all female subjects of childbearing potential who are not postmenopausal > 1 year or surgically sterile. Subjects with a positive urine pregnancy test will be excluded from the study. Subjects will be asked to use contraception during treatment period. If

indicated, (missed period, unprotected sexual intercourse, or Investigator discretion), a urine pregnancy test may be administered subsequently at the discretion of the Investigator.

A procedure appointment will be made for the surgical keloidectomy, and the subject will be instructed to apply Imiquimod 5% daily Monday to Friday the week prior to the scheduled excision date. The cream should be left in place for approximately 8 hours. The treatment area should include a 1 cm margin of skin around the tumor. The diameter of cream droplet applied should range from 5 mm to 10 mm for keloid areas of 0.5 cm to 5 cm, respectively.

The subject will also continue applying 5% Imiquimod daily as instructed for 5 more weeks after surgery. Subjects will be followed-up in the outpatient clinic at 2, 4, and 8 weeks post-excision and by phone 12 weeks post-excision.

6.2 Visit 2 – Procedure visit

Consent for and surgical removal of keloid will be performed in accordance with current standard of care. Keloid excision sample will be sent to pathology per standard of care.

6.3 Visits 3 and 4

Scheduled clinic visits will be at 2 and 4 weeks after the surgical excision (weeks 3 and 5 after initiation of Imiquimod). At these visits, the following will be recorded:

- Subject compliance with therapy – if doses were missed
- The investigator will evaluate for evidence of keloid recurrence
- The investigator will rate the local site reaction on the following scale:
    - None
    - Mild reaction (slight redness, discomfort, no swelling, no ulceration)
    - Moderate reaction (redness, pain and swelling, no ulceration)
    - Severe reaction (redness, pain, swelling, and ulceration)
- The dose of Imiquimod will be adjusted to according to the following algorithm:
    - If intolerable at 5 days per week; stop completely for 1 week then restart but at a
    - reduced dose of 3 days per week. Increase to 5 days per week as soon as tolerable.
    - If intolerable at 3 days per week, a further week off, start again at 3 days per week. Stop if intolerable.
- Any new medication usage and health service usage data will be recorded.

6.4 Visit 5

Last scheduled clinic visit will be at 8 weeks after surgical excision (3 weeks after completing Imiquimod therapy). The investigator will evaluate for evidence of keloid recurrence at this visit and evaluate for any local site reactions per above.

If treatment of the lesion with Imiquimod is stopped because of local toxicity, this will prompt an unscheduled clinic visit so that the dose adjustment algorithm can be implemented.

If at any point during the treatment phase, the keloid is judged to have recurred, then the subject will progress immediately to intralesional injection or re-excision in accordance with current standard of care.

6.5 Phone follow-up (Encounter 6)

Patient will be contacted by phone 12 weeks after surgical excision of keloid (7 weeks after completing Imiquimod therapy). Patient will be asked about recurrence of keloid at excision site and any residual local site symptoms from Imiquimod therapy.

7 Statistical Plan

7.1 Sample Size Determination

In this open-label, single-arm pilot study, 10 patients will be enrolled.
The sample size calculation was based on a historical recurrence rate of 70% at 8 week follow-up after excision alone and an expected 25% recurrence rate with Imiquimod treatment. Using 80 % power and alpha = 5 %, the estimated sample size required is 8. Allowing for a withdrawal rate of approximately 20 %, 10 subjects will have to be enrolled.

7.2 Statistical Methods

Response rate will be presented as a percentage, with 95% confidence intervals. Other endpoints will be presented descriptively, with an appropriate measure of variation (e.g. standard deviation) if necessary. Patient demographics will be summarized.

7.3 Subject Population(s) for Analysis

All subjects who completed the study protocol will be included in the study analysis.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (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)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. 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

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

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

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

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.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be 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 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.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> 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 degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

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 and adverse event.

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

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. 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 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 module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

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 determined that the study treatment or participation is not the 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 after the study period and is considered to be 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

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems <u>within 10 working days from the time the investigator becomes aware of the event:</u>

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

  <u>Unexpected</u> (An event is “unexpected” when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

  AND

Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Reporting Process
Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.4 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 9 Auditing, Monitoring 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 (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Records Retention

All data will be managed behind the Penn, password-protected server. All subject information will be kept in the university REDCAP database. Study documents will be archived after the study's closure.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

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.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, government regulatory bodies, and University compliance and quality assurance 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 accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/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 sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

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. See Attachment for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

Unfunded

12.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

12.3 Subject Stipends or Payments

There are no subject payments or stipends. Cost of keloid excision will be covered by the patient or patient insurance.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the principle investigator for the purposes of performing the study, will be published or passed on to any third party without the consent of the principle investigator or University of Pennsylvania Department of Dermatology. No subjects will be identified in any publications.

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15 Attachments

- Sample Informed Consent Form
- IND exemption letter