

Date: February 13, 2024  
Principal Investigator: Luis Garza, MD-PhD  
Application Number: IRB00105061

**Pilot exploratory study to determine effect of gentle wounding to stimulate hair follicle neogenesis.**

**2/13/2024**

**NCT03491267**

## JHM IRB - eForm A – Protocol

### Pilot exploratory study to determine effect of gentle wounding to stimulate hair follicle neogenesis.

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#### 1. Abstract

Defects in skin wound repair occur in patients with diabetes, pressure ulcers, prolonged immobilization, and venous insufficiency. Wound care costs approximately \$25 billion dollars annually according to the National Institutes of Health. When wounds recur, they frequently recur in the same location as the initial wound indicating the fragility and poor quality of the healing process. Improved wound repair will result in fewer amputations, decreased infection rates, and fewer (re)hospitalizations with corresponding reductions in medical costs and improved quality of life for patients. Therefore, there is a critical need to understand the fundamentals of wound healing not just to speed closure but to enhance full skin regeneration.

Tantalizing reports of a recapitulation of skin embryogenesis after wounding (skin regeneration) were first published in 1946 and was characterized by *de novo* hair follicle formation. Examples of this phenomenon were noted in mice, rabbits and possibly humans(1-4). As published in Nature 2007, Ito and Cotsarelis fully described and characterized this *de novo* follicle neogenesis after wounding in mice (**Wound Induced Hair Neogenesis; WIHN**). These regenerated hair follicles establish a stem cell population, express hair follicle-differentiation markers, produce a functional hair shaft, and successfully transition through all phases of the hair cycle. Furthermore, the hair follicle was not isolated; in fact, sebaceous glands were also re-formed adjacent to the follicles(5), and likely as well blood vessels, nerves and fibroblast stem cell populations. Thus, WIHN proves that the mammalian system is capable of repairing wounds by tissue regeneration. This has applications for enhancing wound repair, curing alopecias, reducing scars but also broad relevance to enhancing regeneration in other organ systems and reducing the heavy burden of fibrosis in human morbidity.

We have done important scientific work in this area with a recent publication in Cell Stem Cell where we identify novel mechanisms which regulate WIHN(6). The present application is to do the very first initial pilot tests in humans to see if any of a diverse combination of mild wounding with FDA approved topicals have the ability to induce WIHN in humans in a small 1x1cm area of the scalp. Our initial focus will be on the scarring alopecia of CCCA with CO2 laser in combination with retinoids. Our recent results in the laboratory have

shown a dramatic synergy between damage related pathways in wounding and FDA approved retinoids to trigger the biochemical changes which engender WIHN.

CCCA is an inflammatory disease of the skin, affecting both men and women, which leads to scarring and permanent alopecia in many cases. To date, therapeutic options are limited, invasive, and often ineffective. In the mouse model, wounding has been shown to generate hair follicles de novo and regrowth of terminal hairs has been shown. This is thought to be related to stem cells assuming a hair follicle phenotype in the setting of known molecular markers of follicle differentiation, including in the *Wnt* pathway. CO<sub>2</sub> laser resurfacing is commonly performed for scar revision and rejuvenation of the skin. It is an ablative laser, and operates by producing soft tissue vaporization and thermal changes to the skin. To date, this treatment modality has not been trialed for the treatment of CCCA. We also hypothesize the addition of topical retinoids such as tretinoin (Retin-A) which are routinely used for acne and overwhelmingly safe will augment this result. Therefore, our initial hypothesis is that we may demonstrate follicular regeneration in response to wounding with the CO<sub>2</sub> laser and topical retinoids in CCCA.

## **2. Objectives**

Primary objective: To determine if CO<sub>2</sub> laser ablation with retinoids of affected scalp in central centrifugal cicatricial alopecia leads to regeneration of hair follicles and gross clinical evidence of regrowth.

Secondary objective: To determine if CO<sub>2</sub> laser ablation with retinoids of affected scalp in central centrifugal cicatricial alopecia leads to up-regulation of molecular markers associated with hair follicle regeneration.

## **3. Background**

Central centrifugal cicatricial alopecia (CCCA) is a scarring, inflammatory alopecia seen more commonly in women of African descent. The distinct pathophysiology of CCCA is poorly understood, but it is known to involve inflammation directed at the upper part of the hair follicle where the stem cells and sebaceous gland are located. If the stem cells and sebaceous gland are destroyed, there is no possibility for regeneration of the hair follicle, and permanent hair loss results. This form of scarring alopecia occurs mainly on the vertex of the scalp, and spreads peripherally, and can lead to baldness. In our dermatology clinics, we see 5-10 patients per week for evaluation and treatment of CCCA.

Currently, treatment is focused on decreasing inflammation and halting the progression of disease. This typically consists of topical and intralesional corticosteroid therapy and anti-inflammatory antibiotics. Hair transplantation is the only treatment option for patients with end-stage CCCA, and has been performed in a small number of patients but the results have been

disappointing with low graft survival rates and slow regrowth of the transplanted hair. In addition, hair transplantation of the curved hair follicles found in patients of African descent is difficult and requires specific expertise(7).

A study by Ito et al showed de novo hair follicle formation after wounding in genetically normal adult mice. The regenerated hair follicles were fully functional, in that they established a stem cell population, expressed known molecular markers of follicle differentiation, and produced a hair shaft that progressed normally through all stages of the hair follicle cycle. It is hypothesized that the regenerated hair follicles likely arise when epithelial cells in the wound assume a hair follicle stem cell phenotype, possibly under the influence of *Wnt* signaling(5).

The CO<sub>2</sub> laser has been used extensively in dermatological surgery over the past 30 years and is now recognized as the gold standard for soft tissue vaporization. CO<sub>2</sub> laser beam heats and vaporizes the skin tissue, instantly removing the superficial layers of the skin. Each fractional micro-spot creates a thermal zone. Intact cells around the treated area help during the healing process which in turn, induces cell regeneration (8). This likely occurs through dsRNA released during wounding(6). We have recently found that retinoids, such as the tretinoin (retin-A) used in acne, can synergize with dsRNA and promote extra Wnt signaling (Figure 1).

We therefore hypothesize that wounding of the area of scarring alopecia in CCCA, using a fractionated CO<sub>2</sub> laser in combination with retinoid acid, will induce hair follicle regeneration.

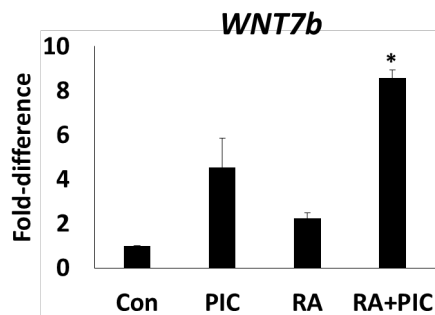


Figure 1: Motivation for current study: Wnt7b is known to trigger WIHN. It is increased in human keratinocytes in culture by PIC which is dsRNA released from damage such as laser. RA is retinoic acid and synergizes with damage to cause even more Wnt7b synthesis.

#### 4. Study Procedures

- a. Study design, including the sequence and timing of study procedures

In order to be eligible for the clinical study, subjects must have been seen in the dermatology clinic at Johns Hopkins Hospital or from patient populations participating in Johns Hopkins Cutaneous Translational Research Program (CTReP) research studies. Interested study participants will be

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evaluated after their routine clinical care visits. Participants will also be recruited from other Johns Hopkins patient populations via the use of fliers. Study procedures will be conducted at the CTReP office located at the Johns Hopkins Outpatient Center. Recruitment may include Johns Hopkins University employees or students, but these populations will not be specifically targeted for recruitment. Interested individuals will be interviewed to ensure they meet basic criteria for participation.

The anticipated enrollment is about 30 subjects. During the first visit, the expected timeline will be discussed. We will also discuss in detail the laser procedure and expectations with regards to healing time and symptoms in the post-procedure period. We will attempt to answer all questions to the subject's satisfaction. If the subject is agreeable, documentation will be obtained of their consent to participate in the study and the subject's agreement to be contacted in for future research studies. After informed consent is obtained, subjects will be formally enrolled.

We will have an optional washout period for subjects under therapies which might influence WIHN. Participants will be assigned treatment on the left or right side of the scalp on the basis of the individual's alopecia. There is no randomization ratio as the treatment area will depend mostly on the participant's scalp. The participant and team members present during the visit will not be blinded to the treatment; however, the only people blinded to the area where treatment occurred will be the hair count evaluators. After selecting the treatment area, we will trim the hair within the selected site to facilitate better photography and more accurate hair counts. We will carefully identify an area without hair including using noninvasive imaging such as photography for documentation. We will then specifically mark the area with tattoo ink to monitor the exact site for future hair growth.

We are already approved by the IRB for the use of tattoo ink in skin research (NA\_00068684 "Feasibility study for fibroblast autologous skin grafts: biopsy of skin fibroblasts, expansion in cell therapy core, topical injection of fibroblasts, and subsequent removal of graft for laboratory studies).

Small tattoos on the skin are routinely performed by radiation oncology to orient radiation fields. These tattoos are permanent for patients, but are subtle and difficult to notice without a trained eye. (See Figure 2) Dr. Garza, the PI, received instruction from Dr. Phuoc Tran, MD-PhD and Valerie Briner, Assistant Chief Therapist for Radiation Oncology at the Johns Hopkins Hospital. Dr. Garza himself has already tattooed 3 human subjects. For visible tattoos, we will remove the tattoo at the final visit biopsy and therefore the subject will be left with no visible mark and only temporarily have the visible tattoo. Also, we will strictly follow the clinical protocol which has proven extremely safe and easy, without complications in its routine clinical use.

1. We will use the exact same pigment used by Radiation oncology which is sold for human use (Black Tattoo ink, Reorder #VIP-1PI, by VanArsdale Innovative Products, Inc.). In this case a single tattoo dot will be made and it will be removed at the end of the study by

biopsy. Alternatively, in the case that there is concern whether tattoo ink will modify WIHN response at the exact site, we will make 4 small tattoos to mark the boundary of the CO2 treated area. In this case, we will use tattoo ink which is only visible by ultraviolet light and is in clinical use(9). Examples of this include SkinCandy Blacklight Invisible Ultraviolet Tattoo Ink (SkinCandy Tattoo Supply; Burbank, CA). In the case of using invisible tattoos we will not remove them by biopsy unless requested by the subject.

2. A single drop will be dropped into a sterile 25gX5/8" needle at the open hub which normally connects with the syringe. A portion of the drop volume is pulled by capillary action to the needle tip.
3. The needle is then inserted gently into stretched human skin with the bevel up at a 30 degree angle to the skin, and only to a depth where the full bevel is covered. The pigment leaches out again only by capillary action and is not pushed out. The needle is immediately removed after insertion. The estimated amount of pigment solution deposited must be much lower than a droplet size of 0.025ml(10).
4. No local care is necessary. The tattoo will be performed at the time of enrollment allow time for it to fully integrate without inflammation in to the skin before laser treatment. If a subject is unhappy with the pigment in their scalp and wish it to be removed by punch biopsy, we will do so at any time.



Figure 2: Subtlety of tattoos used in current Dermatology IRB study NA\_00068684. In both African American and Caucasian subjects, the tattoo is barely visible

**Biopsies:** In this exploratory study we will offer optional baseline biopsies of the affected scalp and after CO2 treatment. Each patient will serve as her own control, as we will compare the treated biopsy to the untreated biopsy. There will be no more than 6 total biopsies total during the study, each either shave or maximum 6mm punch type. Patients may either opt for all or no biopsies.

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CCCA diagnosis will be solely clinical; however, any pre-treatment biopsies collected may be a diagnostic biopsy. If needed, the pre-treatment biopsy can aid in clinically diagnosing alopecia as CCCA specifically.

**Laser:** CO2 laser treatments will occur for no more than three times per week and for no longer than 6 months. Anesthesia will be optional and include injected lidocaine with epinephrine or topical lidocaine/prilocaine or topical lidocaine/tetracaine. The pre-defined area up to 1cm<sup>2</sup> will be treated with a fractionated CO<sub>2</sub> laser, using standard protocol. The CO<sub>2</sub> laser being used has a maximum depth of 700 microns; however, this study procedure will have a depth closer to approximately 500 microns. Skin directly outside the treatment area will not receive exposure to laser light. Subjects will be instructed to return on-site following laser treatment for clinical evaluation and biopsy. Any type of noninvasive imaging such as photography may be obtained at each study visit.

**Wounding alternatives to laser:** If the subject wishes not to have laser treatment we will test other mechanisms of wounding such as gentle curetting. A 4-0 or similar sized curette will be used to scrape of the epidermis of anesthetized skin. This the equivalent to a child having a mild scrape to her knee with only removal of the epidermis and no damage to the dermis. The curettage will occur with the same parameters as laser otherwise.

**FDA approved topical medications:** Topicals to test include FDA approved retinoids such as tretinoin 0.025 to 0.1% of any formulation (such as cream or foam or solution). They will be applied no more frequently than recommended for typical skin use and with no greater quantity. They will be applied at any time either before and/or after CO<sub>2</sub> laser to determine maximum efficacy.

**Photography:** Standardized digital photographs will be obtained by study staff using a digital camera and software under standard photographic conditions. Photograph files will be coded to remove personal identifiers and stored on a secure hard drive in CTReP. They might include dermatoscope photography as well as other non-invasive photography such trichograms or confocal microscopy or optical coherent tomography.

These dermatoscopic images will be taken at every study visit which will also aid in documenting any pigmentary changes that arise at or around the treatment site. It might help monitor the extent of these changes. In regard to recovery time, this will vary based on the individual.

**Clinical assessment:** Clinical assessments will be performed to 1) record baseline skin findings, 2) identify test areas suitable for study treatment and biopsy 3) identify any occurrence of any adverse events 4) presence and growth of hair.

**Hair Counts:** Standardized global photographs of the area of the scalp will be taken for macrophotographs. Patient positioning and photographic distance will be consistent. Prior to hair

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counts at each visit, we may trim the hair within the treatment site in order to obtain more accurate hair counts and clearer photographs. At the end of the study, an expert panel of dermatologists will evaluate hair growth or loss from baseline by comparing baseline with follow-up photographs of each subject by using a 7-point standardized scale.

**Laboratory studies:** Laboratory analysis of biopsy specimens will include molecular mediators of wound healing and follicular regeneration. Other laboratory studies may include microarrays, histopathology, and protein purification, amongst others. Remaining tissue material may be de-identified and stored for use in future studies.

CCCA specimens in women who don't have excessive scarring tend to have higher number of miniaturized follicles; however, separating a component of female pattern alopecia or traction alopecia might not be possible. Barring unusual endocrine conditions affecting hormones, there is some degree of hair loss in all vertex scalps. There will be baseline dermatoscopic photographs generated to document the absence of follicles due to scarring. Clinical photographs will continue to be collected after treatment as well. Half of the biopsy specimen using Hematoxylin and Eosin (H&E) will be used for careful visualization of the new structure and retain the remaining biopsy tissue for ribonucleic acid (RNA). Such biopsies might allow for categorizing based on hair follicle size as well as the number of pre- and post-treatment hair follicles; however, this might not be the primary goal.

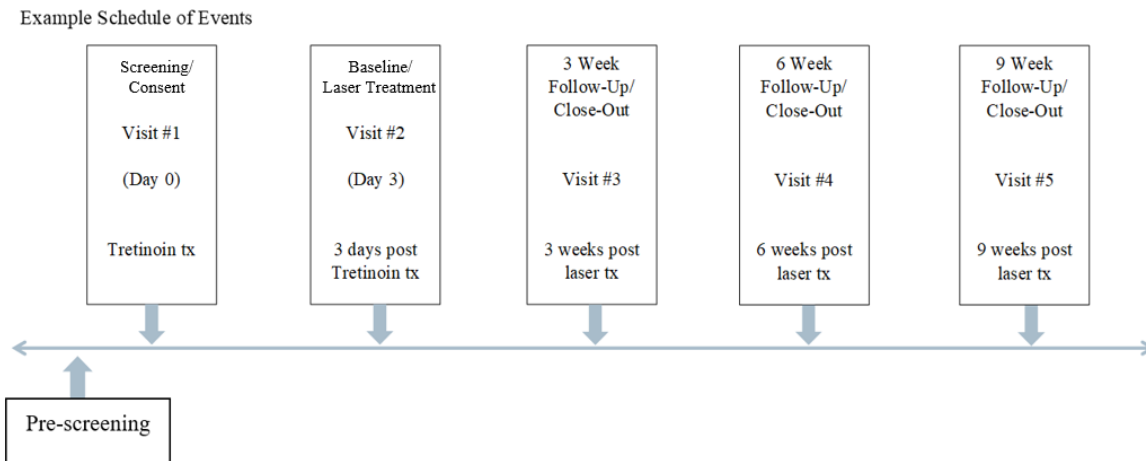
- b. If your study involves data/biospecimens from participants enrolled under other research studies with a written consent or under a waiver of consent, please list the IRB application numbers for those studies. Please note: Certificate of Confidentiality (CoC) protections applied to the data in source studies funded by NIH or CDC will extend to this new study if the funding was active in 2016. If this situation applies, Section 36, question 6 in the application will need to be answered "Yes" and "Hopkins Faculty" should be selected in question 7. No other documents are required.

N/A

- c. Study duration and number of study visits required of research participants.

Although laser will be used for a maximal period of 6 months, we will allow up to 2 years to complete the study for a final follow-up to assess for hair growth. The number of total visits will not exceed 40 over a period of 6 months with the exception of a final follow-up visit. We will determine the length based on the time needed for response. At every visit, hair follicle neogenesis will be assessed and if it occurs, then therapy will stop and we will conduct biopsies.

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- d. Blinding, including justification for blinding or not blinding the trial, if applicable.

Clinical evaluators performing hair counts will be blinded to type of treatment administered. The primary evaluator will not be blinded to which visit the hair count belongs to; however, the subsequent evaluator(s) will be blinded to both the treatment administered and study visit.

- e. Justification of why participants will not receive routine care or will have current therapy stopped.

Participants might be asked to stop treatment with and allow for washout period of topical, intralesional, and systemic therapies which might alter the normal expected dynamics of wound healing, altering not only the healing process but also possibly cell-signaling pathways.

- f. Justification for inclusion of a placebo or non-treatment group.

N/A

- g. Definition of treatment failure or participant removal criteria.

Any clinical findings determined by the Investigator to be important and/or unusual will be referred to as an adverse event (AE). Study participants are asked to contact clinic staff immediately if they experience a reaction at any time during the study. Expected reactions may be documented in a problem events log. The Investigator will use his discretion to remove participants from the study and all problem events will be reported to the IRB.

- h. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Participants who request to be removed from study, even after signing the informed consent form, will be removed from the study. The investigator may remove a participant from the study if the participant does not comply with study rules and procedures, behaves inappropriately in

clinic (e.g., hostility towards study staff), or meets an element of the exclusionary criteria during their participation in the study. Efforts would be made to replace such participants as to meet study enrollment goals and obtain adequate analysis. Study participation could end prematurely if a participant is lost to follow-up. A participant is deemed lost to follow-up if they do not answer phone calls, emails, and text messages from study team members. Concerted efforts will be made to contact participants; however, any data collected prior to this point would be used. Participants will be paid for all the visits they kept.

- i. If biological materials are involved, please describe all the experimental procedures and analyses in which they will be used.

This study will involve the use of RNA, DNA, protein, lipid, metabolite and histology profiling. Laboratory analysis of biopsy specimens will include molecular mediators of wound healing and follicular regeneration. Other laboratory studies may include microarrays, histopathology, and protein purification, amongst others. Remaining tissue material may be de-identified and stored for use in future studies.

## **5. Participant Selection**

### **5.1 Inclusion and Exclusion Criteria**

Participants must fulfill all of the criteria listed below:

#### Inclusion criteria

Subjects who meet the following inclusion criteria will be included in the study:

1. Male or female subject is 18 years of age or older at the screening visit;
2. The subject is healthy, as determined by the investigator based on a medical evaluation including medical history;
3. The subject has clinical diagnosis of CCCA;
4. The subject's CCCA is of grades 2, 3 or 4, as assessed at the time of the screening visit.
5. The subject is willing and able to comply with the requirements of the protocol. In particular, subject must adhere to the visits schedule, concomitant therapy and hair processing prohibitions, subject instructions, and biopsy procedures;
6. The subject is willing to comply with the month-long washout period if deemed necessary;
7. The subject has understood and signed an Informed Consent Form approved by the IRB prior to any investigational procedure

### Exclusion criteria

Any subject who is meeting one or more of the following exclusion criteria at the screening visit and/or at the baseline visit will not be included in this study:

1. The subject has an underlying known disease, a surgical or medical condition that in the opinion of the investigator might put the subject at risk
2. The subject presents with any disease known or described to potentially interfere with a normal wound healing process
3. The subject is pregnant or breastfeeding at the time of enrollment or is planning to become pregnant at any point during the study period (by self-report)
4. The subject has a past history of coagulation trouble
5. The subject has a past history of abnormal healing (hypertrophic scars/keloids within the past 10 years)
6. The subject has an underlying dermatological disease that in the opinion of the investigator could interfere with the study evaluations
7. The subject has scars, sunburn, either damaged or broken (cuts or abrasions) skin or other blemishes, or tattoos on the scalp in the treatment area
8. The subject is unwilling or unable to refrain from specific types of chemical hair styling and processing, including perms, straighteners, relaxers, dyes, weaves
9. The subject has a known allergy or sensitivity to any local anesthetic drug (e.g. lidocaine) or a local antiseptic planned to be used for the laser and/or biopsy procedures
10. The subject is in an exclusion period from a previous study or is participating in another clinical trial
11. The subject is an adult under guardianship or is hospitalized in a public or private institution, or is deprived of freedom
12. The subject is unable to communicate or cooperate with the Investigator due to language problems, poor mental development, or impaired cerebral function

### 5.2 Informed Consent

Interested potential participants will be given an appointment in the Cutaneous Translational Research Unit (CTReP) located at the Johns Hopkins Outpatient Center. Consent appointments are performed by appropriately trained study team members. During the visit, they will be given the consent form and as much time as needed for them to review and ask questions about the study. To assess for understanding, the team member will ask the participant to briefly summarize the study. A copy of the consent form may be provided to them before the visit to allow them ample time to review the document. No screening or study related activities will take place until informed consent has been obtained. Participation in the study is completely voluntary.

## **6. Drugs/ Substances/ Devices**

- a. The rationale for choosing the drug and dose or for choosing the device to be used.

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Lidocaine, prilocaine and tetracaine are anesthetics. The lidocaine/epinephrine combination used in the study will be the same drug provided to the entire Johns Hopkins Dermatology Department. Up to 4cc of lidocaine/epinephrine may be administered. There will not be a separate batch of this drug for the sole purposes of this study as the purpose of this study is unrelated to the use of the drug and is unlikely to interfere with study results. Lidocaine/prilocaine topical (EMLA) is an alternative anesthetic that will be offered to subjects before laser treatments. If this is not adequate and subjects also do not prefer lidocaine/epinephrine injections then we will offer topical Lidocaine 23%/Tetracaine 7% ointment which is in routine use in laser therapies. Up to 5cm<sup>2</sup> may be applied topically before each session.

The CO<sub>2</sub> laser treatment will be administered using standard settings on the eCO<sub>2</sub> Plus laser system, Lutronic Inc. The Lutronic eCO<sub>2</sub> Plus laser system will be obtained by the Johns Hopkins Dermatology department and will be evaluated and approved by the department of Clinical Engineering.

Topical FDA approved retinoids intended for skin use such as Tretinoin will be prescribed at no higher amount than used in clinical practice, such as tretinoin 0.025 to 0.1% of any formulation (such as cream or foam or solution). They will be applied no more frequently than recommended for routine clinical use and with no greater quantity. They will be applied at any time either before and/or after CO<sub>2</sub> laser to determine maximum efficacy.

- b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

N/A

- c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

N/A

## **7. Study Statistics**

The data collected in this study will be used to inform the design of future study related to the use of laser therapies for scarring alopecia.

Data collected throughout the duration of a participant's enrollment will be analyzed. If a participant has been removed, discontinued, or withdrew from the study, the data collected prior to the removal, discontinuation, or withdrawal will still be analyzed. The same will hold true for participants that are lost to follow-up. If a participant is deemed lost to follow-up, any data (e.g., clinical photographs, CRFs, hair counts, etc.) collected prior to this will continue to be used in data analysis. If a protocol deviation occurs, it may need to be reviewed on a case-by-case basis to determine if data collected may be used for analysis.

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a. Primary outcome variable.

The primary outcome variable is hair follicle neogenesis as determined by hair counts or confocal microscopy.

b. Secondary outcome variables.

The secondary outcome variable is the up-regulation of molecular markers associated with hair follicle regeneration.

c. Statistical plan including sample size justification and interim data analysis.

The results from this exploratory study will be used to determine an effect size for the purpose of future sample size power calculations. At this time most of our data has been acquired from mice; therefore, not enough data exists to include sample size justification.

The population for analysis will include all participants that took part in the study. At each visit, participants will have clinical photographs taken to be analyzed. Such analysis will consist of hair follicle counting for each image which can then be compared to determine if hair regrowth occurred.

d. Early stopping rules.

If a participant has a serious adverse event thought to be possibly, probably or definitely related to the study procedures, we will stop and consult with the IRB before proceeding.

## 8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

**Laser:** There are risks associated with ablative laser therapy. The most common local side effects are discomfort during the procedure (10% risk of occurrence), temporary swelling (10% risk of occurrence), acute sensitivity to sun exposure (0.1%), increased sensitivity to use of cosmetic products (0.1%) and erythema (10%). There is also an associated risk of both hyper- and hypopigmentation of the skin in the treated area (1%). Finally, there is a risk of eye injury related to the CO<sub>2</sub> laser therapy. Exposure to the invisible carbon dioxide laser beam (10,600 nm) can cause damage to the cornea or sclera. Other potential complications of this treatment are infection, activation of herpetic lesions (0.001% risk of occurrence), and scar formation (0.001% risk of occurrence). Preventative measures are taken to minimize these risks.

**Biopsy:** There is a risk of potential bleeding (10% risk of occurrence) and slight risk of infection from biopsies (0.1% risk). As with any cut in the skin, a scar will develop (5% risk of occurrence), although its appearance will likely fade over time. As with any biopsy, there is risk of pain or discomfort from the procedure.

**Wounding alternatives to laser:** There is a risk of potential bleeding after conclusion of the visit (20% risk of occurrence in individuals) and slight risk of infection from curettage (0.01% risk occurrence). Gentle curettage is the equivalent to a child having a mild scrape to her knee with only removal of the epidermis and no damage to the dermis. As with any cut in the skin, a scar will develop, although its appearance will likely fade over time (1% risk of occurrence). As with any scrape in the skin, there is risk of pain or discomfort from the procedure.

**Tattoos:** The tattoo markings will be permanent and will have the appearance of a dark freckle. While the markings are being created, the needle may cause discomfort, like a small pinch or insect bite.

**Retinoids:** Topical retinoids can cause some redness and sometimes some scaling (5% risk of occurrence). There is also a risk of burning, itching, dryness, lightening or darkening of the skin.

**Local Anesthesia:** Although very rare (less than 1% of individuals), it is possible that a participant may have an allergic reaction to the local anesthetic (lidocaine) used to numb the skin prior to a biopsy, laser, or curettage. A reaction of this kind would cause swelling and a rash on the participant skin where the anesthetic was injected. Potential side effects of EMLA (5% risk of occurrence) are usually mild, transient, and include local skin reactions such as edema, paleness (pallor or blanching), alterations in temperature sensation, erythema, itching, or rash. Hyperpigmentation of the skin at the site of application has rarely been reported. With much larger doses and exposure times than we propose to use here, there are rare reports of severe complications such as allergic and anaphylactoid reactions, cardiotoxicity, methemoglobinemia, or central nervous system toxicity. For this reason, we will use a minimal dose and time with supervision in the clinic.

b. Steps taken to minimize the risks.

**Laser:** To prevent eye injury, the operator, participant and all other parties within the secured treatment room will wear protective eyewear provided by CTReP. The provided eyewear and has wrap-around shields, meets ANSI/ISEA Z87.1-2003 standards, and offers protection from CO<sub>2</sub> lasers (10,600 nm). To minimize other risks associated with laser use standard precautions will be taken using the same protocol used in our clinics for patients undergoing therapies using ablative laser. We will screen carefully for keloids in our exclusion criteria, but in rare individuals with no prior keloid history, keloids or excessive scarring may develop at the treated site. Caution and careful attention to settings is paramount in performing the technique safely and all treatments will be performed by trained providers. Anesthesia is offered prior to treatment to minimize discomfort.

**Biopsy:** Only appropriately trained individuals will perform biopsies and will use antiseptics on the skin prior to biopsies. Anesthesia is offered prior to biopsy to minimize any discomfort. We will screen carefully for keloids in our exclusion criteria, but in rare individuals with no prior keloid history, keloids or excessive scarring may develop at the treated site.

**Wounding alternatives to laser:** Only appropriately trained individuals will perform wounding procedures and will use antiseptics on the skin prior. We will screen carefully for keloids in our exclusion criteria, but in rare individuals with no prior keloid history, keloids or excessive scarring may develop at the treated site. Anesthesia is offered prior to treatment to minimize discomfort.

**Tattoos:** Tattoo markings will be optional and may also be removed if biopsied after placement.

**Retinoids:** A small amount of retinoid will be applied for a short duration.

**Local Anesthesia:** Persons who have previously experienced an allergic reaction to local anesthetics (e.g. with dental procedures) will be ineligible for study participation. For EMLA we will use a minimal dose and time with supervision in the clinic.

- c. Legal risks such as the risks that would be associated with breach of confidentiality.

There are minimal risks with regards to confidentiality as all participant information will be de-identified. Since this is an exploratory study, no confidential nor protected information would be taken outside the standard.

- d. Financial risks to the participants.

There is slight financial risk to the participants in the rare event that the aforementioned complications occur requiring additional medical care.

## **9. Adverse Event Capture and Reporting Requirements**

### **9.1 Definitions**

#### **9.1.1 Adverse Event (AE)**

An adverse event (AE) is defined as an unusual or undesirable symptom or sign that occurs in participants during the clinical study. Adverse events may include, but are not limited to, clinically significant laboratory or clinical test results, concomitant illness, accidents, medical occurrences or worsening of existing medical conditions that arose during study participation.

#### **9.1.2 Serious Adverse Events (SAEs)**

A Serious AE (SAE) is any untoward medical occurrence that at any dose produces any of the following outcomes:

- Results in death
- Is life threatening (participant was at risk of death at the time of the event; not an event that might have hypothetically caused death if it were more severe)

- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see NOTE below for exceptions)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the participant, or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

NOTE: The following hospitalizations are not considered SAEs:

- Admissions as per protocol for a planned medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for purposes other than remedying ill health state and was planned prior to entry into the study. Appropriate documentation is required in these cases
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative)

#### 9.1.3 Non-Serious Adverse Events

A non-serious adverse event is any AE not classified as serious (as described in previous section). All non-serious AEs will be reported to the IRB on an annual basis with the continuing review.

#### 9.1.4 Unanticipated Problems (UPs)

An unanticipated problem (UP) is defined in accordance with the Office for Human Research Protections (OHRP) guidelines. UPs are defined as any incident, experience, or outcome that meets **all** the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by procedures involved in the research); and
- Suggests that the research places participants or others at greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

### 9.1.5 Protocol Deviations

A protocol deviation is generally an unplanned excursion from the protocol that is not implemented or intended as a systemic change. Protocol deviations include, but are not limited to, the following:

Enrollment or randomization of an ineligible participant

- Failure to obtain informed consent
- Unreported SAEs
- Improper breaking of the blind
- Use of prohibited medication
- Mishandled samples
- Multiple visits missed or outside of study windows

### 9.2 Adverse Event Grading

Adverse events will be identified and graded using the schema described below. The investigator will evaluate the severity of any adverse event using the following definitions:

- Mild: The event may be noticeable to participant; does not influence daily activities; usually does not require intervention.
- Moderate: The event may be of sufficient severity to make the participant uncomfortable; performance of daily activities may be influenced; intervention may be needed.
- Severe: The event may cause severe discomfort; usually interferes with daily activities; participant may not be able to continue in the study; treatment or other intervention usually needed.

AEs graded as 'severe' which are possibly, probably, or definitely attributable to the use of the investigational drug will be recorded and monitored until the event has resolved to meet the definition of 'mild'. All participants will receive care for all adverse events according to good clinical practice as per the standard of care at Johns Hopkins.

### 9.3 Attribution of Adverse Events

All adverse events will be further evaluated for attribution as per the following:

- Unrelated: The adverse event is clearly not related to the investigational agent.
- Possibly: The adverse event is possibly related to the investigational agent.
- Probably: The adverse event is probably related to the investigational agent.
- Definitely: The adverse event is definitely related to the investigational agent.

### 9.4 Adverse Event Capture and Reporting

#### 9.4.1 Routine Adverse Event Capture and Reporting

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The collection of all AE information begins after the participant signs the informed consent form and continues during the clinical study until 30 days of receiving the last dose of study medication.

If an ongoing AE changes in its intensity or in its perceived relationship to investigational product, a new AE entry for the event will be completed. Adverse events will be followed to resolution or stabilization or reported as SAEs if they become serious. Follow-up is also required for AEs that cause interruption or discontinuation of investigational product, or those that are present at the end of study participation. Participants with AEs at study completion will receive post-treatment follow-up as appropriate.

While AEs typically include on-site clinical evaluations, telemedicine evaluations for low-grade AEs might be accommodated on a case-by-case basis to alleviate financial burden and time constraints participants may face.

All identified AEs will be recorded and described on the appropriate Case Report Form (CRF). Non-serious adverse events will be logged and reported to the IRB on an annual basis.

#### 9.4.2 Serious Adverse Event Capture and Reporting

All SAEs, regardless of causality will be reported to the IRB per the JHM IRB reporting requirements. Prompt reporting to the JH SOM IRB of unanticipated problems and SAEs will occur as soon as possible after the PI learns of the event, but in all cases within 10 working days apart from death of a JHM participant. In this instance, reporting requirements defined in [Policy 103.6\(b\)\(i\) will be followed](#).

Study deviations which are considered emergency deviations to protect the well-being of the participant will be reported promptly to the PI and the JH SOM IRB as soon as possible, but no later than 5 days after the emergency event occurred. Minor or administrative deviations will be reported to the JH SOM IRB annually at the time of the continuing review.

All SAEs will be immediately reported to our named Internal Data and Safety Monitor, Dr. Anna Chien, MD. Dr. Chien is a dermatologist with extensive experience in clinical trials as a PI on more than 10 active IRB protocols. She will provide independent authority to stop the trial, remove any participants from the trial, and otherwise maximally ensure patient safety within the limits of the protocol.

#### 9.4.3 Unanticipated Problem Reporting

If UPs occur during the study, the study team will report them to the IRB, SO, and the NIAMS.

All events that meet the requirements of a UP are reported to the IRB in accordance with their policy, within 10 days of participant disclosure to a clinical site.

#### 9.4.4 Protocol Deviations Capture and Reporting

Protocol deviations that are caused by the patient are self-reported by the patient. Protocol deviations caused by the study team are self-reported by the responsible study team member(s). Patient-caused protocol deviations are captured via phone calls or email exchanges with the patient and noted on the Case Report Forms (CRFs). All protocol deviations are entered into a protocol deviation log. This log includes the date of the deviation, subject study ID, description and cause of deviation, corrective action plan (e.g., submit change in research, re-educate staff, develop checklist, etc.), and if applicable, the sponsor notification date.

Pregnancy is part of the exclusion criteria for the study, so any participant that self-reports a pregnancy will no longer be eligible for the study. The PI will be notified of any reported pregnancies and determine the appropriate course of action. Relevant data will be documented as needed.

Protocol deviations are reported to the IRB in accordance with their policy, within 10 days of identification of the event.

#### 9.5 NIAMS Reporting Requirements

A NIAMS-appointed safety officer (SO) will provide safety oversight.

- All AEs should be reported in aggregate to the NIAMS and the SO (through the NIAMS Executive Secretary) as part of the routine data and safety monitoring report.
- All SAEs (regardless of relatedness or expectedness) should be reported to the NIAMS and the SO (through the NIAMS Executive Secretary) within 48 hours of the site PI becoming aware of the event.
- All UPs should be reported to the NIAMS and the SO (through the NIAMS Executive Secretary) within 48 hours of the site PI becoming aware of the event.
- Protocol deviations impacting participant safety should be reported to the NIAMS and the SO (through the NIAMS Executive Secretary) within 48 hours of the site PI becoming aware of the event; all other deviations that do not impact participant safety should be reported as part of the routine data and safety monitoring report.
- Serious or continuing noncompliance should be reported to the NIAMS Program Officer and Grants Management Specialist within 3 business days of IRB determination.

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- Any suspension or termination of IRB approval must include a statement of the reason(s) for the action and should be reported promptly to the NIAMS Program Officer and Grants Management Specialist within 3 business days of receipt by the investigator.

#### **10. Benefits**

- a. Description of the probable benefits for the participant and for society.

It is unclear whether there will be direct benefit to the participant as a result of this study. In theory, the participant may enjoy hair follicle regeneration and regrowth of terminal hair in the small treatment area as a result. This, however, cannot be guaranteed.

#### **11. Payment and Remuneration**

- a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

They will receive \$50 for each biopsy completed. Up to 6 biopsies may be completed. Participants will also receive \$50 for each laser treatment session, with a maximum of 40 visits. Thus, total potential reimbursement is \$2,300 distributed at completion of study.

#### **12. Costs**

- a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

All costs will be covered by Lutronic.

#### **13. Transfer of Materials**

N/A

#### **14. Statement of Compliance**

This study will be conducted in compliance with the protocol, International Council for Harmonization/Good Clinical Practice requirements (ICH/GCP), and applicable state, local, and federal regulatory requirements.

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## Appendix

### I. COVID-19 Mitigation Strategy

The list below serves as a mitigation action plan for the study team during the COVID-19 Pandemic and is subject to change without delay per evolving needs and requirements relating to the COVID-19 Pandemic.

1. To reduce the risk to study team members, the study team will engage in JHH mandatory and additional COVID-screening prior to all study visits, including questions about onset of any new COVID symptoms or known exposures. In total, this will occur at least twice and may happen either: 1 day before subject visit via study team initiated contact; with the use of the screening survey sent to the subject via email/EPIC and/or or upon entry into JHOC on the day of their visit; and/or at the start of the visit.
2. PPE will be worn by staff, including masking, face shields, and gloves at all times. Participants will also wear masks as per institutional guidelines. Participants are also asked to wash their hands or use hand sanitizer immediately upon entering the room.
3. Most study visits will be relatively brief; the longest is around 1 hour. Study staff will continue to perform study visits in a timely manner to decrease length of exposure.
4. The study team will make an effort to link study visit timing to SOC clinic visits or other visits to campus that may already be occurring for the participant, when possible. All visits occur in JHOC, in clinic space within the department of dermatology. These spaces are routinely wiped down according to CDC recommendations with 70% ethanol.
5. Study visits are scheduled to avoid multiple subjects in the waiting room at the same time. Subjects will not wait longer than 5 minutes.
6. Social distancing will be maintained as best possible during all study related activities and a minimal number of study staff will be present for each visit.
7. In an effort to decrease in person visits, if biopsies have been obtained, subjects may be offered a disposable suture removal kit to perform at home suture removal.