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A PROSPECTIVE SINGLE-SUBJECT TRIPLE-BLINDED SINGLE-CENTER STUDY OF LASER-ASSISTED 5-FLUOROURACIL VERSUS LASER-ASSISTED CORTICOSTEROID TREATMENT FOR KELOIDS

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NYULMC Study Number:	*s-20-01172*

Initial version: December 30, 2020

Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), 21 CFR Parts 50, 56, 312, and 812 as applicable, any other applicable US government research regulations, and institutional research policies and procedures. The International Conference on Harmonisation ("ICH") Guideline for Good Clinical Practice ("GCP") (sometimes referred to as "ICH-GCP" or "E6") will be applied only to the extent that it is compatible with FDA and DHHS regulations. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

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List of Abbreviations

AE	Adverse Event/Adverse Experience
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
IRB	Institutional Review Board
N	Number (typically refers to participants)
PI	Principal Investigator
US	United States
5FU	5-fluorouracil
TAC	Triamcinolone
POSAS	Patient and Observer Scar Assessment Scale

Protocol Summary

Title	A Prospective Single-Subject Triple-Blinded Single-Center Study of Laser-Assisted 5-Fluorouracil Versus Laser-Assisted Corticosteroid Treatment for Keloids
Short Title	Treatment of Keloids with Fractional Erbium Laser-Assisted 5-Fluorouracil Versus Laser-Assisted Corticosteroid
Brief Summary	This is a single-subject split-scar study with a target sample size of 20. There are two interventions: fractional erbium:YAG-assisted drug delivery of 5-fluorouracil and fractional erbium:YAG-assisted drug delivery of triamcinolone acetonide. Patients will undergo treatments in a series of 4 treatments at approximately 4 week ± 1 week intervals. We will continue to follow them after the end of the interventional treatment to monitor for safety and continued benefit.
Phase	n/a
Objectives	Primary Objectives 1. To estimate the mean difference between Sites treated with laser-assisted 5-FU delivery versus laser-assisted TAC in the changes from the first POSAS score (before initiating treatment series) to the final POSAS score. 2. To estimate the mean difference between Sites treated with laser-assisted 5-FU delivery versus laser-assisted TAC in the modified Hamilton score at the final visit Secondary Objectives The secondary objective is to assess the differences between Sites treated with laser-assisted 5-FU delivery versus laser-assisted TAC in the changes in size of keloids post treatment Exploratory objective The exploratory objective is to assess the differences between Sites treated with laser-assisted 5-FU delivery versus laser-assisted TAC in the patients subjective experiences, such as pain related to treatment, healing time.
Methodology	Triple-blinded prospective study

Endpoints	Primary Endpoint 1. The first primary endpoint is the POSAS score in Site A and Site B at first visit (pre-treatment) and last visit (post-treatment). 2. The second primary endpoint is a modified Hamilton score based on comparing clinical photographs of the scarred skin and healthy skin next to each other in Site A and Site B. Secondary Study Endpoints The secondary endpoint is measurements of the scar in both Sites of the keloid. The measurements will be calculated as the total volume (length x width x height) in cm^3 of the scar, or length x width in cm^2 if the scars are flush with the surrounding skin scar surface will be differences in change in size for in the two Sites of the keloid treated with laser-assisted 5-FU delivery as compared to laser-assisted TAC. Exploratory Endpoints Patient's subjective experiences measured as follows: 1. Patient perceived side effects after each treatment at visits 1, 2, 3, and 4 in Site A and Site B, in terms of dyspigmentation, pain, itch, swelling. 2. Pain grade after each laser treatment at visits 1, 2, 3, and 4, measured on a scale of 1 to 10 (10 being the worst pain), in Site A and Site B, recorded at the subsequent follow up visit. 3. Perceived speed of healing at final visit (visit 5) will be compared between both Sites using the following answers: Site A healed faster than Site B, Site B healed faster than Site A, Same speed of healing in both Sites
Study Duration	2 years
Participant Duration	1 year
Duration of IP administration	Up to 6 months
Population	Healthy men and women ages 18-89 in the United States.
Study Sites	NYU Dermatologic Surgery Associates.
Number of participants	20 participants.
Description of Study Agent/Procedure	The intervention will be a split-scar fractional ablative erbium laser-assisted delivery of 50mg/mL of 5-fluorouracil solution to one half of the scar and 10 mg/mL of triamcinolone acetonide solution to the other half of the scar. Patients will undergo 4 treatments.
Reference Therapy	N/A
Key Procedures	Laser therapy as above.

Statistical Analysis	Demographic data, baseline scar characteristics, POSAS scores, and modified Hamilton scores will be summarized at baseline and consecutive visits as appropriate. For the primary analysis, a two-sided paired Wilcoxon Signed-Rank test at a 0.05 significance level will be used to compare Site A and Site B of the keloid in the change from the first POSAS score (before initiating treatment series) to the final POSAS score. Similarly, a two-sided paired Wilcoxon Signed-Rank test at a 0.05 significance level will be used to compare the modified Hamilton score in Site A and Site B of the keloid. For the secondary analysis, measurements of the scar will be summarized by visit and compared between the two Sites of the keloid. For the exploratory analysis patient's subjective experiences will be summarized and compared between both Sites of the keloid as follows: 1. Patient perceived side effects after each treatment will be summarized by type (dyspigmentation, pain, itch, swelling), visit (1, 2, 3, 4) and treatment Site (A, B). 2. Pain after each laser treatment will be summarized by grade (scale of 1 to 10 (10 being the worst pain)), visit (1, 2, 3, 4) and treatment Site (A, B). 3. Speed of healing after completion of laser treatments at final visit (visit 5) will be compared between both Sites (Site A healed faster than Site B, Site B healed faster than Site A, Same speed of healing in both Sites)

1 Key Roles

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2 Introduction, Background Information and Scientific Rationale

2.1 Background Information and Relevant Literature

Scars are ubiquitous and create significant morbidity for millions of people across all demographics.[1] Pathologic scarring in particular may result in diminished quality of life due to cosmetic appearance, disfigurement, pruritis, pain, restricted functional capabilities, and decreased emotional health.[1-3] Pathologic scar treatment may also represent a significant financial burden for patients, with high costs associated with the often multiple medical and surgical interventions necessary to return functionality and cosmetic appearance to the site.[1]

Keloids are pathological scars characterized as an overgrowth of scar tissue past the original wound boundaries, but unlike malignant lesions, they are confined to the dermis and thus are considered benign.[4] Keloids are common in genetically predisposed individuals, and their treatment is especially challenging: once keloids are formed, they never regress spontaneously and commonly recur after treatment.[5,6] In fact, in individuals predisposed to keloid formation, surgical debulking can result in the generation of an even larger scar.[1]

The pathophysiology of keloid formation is still incompletely understood and the most commonly used therapeutic options return inconsistent and suboptimal results.[4] There is a need for clinicians to complement current treatment options with new modalities in order to better manage pathological scarring, improve patient experiences, and decrease costs.[1]

Common treatments for keloids include intralesional corticosteroid injections, silicone sheeting, cryotherapy, surgical manipulation, and radiotherapy.[7] Intralesional triamcinolone acetonide (TAC) injection, the most commonly utilized first-line therapy, has been shown to inhibit TGF-β1 expression and induce apoptosis in fibroblasts.[8-10] Though it is the prototypical treatment, the efficacy of intralesional TAC is not completely satisfactory in the treatment of keloids.[11] Additionally, intralesional TAC treatments involve injecting into the mid-dermis where there are thick collagen bundles, which frequently causes extreme pain for patients.[12]

Low-dose 5-fluorouracil (5-FU) has also emerged as a method of keloid treatment.[13] 5-FU is a pyrimidine analogue and antineoplastic agent that halts fibroblast proliferation and induces fibroblast apoptosis.[14,15] Furthermore, 5-FU and TAC have been used off-label mixed together and injected into keloids with good results and no known adverse drug interaction; in fact, they are synergistic.[7,16]

More recently, there has been increased interest in and usage of laser for treatment of pathological scarring. Ablative fractional laser (AFXL) therapy causes micropatterns of thermal injury to the dermis, thus stimulating the complex process of tissue remodeling; however, these microscopic thermal wounds generally heal without scarring.[17-19] Fractional laser devices have demonstrated benefits in the debulking and resurfacing of scars and keloids without adverse events, and are associated with functional improvements, as well as cosmetic benefits.[20,21]

There is growing evidence that combination therapy regimens are more effective than monotherapy.[22,23] Multimodal combination therapies with synergistic effects have an advantage in that the agents can be used at lower doses compared to monotherapy, thus improving the side effect profile.[22] For example, the combination of 5-FU and TAC has been shown to have a higher scar response rate, as well as a reduction of undesirable effects when compared to each alone.[7] Post-laser therapy with topical corticosteroids has been demonstrated to enhance the effects of fractional lasers. This is likely through efficient delivery and uniform distribution of the corticosteroid to the desired level of the skin through the microscopic channels created by laser ablation.[8,12] Additionally, the combination of AFXL and topical triamcinolone may reduce the incidence of pain compared to intralesional corticosteroid injection monotherapy.[12]

Laser-assisted 5-FU and laser-assisted TAC have shown similar efficacies in treating hypertrophic scars, though laser-assisted 5-FU has fewer side effects.[1] Laser-assisted topical triamcinolone is associated with the side effects of topical corticosteroids, including sporadic erythema, telangiectasias, hyperpigmentation, hypopigmentation, and a risk of dermal atrophy and fat atrophy.[8,24]

Building upon previous research, in our study we will investigate the effectiveness of laser-assisted 5-FU and laser-assisted triamcinolone in the treatment of keloids. We will measure “effectiveness” by taking measurements of the height, width, and length of treated lesions prior to, during, and after laser-assisted drug delivery. Analyzing keloids on height, width, and length with calipers allows for 3-dimensional statistical analysis of the effects of the treatments. Similar scar measurement protocols have been used in other studies on pathological scarring.[1]

2.2 Name and Description of the Investigational Agents

In this study we will be utilizing laser-assisted drug delivery of 5-FU and TAC. The laser we will be using is Sciton Joule (see Figure 1 below), the ProFractional XC handpiece, which is a fractional ablative Erbium:yttrium-aluminum-garnet (Er:YAG) laser with a wavelength of 2940nm. Sciton Joule ProFractional System is FDA approved for use in soft tissue (skin, cutaneous tissue, subcutaneous tissue, striated and smooth tissue, muscle, cartilage meniscus, mucous membrane, lymph vessels and nodes; organs, and glands) such as, but not limited to: Dermatology and Plastic Surgery: Skin resurfacing Treatment of wrinkles; Epidermal nevi; Telangiectasia; Spider veins; Actinic chelitis; Keloids; Verrucae; Skin tags; Anal tags; Keratoses; Scar revision (including acne scars). The use of fractional Er:YAG in keloids is FDA approved. The use of fractional Er:YAG for transdermal delivery of medications is off-label.
5-FU is a chemotherapeutic agent that can inhibit fibroblast proliferation. TAC is an anti-inflammatory corticosteroid that inhibits multiple inflammatory cytokines. Both topical and intralesional TAC ranging from 1-40 mg/mL is FDA-indicated for skin disorders, including keloids. Off label, 5-FU 50mg/mL solution is often mixed with TAC 10-40mg/mL by the physician and injected simultaneously into hypertrophic scars and keloids for synergistic effect, as previously mentioned.

Er:YAG-assisted delivery of 5-FU 50mg/mL solution or 5% cream has been performed safely in scars,[25] as well as other cutaneous diseases such as vitiligo[26] and Bowen’s disease.[27] Topical 5-FU 5% cream is FDA-approved for actinic keratoses and superficial basal cell carcinomas, and because topical 5-FU often leads to cutaneous erosions on the area of application, continued application of 5-FU cream for two weeks, as is FDA-indicated, results in application of 5-FU over open wounds. In addition, intravenous 5-FU at a higher dose of 10-30mg/kg/day depending on the disease is FDA-approved for breast, colorectal, gastric and pancreatic cancer. Given the extensive data of safe use of 5-FU in scars in the dermatologic literature, both FDA-indicated and non-FDA-indicated conditions, such as keloids, and the FDA-approved indication of systemic use in cancer patients in much higher doses, the localized and relatively superficial introduction of 1mL of 5-FU 50mg/mL by fractional Er:YAG does not present a significant risk to the health, safety, or welfare of a subject. Additionally, as fractional Er:YAG does not involve use of an implant, is not purported or represented to be for a use in supporting or sustaining life, and is not for a substantial use in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health in a way that would present a potential for serious risk to the health, safety, or welfare of a subject, the use of the Sciton Joule ProFractional System in this study is non-significant risk (NSR) in accordance with 21 CFR 812.3(m).

The maximum depth of ablation by ProFractional system is 1.5 mm, which is more superficial than when a needle is inserted to inject 5-FU or TAC intralesionally in the clinic. Furthermore, we do not plan to ablate deeper than 1 mm in our study. Fractional ablation with the Sciton Joule ProFractional system using 1mL of 5-FU 50mg/mL solution or 10mg/mL of TAC therefore does not involve a route of administration or dosage level that significantly increases the risks associated with the use of the two drugs.
Additionally, as the study involves the use of lawfully marketed drugs and the study:

is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;

is not intended to support a significant change in the advertising for the product;

is conducted in compliance with the requirements for institutional review set forth in part 56 and with the requirements for informed consent set forth in part 50; and

is conducted in compliance with the requirements of §312.7,

this study meets the criteria for IND exemption in accordance with 21 CFR 312.2(b)(1)

Figure 1. Sciton Joule Laser

2.2.1 Clinical Data to Date

In 2013, Waibel *et al.* used ablative fractional photo-thermolysis CO_2 laser treatments to aid corticosteroid delivery in hypertrophic scars. Three to five laser treatments at 2-month to 3-month intervals were combined with a thin topical layer of TAC suspension of 10 or 20 mg/mL, depending on the location and thickness of the skin. They observed an overall scar improvement, with the therapy having the greatest impact on texture but also improved dyschromia and overall appearance.[8]

Park *et al.* compared laser-assisted topical corticosteroid with intralesional corticosteroid injections in keloids. First, the entire keloid was treated with fractional ablative Er:YAG laser. The lesions were subsequently divided into two halves, with the first half receiving intralesional TAC injection (10 mg/mL) and the second receiving topical application of desoxymethasone 0.25% ointment. Four treatments at 6-week

intervals were conducted. They found a statistically significant improvement in the Vancouver Scar Scale (VSS) for both modalities, but the corticosteroid injection was associated with much more severe pain.[12]

In 2019, Waibel *et al.* compared clinical outcomes of hypertrophic scars treated with laser-assisted corticosteroid delivery versus laser-assisted 5-FU delivery. Subjects underwent 3 treatment sessions at 1-month intervals consisting of fractional ablative CO_2 laser treatment followed by postoperative application of either TAC solution (20 mg/mL) or 5-FU solution (50 mg/mL). Scars treated with both modalities demonstrated reduction in overall scar surface area. There was no statistically significant difference between the arms, suggesting that neither agent possesses superior efficacy over the other. However, 5-FU was not associated with the side effect risks that topical corticosteroids foster such as dermal atrophy or telangiectasia formation.[1]

Sabry *et al.* evaluated the effectiveness of combining ablative fractional CO_2 laser and topically applied 5-FU or verapamil hydrochloride in the treatment of hypertrophic scars and keloids. They found that combination therapy of ablative fractional CO_2 and topical 5-FU or verapamil hydrochloride is more effective than ablative fractional CO_2 monotherapy. Significant cosmetic improvement was seen in all subjects based on VSS score. The improvements achieved in scar pliability and erythema were especially notable. In contrast to laser-assisted delivery of 5-FU or verapamil hydrochloride, CO_2 monotherapy showed no significant improvement in scar height. Pigmentation showed minimal improvement in all three groups. They observed no negative side effects with 5-FU topical treatment.[28]

2.2.2 Dose Rationale (if applicable)

5-FU comes in one available dose (50mg/mL) and we will be using 10 mg/mL of TAC, due to the higher risk of adverse effects such as dyspigmentation and atrophy with higher doses.

2.3 Rationale

The primary objective of this study is to determine the differences in clinical outcome and side effect profile for keloids treated with laser-assisted 5-FU delivery as compared to laser-assisted corticosteroid delivery. Transdermal delivery of drugs is limited by permeation through the stratum corneum, where high-molecular weight and hydrophilic compounds often cannot penetrate.[29-31] Topical medications have been found to have a total absorption with only 1% to 5% bioavailability of the applied dose. Intralesional injection requires multiple needle punctures, which can be difficult to tolerate. Laser-assisted drug delivery can improve cutaneous penetration while being less painful.[12]

We hypothesize that efficacy is greater in keloids treated with laser-assisted delivery of 5-FU as compared to those treated with laser-assisted delivery of TAC 10mg/mL, and we expect to see less adverse effects such as hypopigmentation with 5-FU.

Er:YAG laser operates at a wavelength of 2940 nm and is an ablative laser as its energy is highly absorbed by water-containing tissue.[9,32] Er:YAG has been shown to enhance permeation of 5-FU by 53-fold to 133-fold in mouse models.[33] Er:YAG has also been shown to increase the penetration of lipophilic and hydrophilic compounds through the skin.[34] Er:YAG laser has demonstrated safety as a transdermal transport enhancer, with studies showing the depths of the stratum corneum and epidermis recovering to normal conditions within 3-5 days.[33,34] Once the stratum corneum and epidermis are surpassed, the depth of the ablation does not affect drug delivery.[35,36] Of importance in our study, fractional Er:YAG laser is associated with decreased risk of post-inflammatory hyperpigmentation (PIH) as compared to CO2 laser.[37,38] Skin of color (Fitzpatrick skin types IV, V, VI) is more prone to developing keloids and has a generally increased risk of PIH compared to lighter skin types. As such, the fractional erbium laser is the safer choice for our target population.

5-FU will be used at the dose of 50 mg/mL and TAC will be used at the dose of 10mg/mL; the amount will depend on the size of the keloid treated.

2.4 Potential Risks & Benefits

2.4.1 Known Potential Risks

Known potential risks of laser treatment include pain, bleeding and swelling. Known potential risks of laser-assisted delivery of each medication is no different than the potential risks of injecting each medication intradermally. There is no known risk of cross-reaction if both 5-FU and TAC are injected in the same scar; in fact, both have been mixed and injected into keloids at the same for enhanced clinical efficacy.[16] Keloids treated with erbium laser have been shown to have a good safety profile.[37,39] As aforementioned, 5-FU can be applied on open wounds for actinic keratoses as approved by the FDA (sometimes followed by TAC in case of brisk reaction) or even given intravenously in even higher doses for GI malignancies.[40,41]

TAC is used to treat a wide variety of dermatologic conditions (including open wounds/ulcers like pyoderma gangrenosum) and is the prototypical corticosteroid used to treat pathological scarring.[42] Topical side effects include dermal atrophy, fat atrophy, erythema, telangiectasias, hypopigmentation, hyperpigmentation, scarring, easy bruising, and certain inflammatory skin conditions.[8,29]

5-FU is an anti-metabolite widely used in cancer chemotherapy, both intravenously (FOLFOX in gastrointestinal malignancies) and topically.[40] Sabry *et al.* found that topical application of 5-FU after laser sessions showed no side effects, though others report topical side effects of 5-FU include local pain, dermatitis, pruritus, burning, photosensitivity, pigmentation changes, and scarring.[25,29] A review of intralesional injections of 5-FU found negative side effects that may include pain at the injection site, ulceration, burning, and hyperpigmentation; no systemic complications such as anemia, leukopenia, and thrombocytopenia were reported.[37]

2.4.2 Known Potential Benefits

Potential benefits include decrease in keloid size, improved skin texture overlying the keloid, improved pliability and mobility, and decrease in symptoms of itch and/or pain due to keloid.

3 Objectives and Purpose

3.1 Primary Objectives

As noted in section 4.1, Site A and Site B will be randomized to either laser-assisted delivery of 5-FU or laser-assisted delivery of TAC. We hypothesize that there is a significant difference between the two treatments with respect to the clinical outcomes of the POSAS scar scores and the Hamilton scores. Hence, the primary objectives are:

1. To estimate the mean difference between both Sites in the changes from the first POSAS score (before initiating treatment series) to the final POSAS score.
2. To estimate the mean difference between both Sites in the modified Hamilton score at the final visit.

3.2 Secondary Objective

The secondary objective is to assess the differences between both Sites in change in size of keloids post treatment.

3.3 Exploratory objective

The exploratory objective is to assess the differences between both Sites in patients subjective experiences, such as pain related to treatment, healing time.

4 Study Design and Endpoints

4.1 Description of Study Design

This study will be a triple-blinded single-center study of keloid therapies. The intervention will be laser-assisted drug delivery of TAC or 5-FU. When selecting patients, preference will be for two separate scars; however, if a patient presents with a single long scar, we will treat one half of the scar with laser-assisted delivery of TAC and the other half of the scar with laser-assisted delivery of 5-FU. The keloid on the patient's right side or the patient's right half of the keloid (in a single keloid) or the superior keloid (of 2 keloids) will be designated Site A. The keloid on the patient's left side or the patient's left half of the keloid (in a single keloid) or the inferior keloid (of 2 keloids) will be designated Site B. Randomization of which treatment (5-FU or TAC) will be applied to Site A will be performed in RedCap by statistician (BL). The treating physician (NL) will enter the patient ID in RedCap, answer a number of eligibility verification questions and retrieve the assignment for that patient. No patient-identifiable data will be entered in RedCap. All patient data will be entered in paper forms. All patients will undergo 4 treatments total. The patients will be blinded to the treatment. Their eyes will be shut and they will have protective laser goggles on during the treatment portion of their visit. The treating physician will treat Site A and Site B based on the randomized treatment allocation. The treating physician (NL) will not be blinded to the medication being administered after laser treatment nor to the AEs assessment. The treating physician cannot be blinded to the treatments, because TAC is an opaque white solution and 5-FU is a clear colorless solution. A blinded dermatologist (DG) will record the POSAS every visit. Physician observers blinded to the treatment allocation will grade the photographs taken at the final follow-up visit using a modified version of a photograph-based scale, the Hamilton Scale. De-identified data (aside from the patient ID) will be entered in Excel for purposes of statistical analysis.

COVID-19 Safety Precautions

In light of the COVID-19 pandemic, the study team will utilize a verbal recruitment template and COVID19 information sheet to make patients aware of risks of completing an in-person research study. The study team and patients will be protected by complying with all NYU safety measures, including screening, social distancing, PPE, cleaning/disinfecting.

<u>Daily symptoms screening for research personnel</u>

- Each faculty member, employee, and student will complete a "Daily Symptom Check" one to three hours ahead of the start of their time as part of the research study.

<u>Screening research participants for symptoms</u>

- The study team will be responsible for screening research participants in alignment with clinical screening of patients: upon scheduling, appointment reminders, and arrival at the research location.
- Patients will be asked 1. Do you have two or more Covid-19-like symptoms (cough, shortness of breath, fever, chills, shaking chills, muscle pain, headache, sore throat, new loss of taste or smell)? 2. Have you or anyone in household tested positive in the last 10 days? If either question is answered yes, the participant cannot come to their in-person appointment.
- During the screening process study teams must notify research participants that they are required to wear a mask/face covering.
- Upon arrival to NYULH locations, research participants are subject to the same screening procedures as patients.

<u>Social distancing</u>

- Office visits will be staggered to minimize number of individuals in waiting areas
- Waiting rooms/public areas will have seats spread to 6 feet of separation
- The number of study personnel in exam rooms will be minimized

<u>Personal Protective Equipment for research personnel</u>

- All NYU Langone staff, faculty, students, and trainees will wear a face mask at all times
- Researchers will also follow any additional PPE guidelines for our clinical area
- Masks will be worn in all public and work spaces and patient care areas, worn correctly to cover the mouth and nose

- Only NYU Langone-issued masks or personal protective equipment will be worn, no cloth masks or non-NYU Langone issued masks or PPE will be utilized

Personal Protective Equipment for research participants

- All participants and visitors >4 years of age will be offered a face mask upon entry to the ambulatory site
- Patients will be informed to arrive with a face mask during the pre-visit phone call
- Masks will be worn throughout participant's appointments

Cleansing and disinfecting

- All personnel will wash hands or use hand sanitizer often, especially when entering work areas from public spaces and after contacting high-touch surfaces like doorknobs and handles, refrigerator and freezer handles, and elevator buttons

4.2 Study Endpoints

4.2.1 Primary Study Endpoints

We will measure the non-blinded POSAS score at every visit. The POSAS was created in 2004 and takes both the patient and the provider perspectives into account. The POSAS scar scale has been found to be more reliable and less variable than other scar scales utilized in clinical practice[43].
POSAS has two components, the observer (physician) portion and the patient portion. The observer grades on a scale of 1 to 10, 10 being the worst scar imaginable, six measures: vascularity, pigmentation, thickness, relief, pliability and surface area. The patient answers six questions regarding the characteristics of the scar, with each question being on a scale of 1 to 10. The scores of each category are summed to get the total POSAS score, with 120 being the highest score and representing the worst scar.

1. The first primary endpoint is the POSAS score in Site A and Site B at first visit (pre-treatment) and last visit (post-treatment).

2. The second primary endpoint is a modified Hamilton score based on comparing clinical photographs of the scarred skin and healthy skin next to each other in Site A and Site B.

4.2.2 Secondary Study Endpoint

The secondary endpoint is measurements of the scar in both Sites of the keloid. The measurements will be calculated as the total volume (length x width x height) in cm^3 of the scar, or length x width in cm^2 if the scars are flush with the surrounding skin scar surface in the two Sites of the keloid.

4.2.3 Exploratory Endpoints

Patient's subjective experiences measured as follows:

1. Patient perceived side effects after each treatment at visits 1, 2, 3, and 4 in Site A and Site B, in terms of dyspigmentation, pain, itch, swelling.
2. Pain grade after each laser treatment at visits 1, 2, 3, and 4, measured on a scale of 1 to 10 (10 being the worst pain), in Site A and Site B, recorded at the subsequent follow up visit.
3. Perceived speed of healing at final visit (visit 5) will be compared between both Sites using the following answers: Site A healed faster than Site B, Site B healed faster than Site A, Same speed of healing in both Sites

5 Study Enrollment and Withdrawal

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Age 18-89
2. Has one large keloid scar or at least two similar but separate keloid scars
3. Keloid present for at least 1 year

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Currently pregnant
2. Currently breastfeeding
3. Have taken oral retinoids within 6 months of study initiation
4. Has had keloid treatment within 1 month of study initiation
5. Has active infection at treatment site
6. Has active malignancy
7. Presence of pedunculated keloid(s) or keloid(s) that are judged to be best treated with surgical excision first
8. Hypertrophic scars
9. Known hypersensitivity to TAC or 5-FU
10. Chronic systemic corticosteroid or immunosuppressive medication use
11. Has intolerance to anesthesia
12. Has known connective tissue disease
13. Has known infectious disease

5.3 Strategies for Recruitment and Retention

Participants will be recruited from the outpatient clinics of NYU Dermatology Faculty Group Practice. We will reach out to clinicians via word of mouth and email for subject referrals. Please see our email template and recruitment flyer attached as appendices. We will not utilize NYULMC media services or social media. Clinician-referred patients who are possibly eligible for participation in the study will be instructed to email research coordinator SC for pre-screening and permission to access electronic medical records. SC will describe the study and pre-screening process to potential subjects via telephone using an IRB-approved script and obtain verbal consent to ask the pre-screening questions and give permission for the study investigators to review the patient's electronic medical record. If a patient is deemed ineligible or they pass pre-screening but later decide not to participate in the study, the pre-screening data will be immediately destroyed. Target sample size is 15, we will attempt to screen 30-40 in order to obtain the target enrollment.

5.4 Duration of Study Participation

Duration of study participation will be 1 year.

5.5 Total Number of Participants and Sites

Recruitment will end when approximately 30 participants are enrolled. It is expected that approximately 30 participants will be enrolled in order to produce 20 evaluable participants (projected accrual is 20).

The site of this study will be at 222 East 41st Street, New York NY 10017. Treatments will take place on the 24th floor.

5.6 Participant Withdrawal or Termination

5.6.1 Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

Participants will be terminated from the study if there is no clinical response noted by Visit #3.

5.6.2 Handling of Participant Withdrawals or Termination

If a participant withdraws from the study, the investigator(s) will ask if the information already collected can still be used as part of the study.

5.7 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the primary investigator. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.
Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants

Study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the IRB and/or FDA.

6 Study Agent (Study drug, device, biologic, vaccine etc.) and/or Procedural Intervention

6.1 Study Agent(s) and Control Description

5-FU is a chemotherapeutic drug. This medication is kept routinely in the dermatology clinic for intradermal injection. The needles and syringes involved will be disposed of in the labeled clinic sharps container.

For TAC solution, the needles and syringes involved will be disposed of in the labelled clinic sharps container.

6.1.1 Acquisition

Both 5-FU and TAC are routinely available at the NYU Dermatology Faculty Group Practice. These medications are ordered from the Henry Schein medical supplier.

6.1.2 Formulation, Appearance, Packaging, and Labeling

5-FU is a opaque white liquid that comes in a clear glass bottle. This medication comes in the dose 50mg/mL.

TAC solution is a clear colorless solution that comes in a clear glass bottle in the dose 10mg/mL. We will use this medication at the dose 10mg/mL.

6.1.3 Product Storage and Stability

Both 5-FU and TAC are stable at room temperature.

6.1.4 Preparation

Neither 5-FU or TAC will need to be reconstituted; as such, both medications will be drawn up as is.

6.1.5 Dosing and Administration

5-FU will be used at the dose of 50 mg/mL and TAC will be used at the dose of 10mg/mL.

6.1.6 Route of Administration

Planned route of administration is laser-assisted delivery topically

6.1.7 Dose Adjustments/Modifications/Delays

Dose adjustments will be not be made to TAC or 5-FU. Adjustments will be made to the laser settings (density, depth) depending on the thickness of the keloid at the time of treatment.

6.1.8 Duration of Therapy

Therapy duration is 4 total treatments spaced 4 weeks ± 1 week apart.

6.1.9 Tracking of Dose

Dose will be fixed at 50 mg/mL for 5-FU and 10 mg/mL for TAC.

6.1.10 Device Specific Considerations

The laser utilized in our study is the Sciton Joule. Settings depend on the keloid thickness and will range from 400micron – 1mm in depth, 5.5-11% density, single pass, without coagulation. Contraindications to using this device are as follows:

- Patients intolerant of anesthesia
- Patients with infectious disease
- Patients with connective tissue disease
- Patients with history of immunocompromise
- Patients who are pregnant
- Patients who have used isotretinoin within the past year
- Patients with a medical condition that may affect wound healing
- Patients who use anticoagulant medications

6.2 Study Procedural Intervention(s) Description

The procedural intervention in this study is laser-assisted drug delivery with use of fractional ablative erbium laser.

6.2.1 Administration of Procedural Intervention

Participants will be anesthetized with intralesional 1% lidocaine with epinephrine 1:100,000. Patients will be provided safety goggles. All personnel present during the laser treatment will don the appropriate laser goggles protective for 2940 nm. The PI (NL) will then administer the fractional ablative erbium laser intervention to the entire keloid of interest. After treatment of the keloid, a fixed amount of 5-FU solution and TAC solution will each be gently rubbed over two separate keloids or halves of the laser-treated area(s). Site A (which represents the right keloid or right half of the keloid or superior keloid) will be treated with 5-FU, and Site B (which represents the left keloid or left half of the keloid or inferior keloid) will be treated with TAC. Only after the application of these two medications will patients' safety googles be removed. Patients will not be made aware of which medication was applied to which half of the lesion. The patients will be observed for 10 minutes after the procedure. Each intervention will last about 30 minutes. The entire visit will last about 1 hour.

6.2.2 Procedures for Training of Clinicians on Procedural Intervention

The procedural intervention will be provided by a single operator (NL) for standardization purposes. NL completed a procedural fellowship and was trained in Mohs surgery and was trained specifically on the Sciton Joule during fellowship.

7 Study Procedures and Schedule

7.1 Study Procedures/Evaluations

7.1.1 Study Specific Procedures

- Medical history (from both interview and medical records): history of keloids, known allergies (describe what is included for history, e.g., time-frame considerations, whether history will be obtained by interview or from medical records)
- Medication history: currently taken prescription and over the counter medications
- Urine pregnancy test if female of child-bearing age at every visit.
- Physical examination of lesion of interest.
- Photography of lesion of interest
- Measurement of lesion of interest
- Counseling procedures regarding what to expect during and after interventional treatment including post-procedure care

- ***Study Schedule Research coordinator (SC will assist with fielding patient calls and organizing patient scheduling.***
- Patients will be scheduled in the PI's clinic.
- Patients will not be charged for treatments.

7.1.2 Pre-Screening Phone Call (Dermatology Clinical Studies Unit, 240 East 38th Street, 11th floor, New York, NY 10016)

- Review verbal consent to pre-screen
- Ensure patient provided consent for study team to review medical record, including medical history and photos
- Assess inclusion and exclusion criteria
- Collect basic demographic information including:
    - Full Name _____
    - DOB: _____
    - Sex: _____
    - Email Address: _____
    - Current Permanent Address: _____
    - Home Phone (if applicable): _____
    - Mobile Phone (if applicable): _____
    - Emergency Contact information if they agree to provide

      The collection of basic demographic information is required in order to properly schedule their research screening visit (visit 0) in EPIC for the doctor's EPIC schedule.
- Schedule Screening (Visit 0)

7.2 *Screening (Visit 0,* to be completed at 222 East 41st Street, New York NY 10017)

- Obtain informed consent of potential participant verified by signature on study informed consent form. Informed consent will be obtained by the PI, sub-I (DG), study team member (LA) or research coordinator (SC) only.
- Verify inclusion/exclusion criteria.
- Pregnancy urine test for non-menopausal women.
- Obtain demographic information, medical history, medication history, alcohol and tobacco use history.
- Randomization of either which side of lesion (Right or Left) or which lesion (if two comparable lesions present) will be treated with laser-assisted TAC v laser-assisted 5-FU.
- Subjects Passing Screening will immediately have visit one as outlined in Visits 1-4

7.2.1 Treatment Visits

Visits 1 - 4

- Urine pregnancy test if female of child-bearing age. (visits 2-4)
- Physical examination of lesion of interest.
- Photography of lesion (keloid) of interest, taken before any treatment is performed
- Measurement (height, length, width) of lesion (keloid) of interest performed at the start of the visit, before treatment
- Completion of POSAS (including monitoring of adverse events) by patient and providers at the start of the visit
- Administer the study treatment (to be performed by PI): laser assisted drug-delivery treatment of both 5-FU and TAC.
- Monitoring of adverse events
- Patients will be asked about side effects (non-serious adverse events) from laser treatment on visits 2-4
- Patients will be asked to grade their post-procedure pain on a scale of 1 to 10 on visits 2-4
- Patients will be asked which side healed faster after the laser treatment on visits 2-4
- Post-treatment instructions will be provided to patients on an after-treatment instruction sheet: gentle cleansing of area with soap and water twice a day, apply petrolatum jelly and keep area covered for 1 week, avoid sun exposure to treated area(s) for study duration.
- Visits will take place every 4 weeks ± 1 week.

7.2.2 Final Study Visit

Visit 5

- Photography of lesion (keloid) of interest to be reviewed by blinded physician observers
- Measurement (height, length, width) of lesion (keloid) of interest
- Completion of POSAS (including monitoring of adverse events)
- Patients will be asked about side effects (non-serious adverse events) from laser treatment
- Patients will be asked to grade their post-procedure pain on a scale of 1 to 10
- Patients will be asked which side healed faster after the laser treatment

7.2.3 Withdrawal/Early Termination Visit

Photography and lesion measurements (length, width, height) should be done at a termination visit if a subject withdraws or if early termination occurs, provided the participant is willing.

7.2.4 Unscheduled Visit

Unscheduled visits pertaining to the lesion of interest will be documented as an extra visit.

7.3 Prophylactic Medications, Treatments, and Procedures

Intralesional 1% lidocaine with epinephrine 1:100,000 will be used for anesthesia prior to each laser-assisted drug delivery treatment session.

8 Assessment of Safety

8.1.1 Definition of Adverse Events (AE)

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

8.1.2 Definition of Serious Adverse Events (SAE)

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***.

8.1.3 Definition of Unanticipated Problems (UP)

Unanticipated Problems Involving Risk to Subjects or Others
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).

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other

unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.2 Classification of an Adverse Event

8.2.1 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 Relationship to Study Agent

The clinician's assessment of an AE's relationship to study agent (drug, biologic, device) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- ***Related*** *– The AE is known to occur with the study agent, there is a reasonable possibility that the study agent caused the AE, or there is a temporal relationship between the study agent and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study agent and the AE.*
- ***Not Related*** *– There is not a reasonable possibility that the administration of the study agent caused the event, there is no temporal relationship between the study agent and event onset, or an alternate etiology has been established.*

8.2.3 Expectedness

Dr. Nayoung Lee (PI) will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.3 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor (KL). All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate RF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time

during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

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 IRB 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 IRB 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.

9 Data Safety Monitoring Plan

The safety monitor (KL) will be conduct quarterly reviews of aggregate data and adverse events. KL is currently a PI for a Pfizer-sponsored phase 2A trial on hidradenitis suppurativa and a PI for a multi-center trial studying an oral BTK inhibitor in pemphigus vulgaris sponsored by Principia Biopharma. She is also a Co-PI for three sponsored clinical trials androgenetic alopecia, alopecia areata and dermatomyositis. KL will review all visit source documents and photographs every quarter to ensure adherence to the protocol and monitor adverse events.
The study will be prematurely terminated in the event of any unexpected, significant, or unacceptable risk to participants. A summary of the outcomes of these reviews along with all AEs and deviations will be submitted to the IRB on an annual basis with the Continuing Review submission.

10 Statistical Considerations

10.1 Sample Size Considerations

We will recruit and screen 30-40 participants with a projected accrual of 20 evaluable patients.

A sample size of 20 patients with a significance level (alpha) of 0.05 using a two-sided paired Wilcoxon Signed-Rank test (assuming that the actual distribution of paired differences is normal) can achieve 80% power to detect the following means of the paired difference between both Sites in the change from the first POSAS score (before initiating treatment series) to the final POSAS score.
We present in the table below a number of detectable means based on various estimated standard deviations (SD) of the paired differences between both treatments in the change from the first POSAS score to the final POSAS score. The estimated SD's are based on various assumptions of Pearson's correlation coefficients between the first POSAS score and the final POSAS score and between both treatments of the keloid (we assumed that the correlation between the first POSAS score and the final POSAS score is similar to the correlation between both treatments). Additionally, for an estimation of

standard deviation of the POSAS score, we used the difference in its range divided by 4, i.e. (120-12)/4=27.
:

Correlation coefficient between the first POSAS score (before initiating treatment series) and the final POSAS score and between both treatments	Estimated SD of the paired difference between both treatments in the change from the first POSAS score (before initiating treatment series) to the final POSAS score	Detectable mean of paired difference between both treatments in the change from the first POSAS score (before initiating treatment series) to the final POSAS score
0.1	48.6	|33.0|
0.25	40.5	|27.5|
0.5	27.0	|18.4|
0.75	13.5	|9.2|
0.9	5.4	|3.7|

10.2 Statistical Methods

10.2.1 General Methods

Continuous variables will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum). Categorical variables will be summarized showing the number and percentage (n, %) of patients within each classification. Graphical displays will be provided where useful in the interpretation of results.

10.2.2 Demographics and Baseline Characteristics

Demographic data (gender, age [years], age group, race, ethnicity), baseline scar characteristics and POSAS scores at baseline will be summarized.

10.2.3 Primary Analysis

Prior to the analysis, distributions of the POSAS scores will be summarized by visit and the distributions of the modified Hamilton scores will be summarized at the final visit.

A two-sided paired Wilcoxon Signed-Rank test at a 0.05 significance level will be used to compare Site A and Site B of the keloid in the change from the first POSAS score (before initiating treatment series) to the final POSAS score.

A two-sided paired Wilcoxon Signed-Rank test at a 0.05 significance level will be used to compare the modified Hamilton score in Site A and Site B of the keloid.

10.2.4 Secondary Analysis

The measurements of the scar will be defined as the total volume (length x width x height) in cm^3 of the scar, or length x width in cm^2 if the scars are flush with the surrounding skin scar surface.

Measurements of the scar will be summarized by visit and compared between the two Sites of the keloid.

10.2.5 Exploratory Analysis

Patient's subjective experiences will be summarized and compared between both Sites of the keloid as follows:

1. Patient perceived side effects after each treatment will be summarized by type (dyspigmentation, pain, itch, swelling), visit (1, 2, 3, 4) and treatment Site (A, B).

2. Pain after each laser treatment will be summarized by grade (scale of 1 to 10 (10 being the worst pain)), visit (1, 2, 3, 4) and treatment Site (A, B).

3. Speed of healing after completion of laser treatments at final visit (visit 5) will be compared between both Sites (Site A healed faster than Site B, Site B healed faster than Site A, Same speed of healing in both Sites)

10.1 Measures to Minimize Bias

10.1.1 Enrollment/Randomization/Masking Procedures

The keloid on the patient's right side or the patient's right half of the keloid or the superior keloid will be designated Site A. The keloid on the patient's left side or the patient's left half of the keloid or the inferior keloid will be designated Site B. Randomization of which treatment (5-FU or TAC) will be applied to Site A will be performed in in RedCap by the study statistician (BL). The treating physician (NL) will enter the patient ID in RedCap, answer a number of eligibility verification questions and retrieve the assignment for that patient. No patient-identifiable data will be entered in RedCap. All patient data will be entered in paper forms. Study participants will be blinded to the type of drug delivered via laser. Because they will need to wear protective opaque metal laser eye shields during the treatment, they will not be able to see the treatment being performed. The providers involved in the intervention (NL, LA) will not be blinded to the type of drug delivered, given that the two drugs are clearly identifiable based on their color. For this reason, a separate blinded dermatologist (DG) will complete the POSAS score at each visit. NL or LA will indicate which keloid is Site A and which is Site B with a surgical marking pen, when DG is not present. DG will then enter the treatment room and grade each Site A and Site B with the observer portion of the POSAS score. We will also have multiple blinded dermatologist observers on faculty at NYU rate non-identifiable photographs of the keloids that have undergone 4 treatments and complete the Hamilton scar. De-identified data (aside from the patient ID) will be entered in Excel for purposes of statistical analysis.

Source Documents and Access to Source Data/Documents
Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents. If this is the case, it should be stated in this section what data will be collected on CRFs and what data will be collected from other sources.

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, 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 Quality Assurance and Quality Control

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

12 Ethics/Protection of Human Subjects

12.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

12.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

12.3 Informed Consent Process

12.3.1 Consent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product. The consent form will be submitted with this protocol.

12.3.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document in addition to the key information sheet. The investigator (PI and/or co-investigator) will explain the research study to the participant and answer any questions that may arise. Informed consent to review medical records to assess exclusion and inclusion criteria will take place over the phone and subject privacy will be protected. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. At the screening visit, participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document

will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A copy of the signed informed consent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

12.4 Participant and Data 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.

Participant confidentiality is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

The study monitor, representatives of the IRB or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at NYU Langone Medical Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NYU Langone Medical Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NYU Langone Medical Center. Photographs will be non-identifiable in content and labeling. Photographs will be stored on a secured drive on a password protected computer in Room 24-033 in 222 E 41st St and only representative non-identifiable photos will be used for publication and/or teaching purposes.

13 Data Handling and Record Keeping

13.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the PI. The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Data to be recorded include patient's age, gender, keloid measurements (length, width, height), scar scores. All data will be stored on paper to be stored in a locked file cabinet in the PI's office at 222 E 41st st, and only the PI (NL) will have a copy of the key. All source documents will be moved to a double-locked storage space in the DCSU after study completion.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF will be consistent with the source documents or the discrepancies will be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered on paper to be stored in a locked file cabinet. Clinical data will be entered directly from the source documents. De-identified data (aside from the patient ID) will be entered in Excel for purposes of statistical analysis.

13.2 Study Records Retention

Study documents will be retained for 5 years after final reporting/publication.

13.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

The PI/study will identify and report deviations within 30 working days of identification of the protocol deviation, or within 30 working days of the scheduled protocol-required activity per IRB guidelines.

All protocol deviations will be addressed in study source documents.

13.4 Publication and Data Sharing Policy

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee's responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in

ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials":

- Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations of a product subject to FDA regulation;
- Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation (other than small feasibility studies) and pediatric postmarket surveillance studies.

14 Study Finances

14.1 Funding Source

We have received funding from the Rudin Family pilot grant and the Skin of Color Society grant.

14.2 Costs to the Participant

There are no costs to the participant as part of this study.

14.3 Participant Reimbursements or Payments

There are no participant reimbursements or payments.

15 Study Administration

15.1 Study Leadership

The Study Team will govern the conduct of the study. The Steering Committee will be composed of the Principal Investigator, the Sub-investigator, and the Research Fellow. The Study Team will meet virtually or in person at least biannually.

16 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial.

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the NYU Langone Conflict of Interest Management Unit (CIMU) with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

17 References

1. Waibel JS, Wulkan AJ, Rudnick A, Daoud A. Treatment of Hypertrophic Scars Using Laser-Assisted Corticosteroid Versus Laser-Assisted 5-Fluorouracil Delivery. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 2019;45:423-30.
2. Rumsey N, Clarke A, White P. Exploring the psychosocial concerns of outpatients with disfiguring conditions. Journal of wound care 2003;12:247-52.
3. Brown BC, McKenna SP, Siddhi K, McGrouther DA, Bayat A. The hidden cost of skin scars: quality of life after skin scarring. Journal of plastic, reconstructive & aesthetic surgery : JPRAS 2008;61:1049-58.
4. Wolfram D, Tzankov A, Pülzl P, Piza-Katzer H. Hypertrophic scars and keloids--a review of their pathophysiology, risk factors, and therapeutic management. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 2009;35:171-81.
5. Stucker FJ, Shaw GY. An approach to management of keloids. Archives of otolaryngology--head & neck surgery 1992;118:63-7.
6. Chen MA, Davidson TM. Scar management: prevention and treatment strategies. Current opinion in otolaryngology & head and neck surgery 2005;13:242-7.
7. Khan MA, Bashir MM, Khan FA. Intralesional triamcinolone alone and in combination with 5-fluorouracil for the treatment of keloid and hypertrophic scars. JPMA The Journal of the Pakistan Medical Association 2014;64:1003-7.
8. Waibel JS, Wulkan AJ, Shumaker PR. Treatment of hypertrophic scars using laser and laser assisted corticosteroid delivery. Lasers Surg Med 2013;45:135-40.
9. Cavalie M, Sillard L, Montaudie H, Bahadoran P, Lacour JP, Passeron T. Treatment of keloids with laser-assisted topical steroid delivery: a retrospective study of 23 cases. Dermatol Ther 2015;28:74-8.
10. Boyadjiev C, Popchristova E, Mazgalova J. Histomorphologic changes in keloids treated with Kenacort. J Trauma 1995;38:299-302.
11. Mustoe TA, Cooter RD, Gold MH, et al. International clinical recommendations on scar management. Plastic and reconstructive surgery 2002;110:560-71.
12. Park JH, Chun JY, Lee JH. Laser-assisted topical corticosteroid delivery for the treatment of keloids. Lasers in medical science 2017;32:601-8.
13. Haurani MJ, Foreman K, Yang JJ, Siddiqui A. 5-Fluorouracil treatment of problematic scars. Plastic and reconstructive surgery 2009;123:139-48; discussion 49-51.
14. Shridharani SM, Magarakis M, Manson PN, Singh NK, Basdag B, Rosson GD. The emerging role of antineoplastic agents in the treatment of keloids and hypertrophic scars: a review. Annals of plastic surgery 2010;64:355-61.
15. Huang L, Wong YP, Cai YJ, Lung I, Leung CS, Burd A. Low-dose 5-fluorouracil induces cell cycle G2 arrest and apoptosis in keloid fibroblasts. The British journal of dermatology 2010;163:1181-5.
16. Alexandrescu D, Fabi S, Yeh LC, Fitzpatrick RE, Goldman MP. Comparative Results in Treatment of Keloids With Intralesional 5-FU/Kenalog, 5-FU/Verapamil, Enalapril Alone,Verapamil Alone, and Laser: A Case Report and Review of the Literature. Journal of drugs in dermatology : JDD 2016;15:1442-7.
17. Hantash BM, Bedi VP, Kapadia B, et al. In vivo histological evaluation of a novel ablative fractional resurfacing device. Lasers in surgery and medicine 2007;39:96-107.
18. Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers in surgery and medicine 2004;34:426-38.
19. Wind BS, Meesters AA, Kroon MW, et al. Formation of fibrosis after nonablative and ablative fractional laser therapy. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 2012;38:437-42.
20. Hultman CS, Edkins RE, Wu C, Calvert CT, Cairns BA. Prospective, before-after cohort study to assess the efficacy of laser therapy on hypertrophic burn scars. Annals of plastic surgery 2013;70:521-6.
21. Anderson RR, Donelan MB, Hivnor C, et al. Laser treatment of traumatic scars with an emphasis on ablative fractional laser resurfacing: consensus report. JAMA dermatology 2014;150:187-93.

22. Lee YI, Kim J, Yang CE, Hong JW, Lee WJ, Lee JH. Combined Therapeutic Strategies for Keloid Treatment. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 2019;45:802-10.
23. Heppt MV, Breuninger H, Reinholz M, Feller-Heppt G, Ruzicka T, Gauglitz GG. Current Strategies in the Treatment of Scars and Keloids. Facial plastic surgery : FPS 2015;31:386-95.
24. Sklar LR, Burnett CT, Waibel JS, Moy RL, Ozog DM. Laser assisted drug delivery: a review of an evolving technology. Lasers in surgery and medicine 2014;46:249-62.
25. Tawfik AA, Fathy M, Badawi A, Abdallah N, Shokeir H. Topical 5 fluorouracil cream vs combined 5 fluorouracil and fractional erbium YAG laser for treatment of severe hypertrophic scars. Clin Cosmet Investig Dermatol 2019;12:173-80.
26. Abdelwahab M, Salah M, Samy N, Rabie A, Farrag A. Effect of Topical 5-Fluorouracil Alone versus Its Combination with Erbium:YAG (2940 nm) Laser in Treatment of Vitiligo. Clin Cosmet Investig Dermatol 2020;13:77-85.
27. Wang KH, Fang JY, Hu CH, Lee WR. Erbium:YAG laser pretreatment accelerates the response of Bowen's disease treated by topical 5-fluorouracil. Dermatol Surg 2004;30:441-5.
28. Sabry HH, Abdel Rahman SH, Hussein MS, Sanad RR, Abd El Azez TA. The Efficacy of Combining Fractional Carbon Dioxide Laser With Verapamil Hydrochloride or 5-Fluorouracil in the Treatment of Hypertrophic Scars and Keloids: A Clinical and Immunohistochemical Study. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 2019;45:536-46.
29. Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. Advances in lipid research 1991;24:1-26.
30. Morrow DI, Garland MJ, McCarron PA, Woolfson AD, Donnelly RF. Innovative drug delivery strategies for topical photodynamic therapy using porphyrin precursors. Journal of environmental pathology, toxicology and oncology : official organ of the International Society for Environmental Toxicology and Cancer 2007;26:105-16.
31. Zaleski-Larsen LA, Fabi SG. Laser-Assisted Drug Delivery. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 2016;42:919-31.
32. Manaloto RM, Alster T. Erbium:YAG laser resurfacing for refractory melasma. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 1999;25:121-3.
33. Lee WR, Shen SC, Wang KH, Hu CH, Fang JY. The effect of laser treatment on skin to enhance and control transdermal delivery of 5-fluorouracil. Journal of pharmaceutical sciences 2002;91:1613-26.
34. Lee WR, Shen SC, Lai HH, Hu CH, Fang JY. Transdermal drug delivery enhanced and controlled by erbium:YAG laser: a comparative study of lipophilic and hydrophilic drugs. Journal of controlled release : official journal of the Controlled Release Society 2001;75:155-66.
35. Haak CS, Farinelli WA, Tam J, Doukas AG, Anderson RR, Haedersdal M. Fractional laser-assisted delivery of methyl aminolevulinate: Impact of laser channel depth and incubation time. Lasers in surgery and medicine 2012;44:787-95.
36. Forster B, Klein A, Szeimies RM, Maisch T. Penetration enhancement of two topical 5-aminolaevulinic acid formulations for photodynamic therapy by erbium:YAG laser ablation of the stratum corneum: continuous versus fractional ablation. Experimental dermatology 2010;19:806-12.
37. Alexis AF. Lasers and light-based therapies in ethnic skin: treatment options and recommendations for Fitzpatrick skin types V and VI. The British journal of dermatology 2013;169 Suppl 3:91-7.
38. Woolery-Lloyd H, Viera MH, Valins W. Laser therapy in black skin. Facial plastic surgery clinics of North America 2011;19:405-16.
39. Ali FR, Forbat E, Al-Niaimi F. Laser Treatment of Keloid Scars. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 2017;43:318.
40. Prince GT, Cameron MC, Fathi R, Alkousakis T. Topical 5-fluorouracil in dermatologic disease. International journal of dermatology 2018;57:1259-64.
41. Prince GT, Cameron MC, Fathi R, Alkousakis T. Intralesional and Laser-Assisted 5-Fluorouracil in Dermatologic Disease: A Systematic Review. Journal of drugs in dermatology : JDD 2018;17:274-80.
42. Manuskiatti W, Fitzpatrick RE. Treatment response of keloidal and hypertrophic sternotomy scars: comparison among intralesional corticosteroid, 5-fluorouracil, and 585-nm flashlamp-pumped pulsed-dye laser treatments. Archives of dermatology 2002;138:1149-55.
43. Nguyen TA, Feldstein SI, Shumaker PR, Krakowski AC. A review of scar assessment scales. Seminars in cutaneous medicine and surgery 2015;34:28-36.

18 Attachments

These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

Please see the sample consent form, COVID19 information documents, and key information sheet attached.

19 Schedule of Events

Activity	Screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Study team procedures						
Informed Consent	X					
Urine pregnancy test (if applicable)	X	X	X	X	X	X
Medical History	X					
Physical Exam	X	X	X	X	X	X
Randomization	X					
Study drug/device dispensation		X	X	X	X	
Dermatology assessments						
Photography		X	X	X	X	X
Caliper measurements		X	X	X	X	X
POSAS		X	X	X	X	X