IND Application Section 6: Protocols

Protocol Title: A Phase 1 Single Center Trial of Gene Transfer for Recessive Dystrophic Epidermolysis Bullosa (RDEB) using the drug LZRSE-Col7A1 Engineered Autologous Epidermal Sheets (LEAES).

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1. Abbreviations List:

AEs: adverse events (defined in section 16) and in Safety Monitoring Plan
ALT (SGPT): alanine aminotransferase, included in metabolic panel
AST (SGOT): aspartate aminotransferase, included in metabolic panel
BCIP/NBT: 5-bromo-4-chloro-3'indolyphosphate p-toluidine salt and nitro-blue tetrazolium chloride, substrate used in Stanford Dermatology Research Lab
CBC: complete blood count
cDNA: complementary deoxyribonucleic acid
CITI: Collaborative IRB Training Initiative
CLIA: Clinical Laboratory Improvement Amendments
COL7A1: Collagen 7 gene
CTC: Common Toxicity Criteria
DDEB: dominant dystrophic epidermolysis bullosa
DEB: dystrophic epidermolysis bullosa
DIF: direct immunofluorescence
D-MEM: Dulbecco's Modified Eagle Medium, media used in Stanford Dermatology Research Lab
DNA: deoxyribonucleic acid
DSMB: data safety monitoring board
EB: epidermolysis bullosa
EBA: epidermolysis bullosa acquisita
ECG: electrocardiogram
eCRF: electronic case report forms
EDC: electronic data capture
EDTA: ethylenediamine tetraacetic acid

ELISA: enzyme-linked immunosorbent assay
EM: electron microscopy
EMLA: Eutectic mixture of local anesthetic
FACS: Fluorescence-activated cell sorting
FCS: fetal calf serum, used in Stanford Dermatology Research lab
FDA: Food and Drug Administration
HIPAA: Health Insurance Portability and Accountability Act
HIV: human immunodeficiency virus
IAW: in accordance with
ICF: informed consent form
IEM: immunoelectron microscopy
IF: immunofluorescence
IIF: indirect immunofluorescence
IND: Investigational New Drug application
IP: Internet Protocol
IRB: Institutional Review Board
IUVPF: Indiana University Vector Production Facility
Keratinocyte-SFM: Keratinocyte serum free media, used in Stanford Dermatology Research Lab
KGM: keratinocyte growth media, used in Stanford Dermatology Research Lab
LEAES: LZRSE-Col7A1 Engineered Autologous Epidermal Sheets
LLN: Lower limit of normal
LPCH: Lucile Packard Children's Hospital
MCH: mean corpuscular hemoglobin, included in complete blood count
MCHC: mean corpuscular hemoglobin concentration, included in complete blood count
MCV: mean cell volume, included in complete blood count
MOOP: Manual of Operating Procedures
mRNA: messenger ribonucleic acid
NC1: non-collagenous region 1 of the collagen 7 molecule
NCI: National Cancer Institute
NIH: National Institute of Health
OD: optical density
PCP: primary care practitioner
PCR: polymerase chain reaction
PHI: protected health information
qPCR: quantitative polymerase chain reaction
qRT-PCR: quantitative real time polymerase chain reaction
R01: NIH grant funding mechanism
RAC: Recombinant DNA Advisory Committee
RCR: replication competent retrovirus
RDEB: recessive dystrophic epidermolysis bullosa
RDW: red blood cell distribution width, included in complete blood count
REDCap: Research Electronic Data Capture
RNA: ribonucleic acid
SCC: squamous cell carcinoma
VPN: Virtual private network
WBC: white blood cell
WNL: within normal limits

2. Introduction:

2.A. Objective:

The primary objective of this protocol is to evaluate the safety of autologous skin grafts transduced with a retroviral vector containing the gene encoding type VII collagen (LEAES) in subjects with RDEB.

2.B. Purpose:

The purpose of this study is to achieve proof-of-concept for this general approach to cell-based gene therapy in humans and to set the stage for further therapeutic extension in RDEB.

2.C. Protocol summary:

Recessive dystrophic epidermolysis bullosa (RDEB) is a severe inherited blistering skin disease caused by absence of a protein known as type VII collagen. Patients with RDEB develop large, severely painful blisters and open wounds from minor trauma to their skin. This trial will create a graft, which we call "LEAES," of the patient's own skin that has been genetically engineered in our lab to

express this missing protein. We will basically take a subject's own cells, correct them in culture, and then transplant the corrected cells back onto them.

2.D. Study end points:

LEAES grafts will be evaluated at 12 weeks, 25 weeks, and 52 weeks after grafting for expression of type VII collagen and presence of anchoring fibrils. Secondary endpoints include evaluation at 12 weeks, 25 weeks, 52 weeks, and yearly thereafter for appearance, durability, and ease of blistering. Subjects will continue to be followed for safety in a separate long-term follow-up protocol under this IND.

2.E. History of protocol and oversight:

Stanford's Administrative Panel on Human Subjects in Medical Research, also called the Institutional Review Board (IRB), and the Administrative Panel on Biosafety will review all protocols and processes related to this study.

We obtained IRB approval and began the screening process on August 7, 2007 (IRB protocol # 8557, ClinicalTrials.gov, Identifier NCT00533572). We consented, biopsied and collected blood to screen specifically for gene transfer on one subject under this protocol in March 2008. At the request of the Food and Drug Administration (FDA), we ceased all screening procedures for gene transfer on June 6, 2008 (Protocol Amendment 1). Protocol 8557 has since been closed and no additional subjects have been enrolled.

We subsequently changed the process for subject selection for gene transfer. We will now select candidates for the gene transfer trial from a pool of subjects who have completed a separate research study on the characteristics of EB patients. These “characteristics” protocols were approved by the Stanford IRB (protocols 17158 and 15898), and this has been communicated with the FDA (Protocol Amendment 2). We will refer to the “Characteristics” protocol as “Pre-screening” throughout this document to determine eligibility for gene transfer. Protocol 15898 was closed in May 2014.

We initially submitted our IND application to the FDA in May 2008. We were placed on “clinical hold” in June 2008, as the FDA requested additional information. The clinical hold was removed August 28, 2009. We have made two protocol revisions (including this document). A list of the changes to the clinical protocol is included with this amendment. A table of our IND protocol amendments and a listing of the dates that they were submitted to the FDA is below:

Table 1: IND Amendments for RDEB Gene Transfer Clinical Protocol

	Date submitted	Comments
Initial IND Application	May 2008	Placed on “Clinical Hold”
IND Amendment 1	June 2008	Halting of screening procedures
IND Amendment 2	Jan. 2009	Notification of planned Characteristics studies
IND Amendment 3	July 2009	Response to “Clinical Hold.” Clinical hold removed Aug. 28, 2009.
IND Amendment 4	July 2009	Response to FDA Comments.
IND Amendment 5	Oct 2010	IND Annual report and Amendment 5, to reconcile FDA protocol with IRB approved protocol 14563
IND Amendment 6	October 2011	Updated protocol including suggestions from NIAMS, anesthesiology.
IND Amendment 7	November 2011	Additional information about 9CFR testing provided, per FDA request
IND Amendment 8	October 2012	IND Annual Report, replacement products in CMC
IND Amendment 9	December 2012	Updated 1572 with new investigator address

IND Amendment 10	December 2012	Additional sterility testing, added possibility of hospitalization post-grafting
IND Amendment 11	January 2013	Re-submission of documents from Amendment 10, with tracked changes and final versions
IND Amendment 12	July 2013	Addition of C1 mimetic to CMC, addition of biopsy for FACS to clinical protocol
IND Amendment 13	September 2013	2013 IND Annual Report
IND Amendment 14	February 2014	Revision of wound dressing protocol, grafting protocol, CMC revision
IND Amendment 15	June 2014 (this amendment)	Removal of C7 antibodies by ELISA as exclusion criteria, replaced by IDIF and DIF analysis; removal of parental mutation confirmation
IND Amendment 16	September 2014	2014 IND Annual Review
IND Amendment 17	January 2015	Change of PI from Alfred Lane to Jean Tang
IND Amendment 18	October 2015	2015 IND Annual Review

3. Name and address and statement of qualifications of each investigator:

This information is included in Form 1572

4. Criteria for subject selection:

The information below defines the inclusion and exclusion criteria for this study. Some RDEB subjects may already have documented results for some of the testing listed below. At the discretion of investigators and the EB physician, we may elect not to repeat previous testing that is documented and meets the screening requirements listed below. This would be in order to limit the number of skin biopsies and quantity of blood required from the subject.

4.A. Number of subjects:

We plan to graft 5 adult subjects under this protocol.

4.B. Characteristics Studies (Pre-Screening):

Under IRB approved pre-screening protocols 15898 (ClinicalTrials.gov Identifier NCT00533572) and 17158 (ClinicalTrials.gov Identifier NCT01019148), we have completed an initial phone screen or discussion in clinic for over 100 subjects interested in our clinical trials, as part of a research study in which we are seeking to determine the characteristics of patients with dystrophic EB. However, this information will also help us to identify a candidate for gene transfer.

The reason that two protocols existed for the purpose of determining the characteristics of DEB patients is that these two protocols have slightly different inclusion/exclusion criteria and are funded by different sources. As of May 2014, protocol 15898 is closed.

4.B.i. Plan for recruitment:

Subjects are currently being evaluated for their characteristics (pre-screening) through IRB Approved Protocol 17158. Based on the results of the pre-screening, subjects may meet the criteria to enroll in the gene transfer study. If they meet the criteria, they will be invited to participate in the gene transfer protocol. There may be subjects in the pre-screening studies who do not meet criteria to enroll in gene transfer, in which case they would not be invited to participate in the gene transfer trial.

We cooperate with national and international networks of families, researchers, and physicians who care for children and adults with EB and plan to use these groups to recruit

subjects for this study. In addition we will use our email listserv (ebcarenetwork@lists.Stanford.edu) to inform our national communities that we are recruiting for this study. Our study is also listed on http://www.ClinicalTrials.gov (CT.gov Identifier NCT01263379) and http://clinicaltrials.stanford.edu. We initially will limit enrollment to subjects from the USA. Similar information is posted on the Department of Dermatology Epidermolysis Bullosa webpage called Stanford EB Research Update at http://dermatology.stanford.edu/research/research.html. Our updates for the listserv and the website have been approved by the Stanford IRB.

We have a database of people who have contacted us regarding our epidermolysis bullosa research. Each one has completed a phone screen through the Characteristics study, in which they have requested to be added to a database of people contacted about future studies. A letter (approved by the Stanford IRB) will be mailed to them to inform them that we are recruiting for the gene transfer study and letting them know about our process for recruitment.

4.B.ii. Identifying subjects eligible for gene transfer (based on Pre-Screening):

A specific subset of individuals with RDEB will be selected for this clinical trial. RDEB subjects will initially be required to express the NC1 amino-terminal fragment of collagen VII (NC1[+], approximately 75% of our patients), be genotyped with confirmed recessive COL7A1 mutations, and have no evidence of an immune response to type VII collagen. .

As described in the Introductory Statement (Section 3-4) of the original IND application and the IND Annual Report, depending on the mutations involved, some RDEB patients express the amino-terminal fragment of type VII collagen (NC1[+]) and some do not (NC1[-], approximately 25% of our patients). [1] As the NC1 domain is generally accepted to be the most antigenic region on the type VII collagen molecule, we expect that NC1[+] subjects will be less likely to develop autoimmune reactions to sites of grafted autologous keratinocytes that express type VII collagen since their immune system should have already become tolerant to NC1 epitopes.

Non-CLIA data will not be shared with the subject, except as described below. Clinically relevant non-CLIA results may be included in the subject's medical record if investigators and the EB physician feel that they are important for the subject's medical care.

4.B.iii. Procedures performed under characteristics study (Pre-screening):

Subjects who arrive at Stanford for pre-screening will be examined to confirm the clinical diagnosis of RDEB. We will examine the subject's medical records to determine which testing has been performed previously. In the pre-screening study, we will obtain a complete history, perform a physical and skin examination, as well as obtaining photographs of the subject's skin. We may elect not to repeat previous testing that is documented and meets our screening requirements in order to limit the number of skin biopsies and quantity of blood required from the subject.

4.B.iii.1. Skin biopsies:

Subjects will be asked to donate 5 skin biopsies from non-wounded skin:
- Two 6mm biopsies, for tissue culture, to determine NC1 status (described below)
- One 3 mm biopsy will be sent for Immunoelectron Microscopy (IEM): This test will screen for type VII collagen by IEM using gold labeled mAb LH24 antibody which recognizes the collagenous region near the NC2 domain of type VII collagen (Gift of Dr. I. Leigh).[2]
- One 4 mm biopsy for indirect and direct immunofluorescence (IIF and DIF, respectively): The biopsy tissue will be screened for multiple epidermal and BMZ antigens (collagen XVII [BP180], collagen IV, collagen VII, laminin-332 gamma 2 chain) which should be positive and also for LH 7.2 mAb which recognizes the NC1 portion of

type VII collagen.[3,4] This biopsy will also be analyzed by direct IF for presence of IgG, IgM, IgA, and complement at the basement membrane.
- One 3 mm biopsy for electron microscopy (EM): This test will evaluate anchoring fibrils, to confirm the diagnosis of dystrophic EB. The EM biopsy should show absent or significantly defective anchoring fibrils.

The Stanford University Dermatopathology Laboratory is CLIA certified (http://dermatopathology.stanford.edu/services/epiderm.html) to perform the IIF, DIF and EM diagnostic tests. Physicians in the Departments of Dermatology and Dermatopathology at Stanford University have extensive experience in immunomapping and EM diagnosis for EB.

4.B.iii.1.a. Assessment of NC1 status:

As the NC1 domain is generally accepted to be the most antigenic region on the type VII collagen molecule, we expect that NC1[+] subjects will be less likely to develop autoimmune reactions to sites of grafted autologous keratinocytes that express type VII collagen since their immune system should have already become tolerant to NC1 epitopes. We are concerned that NC1[-] RDEB subjects may have a higher risk of developing autoimmune reactions at sites of grafted autologous keratinocytes that express type VII collagen because NC1 may represent a previously immunologically "unseen" neoantigen. In order to decrease this potential risk, the initial RDEB subjects will be NC1[+].

For those subjects with confirmed RDEB, IF microscopic analysis is not sensitive enough to document or exclude the expression of NC1 protein. Determining NC1 expression will be accomplished by culturing the keratinocytes and extracting the NC1 protein.[1] The skin biopsies obtained for culture will be placed in keratinocyte media containing KGM (Keratinocyte-SFM, Invitrogen Corporation, Carlsbad, CA). Skin biopsies will be washed 3 times in PBS with antibiotics/antimycotics and cut into pieces not bigger than $1cm^2$. Epidermis will be then separated by incubation in 50 caseolytic units/ml dispase (Invitrogen) for 2 hours at 37°C. After incubation in 0.25 mg/ml trypsin/EDTA (Invitrogen Corporation) for 30 minutes at 37°C, a single cell suspension of keratinocytes will be released by gentle pipetting. After neutralization with Dulbecco's Modified Eagle Medium (D-MEM, Invitrogen) with 10% Fetal Calf Serum (FCS, Omega Scientific, Tarzana, CA) cells will be cultured in KGM in cell culture plate at 37°C in a humidified atmosphere. Keratinocyte extracts will be prepared and subjected to denaturing gel electrophoresis on a 6% polyacrylamide gel. After electrophoresis, protein will be transferred to nitrocellulose membrane and incubated with rabbit anti-FNC1 antibodies to human type VII collagen[1], and the mAbs NP 32 and NP185 (gift of Lynn Y.Sakai) to detect NC1 presence[2].

Subjects who have retained expression of NC1 on immunoblots will be considered NC1[+] and subjects who do not have retained expression of NC1 on immunoblots will be considered NC1[-]. Immunoblot of subject cell extracts will be compared side by side with the cell lysates of our previously published NC1[+] patients cells[1]. To be considered for the study, the subject's cells must show an intensity of NC1 staining by densitometry equal to or greater than 25% of the mean of our published cells.

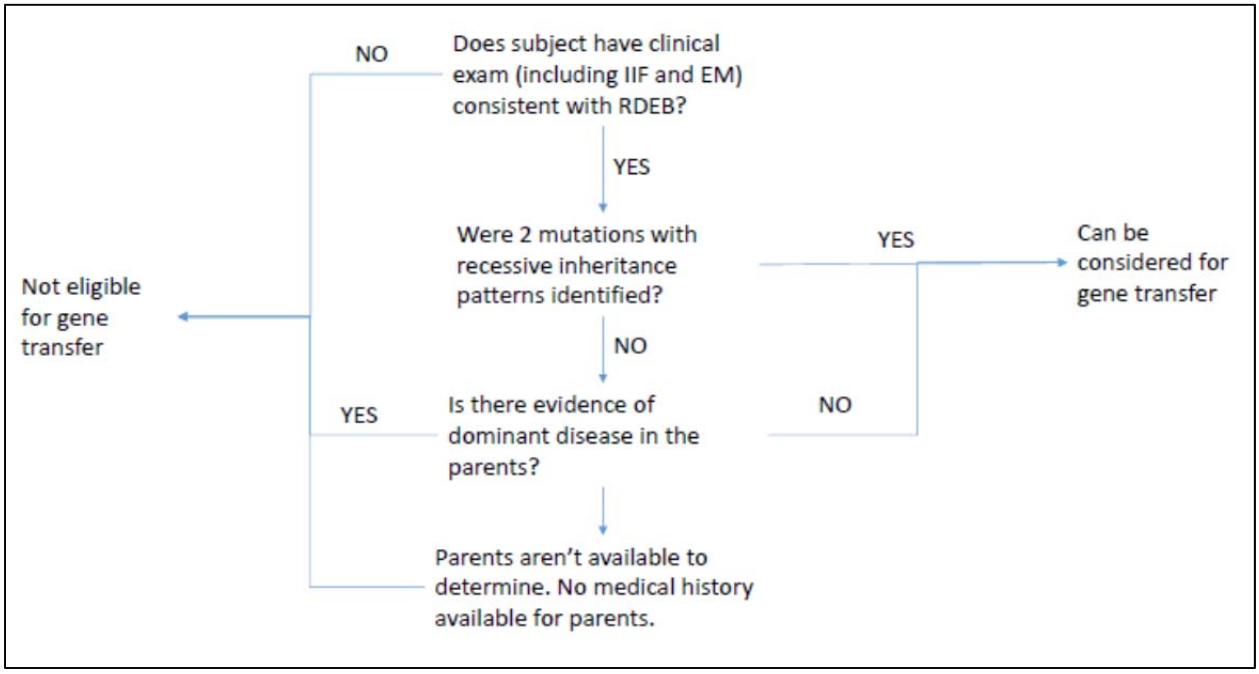
4.B.iii.2. Blood tests:

At the time of the diagnostic skin biopsies, blood will be drawn for the following CLIA tests:

- Complete Blood Count (CBC)
- Complete Metabolic Panel
- Direct and Indirect Bilirubin
- HIV test
- Hepatitis B surface antigen screening
- Hepatitis C antibodies
- IIF on monkey esophagus to rule out circulating antibodies to the basement membrane.
- Genetic testing for COL7A1 mutations (GeneDx, Gaithersburg, MD).

If genetic testing doesn't demonstrate two mutations that have recessive inheritance patterns, we will follow the algorithm depicted in Figure 1.

Figure 1: Parental Genetic Testing Algorithm



4.B.iv. Inclusion/Exclusion Criteria:

Inclusion Criteria for Gene Transfer and Autologous Grafting with LEAES:

1. Clinical diagnosis of RDEB
2. Age 18 years or older, willing and able to give consent
3. Confirmation of RDEB diagnosis by IIF and EM
4. NC1[+]
5. mAb LH24 antibody staining negative, or significantly decreased
6. Two confirmed RDEB type VII collagen mutations with recessive inheritance patterns (or confirmation that parents don't have any evidence of dominant disease)
7. At least 100 to 200cm^2 areas of open erosions on the trunk and/or extremities suitable for skin grafting
8. Able to undergo adequate anesthesia to allow grafting procedures to take place

Exclusion criteria for Gene Transfer and Autologous Grafting with LEAES:

1. Medical instability limiting ability to travel to Stanford University Medical Center
2. The presence of medical illness expected to complicate participation and/or compromise the safety of this technique, such as active infection with HIV, hepatitis B or hepatitis C
3. Evidence of immune response to type VII collagen
4. Active infection in the area that will undergo grafting
5. Evidence of systemic infection
6. Current evidence or a history of squamous cell carcinoma in the area that will undergo grafting
7. Active drug or alcohol addiction
8. Hypersensitivity to vancomycin or amikacin
9. Receipt of chemical or biological study product for the specific treatment of RDEB in the past six months
10. Positive pregnancy test or breast-feeding
11. Clinically significant medical or laboratory abnormalities as determined by investigators and the EB physician

4.B.v. Day -26:

If initial screening criteria have been met, we will ask the subject to return to Stanford to continue the screening procedure and to begin the process of culturing their cells for gene transfer. Subjects will be allowed to bring a companion in order to assist with travel procedures as well as dressing changes. If the subject does not have a companion, or the companion is not able to stay the entire time the subject is at Stanford, we will attempt to provide nursing services.

The time of this visit will be 20-35 days prior to grafting. For simplicity, we will refer to this timepoint throughout the protocol and the consent as “Day -26.” The time variability is related to the speed at which the cultured keratinocytes can grow. It is possible that a subject may not meet criteria (e.g. abnormal labs, unable to undergo anesthesia, cells do not grow, etc.) and would be considered a “screen fail.” Please note that the subject will be considered “enrolled” in the gene transfer study on Day 0, when they receive the LEAES graft.

When the subject comes to Stanford, we will review the gene transfer consent with them. They will have received a copy in the mail before the appointment, so they will have ample time to review it, discuss it with their primary care physician, etc.

4.B.v.1. Skin biopsies for LEAES manufacture

Two 8 mm punch biopsies will be obtained from non-blistered skin for keratinocyte culture, in order to manufacture the LEAES graft. The manufacturing aim is to produce and deliver four to six of the 40 cm^2 to 50 cm^2 sheets for grafting (LEAES). Approximately two to six of the 40-50 cm^2 epithelial sheets will be used in a single grafting session. The maximum total grafting surface area for all the graft sites will be 300 cm^2. Skin biopsies for LEAES manufacture will be labeled with the subject’s name, date of birth, medical record number, and study number.

We anticipate a maximum of 3 grafting sessions over the course of six months. Subjects who do not have initial graft attachment because of wound infections or mechanical causes will have the option (upon approval by the EB physician) of receiving additional grafts in the future prepared from frozen keratinocytes or a new biopsy if necessary.

We will graft two types of wounds. One site will be an acute wound (induced approximately 24 hours prior to grafting, at Day -1, see section 4.B.viii) not to exceed 40-50 cm^2, produced by inducing a blister on intact skin and removing the blister roof just prior to grafting. The other sites will be areas of chronic wounds of approximately 25-50 cm^2 or

greater that have been prepared for grafting. We plan to graft multiple areas of chronic wounds or possibly one large wound with several 40 cm^2 to 50 cm^2 sheets (see Day 0, section 5.A). LEAES grafts will be labeled with the subject's name and study number.

4.B.v.2. Research biopsies

In addition the following biopsies may be obtained from non-blistered, non-traumatized skin near where one of the wounds may be grafted. The biopsies obtained at screening will be used as controls for later biopsies.

We will obtain the minimum number of biopsies necessary; some may be excluded at the discretion of investigators and the EB physician. At this timepoint, we may obtain:

- one 4 mm biopsy for IIF for BMZ antigens to be examined in our research laboratory
- one 4mm biopsy for DIF for immunoreactants in the skin;
- one 4 mm punch biopsy for FACS;
- one 6mm punch biopsy for molecular analysis
- one 3 mm biopsy for EM
- one 3mm biopsy for IEM

4.B.v.2.a. Immunoelectron microscopy (IEM) and electron microscopy (EM):

Both 3 mm biopsies will be shipped over night to one of our collaborators at Shriner's Hospital in Portland, OR who will do the research immunoelectron microscopy (IEM) and electron microscopy (EM).[5] IEM will be performed on one of the 3 mm biopsies using gold-labeled LH24 antibodies to detect and localize delivered type VII collagen protein. Evidence for blistering on an ultrastuctural level will be examined. EM will be performed on the other 3 mm biopsy to determine presence of anchoring fibrils.

4.B.v.2.b. Immunofluorescence (IF), FACS and molecular analysis:

IF analysis for expression of type VII collagen will be performed on one 4 mm biopsy.

Another 4mm biopsy may be used for FACS analysis. FACS analysis will consist of separating T regulatory cells from the skin samples, followed by PCR analysis of their cytokine profile. The investigator may decide to obtain an additional biopsy for the FACS analysis. This sample will be sent to collaborators at UCSF for analysis.

We may do one 6 mm punch biopsy for molecular analysis which will be placed in keratinocyte media containing KGM (Keratinocyte-SFM, Invitrogen Corporation, Carlsbad, CA). This biopsy may be flash frozen in liquid N_2 for future analysis. Analysis will include qPCR to quantify the amount of vector DNA present and qRT-PCR to detect mRNA expression of type VII collagen.

These biopsies will be analyzed in the Stanford Dermatology research lab. The molecular analysis biopsy is not required by the FDA but it was strongly suggested by the NIH review and it may be omitted at times that the investigators think it would add less information (subject to approval of the EB physician).

4.B.v.3. Blood and urine tests

At this time we will also obtain blood for a CBC, Complete Metabolic Panel, and Hepatic Function Testing. If follow-up is required clinically, additional blood tests may be obtained as needed, at the discretion of the EB physician. We will obtain a sample to use as a baseline test to assay cytotoxic T cells (see below). We will also send a blood sample as a baseline test for replication competent retrovirus (RCR) to Indiana University Vector Production Facility

(IUVPF). A blood sample may be obtained for IIF on monkey esophagus analysis to rule out an immune response to type VII collagen, if the IIF test has not been completed recently.

On female subjects of childbearing potential, we will perform a urine pregnancy test to confirm that they are not pregnant.

4.B.v.3.a. Cytotoxic T cell assay

15 mL of whole blood will be collected for the cytotoxic T cell assay. Studies are currently being conducted to determine if a smaller amount of blood can be used for the assay. Testing will be conducted in the Stanford Dermatology research labs. Samples will be labeled with subject number and initials and the date the sample was obtained.

Isolation of T lymphocytes and monocytes from peripheral blood (15mL): Peripheral blood mononuclear cells are isolated from buffy coats or whole blood using Ficoll-Paque (GE Healthcare) density-gradient centrifugation. Adherent monocytes are then recovered after a 2-hour incubation in Petri dishes. $CD4^+$ and $CD8^+$ T lymphocytes are purified together using MACS magnetic cell sorting kits (Miltenyi Biotech), by incubating the nonadherent cells with anti-CD4 and anti-CD8 antibodies conjugated to paramagnetic microbeads.

Th1 (IFN-γ) and Th2 (IL-4) ELISPOT: Ninety-six-well PVDF-filter plates (Millipore) are coated with monoclonal antibody against IFN-γ (BD Pharmingen) or IL-4 (BD Pharmingen), blocked using RPMI medium with 5% human AB serum, and washed with serum-free RPMI. $CD4^+$ and $CD8^+$ T lymphocytes (2×10^5 cells/well), and γ-irradiated monocytes (2.5×10^4 cells/well) are co-incubated on the plate in the presence of 20 UI/ml IL-2 for 40 hours at 37°C, in a humidified, 5% CO_2 in air incubator. The medium contains either 10 µg/ml of recombinant type VII collagen or 3 µg/ml of concanavalin A (Sigma) to stimulate the lymphocytes. The plates are washed and the IFN-γ or IL-4 secreted by individual cells are detected in situ by successively reacting each well with (1) biotinylated anti-IFN-γ or anti-IL-4 monoclonal antibody (BD Pharmingen) at 1 µg/ml, (2) a 1:1000 dilution of streptavidin-conjugated alkaline phosphatase (Roche), and (3) a solution of BCIP/NBT chromogenic substrate (Promega), with suitable intermediate washes. The reaction is halted by washing with water, and spots are counted using a CTL ELISPOT reader. Negative controls are run in parallel using T cells without antigen, and the corresponding scores are subtracted from those of the unknowns.

4.B.v.4. Selection of target chronic wounds

At the first screening visit (Day -26) we will select multiple potential wounds for grafting. At the discretion of investigators, target wound selection may occur at a later visit. We will follow these wounds clinically until the day of grafting/enrollment (Day 0). The decision on which sites to be grafted will not be finalized until Day 0. The determination that target wounds meet all grafting criteria will be made at that time.

The grafted wound areas will be selected by several criteria. The wounds should appear clean with adequate granulation tissue, adequate vascularization, and not appear infected. The surface area should have a smooth texture that can accept a graft.

The duration that the subject thinks that they have each wound will be recorded, but often the subject is unsure of the duration of wounds at specific sites. We believe that the appearance and characteristics of the wound site are more important than the duration as many of the ulcerated areas have been wounded for many years.

The wounds will also need to meet mechanical requirements that decrease trauma to the grafted areas. Joints that are mobile may stretch the graft with movement and force the graft

to tear off before it has adequately attached. Areas that are exposed to constant sheering trauma may limit the ability of the graft to adhere before it has firmly attached. In addition, the areas to be grafted must also be easily covered with effective wound dressings. Sites that cannot be adequately protected by effective wound dressing will be excluded.

Appropriate sites will be on the anterior and/or lateral trunk and/or upper and/or lower extremities in areas protected from frequent trauma or injury. Excluded areas will usually include the face, areas close to mucous membranes (genito-urinary, oral or anal mucosa). Areas over joints and on the back are frequently areas of chronic non-healing wounds. We will consider grafting these areas if we are capable of immobilizing the grafts onto the areas long enough for securing effective graft attachment. The grafting surgeon, investigators, and the EB physician will jointly decide if areas over the joints, the back, and buttocks may be appropriate for grafting on a case-by-case basis. The distance from objective body landmarks will be identified and measured.

4.B.v.5. Assessment of chronic target wounds

The health of target chronic wounds will be determined by investigators and the EB physician. Each wound will be examined for signs of inflammation and infection, and the quality of granulation tissue will be assessed. Wounds appropriate for grafting have a clean, vascularized, nonexudative wound bed, appearing clinically as granulation tissue without drainage. We will avoid any areas that may already contain potential SCC even if that area has been previously biopsied and the biopsy did not document SCC.

Bacterial cultures will be obtained from several wounds for culture and antibiotic sensitivities. Wound cultures may be repeated as needed based upon standard of care and medical judgment of the investigators and EB physician. Chronic wounds in patients with RDEB are oftentimes colonized with bacteria even in the absence of clinical infection. Thus, the diagnosis of an infected wound or cellulitis is made clinically while taking into account the results of the wound culture. Systemic antibiotics will be prescribed for wounds with cellulitis or excessive exudates, with antibiotic choice determined by culture results and judgment of the investigators and EB physician.

The subject may remain in housing near Stanford between Day -26 and Day -7 if specific medical treatment or wound care is needed to prepare for the grafts. They may need to have this treatment at an outside hospital. If additional treatment is not needed, the subject may return home and then come back to Stanford in time for Day -7. This decision will be made by the EB physician.

4.B.v.6. Photographs and wound measurements

Digital photographs will be taken of each site, including a centimeter (cm) scale, to clearly document the location and size of each estimated treated area. A color chart may be included in photographs in order to standardize colors in the photographs. We will also measure the area of each wound using the ARANZ SilhouetteStar digital device, which uses a series of lasers to accurately determine wound measurements, or with the Canfield Vectra system which is able to produce 3D images.. Data captured by the camera systems is stored to track wound progress over time, with easy visualization of the wounds, and calculation of wound progression. We will also measure the distance from the center of the target wound to at least 2 body landmarks (i.e. bony prominence, freckle, etc.).

4.B.v.7. Anesthesia and Plastic Surgery consults, ECG, Echocardiogram

The subject and possibly investigators will meet with an anesthesiologist and plastic surgeon prior to grafting. The plastic surgeon will be the physician who will place the skin

grafts. We will perform an echocardiogram and/or an ECG to determine the cardiac function in preparation for grafting and anesthesia. Special precautions for EB patients will be taken when undergoing the ECG (i.e. use of Mepitel instead of the usual leads), as is commonplace at Stanford/Lucile Packard Children's Hospital. We may also request a cardiology consultation if the ECG or echocardiogram is abnormal.

Additionally, the anesthesiologists will request and review the subject's previous anesthesia records, and their intubation history.

If, in the opinion of the anesthesiologists, cardiologists, grafting surgeon, and/or EB physician, the subject will not be able to undergo adequate local anesthesia, general anesthesia or conscious sedation, the candidate would be considered a screen fail.

In order to limit unnecessary biopsies, we may choose to have subjects undergo these non-invasive tests prior to performing the skin biopsies described in 4.B.v.1 and 4.B.v.2.

4.B.v.8. Adverse event reporting and assessment

We will provide the subject with a paper diary, in which to record all adverse events (AEs). This diary will be reviewed with subjects at every study visit. The diary will be approved by the Stanford IRB. We will also send a letter (approved by the Stanford IRB) to the subject's primary care provider requesting that they inform us immediately of adverse events or any other problems that the subject experiences. We will ask subjects to begin recording adverse events after their first screening visit to Stanford.

At the time that investigators are informed of adverse events, they will be assessed for severity, etiology/causality, and expectedness. Adverse events will be graded according to the National Cancer Institute's Common Toxicity Criteria. At the first screening visit, subjects will be assessed for baseline medical conditions according to these criteria. Any increase in the grade of the condition will be considered an adverse event. Subjects with Grade 2 abnormalities identified prior to Day 0 (other than those specified as exceptions in the Inclusion/Exclusion criteria or by the EB physician) will not receive LEAES grafts and will be considered a "screen fail".

Abnormal laboratory values will be assessed for clinical significance, based on the investigator's judgment, subject to review by the EB physician. Clinically significant, abnormal lab values identified after the subject's initial visit will be captured as AEs, and graded according to the NCI toxicity criteria. EB patients with open wounds have abnormal laboratory values that have not been quantified in enough detail to create normal value limits. Markers of inflammation such as white blood count and sedimentation rate are usually elevated. Anemia is common and is associated with chronic blood loss through wounds as well as anemia associated with chronic disease. Subjects will be followed comparing their own laboratory results over time in order to identify noteworthy deviations from normal for them (as determined by the investigators or EB physician).

4.B.vi. Day -7:

Seven to 14 days before grafting we should have an estimate of the grafting date based upon the status of the growth of the keratinocyte grafts (LEAES). For simplicity, we will refer to this as "Day -7" in the protocol. At Day -7, the target wound areas will again be assessed, possibly photographed and possibly recultured depending on the clinical appearance at the discretion of the investigator and EB physician. Additional blood tests may also be obtained at the discretion of the investigator and EB physician. If necessary, the EB physician may approve additional treatments necessary for the subject to meet grafting inclusion criteria, including the ability to undergo anesthesia. A skin and physical examination will occur, and the subject will be asked about concomitant medications and adverse events.

4.B.vi.1. Wound preparation for grafting

The subject should continue their normal dressing regimen. Site selection for the grafting will be finalized on Day 0 (the date of grafting). Oral antibiotics may be used depending on wound appearance and culture results, at the discretion of the investigator and EB physician.

4.B.vii. Day -3:

Subjects may have their target wounds re-examined at Day -3. The target wound areas will again be assessed, possibly photographed and possibly recultured depending on the clinical appearance. Additional blood tests may also be obtained at the discretion of the investigator and EB physician. If necessary, the EB physician may approve additional treatments necessary for the subject to meet grafting inclusion criteria, including the ability to undergo anesthesia. A skin and physical examination will occur (at the discretion of the EB physician and investigator) and the subject will be asked about concomitant medications and adverse events.

At the discretion of the investigator and EB physician, this visit may be omitted.

4.B.viii. Day -1:

Subjects will have their target wounds re-examined at Day -1. The target wound areas will again be assessed, possibly photographed and possibly recultured depending on the clinical appearance. Additional blood tests may also be obtained at the discretion of the investigator and EB physician. If necessary, the EB physician may approve additional treatments necessary for the subject to meet grafting inclusion criteria, including the ability to undergo anesthesia. A skin and physical examination will occur (at the discretion of the EB physician and investigator), and the subject will be asked about concomitant medications and adverse events.

Patients with RDEB understand how much trauma is necessary to develop a blister and how rapidly blisters can form on their skin. The timing necessary to develop a blister that can be developed into an acute wound will be based upon the suggestions of the RDEB subject. Usually less than 24 hours are necessary. Twenty-four hours or less before grafting we plan to create an acute wound (Study Day -1).

4.B.viii.1. Creation of acute blister

For this process, we will select an area of intact skin on the anterior or lateral torso or upper or lower extremity not to exceed 40-50 cm^2 in size. The area will be marked with a sterile surgical marking pen and photographed with a digital camera and/or Canfield system prior to induction of a blister or applying trauma to the area. .

Either the investigator or the subject will firmly rub the skin within the marked area until they think they have generated enough trauma to develop a blister. The blister may be subclinical at this time but will fill with fluid over the next several hours. This area should not be disturbed until the time of grafting, unless the subject feels the blister is enlarging and extending beyond the marked areas. In this case, a sterile needle may be used to drain the blister, but the roof should not be removed until the time of grafting procedure. At the time of the grafting the roof will be removed and the area grafted as described below. We will provide the subject with written instructions (approved by the Stanford IRB) on how to care for the induced wound.

5. Enrollment/ Grafting:

Subjects will be considered “enrolled” in the gene transfer study when the determination is made that they will undergo grafting with LEAES at Day 0.

685
686 **5.A. Grafting (Day 0):**
687 If needed, additional studies (including blood tests and a urine pregnancy test, if applicable)
688 may be performed prior to grafting on day 0, if deemed necessary by the investigator and EB
689 physician. The subject's enrollment must have been approved by the independent medical
690 monitor, and their inclusion/exclusion criteria will again be verified. A skin and physical
691 examination will occur, and the subject will be asked about concomitant medications and adverse
692 events. Note that this is considered a routine pre-surgical procedure and not a study procedure.
693
694 **5.A.i. Final selection of graft sites**
695 Some of the previously analyzed wound sites may not meet grafting criteria on Day 0
696 because of recent trauma or infection, or they may have healed. On the day planned for grafting
697 we will have the option to choose other sites, to graft fewer sites, to treat an infected area, or to
698 delay grafting. The grafting surgeon along with the EB physician will determine which sites will be
699 grafted and the timing of the grafting, based on clinical judgment and wound appearance.
700 On the day of grafting, which will be considered Study Day 0, the acute and chronic target
701 areas will be identified based on the landmarks, maps and photographs taken at prior visits. Each
702 area will again be examined, assessed, measured and photographed. We will use topography
703 and body maps to draw these exact areas where the grafts are placed. Measurement of the
704 wounds will be done using the ARANZ SilhouetteStar and/or Canfield system.
705 In the operating room, after graft sites have been selected, additional assessments will occur.
706 Assessments will be made prior to and after wound bed preparation. Distance from the wound to
707 body landmarks will be documented. Photographs with a digital camera as well as the ARANZ
708 and/or Canfield will be obtained at these timepoints.
709
710 **5.A.ii. Grafting of LEAES**
711 Grafting will be carried out under local anesthesia, conscious sedation or general anesthesia,
712 depending on the request of the subject and the recommendations of the anesthesiologist,
713 grafting surgeon, investigators and EB physician. Grafts will be labeled with the subject's name
714 and study number to confirm that the correct patient is receiving the correct grafts (even though
715 we will only work with one patient's cells at a time in our tissue culture facility).
716 The grafting process will be modeled after the grafting process of Epicel (Genzyme
717 Biosurgery, Cambridge, MA) and will be as follows:
718 1. The blister roof of the acute wound will be removed.
719 2. All wounds will be gently cleansed with normal saline or providone iodine solution.
720 3. Overhanging epidermis, hyperkeratotic skin, or fibrinous material will be gently
721 debrided with scalpel, scissors, or the Timedsurgery electrosurgical technique[6-8] (or
722 equivalent cauterization technique), or a combination of these at the grafting surgeon's
723 discretion, in consultation with the EB physician.
724 4. Grafts will be applied to the wound beds and affixed with staples, suture, Mepitac,
725 and/or overlying dressing. A layer of topical antibiotics will be applied, with antibiotic
726 choice determined by the grafting surgeon and EB physician.
727 5. Prior to grafting, we will inquire with the subject if they would be willing to let us put a
728 small (~1mm or less) tattoo dot at the corners of the grafts. If the subject has agreed to
729 this, at the discretion of the investigators, grafting surgeon, and EB physician, a small
730 tattoo dot will be placed at the corners of the graft using the following procedure routinely
731 used by radiation oncology:
732 a. A small amount of ink will be placed on the tattoo location using an ink
733 dropper. The dropper will not make contact with the patient's skin.

b. Using a sterile hypodermic needle, a physician will pierce the skin only enough to deliver the ink into the skin.

c. Any ink remaining on the skin will be wiped away to ensure tattoo accuracy.

6. Photographs will be obtained with a digital camera, ARANZ, and/or Canfield system following graft placement.

7. Dressings will consist of a single layer of Interface (silk gauze), Mepitel, Adaptic, Restore, or other equivalent contact layer followed by an absorbent foam dressing (Mepilex, Restore Foam, or Allevyn), which will be held in place with rolled gauze and surgical netting. Dressings will be decided upon by the grafting surgeon, in consultation with the EB physician. The subject will be given written instructions on how to care for their grafts (see section 6) and how to contact study staff.

8. The dressings will stay in place until changed at day +5 to +10. We will plan to see the subject every 3 days (+/- 1 day) following grafting to monitor wound healing if the subject is not hospitalized. If the subject is experiencing problems, we will see them immediately in clinic.

9. At the discretion of the grafting surgeon, investigators, and EB physician, splints may be used to immobilize the grafted areas to prevent any trauma to the grafts.

6. Post-grafting observation (Day +1 - Day +14)

The subject will remain in the local area for observation and frequent examinations for at least 14 days following grafting. Based on the discretion of the grafting surgeon and EB physician, the subject may be admitted to the hospital for observation for several days following grafting. This is in order to minimize movement and immobilize the grafted areas and also to facilitate monitoring of the patient. In the post-grafting period, it is crucial that the graft is not disturbed for at least 5-10 days (until Study Days +5 to +10). We plan to see the subject every 3 days (+/- 1 day) for follow up. This may be as a study visit, or during regular rounds if the subject is hospitalized. If deemed necessary by the investigator or EB physician, we may see subjects more frequently. At these visits, subjects will be assessed for adverse events and changes to concomitant medications. The investigator may perform a physical examination at their discretion and that of the EB physician. If the subject is unable to come to Stanford, investigators may be able to come to the subject's home or hotel to check on their progress. We will also try to provide nursing support for bandage changes.

At the discretion of the grafting surgeon, investigators, and EB physician, the subject may be given prophylactic antibiotics, as is routine following surgery.

If the dressings become moistened with exudates, or if the subject has unexplained fevers over 38.5°C on Study Day +3 or later, the absorbent layers may be changed, but the underlying contact layer should not be removed, irrigated or cleansed. If drainage is excessive or purulent, antibiotics may be used at the discretion of the investigator and the EB physician. The subject will be provided with written instructions (approved by the Stanford IRB) on how to take care of their wounds, and how to contact study staff if they have any problems.

Five to 10 days after placement of the graft, the investigator will perform a physical exam and a skin examination. The investigator and EB physician will determine if any adverse events have occurred.

The outer dressing layers will be removed. At this point, the Vaseline backing of the LEAES grafts will still be attached to the wound. As the sutures dissolve, the Vaseline backing should come off on its own, as the LEAES cells incorporate into the wound beds. The subject and their caretaker will be instructed to let this happen naturally, not to try to remove the gauze.

If results from post-release criteria (i.e. mycoplasma, sterility) are unacceptable, the investigator will be notified immediately and failure investigations will be conducted in accordance with our internal SOPs

(available upon request) and Safety Monitoring Plan. The investigator will discuss treatment options with the EB physician, who will have the authority to determine the appropriate course of action.

At the discretion of investigators and the EB physician, the subject may return on Day +12 (+/- 1 day) for another follow-up visit. At this visit, adverse events will be assessed and concomitant medications will be recorded. Additionally, the investigator and/or EB physician will perform a physical examination and a skin examination. Most likely the LEAES grafts will still be covered by the Vaseline gauze backing. If grafts are visible at this time, they will be assessed and photographed as described below in section 7.E.

7. Post-grafting clinical follow-up:

Please see Schedule of Events (Table 3) for additional clarification on follow up procedures. Routine skin care for RDEB patients will continue throughout the study. Additional skin biopsies, wound cultures, or laboratory tests will be performed as necessary to evaluate variations from the expected protocol, inflammation, infection, or possible SCC growths within the grafted sites at the discretion of the investigator and the EB physician.

7.A. Blood tests

At Study Day +14, 4 weeks, 12 weeks, 25 weeks and 52 weeks after grafting, the subject will return to Stanford University Medical Center where blood will be obtained for CBC and platelets, Complete Metabolic Panel and hepatic function testing, cytotoxic T cell assay, and analysis for an immune response against type VII collagen. Whenever possible, we will draw the minimum (pediatric) amount of blood. Additional blood tests may be added at the discretion of the investigator and the EB physician. If it is not possible to obtain enough blood for all of these studies, tests may be omitted, at the discretion of the investigator and the EB physician.

7.A.i. Replication competent retrovirus analysis

Replication competent retroviral analysis (RCR) will be performed on the blood collected at 12 weeks, 25 weeks and 52 weeks after grafting then yearly thereafter (possibly in a follow-up protocol, as described in section 7.H) for at least 5 years.

7.B. Physical examination, skin examination

At each study visit, the subject will undergo a physical examination and skin examination in addition to the examination of the grafted area.

7.C. Adverse events

The subject will also be asked if they have experienced any adverse events since the previous visit and the study diary will be reviewed. This information will be recorded in the subject's medical record. All adverse events will be recorded, regardless of their attribution to LEAES and will be discussed with the DSMB on a routine basis (section 16.E). Unexpected, harmful, and related adverse events will be reported immediately to the FDA, the Stanford IRB, Stanford Biosafety, and the DSMB, as described in the Safety Monitoring Plan (section 7.E).

7.D. Concomitant medications

At each study visit, the subject will be asked if they have used any concomitant medications or undergone any concomitant therapies. This information will be recorded in the subject's medical record. There are no excluded concomitant therapies or medications in this protocol.

7.E. Photographs and graft evaluation

Photographs will be taken of the grafted sites using the ARANZ SilhouetteStar and/or Canfield Vectra system to generate an accurate image of the graft area and calculate the cm^2 of the wound/graft area.

We will observe the grafts closely in order to separate technical grafting problems from immunological rejection or loss of genetic correction. The graft site will be clinically evaluated with a global score of: 1) 100% to 75% healed, 2) 74% to 50% healed, 3) 49% to 25% healed, 4) 25% to 1% healed, 5) complete graft loss, or 99) unable to determine. It may be several weeks before we are able to adequately evaluate the condition of the grafted areas, as the grafts may still be covered by Vaseline gauze.

The early time points of graft evaluation may not be as accurately recorded because the area of graft acceptance may not clearly be apparent within the wound. We will be able to quantitate the precise surface area of blistering, erosions and graft absence from the digital photographs of the areas.

Blinded observers will confirm the accuracy of the evaluation without knowledge of the duration of the graft or when the digital image was obtained.

We will record the subject's impression of the durability of the grafted sites and the ease of blistering with trauma. We will not physically damage the grafts in order to develop minimum requirements for blistering. Future studies can include methods used to estimate the force necessary to cause blistering in RDEB and RDEB skin grafted with LEAES.

Attention of the clinical examination will also be focused on the sites of the previous biopsies to evaluate healing and scar appearance.

7.F. Skin biopsies

At Study Day +14, 4 weeks, 12 weeks, 25 weeks and 52 weeks after grafting, the graft sites will be identified. Biopsies may be obtained from multiple sites. The following biopsies may be obtained at the discretion of investigators and the EB physician:

- One 4mm punch biopsy for IF to be performed in our research laboratory
- One 4mm punch biopsy for FACS to be performed at UCSF
- One 4mm punch biopsy for DIF to determine if immune complexes are present at the basement membrane. This is a CLIA test which will be performed by the Stanford Dermatopathology Laboratory.
- One 3mm punch biopsy for EM to be performed by Doug Keene, an outside collaborator
- One 3mm punch biopsy for IEM to be performed by Doug Keene, an outside collaborator
- One 6mm punch biopsy for molecular analysis to be performed in our research laboratories

The priority for obtaining skin biopsies will focus on obtaining the IF biopsies for type VII collagen antigens and DIF for immunological response at 12 weeks, 25 weeks and 52 weeks after grafting and EM for presence of anchoring fibrils. Additional biopsies may need to be obtained at the discretion of the investigator and the EB physician. The biopsies may be needed to document presence or absence of grafted tissue in a specific area. Biopsies may also be omitted at the discretion of the investigator and the EB physician.

If the tissue appears abnormal the biopsy will be sent to pathology for histologic evaluation. Additional skin biopsies or laboratory tests will be done as necessary (in consultation with the EB physician) to evaluate variations from the expected protocol, inflammation, or possible SCC growths within the grafted sites.

Based on the success of the graft attachment and/or a sense by the subject that the non-grafted adjacent skin may be more resistant to trauma or may demonstrate healing of wounds that previously would not heal, additional biopsies may be obtained outside of the grafted area (at

the discretion of the EB physician) in order to evaluate potential type VII collagen spreading outside of the grafted area. Biopsies on the periphery of the grafted area may also be obtained if it appears that the graft is spreading outside of the initial graft boundaries.

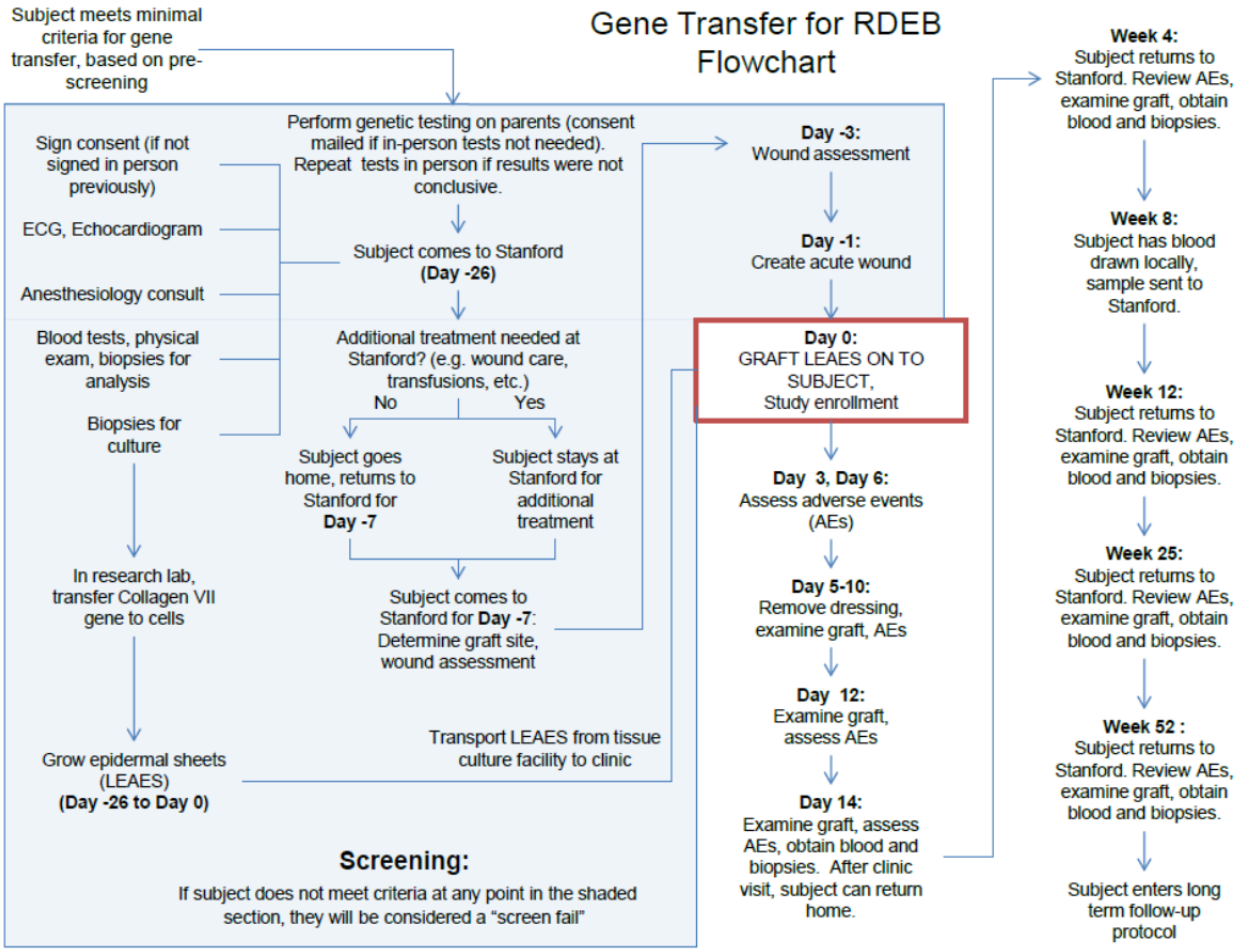
All biopsies will be obtained after local anesthesia and all sites will be marked and photographed to confirm the location of the biopsies. Location of biopsies will be recorded in the source documentation. When multiple grafts are placed on different body sites, additional biopsy sites will be decided based on the graft appearance, previously obtained information, and consultation with the EB physician. Based upon subject preferences and in order to limit the number of procedures, the biopsies may be obtained by one or more surgical elliptical biopsies representing the surface area of the punch biopsies described above.

Research biopsies will be labeled with the subject's study number and possibly initials, anatomical location, and the date the biopsy was obtained. The evaluator of the biopsies will be blinded as to the source of the biopsies, which could be from wounded areas, grafted areas, or non-grafted, non-wounded skin.

7.G. Week 8

At 8 weeks after grafting, blood will be drawn at a laboratory close to the subject's home and shipped to Stanford for CBC and platelets, Complete Metabolic Panel, cytotoxic T cell assay, and for an immune response to type VII collagen. If it is not possible to obtain enough blood for all of these studies, specific tests may be omitted at the discretion of investigators and the EB physician. It will not be necessary for the subject to come to Stanford for the Week 8 visit. However, they may come to Stanford if they prefer. If they come to Stanford for this visit, additional graft assessment and physical examination may be performed.

Figure 2: Flow Chart for RDEB Gene Transfer Protocol:


Gene Transfer for RDEB Flowchart
Subject meets minimal criteria for gene transfer, based on pre-screening
Sign consent (if not signed in person previously)
ECG, Echocardiogram
Anesthesiology consult
Blood tests, physical exam, biopsies for analysis
Biopsies for culture
In research lab, transfer Collagen VII gene to cells
Grow epidermal sheets (LEAES) (Day -26 to Day 0)
Perform genetic testing on parents (consent mailed if in-person tests not needed). Repeat tests in person if results were not conclusive.
Subject comes to Stanford (Day -26)
Additional treatment needed at Stanford? (e.g. wound care, transfusions, etc.)
No
Yes
Subject goes home, returns to Stanford for Day -7
Subject stays at Stanford for additional treatment
Subject comes to Stanford for Day -7: Determine graft site, wound assessment
Transport LEAES from tissue culture facility to clinic
Screening:
If subject does not meet criteria at any point in the shaded section, they will be considered a "screen fail"
Day -3: Wound assessment
Day -1: Create acute wound
Day 0: GRAFT LEAES ON TO SUBJECT, Study enrollment
Day 3, Day 6: Assess adverse events (AEs)
Day 5-10: Remove dressing, examine graft, AEs
Day 12: Examine graft, assess AEs
Day 14: Examine graft, assess AEs, obtain blood and biopsies. After clinic visit, subject can return home.
Week 4: Subject returns to Stanford. Review AEs, examine graft, obtain blood and biopsies.
Week 8: Subject has blood drawn locally, sample sent to Stanford.
Week 12: Subject returns to Stanford. Review AEs, examine graft, obtain blood and biopsies.
Week 25: Subject returns to Stanford. Review AEs, examine graft, obtain blood and biopsies.
Week 52 : Subject returns to Stanford. Review AEs, examine graft, obtain blood and biopsies.
Subject enters long term follow-up protocol

902

Table 2: Schedule of Events for RDEB Gene Transfer:

		Pre-Screening[1]	Screening				Grafting Protocol and Post-Grafting Observation					Post-Grafting Follow Up					
			Day -26[2]	Day -7[2]	Day -3[2]	Day -1	Day 0	Day +3[3]	Day +6[3]	Day +5 - +10[3]	Day +12[3]	Day +14 ±1 day	Week 4 ± 2 days	Week 8 ± 3 days	Week 12 ± 1 wk	Week 25 ± 2 wks	Week 52 ± 2 wks
	Phone Screen, send release to subject, receive release, collect/review medical records	X															
	Informed Consent for Gene Transfer Signed		X														
	Fly Subject & Guest to Stanford	X	X	X									X		X	X	X
	Hotel Stay at Stanford	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
	Inclusion/Exclusion Criteria verified/reviewed		X	X	X	X	X										
	Medical History obtained	X	X														
	Review Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X		X	X	X
	Assess Adverse Events			X	X	X	X	X	X	X	X	X	X		X	X	X
	Physical and Skin Examinations[4]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
	Obtain Digital Photographs	X	X	X	X	X	X			X	X	X	X		X	X	X
	ECG, Echocardiogram		X														
	Anesthesiology Consult		X														
	Urine Pregnancy Test (if applicable)		X				X										
Blood Tests	CBC, Chemistry Panel, Hepatic Function	X	X	X	X	X	X					X	X	X	X	X	X
	HIV, Hepatitis B, Hepatitis C Testing	X															
	Genetic Testing (GeneDx)	X															
	IIF to test immune response	X	X[5]									X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
	Cytotoxic T cell assay		X[5]									X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
	RCR Evaluation		X												X	X	X
Skin Biopsies	EM (3 mm) to Pathology	X															
	IIF (4 mm) to Pathology	X															
	DIF (4mm) to Pathology	X	X									X[5]	X[5]		X[5]	X[5]	X[5]
	Molecular analysis (6 mm)		X[5]									X[5]	X[5]		X[5]	X[5]	X[5]
	IEM (3 mm), Shriner's Hospital	X	X[5]									X[5]	X[5]		X[5]	X[5]	X[5]
	EM (3 mm), Shriner's Hospital		X									X[5]	X[5]		X[5]	X[5]	X[5]
	IF type VII collagen (4 mm)		X									X[5]	X[5]		X[5]	X[5]	X[5]
	FACS (4 mm)		X[5]									X[5]	X[5]		X[5]	X[5]	X[5]
	For LEAES manufacture (8 mm x2)	X	X														
	Keratinocyte culture (6 mm)	X															
	LEAES cultured at Lokey Stem Cell Building		X	X	X	X											
	Create acute wound					X											
	LEAES Grafting						X										
	Graft Assessment									X	X	X	X		X	X	X

[1] Will occur as part of the Characteristics pre-screening study
[2] Date is approximate, depending on the cell culture process.
[3] Observational visits post-grafting. Visits will be at the discretion of the investigator and/or EB physician. Optional if subject is hospitalized.
[4] At the discretion of investigators and EB physician. May also include wound cultures if indicated clinically.
[5] Optional, at the discretion of investigators and the EB physician. Additional biopsies or blood tests may be obtained at the discretion of the investigators and EB physician.

7.H. Long term follow up protocol

After 52 weeks (1 year) subjects will be enrolled in a long term follow-up protocol under this IND, in which we will follow them for life.

Regarding the NIH sponsored DSMB, the end of the DSMB follow-up will be 1 year after the last subject is enrolled.

8. Discontinuation, Withdrawal, Lost to Follow-Up, or Early Termination

Patients who discontinue due to adverse events or protocol deviations/violations (section 15 and section 6 of the Safety Monitoring Plan) shall not be replaced. Any and all patients who discontinue or withdraw from the study after receipt of LEAES will be included in the long-term follow up.

A subject may withdraw at any time, but we would plan to follow them for at least 15 years and up to their lifetime (section 7.H). They will have the option to not follow our suggestions, but we will still attempt to contact them. If a subject does not follow directions or refuses to return for follow up we will still attempt to evaluate them or have their local physician evaluate them.

Collected skin tissue may be used until the subject asks that it not be used.

If subjects withdraw from the study, we will record the date of withdrawal, as well as the reason for withdrawal in our study records. This information will be communicated to the DSMB at each meeting, when we give an enrollment update (see Safety Monitoring Plan, section 8).

If a subject is no longer able to travel to Stanford due to illness, either related to the study treatment, or due to their underlying disease, we will attempt to send a study investigator to the subject, to see them when they visit their PCP.

If we must remove the LEAES graft from a subject, we will record the date of graft removal, and the reason for graft removal in source documents and will present it to the DSMB at the next scheduled meeting, unless LEAES removal is due to a serious adverse event that warrants an ad hoc meeting of the DSMB. If we decide to re-graft the subject (subject to approval of the EB physician), they will restart the study protocol at Day -26 (if they must be re-biopsied) or at Day -7 (if another biopsy is not necessary) in order to prepare the wound beds to receive gene transfer. If we decide not to re-graft, we will contact the subject for safety monitoring every 3 months up to one year following the graft removal, after which we will contact the subject yearly for safety monitoring. Safety monitoring in this case will include communication with the subject as well as the subject's PCP to determine if any AEs or SAEs have occurred.

If the subject withdraws for other reasons, we will continue to attempt to contact them at least once per year to perform safety monitoring as described above. We may elect to contact subjects more frequently if it is deemed necessary by investigators and/or EB physician.

If a subject is lost to follow up, we will document our attempts to contact them, in an effort to show due diligence.

If a subject is terminated early from the study, we will follow the same process as for "withdrawal" of a subject as described above.

9. Time frame between subjects

After grafting the second subject, we will plan to have at least one week between grafting of each subsequent subject and starting cell culture of the next subject. However, we will not work with more than one subject's cells at a time in our tissue culture room in order to reduce the risks of contamination or mistaken identity. If complications develop we will make additional grafting decisions based upon the suggestions of the EB physician and Data Safety Monitoring Board (DSMB), as well as the requirements of the FDA and other regulatory bodies.

Based on the success or failure of the grafting process, we will work with the FDA to determine an appropriate time to begin evaluation and grafting of children less than 18 years of age. A meeting of the DSMB will also take place at that time. If we are unable to identify five adult subjects that meet the

requirements for entry into this protocol we may petition the FDA to allow us to enroll children between the ages of 12 and 18 years of age.

10. Unscheduled visits

Unscheduled visits will be handled at the discretion of the investigator and the EB physician. Study procedures performed at unscheduled visits will be determined by the investigator and the EB physician and may include, but are not limited to physical exam, photographs, graft assessment, skin biopsies, wound cultures, and/or blood draws for lab procedures.

11. Exceptions to protocol

Exceptions to inclusion/exclusion criteria, target chronic wound criteria, or grafting criteria may be made at the discretion of the EB physician and the investigator(s). Exceptions that have been made in advance of a protocol deviation will be considered "planned protocol deviations" and will be reported as described in the Safety Monitoring Plan (section 6).

12. Method for determining dosage

The RDEB keratinocyte preparation, transduction and epidermal sheet production are described in detail in the Chemistry, Manufacturing, and Control section of FDA Investigational New Drug Application 13708 and our Basic Biosafety Application. The investigational drug will be developed in a clean room at the Lokey Stem Cell Building. Only designated, trained personnel will have access to the clean room. We will maintain batch records for each graft that we manufacture. The LEAES grafts will go directly onto the subject once it removed from the Lokey Stem Cell Building tissue culture facility and transported to the facility where grafting is taking place.

The manufacturing aim is to produce and deliver four to six of the 40 cm^2 to 50 cm^2 sheets for grafting (LEAES). Approximately two to six of the 40-50 cm^2 epithelial sheets will be used in a single grafting session. We anticipate a maximum of 3 grafting sessions over the course of six months. Subjects who do not have initial graft attachment because of wound infections or mechanical causes will have the option (upon approval by the investigators and EB physician) of receiving additional grafts in the future prepared from frozen keratinocytes or a new biopsy if necessary.

The maximum total grafting surface area for all the graft sites will be 300 cm^2. We will graft two types of wounds. One site will be an acute wound (induced approximately 24 hours prior to grafting) not to exceed 40-50 cm^2, produced by inducing a blister on intact skin and removing the blister roof just prior to grafting. The other sites will be areas of chronic wounds of approximately 25-50 cm^2 or greater that have been prepared for grafting. We plan to graft multiple areas of chronic wounds or possibly one large wound with several 40 cm^2 to 50 cm^2 sheets.

The amount of area grafted will depend on the appropriate wound surface area available for grafting and the quantity of grafts available. We expect the grafts to remain in place for the life of the subject unless they are lost because of infection, rejection, or they need to be removed because of development of SCC or they are migrating over mucous membranes. If they occur, these adverse events will be discussed immediately with the DSMB (see Safety Monitoring Plan).

The success rate of attaching cultured keratinocytes in epithelial sheets in burn patients ranges from 0 to 100%.[9,10] We will be grafting wound areas that may have existed for months to years. We are unsure of the ability of the old, chronic wounds to accept grafts without additional specialized techniques of graft bed preparation that may need to be developed in the future. We plan to graft between 100 cm^2 to 300 cm^2 areas in order to minimize the risks of graft failure based on grafting techniques. Grafting a surface area of up to 300 cm^2 will require little additional discomfort for the research subjects. We will also graft different locations on the body and wounds with different appearances in order to define the best wound appearance for successful grafting. If an adverse reaction occurs, the grafts can be easily

removed as they are easily accessible and we will have clearly documented their location by photographs and measurements.

13. Observations and measurements

Table 3 describes the time line and expected type of observations and measurements that are planned. Skin biopsies, blood tests, or medical interventions may occur at the discretion of the EB physician because of clinical changes that require evaluation or therapy. Wound cultures and other routine EB care will continue as necessary.

13.A. Skin examinations and photographs

Throughout the study the grafted and non-grafted skin will be examined in addition to examining the non-blistered skin. The specific observations that will be done may include measuring the changes in the wounds using the ARANZ SilhouetteStar, the Canfield system, and/or digital photographs. The digital photographs can be examined in the future by individuals blinded to grafted or not grafted lesions and the timing of the grafting.

13.B. Blood tests

Blood tests will be obtained at the specified intervals and more frequently if necessary. These include but are not limited to CBC, Complete Metabolic Panel, Hepatic Function Tests, Replication Competent Retrovirus (RCR), cytotoxic T cell assay, and IIF evaluating the subject's immune response to type VII collagen as well as additional tests necessary for patient care and evaluation of potential immunological reactions. RCR testing will be performed yearly (at a minimum) for at least 5 years. Subjects will be enrolled in an additional protocol to facilitate long term follow-up (section 7.H).

13.C. Skin biopsies

Skin biopsies will be obtained to observe physical development of the anchoring fibrils using electron microscopy (EM). In addition skin biopsies will be obtained to evaluate expression of type VII collagen, both NC1 and NC2 epitopes, using immunoelectron microscopy (IEM) and immunofluorescent light microscopy (IF). Skin biopsies will also be evaluated for genomic retention of type VII collagen proviral vector sequences (DNA retention) as well as mRNA expression in the LEAES grafts. We may also evaluate skin biopsies for cytokines using FACS. Immune responses to the grafted skin will be evaluated by DIF analysis for immune reactants.

14. Risks

14.A. Risks for investigational drug

Since our toxicological studies in animals were done on an immunological suppressed animal model, we are concerned about human immunological responses in this study. Furthermore, additional risks have been identified in other published gene transfer trials and in the technique of skin grafting. These risks are examined below and were communicated to the FDA in our IND application. We plan to be in frequent communications with the subjects in this study. We expect them to contact us with any concern that may develop.

14.A.i. Anticipated risks

There are several risks anticipated in this trial. Subjects could develop physical difficulties which could destroy individual grafts. These events include wound infections or physical trauma which removes the graft before it has attached.

14.A.ii. EBA/Immunologic graft rejection

A subject could develop autoantibodies to type VII collagen (EBA). If that happens we expect the individual to form blisters under the grafts and lose the graft. We expect immunological graft rejection to initially appear as redness and possibly spontaneous blisters within the grafts. We will evaluate inflammation within the grafted sites with a routine biopsy for Hematoxylin and Eosin (H&E) and Direct Immunofluorescence (DIF) as we would any inflammatory lesion in the skin in a patient. Immunological rejection will be immediately reported to the DSMB, FDA and IRB and additional subjects will not be enrolled until approved by the DSMB. Immunologic rejection will initially be treated with potent topical steroids. As clinical experienced is gained, combinations of systemic and topical therapy will be considered. If we see no signs of immunological response in the NC1[+] subjects we will consider NC1[-] subjects in future. The decision to enroll NC1[-] subjects or to use systemic anti-rejection medications would first need to be approved by our EB physician. Subsequently we would need to have review and approval by the FDA, RAC and IRB before proceeding with enrollment of NC1[-] subjects.

If a subject does develop EBA, we will consider use of immune suppression to protect the grafts. Immune suppression may increase the subject's overall risk of infection. The decision to use immune suppression or another alternative treatment will require approval from our EB physician. This adverse event would be reported expeditiously to the DSMB, the IRB, and the FDA (see Safety Monitoring Plan sections 5.B and 7.E).

14.A.iii. Cancer

We know from other retroviral studies that a risk of inducing cancer is possible. Gene transfer of keratinocytes may increase the risks of SCC dependent upon the site of retrovirus vector integration as was seen in autologous bone marrow cells developing leukemia.[11,12]

Since the subject with RDEB is already at increased risk for SCC which can be metastatic and lethal, we are concerned of an increased risk with gene transfer. Grafted sites that develop thickening or an abnormal appearance will be biopsied. Identified SCC will be removed and the subject will be evaluated for metastasis. Throughout this trial we will be observing the subjects for development of squamous cell carcinoma (SCC) in the grafted sites as well as other sites on their body. We will consult with the EB physician and biopsy any suspicious lesions in order to identify any lesion that could potentially be a SCC.

If an SCC is identified, it will be removed surgically. In order to assess if the SCC contains proviral genome, approximately a 100 mg SCC biopsy sample will be taken and used for genomic DNA and total RNA purification. The sample will be stored in RNA Later solution upon processing to prevent RNA degradation. The genomic DNA and total RNA will be purified. qPCR and qRT-PCR will be performed using multiple primer sets specific for the proviral genome extended packaging sequence (5' primers) and sequence within exon 2 of Col7A1 cDNA (3' primers).

We are aware that SCC is a common complication of RDEB subjects over the age of 15 years old. It will be important for us to confirm if the SCC is related to the grafted cells or related to the underlying disease process. Presence of SCC containing proviral genome will require that we graft no additional subjects and the DSMB will be informed expeditiously as well as the FDA and IRB (see Safety Monitoring Plan sections 5.B and 7.E).

14.A.iv. Advancing epithelial surfaces/migration of graft over mucous membranes:

Advancing epithelial surfaces from a grafted area over an ungrafted area will be of concern, and treatment options (including the decision to remove the graft) will be made by investigators and the EB physician. This will be reported expeditiously to the DSMB, FDA and IRB (see Safety Monitoring Plan sections 5.B and 7.E). There is also a remote risk that the grafts may expand

beyond the wound area and possibly migrate to cover mucous membrane areas. In order to avoid this possibility we will attempt to graft areas away from the mucous membranes. If migration does become a problem, we may be required to surgically remove portions of the graft. If this occurs, we will consult with the EB physician and notify the FDA and other regulatory bodies. We will not enroll any additional subjects until details of the affected subject are understood and the extent of the spread is recognized.

A successful grafting outcome may be present if the graft advancement is only onto the adjacent chronically wounded surface and not onto contiguous unwounded skin. The DSMB will approve enrolling additional subjects after appropriate consultation with the FDA and IRB.

14.A.v. Systemic infection

Because they have many open wounds, RDEB patients have frequent cutaneous infections and occasional systemic infections. Any subject who has a severe infection will be treated for the infections as is standard care in consultation with the EB physician. Systemic infections thought to be associated with the grafting will be reported immediately to the DSMB, FDA and IRB. The DSMB will have the authority to terminate or delay enrollment of additional subjects if concerns exist that the grafts may have had an association with the source of the infection.

14.B. Risks for commercially available drugs

Local anesthesia will be done prior to any biopsy procedures. We are currently planning to do the grafting procedures using local anesthesia, general anesthesia, or conscious sedation. A pediatric anesthesiologist experienced with EB patients will assist with this procedure. As described below (section 14.B.ii), the anesthesiologist will go over the risks of the drugs used for conscious sedation or general anesthesia. The anesthesiologist will review the subject's intubation history and other tests to verify that they meet the requirements to undergo anesthesia (section 4.B.v.7).

14.B.i. Commercially available drugs used for biopsy

EMLA Cream (lidocaine 2.5% and prilocaine 2.5%) [One gram contains lidocaine 25 mg, prilocaine 25 mg] or LMX 4 Cream (Lidocaine 4%) [One gram contains lidocaine 40 mg] topical anesthetic creams, or similar formulations will be used for local anesthesia prior to biopsies. EMLA Cream is a prescription drug and LMX 4 is over-the-counter. They are usually applied 30 minutes to an hour before the procedure.

Usually only 4 to 5 grams of cream are needed prior to the multiple biopsy procedures. If we assume 100% absorption then the total dose would be 200 mg of lidocaine for LMX 4 and 125 mg of lidocaine for EMLA. The absorption through the intact skin that will be biopsied will be much lower than 100%.

The suggested maximum dose of infiltrated lidocaine over a 2 hour period, assuming 100% absorption of the injection, is 300 mg alone and 500 mg if injected with epinephrine containing solution. Topically to intact skin larger quantities can be applied. In addition to the topical lidocaine, 1% lidocaine with epinephrine (10 mg/ml with epinephrine) will be injected to the sites where the biopsy will be done. After the topical lidocaine this usually only requires about 3 ml (30 mg) injected. These doses are under the minimum toxicity doses.

It has been described that a solution of 1% lidocaine hydrochloride with epinephrine buffered with 8.4% sodium bicarbonate decreases pain associated with the injection of local anesthetic.[13] We may elect to add sodium bicarbonate to the lidocaine in a 10:1 dilution when performing biopsies. This will be at the discretion of the investigator, subject to approval of the EB physician.

14.B.ii. Commercially available drugs used for grafting

For the grafting procedure we will need to anesthetize a larger surface area. For this procedure we will assume greater absorption of the topical anesthesia so a much smaller quantity will be used. In addition we expect that the grafting will be done over a period longer than one hour which will allow some metabolism of the lidocaine.

The main local anesthetic will be injected 1% lidocaine with epinephrine. This will be injected about 10 minutes before the grafting to the specific site. By using a 30 G needle and a 1 cc syringe we are able to anesthetize large areas with a small amount of injected anesthesia. We assume that each 50 cm^2 graft surface area will require 3 ml to 4 ml of injected 1% lidocaine with epinephrine (10 mg/ml with epinephrine) for a total injection of 30 mg to 40 mg of lidocaine. If we do graft six grafts we should only inject between 180 to 240 mg of lidocaine with epinephrine (24 ml of the 1% solution), well below the 500 mg maximum. Ten to 30 minutes prior to injecting, approximately 1/5 of a gram of EMLA Cream or LMX 4 Cream can be applied to one corner of the grafted area. This allows the initial site of the needle insertion to be anesthetized prior to the first injection. By injecting slowly with the 30 G needle and 1 cc syringe the lidocaine anesthesia can be extended through the 50 cm^2 surface area with little or no discomfort.

If general anesthesia or conscious sedation are recommended by the EB physician or grafting surgeon, or at the request of the subject, the risks will be explained to the subject by the anesthesiologist who will be performing the procedure. LPCH has a team of pediatric anesthesiologists who are experienced with anesthesia for children and adults with all severe forms of EB. We will rely on the anesthesiologist to review with the subject the medications that will be used during the procedure.

14.C. Risks for procedures to be performed

Risks of the gene transfer procedure are as described above, under the section "Investigational Drugs."

14.C.i. Blood draws

Blood drawing is usually accomplished with the risks of pain and occasionally bruising at the site of the blood drawing. The subjects with RDEB commonly have great difficulty finding veins for blood drawing because of the scars and open wounds. We may try to use topical anesthesia prior to the blood drawing, if possible, in order to limit the pain of blood drawing.

14.C.ii. Skin biopsies

Skin biopsy is a common procedure performed in our dermatology clinics. Infection of a skin biopsy is a rare event occurring less than 1% of the time. Patients with RDEB frequently have wound infections secondary to their large surface areas of open wounds. The subjects in this trial will have an increased risk of infection at the biopsy site because of their potentially infected wounds at sites distant to the biopsy site.

It is easier than expected for an experienced surgeon to biopsy a patient with RDEB. The members of our team have completed many diagnostic and research biopsies on RDEB patients and RDEB research subjects without major complications or chronic wound formation. Although the epidermal-dermal junction is extremely fragile, the dermal strength is normal. Wounded or non-wounded skin can be sutured easily during most biopsy procedures. These wounds heal and remain closed after the sutures are removed. For punch biopsies of 3 mm or less we have found that sutures are not needed and the wounds will contract normally. For biopsies of 4 mm or larger sutures are frequently beneficial. Occasionally we may elect to do an elliptical biopsy rather than punch biopsies for keratinocyte culture or molecular analysis. This decision will depend on the mobility of the research subject's skin and the location of the biopsy. The most important

technique during the biopsy procedure is to minimize lateral trauma during the biopsy and suture removal process.

The success of genetically corrected keratinocyte grafts in this application will depend on many factors. The design of this trial, including the frequency of skin biopsies, was calculated for maximum patient protection as well as maximum scientific value to improve and refine future therapeutic efforts. The 6 mm biopsies for tissue culture are needed to collect a large enough surface to isolate adequate keratinocytes for culture. The 4 mm biopsies for IF and 3 mm biopsies for EM are specifically done in order to maximize the diagnostic IF or EM tissue preparation techniques and minimize the trauma to the biopsied tissue during the procedure. These are the smallest biopsy techniques that will consistently give adequate tissue for evaluation. Based upon the condition of the grafts and the medical judgment of the investigator and the EB physician, we may obtain these biopsies from the same graft or from several grafted areas.

The specific biopsies of the grafted skin were also chosen to minimize the required trauma while obtaining adequate information. Again, the 4 mm biopsy for IF, the 6 mm biopsy for molecular analysis, and the two 3 mm biopsies for EM and IEM are the smallest tissues that will give adequate information with minimal trauma to the biopsied tissues. Our protocol and consents are designed so that we have flexibility and will try to biopsy as infrequently as possible and to obtain as small amount of tissue as possible in order to document subject safety and success or failure of the grafting process. We will try to use photographs and observations as effectively as possible in order to limit biopsies. On the other hand, biopsies can give precise detail and information that clinical observation cannot provide.

14.C.iii. Echocardiogram

An echocardiogram is a safe, non-invasive procedure. It requires the patient to lie down, and a cool gel will be applied to their skin. This gel should not cause any pain, and will prevent EB skin from forming blisters due to the echo transducer, allowing cardiologists to visualize the heart and its function. There are no additional risks anticipated with this procedure.

14.C.iv. Electrocardiogram (ECG)

An ECG is a safe, non-invasive procedure used to measure electrical activity of the heart. However, EB patients have some special requirements for ECGs. Instead of the usual sticky leads, ECG technicians will use a piece of Mepitel or defibrillator pads, in order to prevent the skin from tearing. They are familiar with this procedure for EB patients. There are no other anticipated risks with this procedure.

14.D. Privacy and confidentiality

In order to protect the privacy interests of participants, study appointments will take place in an isolated, private examination room. Only authorized personnel will be allowed to conduct study visits. Phone interviews will only take place if study personnel are in a private location. If the subject does not have enough privacy during the phone call, the call will be postponed until the subject is comfortable with their privacy level.

Hard copies of documentation containing PHI will be stored in a secure, locked cabinet. Only authorized personnel will have access to this cabinet. Any electronic information will be kept on a secure, password protected computer. Only authorized study personnel will have access to this electronic data. Data and coded specimens will be coded with the subject's initials and a unique study code number. Only authorized personnel will have access to this code. It will be kept on a secure, password protected computer accessible only to authorized study personnel. Whenever possible, subjects will be referred to by their study code only.

For non-CLIA testing, specimens will only be identified with subject initials and a unique study number. Only authorized personnel will have access to the code. For CLIA approved testing, specimens will be identified with the subject's name, medical record number, and date of birth. This information will be made available to the subject and may be included in the subject's medical record. Only research staff (listed on this protocol) will have access to data or specimens. All study staff have completed the appropriate training, including HIPAA and CITI training, and blood borne pathogens training as necessary.

Grafts will be labeled with the subject's name, and study number.

Data related to this study may be transferred via email between study personnel, or between study personnel, however any email correspondence that contains PHI will be sent via Stanford's secure email service. PHI may be sent to medical records, but it will be sent via secure email, accessible only to authorized personnel.

The investigator will permit direct access to source data and documents by the FDA, and other applicable regulatory authorities. The access may consist of study-related monitoring, audits, IRB reviews, and FDA/regulatory authority inspections.

15. Protocol deviations/violations

Any unplanned excursion from the protocol can be referred to as a protocol deviation or violation. For our purposes, the terms "deviation" and "violation" are synonymous. A protocol deviation/violation may be intended or not intended.

The EB physician will grant "protocol exceptions" if necessary. Investigators may request these exceptions from the EB physician. These exceptions will be considered intended protocol deviations/violations. (See section 11, and section 6 of the Safety Monitoring Plan for additional information including timelines for reporting exceptions).

The EB physician, investigators, study staff, and the independent study monitor will work together to determine if any protocol deviations/violations have occurred, and to report them as necessary (see Safety Monitoring Plan, section 6). All protocol deviations/violations (whether intended or unintended) will be reported to the Stanford IRB at Continuing Review (see Safety Monitoring Plan, section 6) and to the DSMB during their regularly scheduled meetings.

Please note that the Stanford IRB only requires immediate notification of protocol deviations/violations if intended to eliminate apparent immediate hazard to a research participant, or if harmful (caused harm to participants or others, or placed them at increased risk of harm – including physical, psychological, economic, or social harm), or if a possible serious or continued noncompliance.

16. Minimizing risks and monitoring for adverse events

The schedule of events (Table 2) describes the time line and expected type of observations, laboratory tests, and measurements that are planned. Additional testing and patient care will occur as necessary, in consultation with the EB physician. Long term follow up is also planned as described above (section 7.H).

All treatments will be at the discretion of the investigator, subject to approval of the EB physician. If the graft is removed because of infection or trauma, we will consider re-grafting. If lesions occur that are indurated or unusual we will perform diagnostic skin biopsies and treat the lesions as appropriate. If the grafted area expands over mucous membranes we will consider surgical removal of that portion of the graft. If the subject develops EBA or cell mediated inflammation we will evaluate the reaction and treat topically or systemically under the recommendations of the investigator and the EB physician.

In order to verify that subjects are able to undergo anesthesia for grafting, they will meet with the anesthesiologists prior to grafting. The anesthesiologists will review the subject's previous intubation records. Additionally, a cardiology consult may be required, in which the subject may undergo an

echocardiogram as well as an ECG. They may also need additional lab studies to verify that they will be able to tolerate anesthesia.

16.A. Definitions

Please note that the information listed here is identical to that listed in the Safety Monitoring Plan (section 7.B). These definitions are in accordance with the FDA final rule for 21 CFR 312 and 320, effective March 28, 2011.

Adverse event (AE): any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. AEs include any new events not present during the pre-intervention period or events that were present during the pre-intervention period, which have increased in severity.

Life threatening AE: Any AE or adverse gene transfer product experience that places the subject, in the view of the investigator, its occurrence places the subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious Adverse Event (SAE): Any adverse event that, in the view of the investigator, results in any of the following outcomes:

- Death
- a life-threatening adverse event
- requiring inpatient hospitalization or prolongation of existing hospitalization. Note: hospitalization per protocol after grafting (if it is in the best interest of the patient as judged by the treating physician) will not be considered an SAE. However if there is an event triggering the decision to hospitalize the patient, this will be considered an SAE.
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Additionally, any finding from tests in laboratory animals that suggests a significant risk for human research participants, including reports of mutagenicity, teratogenicity, or carcinogenicity will be reported as a serious adverse event.

Associated with the use of LEAES: There is a reasonable possibility, according to the investigator or EB physician, that the experience may have been caused by the gene transfer product (LEAES). Including:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture).

- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Unexpected AE: Any adverse event, the specificity or severity of which is not consistent with the risk information described in the Clinical Protocol, or what is expected based on the medical judgment of the investigator and/or EB physician.

Severity: Severity of the AE will be determined by the investigator and the EB physician. Severity may be mild, moderate, or severe.

Nonconformance: any departure from approved manufacturing standard operating procedures or specifications whether or not LEAES product quality is affected. It is possible that a nonconformance can result in an AE or SAE, in which case the AE will be reported in accordance with the processes described in section 7.E of the Safety Monitoring Plan.

Protocol deviation/violation: Any unplanned excursion from the protocol can be referred to as a protocol deviation or violation. For our purposes, the terms “deviation” and “violation” are synonymous. A protocol deviation/violation may be intended or not intended. A protocol exception is an example of an intended protocol deviation. Please see section 6 of the Safety Monitoring Plan for more information on protocol exceptions.

16.B. Triggers for temporary hold, DSMB review, and regulatory reporting:

The following will require reporting to the DSMB for an ad hoc meeting and will initiate a temporary hold on the study:

- Occurrence of an SAE related or possibly related to the study intervention
- Milestone reached (i.e., recommendation needed for grafting of second participant or moving from grafting adults to pediatric population)
- Outside information is discovered that may affect the study and its participants
- Consideration of graft removal

Corrective actions/treatment will occur at the discretion of the investigator and the EB physician. They are subject to urgent review by the DSMB, IRB, FDA, and other regulatory bodies (see Safety Monitoring Plan, section 7.E). Please note that the following are examples of SAEs that would be related or possibly related to the study intervention, listed as a trigger for DSMB review above.

- Evidence of allergic reaction at graft site
- Evidence of graft rejection
- Evidence of malignancy at graft site
- Evidence of systemic infection
- Evidence of advancing epithelial surfaces
- Death of a study subject

16.C. Patient advocate

Because of the severity of this disease and the complex nature of this study we will offer additional help for the subjects in deciding whether or not to enter or to continue with this research

study. We will ask that each subject communicate with a patient advocate. The advocate will not have any investment in the outcome of our research and will be knowledgeable about all the risks and benefits of participation in this research trial.

16.D. EB Physician

A dermatologist with expertise caring for patients with RDEB has been designated as the EB physician for this study. The EB physician will be involved with the routine care and problems that a patient with RDEB faces in their daily life. The EB physician will have the following responsibilities:

- Exceptions:
  - Review/approve major and minor exceptions to clinical protocol (e.g., lab results not within stated limits, additional clinical abnormalities not specified in protocol, exceptions to target wound criteria. The EB physician will also write a brief justification for the exception, for documentation purposes.
- Treatments:
  - Determine treatments for complications, either due to underlying disease (e.g., low hemoglobin or hematocrit, wound infection, other medical conditions) or grafting with LEAES (adverse events, wound infection, post-release criteria out of specification).
  - Determine if subject needs to remain at Stanford between Day -26 and Day -7 for additional wound or systemic treatments.
  - Determine if additional (unscheduled) visits are needed. Determine which procedures will occur at unscheduled visits.
  - Determine corrective actions for adverse events.
  - Determine whether to re-graft, or perform additional grafting sessions.
- Assessments:
  - Review investigators' assessment of adverse events (e.g., severity, causality).
  - Assess laboratory results for clinically significant abnormalities.
  - Assess vital signs and physical exam for clinically significant abnormalities.
- Study visits:
  - Assist with/conduct study visits caring for the common problems and complications associated with routine care of a patient with RDEB.
  - Assist with grafting procedure, consult with grafting surgeon on graft technique, which sites to be grafted, timing of grafting, and which dressings to use on the graft.
  - Determine which laboratory studies are needed at study visits and in order to prepare for grafting (e.g., additional blood tests, fewer blood tests, urine pregnancy test, omit cytotoxic T cell assay, additional skin biopsies, fewer skin biopsies, determine which graft to obtain biopsies from).
  - Determine if subject meets all requirements to undergo grafting (in consultation with grafting surgeon, investigators, anesthesiologists, cardiologists, etc.).
- Other:
  - Act as a resource for investigators. Provide recommendations in the best interest of the subject. Provide medical oversight for trial.

16.E. Data Safety Monitoring Board

For detailed information on the Data Safety Monitoring Board, please see the Safety Monitoring Plan, which describes the triggers for ad hoc DSMB review (section 4.A.i.1.a), as well as the process for reporting adverse events to each of the regulatory bodies (section 7.E), including the DSMB.

Briefly, the DSMB will meet in person prior to the enrollment of the first subject in the Phase 1 trial in order to review all the safety plans and information. Meeting frequency will occur as described in the DSMB charter.

As described in the Safety Monitoring Plan (section 4), the DSMB will be informed of any complications and variations in the trial as the trial progresses. The DSMB will monitor the gene transfer clinical trial and have the ability to stop or delay the trial.

The DSMB will be involved in the decision whether or not to enroll a second subject in the trial. This decision will be made either during a routine DSMB meeting or a meeting specific to this issue.

Periodically the DSMB will need to have an ad hoc meeting and will be required to consult with the investigators to determine whether or not to proceed with the study. The situations in which an ad hoc meeting would occur are listed in the Safety Monitoring Plan, and include notification of serious adverse events related or possibly related to the study intervention.

A complete listing of the reports that will be reviewed by the DSMB at each meeting are included in the Safety Monitoring Plan (section 8).

16.F. Independent study monitor

This trial will have a paid Consultant for Regulatory Compliance who will monitor for data integrity and good clinical practice. Monitoring will take place at regularly scheduled intervals.

A pre-study site evaluation and initiation visit will be conducted prior to study start to document the appropriateness of the site, to ensure that all documentation required for study start is in place, to review study protocol with staff involved in the trial, and to discuss Good Clinical Practices (GCPs) and study visit documentation.

A monitoring visit will occur after the first visit by the first patient. Monitoring visits will occur at a minimum of every eight weeks unless the volume of the study dictates more frequent monitoring.

The monitor will have access to all study documents. They will review all signed consent forms, entry criteria, source documentation, adverse events, efficacy parameters, and product accountability logs. The monitor will identify any protocol deviations not previously reported (e.g. enrollment of ineligible participant, failure to obtain informed consent, entering of participant into another study, failure to keep IRB approval up to date, etc.). The monitor will also review documentation of reported protocol deviations, including exceptions (see Safety Monitoring Plan, section 6).

All protocol deviations will be recorded in the protocol deviations log (see Manual of Operating Procedures [MOOP], section 29.c) and reported to the DSMB at their regularly scheduled meetings (see Safety Monitoring Plan, section 8). Investigators will ultimately determine if the deviations require reporting to regulatory agencies. See section 7.E.iii of the Safety Monitoring Plan for information on reporting protocol deviations to the Stanford IRB and section 6 of the Safety Monitoring Plan for information on intended deviations (exceptions).

The monitor will also be responsible for ensuring rights/safety of participants, confirming GCP guidelines are followed, ensuring maintenance of required documents, verifying adherence to protocol, monitoring quality of data collected, ensuring accurate reporting and documentation of AEs, ensuring ICF obtained and documented IAW IRB/FDA regulations, information on forms is complete/accurate, there are no omissions in forms, missing examinations are indicated on forms. The monitor will also ensure that the regulatory binder is complete and up to date (see MOOP, section 28).

At the monitoring visit, the monitor will complete the monitoring log (see MOOP, section 29.g). The monitor will review their findings with the PI and will send a letter following the visit documenting any action items, or other items outstanding.

16.G. Independent medical monitor

An independent medical monitor will be responsible for verifying subject eligibility prior to their enrollment in the study. The medical monitor will have access to all study documents, including results of the Characteristics study, preliminary data from Day -26 visit and any other pre-grafting visits, and non-study medical records.

17. Study termination:

The study protocol does not have a specific end date. Individual subject participation will end one year after grafting, at which time they will be enrolled in a separate follow up protocol, as stated previously in the clinical protocol (section 7.H). As previously stated, we plan to enroll 5 subjects over 5 years, based on the amount of funding received from the NIH. The responsibilities of the NIH supported DSMB will end 1 year after grafting of the last subject supported by the NIH grant.

If the gene transfer trial terminates at the request of the DSMB, FDA, IRB, or other regulatory body because of severe complications, all the subjects will be informed and we will attempt to treat the associated complications as best as possible with surgical or medical interventions at the discretion of investigators and the EB physician. We will follow any additional instructions from the EB physician, DSMB, FDA, or other regulatory bodies.

18. Data management plan:

Paper source documents will be prepared prior to study start. These source documents will be used in clinic to collect data for the study.

REDCap, an electronic data capture (EDC) system, will be utilized in this study as an electronic case report form (eCRF). REDCap is a HIPAA-secure database system available through Stanford at http://redcap.stanford.edu.This system can only be accessed by authorized users, and only users with permission to view identifiers will be able to view PHI. Users must sign into REDCap using their Stanford University ID and password. Additionally, users may only access the database from a Stanford IP address, or via a Virtual Private Network (VPN). Prior to study start, a database will be designed for this study that incorporates the data points collected in the source documentation.

After each study visit, data will be transcribed from paper source documents into the electronic data capture (EDC) REDCap system. Hard copies of source documentation will also be retained and stored in the subject binder. Photographs, laboratory reports, and dictations will be uploaded to REDCap.

Study staff will be responsible for "cleaning" the source document data and EDC prior to monitoring. This includes but is not limited to: making sure that dates are logical, reasons for concomitant medication have a corresponding adverse event, making sure that GCP is followed, etc.

Every 8 weeks (unless the volume of study dictates more frequent monitoring), the study monitor will verify that the data entered into REDCap matches the data recorded in the source documentation. REDCap data will be printed and the monitor will verify that this information is the same as in the source documentation.

Data from REDCap will be exported to excel, SPSS, or SAS for analysis. When exporting data, PHI will not be included in the dataset.

19. Statistical plan:

Sample size and power calculations: Due to the pilot nature of this first-in-human study, it is not possible to calculate power or sample size. The effect of the treatment is not known at this time.

Sample size (n=5) was chosen based upon funding constraints as well as the orphan nature of RDEB, and the intensive nature of this protocol. Initially we are selecting only adult subjects (n=5), limiting our potential subject population, as many patients with RDEB do not live to the age of 18. It is currently not known how many RDEB patients are over the age of 18. We are developing additional epidemiological studies to determine this. Further, we are selecting subjects who are NC1[+], which is

approximately 75% of those with RDEB. Additionally, subjects must have both parents alive who are willing to undergo genetic testing in order to confirm the genetic mutations.

Variables to consider:

We will determine time to response, with "response" defined as each of the primary outcomes.

19.A. Primary outcomes:

Determined at Week 12, Week 25, Week 52:

- Production of collagen VII for each graft: (determined by IF). Rater will be blinded as to the time point of the biopsy, whether or not it was obtained from grafted skin or non-grafted, non-wounded skin.
- Production of anchoring fibrils for each graft: (determined by EM). Rater will be blinded as to the time point of the biopsy, whether or not it was obtained from grafted skin or non-grafted, non-wounded skin.
- Incidence of adverse events associated with graft (infection, cancer, autoimmune reaction)
- Investigator's assessment of graft for each graft:
  - Global score: 100-75% healed, 74-50% healed, 49-25% healed, 25-1% healed, Complete graft loss, Unable to determine

19.B. Secondary outcomes:

Collected at Day +14, Week 4, Week 12, Week 25, Week 52:

- Subject's impression of graft, for each graft:
  - Overall impression of graft: rated as totally healed, mostly healed, stable, or worsened
  - Durability (compared to non-grafted skin): rated as more durable, no change, less durable
  - Ease of blistering (compared to non-grafted skin): more difficult to blister, no change, blisters easier
- Collagen VII production and presence of anchoring fibrils at Day +14 and Week 4. Note that these measures are primary outcomes at week 12, week 25 and week 52.
- Investigator's assessment of previous biopsy sites (for each graft): healed, healing, scarred, blistered, N/A (no previous biopsy sites), other
- Dimensions of wound at grafting site (for each graft): Dimensions, including length, width, and area (in cm^2), will be obtained using the ARANZ SilhouetteStar and/or the Canfield system. Changes in dimensions between visits as well as changes in dimensions from baseline will be recorded.
  - Dimensions of intact skin (not wounded) at all graft sites
  - Dimensions of erosions/graft absence at graft sites
- Clinically significant changes in laboratory values: Clinical significance will be determined based upon investigator's judgment.
  - Immune response against collagen VII
  - Cytotoxic T cell assay
  - Replication Competent Retrovirus (RCR)
  - Complete Blood Count with Differential:
    - WBC
    - Hemoglobin
    - Hematocrit
    - Platelet Count
    - MCV
    - RDW
    - RBC
    - MCH
    - MCHC
    - Neutrophils, % and abs
    - Lymphocytes, % and abs
    - Monocytes, % and abs

- Eosinophils, % and abs
- Basophils, % and abs
- Metabolic Panel:
  - Albumin
  - Alkaline Phosphatase Total
  - Anion Gap
  - ALT (SGPT)
  - AST (SGOT)
  - Urea Nitrogen
  - Calcium
  - Chloride
  - CO_2
  - Creatinine
  - Glucose
  - Globulin
  - Potassium
  - Sodium
  - Total Bilirubin
  - Total Protein
- Bilirubin:
  - Direct
  - Indirect

- Clinically significant changes in vital signs: blood pressure, heart rate, respiratory rate, temperature
- Clinically significant changes seen in physical exam: i.e. changes in total % surface area wounded
- Blinded investigator's assessment of healing (photographs):
  - Digital photographs of wounded and non-wounded skin obtained at entry into study, target wounds prior to grafting, target wounds immediately after grafting, grafts at Day 14, Week 4, Week 12, and Week 25. If photographs are assessed at Week 52 (or later as part of a follow up protocol), those photographs will be included in the analysis.
  - Photographs will be standardized for size and color.
  - Blinded observers will confirm the accuracy of the investigator's graft assessments without knowledge of the duration of the graft or when the digital image was obtained.
  - In order to confirm the agreement of the observers' ratings, a Cohen's kappa test will be applied.

For the lab values and other outcomes, we will report the mean or median as appropriate at different time points. We will also look at change in the values using an appropriate statistical test (Wilcoxon signed rank test or McNemar's test for paired measures).

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