PROTOCOL TITLE:	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
JHM IRB:	NA_00068684
NCT	NCT01964859
PROTOCOL DATE:	February 08, 2018

PRINCIPAL INVESTIGATOR: Luis Garza, MD, PhD
Associate Professor
Department of Dermatology
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CLINICAL PROTOCOL

AMENDMENT 7

PROTOCOL TITLE:	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
JHM IRB:	NA_00068684
INVESTIGATIONAL AGENT:	Autologous skin fibroblasts
IND/IND EXEMPTION:	*15658*
PROTOCOL VERSION/DATE:	Version 9/ February 08, 2018
PRINCIPAL INVESTIGATOR:	Luis Garza, MD, PhD Associate Professor Department of Dermatology Johns Hopkins School of Medicine Office: Suite 204 Lab: Suite 216 Koch CRBII 1551 Orleans Street Baltimore, MD 21287 Telephone 410-614-6700 (direct line) Fax 410-614-0635 Email: LAG@jhmi.edu

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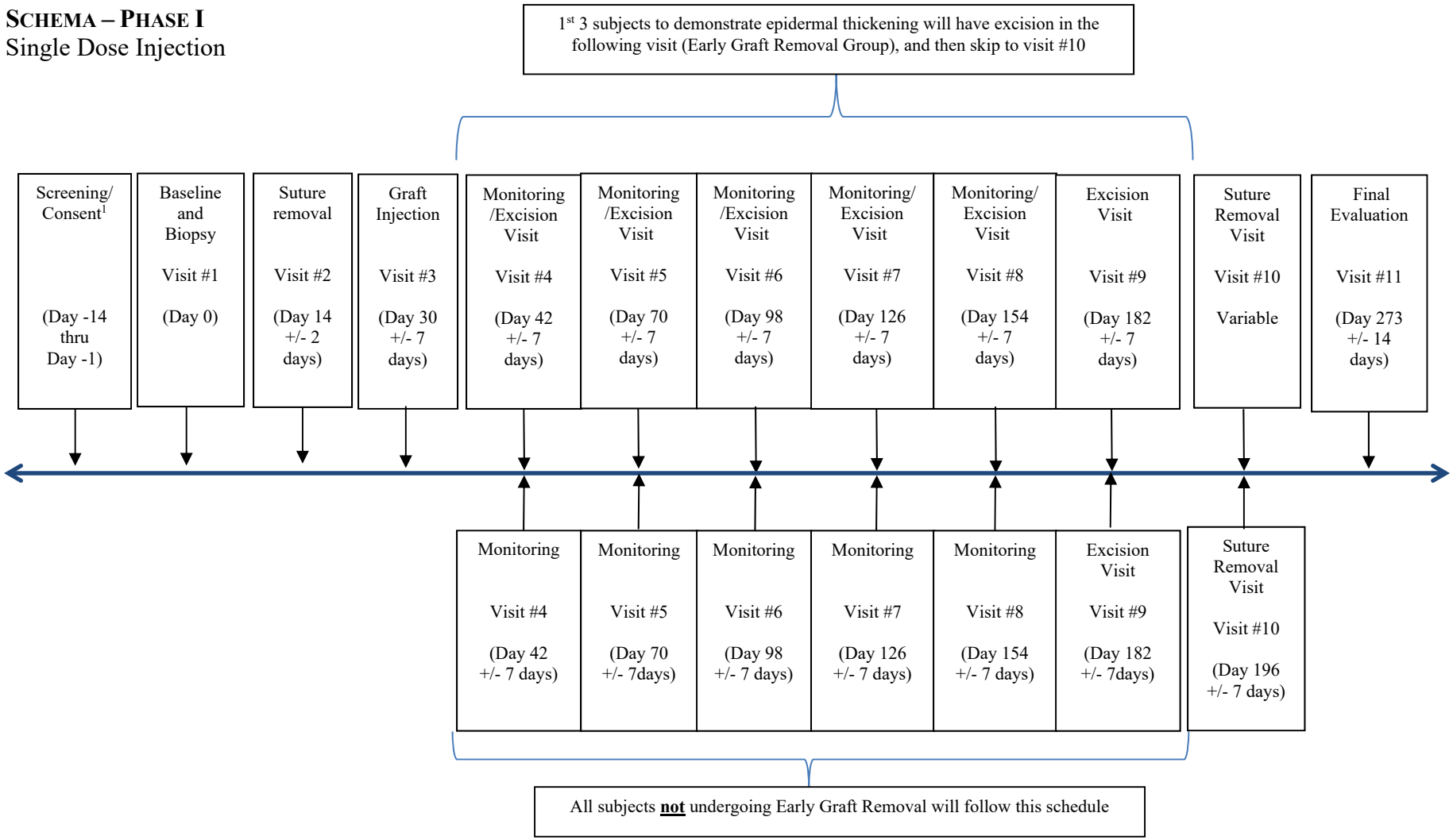
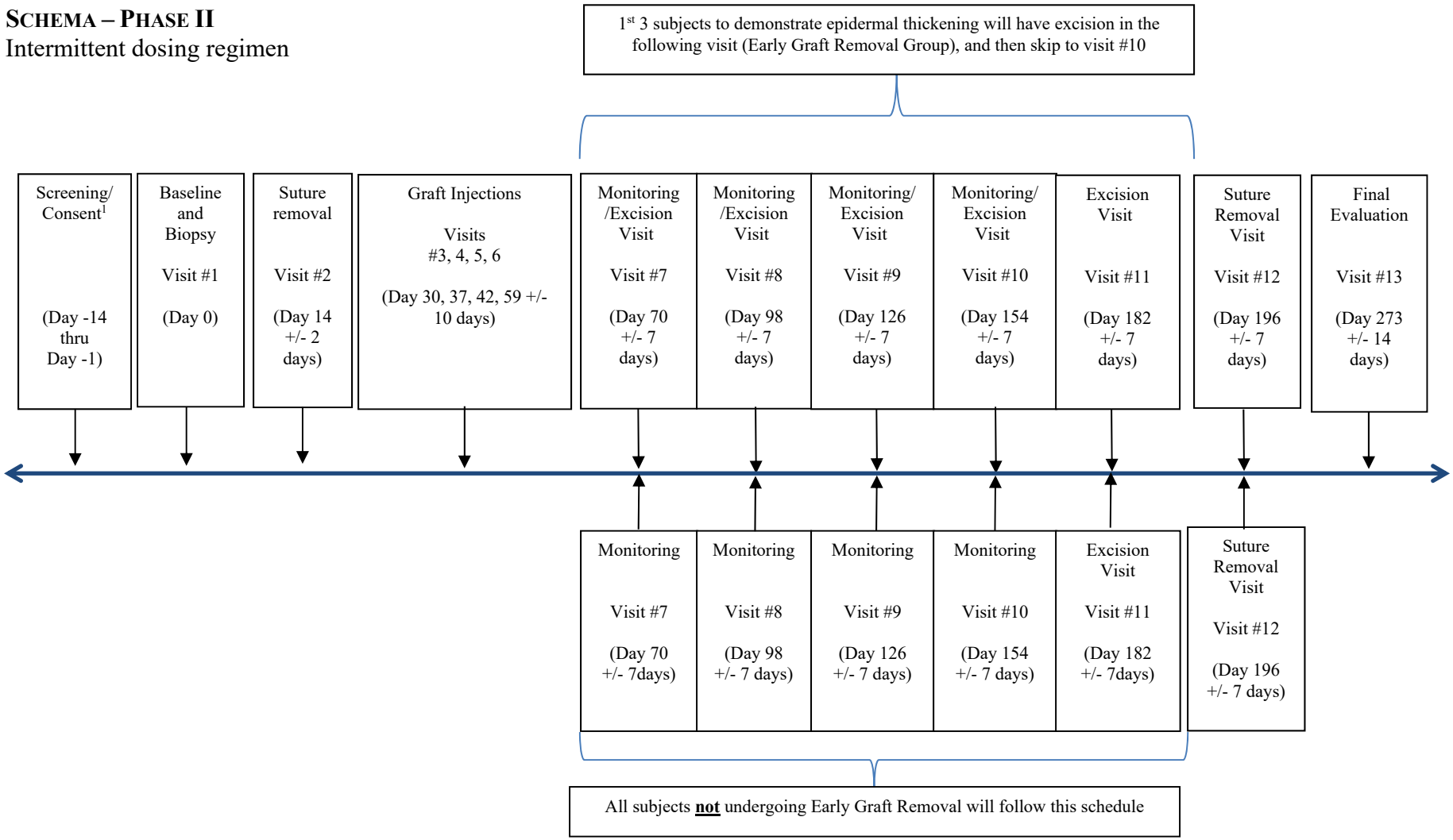


SCHEMA – PHASE II
Intermittent dosing regimen



1 ABSTRACT

Cellular therapy holds great promise in medicine. This proposal will employ autologous fibroblasts in an attempt to help the more than 1.7 million (1 out of every 200) people in the US who have had limb amputations. While improvements in prosthetics have been made, their use is still dramatically limited by pain and skin-breakdown at the stump site. Our long-term goal is to permanently convert the skin at the stump site to **volar type (palmo-plantar)** skin. Volar epidermis is markedly thicker than skin at other locations and **uniquely** expresses Keratin 9 (KRT9) which makes it more friction- and irritant-resistant. We and others have shown that volar fibroblasts have the ability to increase epidermal thickness in 3-D and induce ectopic KRT9 **in non-volar keratinocytes** in both 2-D and 3-D co-cultures. (Given that KRT9 is a suprabasalar keratin, and unique to volar skin, it is an ideal read-out). Therefore our aim is a proof-of-concept demonstration

Hypothesis: Autologous volar fibroblasts have the capacity to induce ectopic volar gene expression such as KRT9 in human subjects. To investigate this, we intend to:

Phase One

1. Inject autologous volar and nonvolar fibroblasts in paired areas of the buttocks, or non-butttocks area. (All non-buttocks areas will exclude genitals, face, or at joints) Perform noninvasive imaging over a maximum period of 6 months to monitor for thickened epidermis/stratum corneum and longevity of effect. Also, if early thickening occurs, then we will remove injected skin area early in ½ of participants for #2.

2. Analyze epidermis by both histology and RT-PCR for features of volar phenotype (KRT9, epidermal thickness, stratum corneum thickness, presence of $PAX9^{high}$, $LMX1b^{low}$, $EMX2^{low}$, $SHOX^{low}$ fibroblasts)

Phase Two

3. Identify optimal methods of delivery that will enhance skin reprogramming and improve conversion of non-volar to volar skin. We propose 2 testing methods with the goal of enhancing volar fibroblast stem cell engraftment: (a) We will investigate the efficacy of multiple, intermittent injections, while holding the total number of injected cells constant. (b) An additional approach is to lightly curette the skin at the injection sites to test if superficial wounding of the epidermis enhances volar fibroblast stem cell engraftment.

If successful, the results of this proposal will be a first to ectopically reprogram human skin identity.

2 PRIMARY OBJECTIVE

1. To determine the safety and tolerability of the use autologous volar fibroblasts to reprogram non-volar to KRT9-expressing volar epidermis in human participants.

SECONDARY OBJECTIVES

1. To measure non-invasively for epidermal thickening in sites of injection of volar fibroblasts compared to both non-volar fibroblast injection and vehicle only injection

2. To measure for molecular changes in excised injected skin (for example increases in KRT9).

3. To describe persistence of epidermal thickening in sites of injection of volar fibroblasts over time.

4. To evaluate the safety and efficacy of an intermittent, serial dosing regimen of the autologous volar fibroblasts.

5. To evaluate the efficacy of superficial wounding with a curette at the injection sites.

3 BACKGROUND

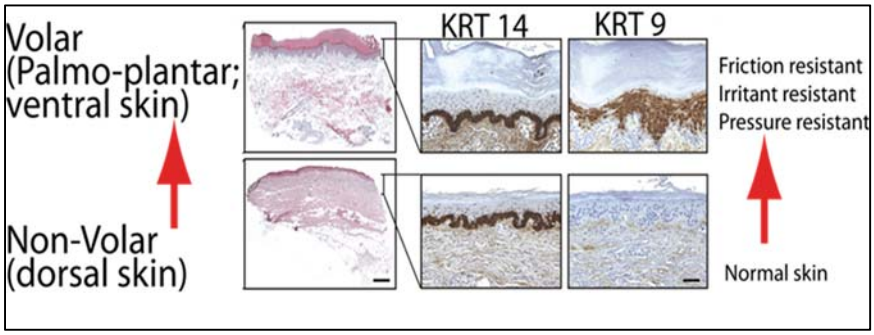


Figure 1: Properties of Volar versus Non-Volar Skin. Note **Keratin 9** expression is limited to thick volar skin.

(Adapted from Rinn et al[1])

Solution: Convert stump skin to weight-bearing volar (i.e. palmoplantar) skin

The goal of this proposal is to make the placement of a prosthetic at a stump site analogous to the placement of a shoe on a foot, for example. We hope to achieve the rarity of skin break-down at the soles of our feet for stump sites in amputation victims. Therefore we propose to convert stump skin to volar type skin with its attendant friction-resistant, irritant-resistant and weight bearing properties.

Properties of Volar Skin (Figure 1)

Volar skin, found at our palms and soles, has many distinguishing features adapted for their location as frequent points of interface with our environment. The primary feature of volar skin is the thick stratum corneum—the very top layer of skin which consists of dead keratinocytes which are cross-linked to form a strong water-impermeable layer. This stratum corneum is approximately 10 times as thick as in other parts of the skin. The thickness of this layer allows for more even distribution of weight across volar skin, resistance to friction, resistance to injury and impermeability to irritants and allergens.

These physical differences are reflected in different proteins which are expressed in volar skin (see Preliminary Studies). The most well studied one is Keratin 9. This structural molecule is unique to volar skin and provides tensile strength to cells (Figure 1).

Fibroblasts: Candidate Therapy to Convert Non-Volar to Volar Skin

The skin is made of two parts, the top epidermis and the bottom dermis. The predominant cell in the epidermis is the keratinocyte. The predominant cell in the dermis is the fibroblast. Fibroblasts have important functions in controlling tissue identity. Evidence for this comes from both animal studies and human studies.

The primary innovation of this investigation is to clinically apply established concepts in developmental biology of the mesenchymal (dermal fibroblast) control of epithelial (keratinocyte) function. Early experiments swapping chicken and duck epithelium and mesenchyme established that mesenchyme controls epidermal identity[3]. We hope to translate this fundamental concept to help improve the quality of life for amputation victims.

Human studies demonstrate similar results. Scalp dermal fibroblasts create long hairs if transplanted to the arm[4]. Part of the mechanism of this effect is that fibroblasts can "remember" their origin position in the body. When human dermal fibroblasts are removed from different areas of the skin, and their gene expression is measured, fibroblasts maintain expression of specific genes which reflect the location of origin of the fibroblasts: Hox or homeobox genes [5-8]. Fibroblasts retained their Hox signatures even after 35 cell doublings in cell culture[9]. The Hox genes control tissue identity as shown most plainly in the case of drosophila (Figure 2). In summary, dermal fibroblasts control skin identity, probably through expression of Hox genes.

Volar Fibroblasts Control Volar Keratinocyte Gene Expression

Volar skin has thickened stratum corneum and epidermis. Early experiments using human cells by Yagamuchi et al confirmed that dermal fibroblasts control KRT9 expression. In this experiment, human plantar fibroblasts can convert trunk keratinocytes into a thickened KRT9 positive epidermis when transplanted to SCID mice[2]. Also, when human non-volar epidermis is transplanted to palm dermis, the epidermis thickens and expresses KRT9, indicating that palmar dermis directs epidermal identity (Figure 3). The specific innovation of this research is to establish the reciprocal concept: human fibroblasts are capable of inducing KRT9 and can induce ectopic volar skin in human participants.
Yagamuchi extended this work to demonstrate that DKK-1 is elevated in volar fibroblasts and has the ability itself to increase epidermal thickness and decrease pigmentation in volar epidermis[10]. Similarly Rinn et al demonstrated that the distal homeobox A13 (HOXA13) is necessary for KRT9 induction and induces Wnt5a which itself also can stimulate KRT9 expression [8].

In conclusion, in the search for therapies to induce ectopic volar skin, we choose to focus on the fibroblasts themselves—which maintain their identity in culture[5], and have a lower regulatory hurdle for use—rather than any particular signaling factor.

Preliminary Studies

Through a seedling grant from DARPA we have verified that volar fibroblasts have the ability to induce KRT9 in cells which do not normally do so. We biopsied human volunteers, cultured either volar or non-volar fibroblasts, and added them to foreskin keratinocytes. We detected that just as in normal human skin (Figure 4), palm or sole fibroblasts could induce ectopic KRT 9 (Figure 5). Given that KRT9 is a suprabasal keratin found only in differentiated volar keratinocytes, this strongly supports their use in inducing ectopic volar skin in amputees.
To verify the accuracy of our assay, we have done extensive microarrays. We discovered a master regulatory gene which controls the volar phenotype—the homeobox gene LMX1b. If we knockdown LMX1b then we can now endow even non-volar cells with the ability to induce KRT9 (Figure 6). We have also verified KRT9 inductivity persists through passage 16 of fibroblasts, with one biopsy yielding more than 100 million fibroblasts in a month.

Besides these in 2-D in vitro assays, there is further proof for the inductive power of fibroblasts. In 3-D cultures human volar fibroblasts can make ectopic thickened epidermis and stratum corneum from foreskin keratinocytes (Fig 7). Similarly, Fig 3 demonstrates that non-volar keratinocytes are turned into volar keratinocytes when transplanted to volar fibroblasts. Also Yamaguchi et al demonstrated that human volar fibroblasts could also convert non-volar to volar keratinocytes in a SCID mouse model[2]. We ourselves have performed similar assays where we add mouse keratinocytes either alone or plus inductive fibroblasts and we can show that fibroblasts direct the development of hair growth. These results provide in vivo evidence on how fibroblasts can change epidermal function and control skin identity.

In the course of these studies we have enrolled 11 participants who have had sole/foot biopsies (NA_00033375). Of 11 biopsies, only a single one had a possible infection which was successfully treated with antibiotics. Otherwise there were no adverse events. Therefore we predict a similar safety profile for the currently proposed study.

Phase One - Proposed therapeutic outline:

1. Biopsy palmo/plantar skin at the sole of the foot and the scalp (or other area with suitable non-volar skin) (note higher KRT9 in native tissue in Fig 4 and KRT9 induction in Fig 5)

2. Expand autologous palmo/plantar fibroblasts

3. Inject autologous fibroblasts into stump site (in the current proposal we will first test healthy participants and not amputees)

4. Monitor for conversion to ectopic palmo/plantar skin

Phase Two – Proposed therapeutic outline:

1. Biopsy palmo/plantar skin at the sole of the foot and the scalp (or other area with suitable non-volar skin) (note higher KRT9 in native tissue in Fig 4 and KRT9 induction in Fig 5)

2. Expand autologous palmo/plantar fibroblasts

3. Inject autologous fibroblasts into stump site (in the current proposal we will first test healthy participants and not amputees)

4. Introduce intermittent dosing regimen of autologous fibroblasts (multiple injections vs. single injection) or RIMSO-50 injection (50% DMSO),and superficial wounding with a curette in a subset of subjects.

5. Monitor for conversion to ectopic palmo/plantar skin.

4 STUDY DESIGN

4.1 DESIGN OVERVIEW

This is an open-label, Phase I, single arm study to evaluate the safety and feasibility of autologous volar fibroblasts to reprogram non-volar to KRT9-expressing volar epidermis in human participants.

We anticipate enrolling 20 patients with full accrual expected within six months for Phase One of the study. To complete Phase Two objectives, an additional 80 subjects will be enrolled. Thus, up to 100 patients may be consented in order to complete enrollment for both Phase One and Phase Two of the study. No part of this protocol will be considered routine care.

4.1.1 BLINDING/RANDOMIZATION

The clinicians will not be blinded, but the research staff who measure epidermal thickness and KRT9 levels will be blinded. It will be impossible to blind the clinicians since they will be injecting the cells versus vehicle. Although we could blind the clinicians to the identity of volar versus non-volar cells, it might be important to judge adverse events and will not impact on endpoint determination—primarily occurring in the laboratory. Another reason not to blind the clinicians is that this is a feasibility trial.

We will blind the research staff by labeling sites/biopsies with numbers (1, 2 or 3) rather than the identity of injected cells at that location. At the injection visit, the numbers are assigned as in the CRF (see Appendix V) with the corresponding identity of cells injected by the clinician. This injection form will not be available to research staff unless there is an adverse event when un-blinding will occur. Blinding will occur for research staff both during measurements of epidermal thickness/area by OCT as well as in the laboratory phase where the removed injection sites will be analyzed by histology and RT-PCR.

The subjects will also only be told the number of each site so that subjects will be blinded as well (See Appendix VI; injection diary).

4.2 PARTICIPANT SELECTION

4.2.1 INCLUSION CRITERIA

Patients interested in study participation must meet all of the following inclusion criteria:

- May be male or female
- Must be between 18 years and 65 years of age
- In the opinion of the investigator, must be medically able to undergo the administration of study material determined by laboratory tests obtained within 2 weeks before baseline for which the investigator identified no clinically significant abnormality.
- Be able to comprehend the informed consent document and provide consent for participation
- Females of childbearing potential must:
    - have a negative pregnancy test at screening
    - agree to not become pregnant or breastfeed for the period of the study through 1 month after completion of the study
    - be willing to use a reliable form of contraception during the study
- Have healthy skin as determined by the PI.
- Be willing and able to comply with the scheduled visits, biopsy/injection procedures, wound care instructions treatment plan, and other study procedures for the duration of the study.

4.2.2 EXCLUSION CRITERIA

Patients meeting any of the following criteria will be ineligible for study participation:

- Having received any investigational drug within 30 days prior to study entry
- An allergy history to any study materials including local anesthetic, antibiotics, antimycotics, dimethyl sulfoxide, human albumin, or bovine constituents, or hetastarch
    - Specifically: subjects allergic to penicillin, streptomycin, amphotericin will be excluded in addition to those listed above.
- Pregnant, lactating, or trying to become pregnant
- A history of keloid formation
- An active nonhealing wound

- Having a significant medical history that the investigator feels is not safe for study participation (for example, some forms of autoimmune conditions, metastatic cancer, infectious diseases such as HIV, HTLV I/II, Hepatitis B, Hepatitis C). Biopsies taken from individuals with infections that are not allowed to enter the cell therapy core will make it such that these individuals cannot participate.
    - Specifically we will exclude those with autoimmune diseases affecting the skin such as lupus.
- Having current skin diseases (i.e. extreme and active eczema, psoriasis, lichen planus) that the investigator feels is not safe for study participation
- A diagnosis of uncontrolled diabetes
- Active smoker during the study
- We will also exclude those who are on chronic immunosuppressive therapies such as oral steroids, but also those on chronic topical steroids in the area of investigation.

4.2.3 PARTICIPANT RECRUITMENT

Participants may be recruited if they are under the direct care of the PI or are referred by other providers who have identified the potential participant, asked their permission to be contacted, and then passed their information to the investigator. Additionally, participants may be recruited from those patients who were previously enrolled in a dermatologic study within the department and who gave their permission to be contacted regarding participation in future studies.

Participants will be approached by their physician after routine clinical encounters, in the privacy of the exam room, regarding their interest in participation in the study. In addition, potential participants may respond to IRB-approved recruitment flyers located throughout Johns Hopkins Department of Dermatology and Johns Hopkins Hospital.

4.3 INFORMED CONSENT

Interested potential participants will be given an appointment in the Cutaneous Translational Research Unit located at the Johns Hopkins Outpatient Center. At the appointment, 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 investigator will ask the participant to briefly summarize the study. The consent form may be provided for them before the appointment to allow them ample time to review the document. Non-English speakers will not be enrolled at this time. No screening or study related activities will take place until informed consent has been obtained.

4.4 PARTICIPANT REMOVAL CRITERIA

Participants will be removed from study if they request to be removed from study after signing consent but prior to administration of their autologous fibroblasts. The investigator may remove a participant from study due to lack of compliance to study directions, inappropriate behavior in clinic such as hostility to staff or the novel acquisition of an exclusionary criteria, for example. Such subjects will be replaced for the goal of attaining an adequate sample size for the study.

4.4.1 PARTICIPANTS WHOSE STUDY PARTICIPATION ENDS PREMATURELY

If a participant withdraws from the study prior to excision of the fibroblast injection sites, we will make a concerted effort to contact that patient. We will stress to the participant that this is an experimental therapy and that excision is the safest endpoint. We have also included this in the informed consent.

Study participation might also end prematurely if laboratory expansion of fibroblasts is not successful. There are many potential reasons for a failure to generate aliquots of fibroblasts of the desired cellular number from both volar and non-volar fibroblasts. These might include for example failure to simply have fibroblasts expand, an accidental infection during cell culture, a failure of release testing or the chance that fibroblasts are expanding so slowly the full cadre are not available before 40 days of expansion. If for any of these reasons either or both fibroblasts populations (volar and non-volar) are not available, then the subject will be discharged early from the study. No repeat biopsies will be done to avoid any potential injury to the patient in the context of a potential human error in sample processing. Subjects will be paid for all of the visits they kept.

4.4.2 TREATMENT FAILURE

For purposes of this study, treatment failure will be defined as no increase in epidermal thickness or induction of volar specific genes such as KRT9.

4.5 STUDY DURATION AND REQUIRED VISITS

Participants will be considered ‘on-study’ once informed consent has been obtained. The duration of the study for each participant will begin at the time of consent and continue until completion of the final study evaluation.

For Phase One participants: At a minimum, there will be 7 study visits, including screening procedures. At a maximum, there will be 12 study visits over a period of 10 months.

For Phase Two participants: At a minimum, there will be 4 study visits, including screening procedures. At a maximum, there will be 14 study visits over a period of 12 months.

4.6 PAYMENT AND REMUNERATION

Phase One: Participants will receive $150.00 per completed milestone (maximum payment of $600). The four milestones are: a) Screening and baseline biopsy; b) Graft injection; c) Graft monitoring and removal; and d) Final evaluation.

Phase Two: Participants will receive $150.00 per completed visit milestone (maximum payment of $600). The four milestones are: a) Screening and baseline biopsy; b) Graft injection; c) Graft monitoring and removal; and d) Final evaluation.

These sums will be provided in cash or gift card form.

Participants will not be paid for missed milestones unless visits are rescheduled within 7 days. Also, participants may forfeit their bonus if they miss more than 2 visits that are not rescheduled within 7 days. Participants may forfeit their bonus for final evaluation if they do not complete the study.

4.7 COSTS

Participants will not be responsible for any study related costs. Dr. Garza's DoD AFIRM2 grant and NIH NIAMS R01 will cover all study costs.

5. STUDY SCHEDULE

5.1 SCHEDULE OF EVALUATIONS

See Appendix I for a table containing the full schedule of study evaluations.

5.2 STUDY VISITS

5.2.1 SCREENING AND INFORMED CONSENT VISIT (TO BE OBTAINED WITHIN 14 DAYS OF BASELINE/BIOPSY VISIT)

During the first scheduled appointment, the research protocol will be discussed with the potential participant. We will review exclusion and inclusion criteria and provide the participant with an informed consent document. After reviewing it together, assessing the patients understanding of the material and answering any questions that they may have, informed consent will be obtained from the subject.

Once informed consent has been obtained, the following evaluations will be done:

- Medication review
- History and limited physical examination
- Vital signs including BP, pulse, and respirations

Screening blood tests obtained at this visit will include:

- Serum HCG for females of child bearing capacity

- Hopkins test code 4133 Donor Serology Panel which requires 2 serum tubes and 1 EDTA tube for the following studies:
    - HIV Screen
        - HIV1 and HIV2 Antibody
        - HIV nucleic acid
        - For the HIV test only, the participant will be given the State of Maryland HIV consent form as part of that process. (If this test is positive, it does not always mean that the participant is infected with the HIV virus. It means the participant will need further testing and the participant will receive counseling).
    - HTLV I/II
        - Antibody test
    - Hepatitis B
        - Hep B Surface Ag
        - Hep B Core Antibody
    - Hepatitis C
        - HEP C Nucleic acid
- For HIV and Hepatitis B and C tests, the law requires us to report positive tests to the health department. Result of the blood sample tests must be negative for the participant to participate in this study.

The participant's next study visit will be scheduled by the study team as soon as all of the screening laboratory test results are received. The participant will be notified by telephone of the appointment.

5.2.2 BASELINE/BIOPSY VISIT (VISIT #1) (STUDY DAY 0)

At this study visit, the following will be obtained:

- History and limited physical examination
- Review of eligibility/screening test results
- AE Assessment

The investigator will identify 1 area on the participant's sole and 1 area with suitable non-volar skin, like the scalp, to biopsy and clinical photographs will be taken of the sites. (See Appendix II). Local anesthesia consisting of lidocaine with epinephrine will be used and the investigator will perform 3 skin biopsies (1 punch of volar sole skin and 2 punches of non-volar skin). These will be a standard 4mm punch style biopsy (or smaller) taken from the participant's skin to a depth of 2mm.

One suture will be placed in each biopsy site to aid healing. The biopsy sites may be from the participant's sole and dorsum of foot (or any site with suitable volar/nonvolar skin). The participant may provide their preference of biopsy sites.

The amount of time taken for the biopsies is about 20 minutes. After the biopsy is completed, it is sent to the Hopkins Cellular Therapy Core (see IND CMC section) where the fibroblasts cells will be grown. (See Appendix III for specimen preparation and handling.)

Before leaving, participants will receive a biopsy site care information sheet (See Appendix IV) with contact information in the event of any adverse event. They will also be given a return appointment for suture removal at this time.

During the baseline visit, the 3 graft injection sites will be identified. To optimize future localization, very small tattoos, the size of a pinpoint or freckle, will be made using tattoo ink. Please see tattoo procedure in section 5.2.4.

5.2.3 SUTURE REMOVAL VISIT (VISIT #2) (STUDY DAY 14 +/- 2 DAYS)

The participant will return to clinic for suture removal. At this study visit, the following will be obtained/performed:

- Vital signs including BP, pulse, and respirations
- Biopsy site/AE assessment
- Biopsy site suture removal

The participant will be given their graft administration visit appointment at this time.

5.2.4 GRAFT INJECTION VISIT
PHASE ONE VISIT #3 (STUDY DAY 30 +/- 7 DAYS) OR
PHASE TWO VISITS #3-6 (STUDY DAYS 30, 37, 42, 59)

The participant will return to the clinic on or near Day 30 for their graft injection.

Participants will have the following assessments done:

- History and limited physical examination
- Females of child bearing potential will have a urine pregnancy test done prior to administration of the investigational cells to ensure that they are not pregnant.
- Clinical photographs will be taken of each of these 3 sites before the investigational product is administered. These photographs will be used throughout the study to document changes in the skin as well as aid in location of the site at subsequent visits. Photograph files will be coded with the participant's unique identifier. The participant will not be identifiable from the clinical photographs.

Together with the study investigator, a site for injection of the subject's grown fibroblasts will be chosen. The injections will be placed by the investigator into the three sites mutually identified (e.g. the three injections will be placed on one of

the participant's buttocks, or non-buttocks area as above). The injection sites will be spaced at least 5 cm from the center of any neighbor and may be divided between left and right buttocks or non-buttocks area as above to ensure a lack of overlap.

Identification of injection sites to optimize future localization will occur through these steps:

- We will identify distances and angle from anatomical landmarks such as the sacroiliac joint.
- We will draw anatomical landmark maps with transparency paper used for projections. We will circle landmarks so that the transparency can be directly placed on the subjects buttocks or non-buttocks area as above in a repeatable orientation, and also identify injection sites.

To further enhance our ability to localize injection sites, we will use a small volume of ($x<0.025$ ml) tattoo ink to mark areas of cellular injection:

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 1)

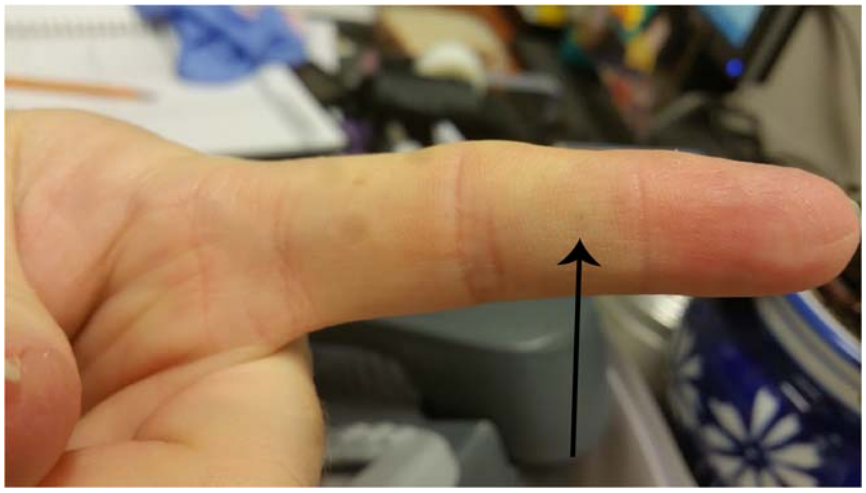


Figure 1: Tattoo mark on figure at end of arrowhead appears vaguely bluish in the subepidermal compartment.

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. We will only tattoo areas which will be removed in the final excision of the area injected with cells. Therefore the subject will be left with no mark and only temporarily have the 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.)

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 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(1).
4.No local care is necessary. The tattoo will be performed at the time of initial biopsy for skin harvesting to allow time for it to fully integrate without inflammation in to the skin before cellular injection. In the rare and unusual case where cells fail to grow or are rejected for any reason and are not available for injection, then the subject will be given the option of a small punch biopsy in those 3 areas of cellular injection to remove the miniscule dot of pigment (See Figure 1). In this way all subjects will be guaranteed the option of full removal of pigment, though normally radiation oncology patients retain the tattoos indefinitely.

1.Tripp GK, Good KL, Motta MJ, Kass PH, Murphy CJ. The effect of needle gauge, needle type, and needle orientation on the volume of a drop. Veterinary ophthalmology. 2015. Epub 2015/02/04. doi: 10.1111/vop.12253. PubMed PMID: 25643934.

If a subject refuses consent for the tattoo marker, but is otherwise willing to complete all other study procedures, then we can use the palpated sacral hiatus, and initiation of the gluteal cleft as 2nd and 3rd universal landmarks. In addition, we will maximize the use of individual landmarks such as nevi, seborrheic keratoses, scars and hemangiomas which are found on human skin. In previous studies that the PI has performed(1), he has used exactly this method for the injection of hyaluronic acid to identify sites months later and had universal success at identifying injection sites. Also, to enhance our ability to identify sites we will not limit injections to one side of the buttocks (or non-buttocks area as above) to enhance the available number of landmarks and ensure proper identification in subsequent visits after the injection. Finally, we will frequently also consult clinical photographs (photographed with rulers) of the injection site taken on the day of injection to optimize our ability to correctly map injection sites.

We will avoid injection into the skin above the ischial tuberosity to minimize discomfort and risks of ulcers

Phase One: Single Dose Injection
After numbing the participant with anesthesia described in Visit 2, two of the sites will be injected with 0.7-0.8 mls of media (5% DMSO, 6% hetastarch and 2.5% human serum albumin) containing 30-40 million autologous fibroblasts into a small area about 3-4mm injection area subepidermally at the level of the epidermis-

dermis junction. The third site will be injected with the same volume of media containing no autologous cells.

Phase Two: Intermittent Dosing Regimen
We do not propose to increase the total number of cells injected, but to inject fewer cells more often. At the PI's discretion, the subject may receive injections of the dosing regimen or injections of 10% DMSO.

Two of the three treated sites will receive cells. A total of 4 injections may maximally occur; the first will always include cells for these sites. The remaining 3 injections may include vehicle and up to 10% DMSO instead of cells administered within 30 days after injection of cellular product.

The third control site will not receive cells but instead be injected with the same volume of media or up to 10% DMSO, containing no autologous cells; it, may receive intermittent administration of vehicle media or up to 10% DMSO with the same schedule as above for the two sites receiving cells. The 10% DMSO media will be RIMSO-50, a FDA-approved product for the use in interstitial cystitis and delivered intravesically. The injections may be spaced in any manner over the 30 days. The dose volume will be determined on the basis of results from Phase One. A range of dose volumes may be tested but the total number of injected autologous fibroblasts will not exceed 30-40 million. As in Phase One, the dose will be prepared by appropriately trained and qualified personnel in the cellular therapy core and administered by the PI. We will be using an FDA approved product (RIMSO-50), because we unexpectedly found in our animal studies that the DMSO in our vehicle actually enhances cellular engraftment. We propose to inject no more than 10% DMSO at a volume of less than 1 ml into the skin not more than 3 times. We will only inject the areas where the vehicle or cells were previously injected (3 approximately 6 mm circles). We confirm that this study will not proceed until this Protocol Amendment is granted approval from the Johns Hopkins Medicine Institutional Review Boards.

The following information will be recorded by the investigator administering the cells (see Cell Administration sheet in Appendix V):

- Vial identification numbers
- Date of administration
- Times of administration
- Total cell dose
- Injection sites

Superficial skin scrapings are routinely done in dermatology and may be employed before or after we inject cells in order to potentially enhance tissue repair and regeneration. They might also occur during graft evaluations in subsequent visits. Scrapes will be done at injection sites using two gentle steady strokes of a sterile disposable #15 blade or curette. This wounding technique was specifically chosen to eliminate bleeding and infection risks.

Participants will be observed for 60 minutes following the cell administration for adverse events. At two points in this interval we will perform blood pressure and pulse measurements to monitor for an allergic reaction

Upon release of the subject, we will include instructions to keep the injection site covered with a padded dressing for 24-48 hours after injection. Especially during that time and afterwards, the subjects will be instructed to avoid manipulating the site for example by scratching, ice or topical remedies. If the subject has discomfort for which they desire therapy, we will ask them to return to the clinic for evaluation by a study team member at which time we will assess for adverse events.

Participants will be instructed to maintain diaries of injection site symptoms and events which they will bring to each subsequent appointment until the graft has been excised. (See Appendix VI for injection site diary.)

5.2.5 GRAFT MONITORING AND REMOVAL

Dependent upon participant response, as many as six monitoring visits will be conducted to evaluate skin thickness and adverse events (see study Schemas, page 3-4). As per the pre-IND discussion with FDA, persistence of any thickening response is of particular interest. In order to assess this, we will divide the subjects such that there will be 2 groups. The first portion will immediately have graft removal once skin thickness increases in order to assess the molecular features of induction of volar skin, and a 2nd portion of subjects will instead be followed for permanence of effect, and undergo graft removal at the conclusion of 10 months of the study.

The method of dividing the subjects will be as follows. At all monitoring visits all injected sites on all subjects will be measured for epidermal thickness by non-invasive confocal imaging or through the non-invasive measurement of skin induration (as long as those injected sites have not been removed through biopsy). The first three subjects who demonstrate a statistically significant increase of measured epidermal thickness on the volar fibroblast injected side will have their grafts removed during the follow-up visit that the increased thickness determined to be statistically significant.

The means of calculating an increase in epidermal thickness of the volar fibroblast injected site will be through a t-test statistical analysis of 3 sequential readings (within a visit) from the three injection sites (no fibroblasts, non-volar fibroblasts, and volar fibroblasts). For a subject to be designated as an “early graft removal”, he/she should have a statistically significantly elevated epidermal thickness in the volar injected site but not the non-volar or vehicle-only injected sites.

If no subjects show an increase, then all subjects will be followed for epidermal thickness till the end of the study and undergo graft removal at the end of the 10 months. If fewer than 3 subjects are identified before the end of the study to have an increase, then the remainder will undergo graft removal at the end of the study. If more than 3 subjects are identified, then those remaining subjects will be

followed through Visit 8 and undergo graft removal at the end of the 10 month period..

"Early Graft Removal" subjects will skip all remaining graft monitoring visits and instead go directly to suture removal visit.

All subjects will have at least one monitoring visit.
At each of the monitoring visits, the study team will obtain:

- Vital signs including HR, RR and BP
- Clinical photographs of the injection sites
- Measurements of injection site thickness and areas of increased thickness via Optical Coherence Tomography (OCT) or SkinFibroMeter. OCT is a non-invasive means of capturing images of superficial layers of the human skin (see Figure 8). From these images, thickness of the epidermal layer will be calculated. 3 independent measurements from each injection site in each subject will be made at every visit so that statistics can be performed (see below). SkinFibroMeter is a non-invasive, portable device utilizes an indenter that is briefly pressed on the skin. The resistance of short-term load from the skin tissue will be read by the device and the induration value of Newton will be calculated. Some visits might not include OCT imaging or SkinFibroMeter given operator and machine availability. Some exceptions can be made for unusual life events for missing a study monitoring visit.
- AE assessment/ symptom diary review

Monitoring will continue on a monthly basis for up to 5 visits in order to assess the participant's progress and for any adverse events. At each monitoring visit, a repeat gentle skin scraping may be peformed.

5.2.5.1 GRAFT MONITORING VISIT (VISIT #7) (STUDY DAY 42 +/- 7 DAYS)

At this visit, the study team will obtain:

- Vital signs including HR, RR and BP
- Clinical photographs of the injection sites
- Thickness measurements/Area of epidermal thickness via Optical Coherence Tomography (OCT)
- Skin induration measurement via SkinFibroMeter
- AE assessment/symptom diary review
- A repeat gentle skin scraping may be performed.

5.2. 5.2 GRAFT MONITORING VISIT (VISIT #8) (STUDY DAY 70 +/- 7 DAYS)

At this visit, the study team will obtain:

- Vital signs including HR, RR and BP

- Clinical photographs of the injection sites
- Thickness measurements/Area of epidermal thickness via Optical Coherence Tomography (OCT)
- Skin induration measurement via SkinFibroMeter
- AE assessment/symptom diary review
- A repeat gentle skin scraping may be performed.

5.2.5.3 GRAFT MONITORING VISIT (VISIT #9) (STUDY DAY 98 +/- 7 DAYS)

At this visit, the study team will obtain:

- Vital signs including HR, RR and BP
- Clinical photographs of the injection sites
- Thickness measurements/Area of epidermal thickness via Optical Coherence Tomography (OCT)
- Skin induration measurement via SkinFibroMeter
- AE assessment/symptom diary review
- A repeat gentle skin scraping may be performed.

5.2.5.4 GRAFT MONITORING VISIT (VISIT #10) (STUDY DAY 126 +/-7 DAYS)

At this visit, the study team will obtain:

- Vital signs including HR, RR and BP
- Clinical photographs of the injection sites
- Thickness measurements/Area of epidermal thickness via Optical Coherence Tomography (OCT)
- Skin induration measurement via SkinFibroMeter
- AE assessment/symptom diary review
- A repeat gentle skin scraping may be performed.

5.2.5.5 GRAFT MONITORING VISIT (VISIT #11) (STUDY DAY 154 +/- 7 DAYS)

At this visit, the study team will obtain:

- Vital signs including HR, RR and BP
- Clinical photographs of the injection sites
- Thickness measurements/Area of epidermal thickness via Optical Coherence Tomography (OCT)
- Skin induration measurement via SkinFibroMeter
- AE assessment/symptom diary review
- A repeat gentle skin scraping may be performed.

5.2.5.6 GRAFT REMOVAL VISIT (VISIT #12) (STUDY DAY 182 +/-7 DAYS)

At this visit, the study team will obtain:

- Vital signs including HR, RR and BP
- Clinical photographs of the injection sites
- Thickness measurements/Area of epidermal thickness via Optical Coherence Tomography (OCT)
- Skin induration measurement via SkinFibroMeter
- AE assessment/symptom diary review
- Graft removal will be performed as was the initial biopsy and is detailed in Appendix VII.

5.2.6 SUTURE REMOVAL VISIT (VISIT #13) (STUDY DAY 196 +/- 7 DAYS OR 14 DAYS +/-2 AFTER EARLY GRAFT REMOVAL)

All participants with the exception of those who undergo early graft removal will return for suture removal at this time point. "Early graft removal" participants will return to have sutures removed 14 days (+/- 2 days) after their graft removal. "Early graft removal" subjects will have fewer graft monitoring visits, but will be expected to return for a final evaluation visit (see Visit 11).

5.2.7 FINAL EVALUATION VISIT (VISIT #14) (STUDY DAY 273 +/- 14 DAYS)

At day 273 (+/- 14 days) after the subject's initial injection the participant will return for the following:

- Graft excision site evaluation/wound healing.
- AE assessment

Subjects who have an "Early graft removal" will be expected to return at this time for monitoring also.

6. RISKS/BENEFITS

6.1 RISKS

6.1.1 AUTOLOGOUS VOLAR FIBROBLASTS

We expect to remove the injected cells, but cellular injections have been rarely associated with mild redness, bruising, swelling, pain, bumps, irritation and itch at the injection site in less than 10% of participants. Injection site reactions may occur including pain, bruising, swelling, erythema, pigmentation changes, induration.

6.1.2 DMSO INJECTION

We have been injecting 5% DMSO in vehicle with no adverse events. When used at very high doses (such as large volumes of 100%), the risks of DMSO include

bad breath, and changes in the skin (like redness, itching, hives, dryness, and rarely blisters). Very rarely people may feel sedated, have headaches or nausea. However, we have not observed any of these effects. Adverse events will be notified to the FDA and IRB.

6.1.3 PUNCH BIOPSY

Common risks of skin biopsies include potential bleeding, pain or discomfort, and a slight risk of infection at the biopsy site. As with any cut in the skin, a scar will develop, although its appearance will likely fade over time.

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 biopsy site.

6.1.4 BLOOD DRAW

Taking blood may commonly cause discomfort, bleeding, or bruising where the needle enters the body.

In rare cases, it may result in fainting. There is also a small risk of infection at the draw site.

6.1.5 LIDOCAINE WITH EPINEPHRINE

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 punch biopsy. A reaction of this kind would cause swelling and a rash on the participant skin where the anesthetic was injected.

6.1.6 FINANCIAL RISK

There is a slight financial risk to the participants in the rare event that the above complications occurs requiring additional medical care.

6.1.7 CONFIDENTIALITY

There is a potential risk to confidentiality. Most participant related data will be stored in our institution's electronic medical record. If members from the IRB or other Federal agencies need to inspect trial records, they will be released. Hence, absolute confidentiality cannot be guaranteed.

6.1.8 TATTOOS

The tattoo markings will be permanent and will have the appearance of a dark freckle until we excise the injection sites during the graft removal visit (see section 5.2.5.6). While the markings are being created, the needle may cause discomfort, like a small pinch or insect bite.

6.2 STEPS TAKEN TO MINIMIZE RISK

6.2.1 AUTOLOGOUS VOLAR FIBROBLASTS

Injected cells will meet all required release criteria for clinical use before injection (see IND application which includes steps of verifying purity, sterility and potency) by the Hopkins Cellular Therapy Core. Additionally, we will remove the injected site via biopsy in order to avoid any theoretical long-term problems from injected cells. Finally, all participants will be closely monitored for adverse events throughout the study.

6.2.2 DMSO INJECTION

We find that DMSO enhances engraftment of cells, and have already been injecting it in our vehicle so we consider it safe. However, to minimize risk, we will only use an FDA approved (for interstitial cystitis and intravesical delivery) of DMSO formulation (RIMSO-50). We will only inject small volumes (maximum 1 ml). We will monitor the participants to ensure safety and to avoid any potential side effects induced by the DMSO.

6.2.3 PUNCH BIOPSY

Only Dr. Garza, a trained dermatologist, will perform the biopsy procedures. 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 biopsy site.

6.2.4 BLOOD DRAW

Blood draws for this study will be done by trained phlebotomists in order to reduce the potential risks associated with the procedure.

6.2.5 LIDOCAINE WITH EPINEPHRINE

Persons who have previously experienced an allergic reaction to local anesthetics (e.g. with dental procedures) will be ineligible for study participation.

6.2.6 FINANCIAL RISK

The study will be paying for all lab work, fibroblast production, product administration and biopsies required for participation.

6.2.7 CONFIDENTIALITY

Every effort will be made to keep the participants' study information and records safe by following university and HIPAA policy for handling PHI. Participants

will be assigned a unique identifier number which will be used to identify the patient's information as well as their cell samples during the study. The results of this research project may be presented at meetings or in publications; however, participants' identity will not be disclosed. There are no legal risks associated with breach of confidentiality as we are working with healthy volunteers.

6.2.8 TATTOOS

The tattoo markings will be completely excised during the graft removal process. Also, the tattoo procedure will be optional.

6.3 BENEFITS

6.3.1 INDIVIDUAL PARTICIPANT

There will be no benefit to the individual participant.

6.3.2 SOCIETY

The practical goal for this research—creating ectopic volar skin at the stump site of amputees—will occur as a second step to the proposed experiments. In this protocol we will test in a proof-of-concept study in healthy individuals the ability to convert non-volar to volar skin. This in essence is an early phase 1 trial we propose in this investigation. The results will identify the degree and longevity of response which will directly inform the design of future trials. The immediate next trial we propose at the completion of this study will be optimizing again in healthy participants the ideal dose and frequency of cellular delivery in a more formal Phase 1/2 human trial (here optimized only in animal and in vitro models). Only after completion of this second healthy participant study will we attempt a trial on the vulnerable population of amputees. In that trial (Phase 2/3) we will scale up the number and volume of cells to inject according to the increased area of the stump. We predict that this will be feasible given the large increases of cell numbers at later doublings of fibroblast cultures and the use of ring-blocks for anesthesia in an out-patient setting. Importantly we will focus later trials on measuring for increased usability of prosthetics. We are funded for these future trials under DoD AFIRM2.

7. ADVERSE EVENT COLLECTION AND REPORTING REQUIREMENTS

7.1. DEFINITIONS

7.1.1. ADVERSE EVENT (AE)

An adverse event (AE) will be defined as an unusual and undesirable symptom or sign that occurs in participants during the clinical study. Adverse events include those clinically significant laboratory values and test results, concomitant illness, accident, medical occurrence or worsening of existing medical condition that emerge during study participation.

7.1.2. SERIOUS ADVERSE EVENT (SAE)

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 (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have 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 (note: reports of congenital anomalies/birth defects must also be reported on the Pregnancy Supplemental Form);
- 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 purpose 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).

7.1.3. NON-SERIOUS ADVERSE EVENT

A non-serious adverse event is any adverse events 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.

7.2. 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* - event may be noticeable to participant; does not influence daily activities; usually does not require intervention.
- *Moderate* - event may be of sufficient severity to make participant uncomfortable; performance of daily activities may be influenced; intervention may be needed.
- *Severe* - 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 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.

7.3. ATTRIBUTION OF ADVERSE EVENTS

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

- Unrelated: Adverse event is clearly not related to the investigational agent.
- Unlikely: Adverse event is doubtfully related to the investigational agent.
- Possibly: Adverse event is possibly related to the investigational agent.
- Probably: Adverse event is probably related to the investigational agent.
- Definitely: Adverse event is definitely related to the investigational agent.

7.4 ADVERSE EVENT CAPTURE AND REPORTING

7.4.1. ROUTINE ADVERSE EVENT CAPTURE AND REPORTING

The collection of all AE information begins at initiation of investigational product 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.

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. FDA will receive an annual report within 60 days of the anniversary of IND approval each year as per regulation.

7.4.2. SERIOUS ADVERSE EVENT CAPTURE AND REPORTING

ALL serious adverse events, regardless of causality will be reported to the IRB per the JHM IRB reporting requirements. Prompt reporting to the JH SOM IBR 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 with the exception of death of a JHM participant. In this instance, reporting requirements defined in Policy 103.6(b)(i) will be followed.

Serious and expected adverse events will also be reported to FDA as soon as possible and no later than 15 calendar days after the sponsor's initial receipt of the formation. Serious unexpected adverse events will be reported immediately upon learning of the event via phone/fax and within 7 days via MedWatch 3500A.

Also, all SAEs will be immediately reported to our named internal 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.

8. STUDY PRODUCT INFORMATION

8.1 AUTOLOGOUS VOLAR FIBROBLASTS

The active component of this therapy is autologous cultured fibroblasts.

PRECLINICAL DATA

The autologous fibroblasts used for this product have not been tested in nonhuman in vivo systems as no suitable model for induction of a volar phenotype exists. Additionally, since this autologous product is derived exclusively from the recipient and will be completely removed at the end of the study, no testing for oncogenic potential will be performed. This approach is supported by the azficel-T clinical safety data in humans.
Other model systems have been discussed to test our therapy prior to moving into humans. However, no ideal animal model exists. The most well-studied human skin equivalent are pigs, but pigs are hooved animals and do not have equivalent volar skin to humans. There are methodological constraints with xenografts that we outline below and we do not believe they represent a useful animal model for testing this therapy.

Xenografts are prepared by taking discarded human skin (eg, from abdominoplasties) and sewing it to the back of an immunosuppressed mouse. The following limitations of the xenograft approach have led to the decision not to pursue this method:

1. Graft preparation obviates applicability

   For successful graft “take,” the human skin must be vigorously scraped so that the base of the tissue will allow perfusion of oxygen and blood to the donor human skin. We are interested in injecting our cells into the dermis. A severely damaged dermis that is required for the xenograft model is not applicable to our therapy.

2. Take rate

   The take rate of human grafts on mice is extremely low. Thus, an unacceptably large number of human grafts and immunosuppressed mice would be required for the following reasons:

   - The size of the grafts would only allow for a single injection and we need at least 2 injections for a comparison between volar and nonvolar fibroblast transplants.
   - Replicates are required for validation.
   - Discarded human skin for these studies is limited.

3. Infiltrating mouse cells

   It has been shown that human grafts are quickly infiltrated with mouse cells, particularly given the preparation to induce the graft to "take." How these mouse cells might alter our phenotype in unpredictable ways also makes the model less relevant.

4. Wound state

   A xenograft is in a chronic "wounded state" given the preparation that is required for the graft to "take." This state is not equivalent to the scenario into which our cells are being transplanted.

PREPARATION

The fibroblasts are cultured, using standard methodologies, from a small punch biopsy that includes the epidermal and dermal layer taken from a volar (palm or sole) or a nonvolar skin site. During and after in vitro expansion, the fibroblasts are harvested and quality control tests are performed. Greater than 95% of the final suspension is composed of fibroblasts as most of the keratinocytes are removed in the initial stages of processing.

Once the final suspension reaches up to 37.5 million fibroblasts, the cell suspension is cryopreserved in freezing media containing human serum albumin, hetastarch and dimethyl sulfoxide (DMSO) (2.5% human serum albumin, 5% DMSO, 6% hetastarch in sterile saline) at a defined fibroblast cell concentration. Before clinical use, cells are thawed and then injected via an intradermal route into the autologous graft site within 24 hours. For Phase Two's intermittent dosing, each dose will be frozen separately and thawed the day of injection. The transplant volume will contain no more than 37.5 million cells in 0.75 mL of freezing media.

DOSE SPECIFICS

Dosing of our therapy has also provided challenges. We chose 37 million cells based on a number of considerations, many from our investigations in optimizing hair follicle neogenesis using mouse cells. It is important to note that this value may require some optimization. Lower dosages may be used, up to 37.5 million cells. We also propose to administer multiple doses intermittently over 30 days (see Schema, page 4). In the assay that closely parallels this work, we try to convert tissue identity and create hair follicle structures from single cell slurries of both keratinocytes and fibroblasts. In these efforts, we discovered a continuously enhanced rate of hair follicle creation with a higher amount of fibroblasts and keratinocytes used[11]. In our present effort, we are not injecting keratinocytes but using the native keratinocytes that are present and extend for a much greater area than the injection area. Therefore, to optimize our efforts, we wish to try an adequate number of fibroblasts in a small area to maximize proximity of the fibroblasts to the superiorly oriented epidermis.

We also have conducted in vitro ratio studies and determined that a 4:1 ratio of fibroblasts to keratinocytes was the optimum for KRT9 induction. Given that a keratinocyte is roughly 5 μm in width, a 4-mm punch biopsy would contain approximately 800 × 800 or 640,000 basal layer keratinocytes and there are roughly 4 layers of cells in the biopsy or 2,880,000 keratinocytes. For a 4-fold increase, this would be a minimum of roughly 7,200,000 fibroblasts. However, we assume that directly thawed fibroblasts will have a 5-fold decreased potency due to thawing and positional effects and, thus, we are interested in injecting around 36 million fibroblasts. Azficel-T injections also use a similar number of cells (18 million). Recent studies testing fibroblasts as therapies for bullous diseases used cells of the same order of magnitude[12].

ADMINISTRATION

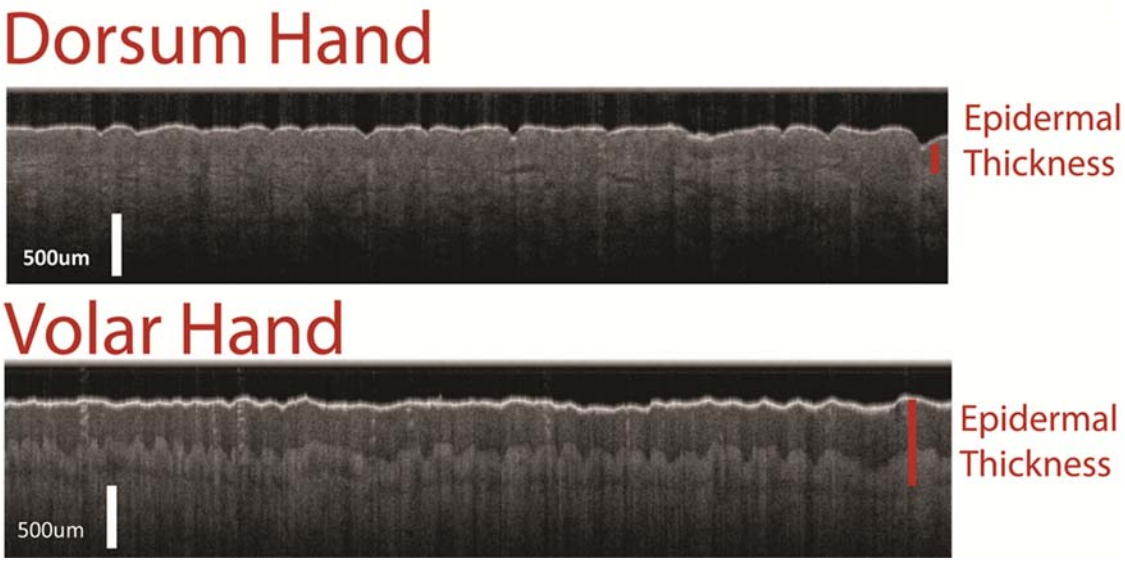
The mode of administration and site of inoculation is based on the finding that there is no known cell-to-cell contact between fibroblasts and keratinocytes. In normal skin anatomy, keratinocytes and fibroblasts are separated by the basement membrane zone. Nonetheless, the basis of this therapy is that fibroblasts dictate tissue identity, specifically keratinocyte phenotype. The mechanism of the interaction between fibroblasts and keratinocytes is not known. Therefore, our intent is to put our transplanted autologous fibroblasts in their most native environment in the dermis to promote our desired phenotype. It is for this reason that we are pursuing intradermal injection.

9. STATISTICAL METHODS

9.1. SAMPLE SIZE JUSTIFICATION

This is a feasibility study and thus sample size is limited due to the experimental nature of the effort. However, if KRT9 increases are as Figure 8 below, then the sample size is justified.

Figure 8: Optical Coherence Technology is non-invasive imaging to monitor for graft site epidermal thickening (images from Drs. Jin Kang and Xuan Liu of Johns Hopkins Department of Electrical and Computer Engineering).



9.2 ANALYSIS PLAN

The first method of analysis will happen during each participant's graft monitoring visits to determine whether they will be one of three "Early Graft Removal" subjects. Three epidermal thickness measurements will be calculated at each monitoring visit for each injection site. We will analyze this data to determine whether volar fibroblasts caused an increase in epidermal thickness. The means of determining whether an increase is statistically significant will be through a t-test statistical analysis of 3 independent readings from the three injection sites (no fibroblasts, non-volar fibroblasts, and volar fibroblasts). We will consider a p value less than 0.05 as significant. For a subject to be designated as an "early graft removal", he/she should have a statistically significantly elevated epidermal thickness in the volar injected site but not the non-volar or vehicle-only injected sites.

For statistical analysis at the conclusion of the study we will average multiple subject's values. We will perform a student's T-test comparing the values of epidermal thickness between at least 3 non-volar fibroblast injections and 3 volar fibroblast injection to determine a p value for a significant difference. We will consider a p value less than 0.05 as significant. We will do the same for KRT9 mRNA values. As more participants complete the study, we will repeat this calculation until our enrollment goal is met.

Power analysis: With a difference of mean assumed to be 2, standard deviation of all samples to be 0.5, alpha 0.06, power of 0.8 then a sample size of 3 participants will be sufficient and our goal will exceed this.

We will perform repetitive interim data analysis as each participant completes the final time point.

We will also calculate average areas of increased epidermal thickness.

9.2.1. PRIMARY OUTCOME VARIABLE

The primary outcome variable for this study will be how well the autologous fibroblasts are tolerated by the participants.

9.2.2 SECONDARY OUTCOME VARIABLES

Secondary outcome variables for this study will be 1) an increase in epidermal thickness as detected by optical coherence tomography in vivo, or by histology, and 2) an increase in KRT9 by RT-PCR, western or immunohistochemistry

9.3 EARLY STOPPING RULES

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

10. REGULATORY CONSIDERATIONS

10.1. CLINICAL TRIAL MONITORING

The PI will review study data on a quarterly basis and ensure that study documents are monitored at least annually for the duration of the study for data accuracy and completeness.

10.2. RECORDS TO BE KEPT

10.2.1 CASE REPORT FORMS (CRFS)

As used in this protocol, the term case report form (CRF) refers to either a paper form or an electronic data record or both, depending on the data collection method used in this study. A CRF is required and should be completed for each included participant.

The investigator has ultimate responsibility for the accuracy, authenticity, and timely collection and reporting of all clinical, safety, laboratory data entered on the CRFs and any other data collection forms. All CRFs must be signed by the investigator to verify that the data contained on the CRFs is accurate. Any corrections to entries made in the CRFs, source documents must be dated, initialed and explained (if necessary) and should not obscure the original entry.

Usually, source documents are the hospital's or the physician's participant medical chart. In these instances the data collected on the CRFs must match the data in the corresponding charts. A CRF, or part of the CRF, may also serve as a source document.

The PI will maintain a file of human subjects' research project documents including (at a minimum) the following items:

- A copy of the original human subjects research application submitted to the JHM-IRB
- A copy of the JHM IRB approved consent form.
- The original of each consent form signed by each participant enrolled in the research.
- A copy of all correspondence with the IRB and other regulatory institutions as appropriate
- A copy of all data derived from the study (case report forms, computer data, adverse event reports, drug/device accountability records etc.)

10.2.2. RECORD RETENTION

To enable inspections and/or audits from regulatory authorities, the investigator agrees to keep records, including the identity of all participating subjects (i.e. information to link records, e.g., CRFs and hospital records), all original signed informed consent forms, copies of all CRFs, serious adverse event forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports).

The study records will be retained by the investigator for 2 years after either 1) a marketing application is approved or 2) if an application is not approved for the agent, until 2 years after shipment and delivery of the biologic for investigational use is discontinued and the FDA so notified" [21 CFR 312.61(c)].

10.2.3. CLINICAL PHOTOGRAPHS

Photographs of cellular injection sites both before injection and after will be retained as scientific data. No identifying photographs will be taken. For example, no photographs of the face will be taken. All photos will be de-identified and will be maintained in a password protected areas.

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APPENDIX I- Phase One (Single Dose)

Visit/ Study Day:	Screening & Consent Day –14 Through Day –1	Baseline Visit 1 Day 0	Suture removal Visit 2 Day 15 (±3 days)	Graft Injection Visit 3 Day 30 (±10 days)	Graft Removal / monitoring Visit 4 Day 42 (±7 days)	Graft Removal / monitoring Visit 5 Day 70 (±7 days)	Graft Removal[3] / monitoring Visit 6 Day 98 (±7 days)	Graft Removal[3] / monitoring Visit 7 Day 126 (±7 days)	Graft Removal[3] / monitoring Visit 8 Day` 154 (±7 days)	Graft Removal Day 182 Visit 9 (±7 days)	Suture Removal[2] Day 196 Visit 10 (±7 days)	Final evaluation Day 273 Visit 11 (±14 days)
Informed consent	X											
Eligibility review	X	X										
Assessments												
Vital signs			X		X	X	X	X	X	X		
History & limited physical	X	X		X								
Medication Review	X											
Clinical Photographs		X		X	X	X	X	X	X	X	X	X
Biopsy/graft excision site assessment			X								X	X
Adverse events/symptom diary review		X	X	X	X	X	X	X	X	X	X	X
Optical Coherence Tomography					X	X	X	X	X	X		
Laboratory Testing												
HIV I Antibody	X											
HIV 2 Antibody	X											
HIV Nucleic acid	X											
HTLV I/II Assay (Abs)	X											
Serum HCG[1]	X[1]											
Urine HCG[1]				X[1]								
Hepatitis B Surface Ag	X											
Hepatitis B, Core antibody	X											
Hepatitis C nucleic acid	X											
Procedures												
Skin biopsy		X										
Suture removal			X								X[2]	
Investigational injections				X								
Excise 3 graft sites; dress graft sites[3]							X[3]	X[3]	X[3]	X		

[1]- Females of child bearing potential only
[2]- 14 days +/- 2 days after early graft removal OR study day 196 +/- 7 days
[3]- ONLY for the first three participants who exhibit statistically significant thickening based upon three OCT measurements

APPENDIX II- Phase Two (Intermittent Dosing Regimen)

Visit/ Study Day:	Screening & Consent Day –14 Through Day –1	Baseline Visit 1 Day 0	Suture removal Visit 2 Day 15 (±3 days)	Graft Injection Visit 3, 4, 5, 6 Day 30,37,42,59 (±10 days)	Graft Removal / monitoring Visit 7 Day 70 (±7 days)	Graft Removal / monitoring Visit 8 Day 98 (±7 days)	Graft Removal[3] / monitoring Visit 9 Day 126 (±7 days)	Graft Removal[3] / monitoring Visit 10 Day 154 (±7 days)	Graft Removal Day 182 Visit 11 (±7 days)	Suture Removal[2] Day 196 Visit 12 (±7 days)	Final evaluation Day 273 Visit 13 (±14 days)
Informed consent	X										
Eligibility review	X	X									
Vital signs			X		X	X	X	X	X		
History & limited physical	X	X		X							
Medication Review	X										
Clinical Photographs		X		X	X	X	X	X	X	X	X
Biopsy/graft excision site assessment			X							X	X
Adverse events/symptom diary review		X	X	X	X	X	X	X	X	X	X
Optical Coherence Tomography					X	X	X	X	X		
HIV I Antibody	X										
HIV 2 Antibody	X										
HIV Nucleic acid	X										
HTLV I/II Assay (Abs)	X										
Serum HCG[1]	X[1]										
Urine HCG[1]				X[1]							
Hepatitis B Surface Ag	X										
Hepatitis B, Core antibody	X										
Hepatitis C nucleic acid	X										
Skin biopsy		X									
Suture removal			X							X[2]	
Investigational injections				X							
Excise 3 graft sites; dress graft sites[3]						X[3]	X[3]	X[3]	X		

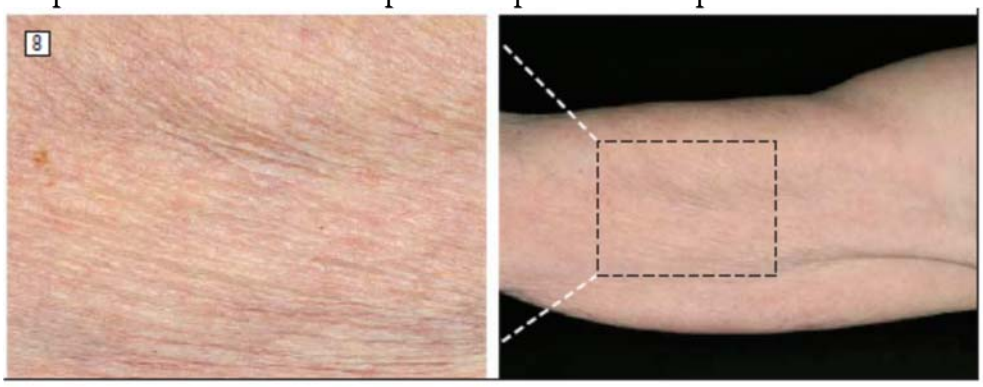
[1]- Females of child bearing potential only

[2]- 14 days +/- 2 days after early graft removal OR study day 196 +/- 7 days
[3]- ONLY for the first three participants who exhibit statistically significant thickening based upon three OCT measurements

APPENDIX II- PHOTOGRAPHY SOP

The study PI or other IRB approved study member will be performing photography in the clinic dark room which is adjacent to patient exam room. Nikon D300s digital handheld including a cross-polarized lens (Canfield, TF X-Polarizer Filter Kit).

1) Ensure patient is gowned or in tank top to ensure clothing is out of the field.
2) Use black backdrop in dark room.
3) Position the subject standing facing the photographer approximately 1 foot in front of the black backdrop.
4) Hold constant flash output and camera-to-arm distance (approximately >5 feet)
5) Ask subject to stand erect, to extend arm 90 degrees from trunk laterally, and to gently place their hand on the vertically placed chain rope.
6) Saved photos should have derm study number, subject number, subject initial, and other pertinent body site info in file name.
7) All photos will be saved on a password protected computer.



APPENDIX III- BASELINE BIOPSY SPECIMEN PREPARATION AND HANDLING

At Johns Hopkins Hospital, two skin biopsies will be taken to yield non-volar skin and volar skin.

1) The chosen biopsy sites will be cleaned as per Departmental Standard of care for skin biopsies which includes alcohol swabs.

2) Local anesthesia will be administered consisting of 1% lidocaine with epinephrine as per standard of care.

3) After allowing time for anesthesia to take effect, the punch biopsies 2-6mm in diameter by 2mm deep will be obtained from the preselected sites.

4) One stitch will be put in each biopsy site to aid healing.

5) The biopsies will be kept separate and placed into labeled sterile 50 ml tubes containing Dulbecco's Phosphate Buffered Saline (DPBS) containing penicillin (100 units/ml), streptomycin (100 ug/ml) and Amphotericin B (0.25 ug/ml) prior to room temperature transport to the Cell Therapy Laboratory. Labling of tubes will include the subject initials, unique participant ID, the date, the biopsy sites, and the identity (volar vs nonvolar).

6) The biopsies will be directly hand delivered to the Cell Therapy Lab and received by lab staff as per CTL protocol.

7) Upon receipt, each biopsy may be processed immediately or the tubes may be placed into a 2-8°C refrigerator for up to 24 hours.

8) Following this, fibroblast isolation will begin as per CTL protocol.

APPENDIX IV-

NA_00068684
BIOPSY SITE INFORMATION SHEET **PARTICIPANT NAME:**_____

PATIENT INSTRUCTIONS FOR BIOPSY SITE CARE

1. LEAVE YOUR WOUND DRESSINGS IN PLACE FOR THE REST OF THE DAY OF THE BIOPSY AND KEEP THEM DRY.
2. CHANGE BAND-AIDS DAILY STARTING THE DAY AFTER THE BIOPSY.
3. SHOWERS ARE FINE STARTING THE DAY AFTER THE BIOPSY. LEAVE THE BAND-AIDS IN PLACE WHILE YOU SHOWER AND CHANGE THEM AFTER YOU DRY OFF.
4. DURING THE TIME PERIOD OF DAILY BAND-AID CHANGES, DO NOT SOAK IN A BATH OR SWIM.
5. THE AVERAGE TIME FOR DAILY BAND-AID CHANGES IS 5 TO 6 DAYS (RANGE IS FROM 1 OR 2 DAYS UP TO 2 WEEKS).
6. BECAUSE WOUNDS ON THE FEET CAN EASILY BECOME INFECTED, CLEANING WITH HYDROGEN PEROXIDE IS PERMITTED. IF THE WOUNDS ARE FINE (I.E., NO SIGNS OF INFECTION), ALL THAT IS REQUIRED IS A DAILY BAND-AID CHANGE.
7. THE WOUNDS MAY OR MAY NOT FORM A SCAB AS THEY HEAL; EITHER WAY IS FINE.
8. CONTINUE TO CHANGE THE BAND-AID S DAILY UNTIL THERE ARE NO OPEN WOUNDS.
9. THE LOCAL ANESTHETIC USED FOR THE BIOPSY WILL USUALLY LAST FOR 1 TO 2 HOURS AFTER THE PROCEDURE. AFTER IT WEARS OFF, YOU MAY HAVE SOME MILD, LOCALIZED SORENESS AND TENDERNESS AT THE BIOPSY SITES OVER THE NEXT DAY OR TWO. YOU MAY FIND REGULAR TYLENOL IS HELPFUL FOR THE DISCOMFORT.
10. REFRAIN FROM DOING EXTREMELY STRENUOUS ACTIVITY FOR THE REST OF THE DAY OF YOUR BIOPSY (SUCH AS RUNNING OR HEAVY LIFTING).
11. ONCE YOU ARE WITHOUT THE BAND-AID, THE BIOPSY SITES MAY LOOK SLIGHTLY RED OR DARKER THAN THE REST OF YOUR SKIN. THIS DISCOLORATION WILL GRADUALLY FADE AND BLEND BACK WITH YOUR NORMAL SKIN COLOR. THIS FADING PROCESS MAY TAKE ANYWHERE FROM A FEW MONTHS UP TO A YEAR.
12. IT IS VERY RARE FOR PEOPLE TO HAVE ANY PROBLEMS DURING THE HEALING PERIOD. IT IS NORMAL FOR THE BIOPSY SITES TO BLEED A LITTLE BIT OR DRAIN PINK FLUID FOR A DAY OR TWO AFTER THE BIOPSIES. THEY SHOULD NOT BLEED EXCESSIVELY (I.E., THROUGH THE BAND-AID) AFTER THAT TIME. THEY SHOULD NEVER DRAIN PUS. IF YOU DO EXPERIENCE PROBLEMS WITH SIGNIFICANT BLEEDING, REDNESS, INFECTION, OR OTHER PROBLEMS, CALL YOUR DOCTOR'S OFFICE.

YOUR RETURN APPOINTMENT FOR SUTURE REMOVAL IS:

DATE: _____

TIME: _____

LOCATION: _____

STUDY TEAM CONTACT INFORMATION:

- **IF YOU EXPERIENCE RED STREAKS GOING UP YOUR LEG, PLEASE CONTACT THE STUDY TEAM IMMEDIATELY**

DURING BUSINESS HOURS MONDAY THROUGH FRIDAY 8 AM-5 PM, CALL 410-502-7546 TO SPEAK WITH SHERRY LEUNG. AFTER 5PM ON WEEKDAYS AND 24 HOURS ON WEEKENDS, CALL (410)955-6070 AND ASK TO HAVE THE DERMATOLOGY RESIDENT ON-CALL PAGED IMMEDIATELY.

APPENDIX V- CELL ADMINISTRATION SHEET

NA_00068684
AUTOLOGOUS FIBROBLAST ADMINISTRATION RECORD **PARTICIPANT NAME:** _____

DATE OF ADMINISTRATION: _____

#	INJECTION LOCATION	VIAL IDENTIFICATION NUMBER	TIME INJECTION BEGUN	TIME INJECTION COMPLETED	CELL DOSE
1					
2					
3					

ADMINISTERED BY: _____

DATE: _____

TIME: _____

APPENDIX VI- INJECTION SITE PATIENT DIARY

NA_00068684 INJECTION SITE PATIENT DIARY **DATE: _____** **PATIENT NAME:_____**

DATE	SITE LOCATION	SITE COLOR	SITE SIZE (e.g. Dime, nickel, etc.)	SITE DISCOMFORT (Use table for code)	PHYSICAL SYMPTOMS (i.e. Fever, headache, skin rash, etc)	COMMENTS
	1					
	2					
	3					
	1					
	2					
	3					
	1					
	2					
	3					
	1					
	2					
	3					
	1					
	2					
	3					

REACTION COLOR CODES		DISCOMFORT CODES	
A	NO REACTOIN	1	NO DISCOMFORT
B	LIGHT PINK	2	SWELLING
C	DARK PINK	3	ITCHING
D	**RED**	4	TENDERNESS
E	**PURPLE**	5	WARMTH
F	**OTHER**	6	OTHER

SITE DIAGRAM

APPENDIX VII- BASELINE BIOPSY SPECIMEN PREPARATION AND HANDLING

At Johns Hopkins Hospital, we will perform final biopsies to remove the skin from the 3 injection sites.

1) The chosen biopsy sites will be cleaned as per Departmental Standard of care for skin biopsies which includes alcohol swabs.

2) Local anesthesia will be administered consisting of 1% lidocaine with epinephrine as per standard of care.

3) After allowing time for anesthesia to take effect, the punch biopsies 3-6mm in diameter by 2mm deep will be obtained from the injected sites.

4) One stitch will be put in each biopsy site to aid healing.

5) The biopsies will be kept bisected with ½ placed in RNA-later and ½ placed in paraformaldehyde. Labeling of tubes will include the subject initials, unique participant ID, the date, the injection #, and the identity (vehicle, volar or nonvolar).

6) The biopsies will be directly hand delivered to the Garza lab.

7) Upon receipt, each biopsy may be processed immediately or the tubes may be placed into a 2-8°C refrigerator for up to 24 hours.

8) Following this, experiments to measure KRT9 expression and histology will begin.

APPENDIX VIII- POST-CELLULAR INJECTIONS INSTRUCTIONS

NA_00068684
INJECTION SITE INFORMATION SHEET PARTICIPANT NAME:_____

PATIENT INSTRUCTIONS FOR BIOPSY SITE CARE

1. KEEP YOUR DRESSINGS ON FOR 48 HOURS ON THE INJECTION SITES
2. DO NOT SCRATCH THE INJECTION SITES.
3. DO NOT PLACE ICE ON THE INJECTION SITES.
4. DO NOT PLACE ANY TOPICAL CREAMS OR REMEDIES ON THE INJECTION SITES.

STUDY TEAM CONTACT INFORMATION:

- **IF YOU EXPERIENCE ANY PROBLEMS AT YOUR INJECTION SITES, PLEASE CONTACT THE STUDY TEAM IMMEDIATELY** AS FOLLOWS:

DURING BUSINESS HOURS MONDAY THROUGH FRIDAY 8 AM-5 PM, CALL 410-502-7546 TO SPEAK WITH SHERRY LEUNG. AFTER 5PM ON WEEKDAYS AND 24 HOURS ON WEEKENDS, CALL (410)955-6070 AND ASK TO HAVE THE DERMATOLOGY RESIDENT ON-CALL PAGED IMMEDIATELY.