PROTOCOL FULL TITLE

Phase I study of lentiviral-mediated *COL7A1* gene-modified autologous fibroblasts in adults with recessive dystrophic epidermolysis bullosa (RDEB).

Trial Acronym: LENTICOL-F

CONFIDENTIAL

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Chief Investigator's Signature

Phase I study of lentiviral-mediated *COL7A1* gene-modified autologous fibroblasts in adults with recessive dystrophic epidermolysis bullosa (RDEB).

Trial Acronym: LENTICOL-F

I, the undersigned, have read and understood the protocol specified above and agree to conduct the study as outlined herein. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the investigational agent and the conduct of the study according to GCP as described in the GCP Directive and to the relevant ethics committee (IRB/IEC) requirements.

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1. Study Synopsis

Title of clinical trial	**Phase 1 study of lentiviral-mediated *COL7A1* gene-modified autologous fibroblasts in adults with recessive dystrophic epidermolysis bullosa (RDEB).**
Protocol Short Title/Acronym	**LENTICOL-F**
Study Phase	1
Sponsors	**Lead Sponsor**: King's College London (King's Health Partners) **Co-Sponsor**: Guy's and St Thomas' NHS Foundation Trust
Chief Investigator	Professor John A McGrath
Medical condition or disease under investigation	Recessive Dystrophic Epidermolysis Bullosa (RDEB)
Purpose of clinical trial	To assess whether intradermal injections of self-inactivating (SIN) lentiviral vector (LV)-mediated *ex vivo* transduced autologous fibroblasts expressing codon-optimised *COL7A1* (investigational medicinal product – IMP), are safe and have potential efficacy in adults with RDEB.
Primary objective	To evaluate the safety of intradermal injections of SIN LV-mediated *COL7A1* gene-modified autologous fibroblasts in adults with RDEB.
Secondary objectives	1. To evaluate the potential efficacy of intradermal injections of SIN LV-mediated *COL7A1* gene-modified autologous fibroblasts in adults with RDEB at week (W) 2, month (M) 3 and M12 after the IMP injections. 2. To evaluate the immune response against the recombinant type VII collagen (C7) at W2, W4, M3, M6 and M12 after the IMP injections compared to baseline.
Trial Design	Phase 1, non-randomised, open-label, single-centre, proof-of-concept study.
Endpoints	**<u>Primary Endpoints</u>** • Adverse events (AEs), Serious Adverse Events (SAEs), Adverse Reactions (ARs) and Serious Adverse Reactions (SARs) at each visit

	over a 12-month follow-up period. **<u>Secondary Endpoints</u>** • Skin biopsy analysis of treated skin at W2, M3 and M12 compared to untreated skin: I. C7 protein expression by immunofluorescence microscopy (IF) II. Morphology of anchoring fibrils at the dermal-epidermal junction (DEJ) by transmission electron microscopy (TEM) III. Vector copy number by quantitative polymerase chain reaction (qPCR) • Serum analysis for: IV. Detection of anti-C7 antibodies by enzyme-linked immunosorbent assay (ELISA) (against NC1 and NC2 domains of C7) and indirect immunofluorescence (IIF) at W2, W4, M1, M3, M6 and M12 post-injections V. Detection of T-cell responses to the full length C7 by enzyme-linked immunosorbent spot (ELISPOT) assay at W4, M6 and M12 post-injections.
Sample Size	5–10 patients
Summary of eligibility criteria	**<u>Inclusion criteria</u>** 1. Clinical and genetic diagnosis of RDEB with confirmed bi-allelic *COL7A1* mutations. 2. A reduced number or morphologically abnormal anchoring fibrils confirmed by TEM. 3. At least 5x3cm of intact skin on the trunk and/or extremities that is suitable for cell injections. 4. Able to undergo local anaesthesia. 5. Subjects aged ≥ 17years and able to give informed consent prior to the first study intervention. **<u>Exclusion criteria</u>** 1. Subjects who received other investigational medicinal products within 6 months prior to enrolment into this study.

	2. Past medical history of biopsy proven skin malignancy. 3. Subjects who have received immunotherapy including oral corticosteroids (Prednisolone >1mg/kg) for more than one week (intranasal and topical preparations are permitted) or chemotherapy within 60 days of enrolment into this study. 4. Known allergy to any of the constituents of the investigational medicinal product (IMP). 5. Subjects with **BOTH**: • positive serum antibodies to C7 confirmed by ELISA **and** • positive IIF with binding to the base of salt split skin. 6. Subjects with positive results for HIV, Hepatitis B, Hepatitis C, HTLV or Syphilis. 7. Subjects who are pregnant or of child-bearing potential who are neither abstinent nor practising an acceptable means of contraception when this is in line with the usual and preferred lifestyle of the subject, as determined by the Investigator, for 12 months after the cell injections.
IMP, dosage and route of administration	**IMP:** SIN LV-mediated *ex vivo* transduced autologous fibroblasts expressing codon-optimised *COL7A1.* **DOSAGE:** 0.8–1.2 million cells suspended in 0.25ml of 0.9% saline per injection over 1 cm^2 of intact skin (3 injections of IMP at a single timepoint). **ROUTE OF ADMINISTRATION:** Intradermal injections.
Active comparator product (Control)	None.
Maximum duration of study participation	32 months (each patient will be followed up for a total of 12 months post-IMP injections and screening will take place up to 4 months prior to the intervention).
Version and date of protocol	Version 4.0, 01/MAR/2016

2. Glossary of Terms

AE	Adverse Event
AR	Adverse Reaction
ATMP	Advanced Therapy Medicinal Product
BEBSS	Birmingham Epidermolysis Bullosa Severity Score
C7	Type VII Collagen
CRF	Case Report Form
DEB	Dystrophic Epidermolysis Bullosa
DEJ	Dermal-Epidermal Junction
DIF	Direct Immunofluorescence
DMC	Data Monitoring Committee
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulphoxide
EB	Epidermolysis Bullosa
EBS	Epidermolysis Bullosa Simplex
EDTA	Ethylenediaminetetraacetic acid
EUD	European Union Directives
FACS	Fluorescence activated cell sorting
FCS	Fetal calf serum
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GOSH	Great Ormond Street Hospital
GSTT	Guy's and St Thomas' NHS Foundation Trust
GTAC	Gene Therapy Advisory Committee
GvHD	Graft versus Host Disease
HIV	Human immunodeficiency virus
HLA	Human Leucocyte Antigen
HTLV	Human T-lymphotropic virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	Institute of Child Health
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier

iPS	Induced Pluripotent Stem Cells
ISA	Integration site analysis
JEB	Junctional Epidermolysis Bullosa
KHP-CTO	King's Health Partners-Clinical Trials Office
LV	Lentiviral Vector
MHC	Major Histocompatibility Complex
MHRA	Medicines and Health Products Regulatory Agency
QP	Qualified Person
qPCR	Real Time Polymerase Chain Reaction
RCL	Replication-competent Lentivirus
RDEB	Recessive Dystrophic Epidermolysis Bullosa
RV	Gamma-Retroviral Vector
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDs	Standard Deviations
SIN	Self-inactivating
SOPs	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UCL	University College London

3 Background & Rationale

3.1 Epidermolysis bullosa (EB)

Epidermolysis bullosa (EB) is a heterogeneous group of inherited disorders characterised by skin blisters and mucosal fragility. EB affects ~1 in 17,000 live births and ~500,000 individuals worldwide (Fine et al. 2014). EB is classified into 4 major subtypes based on the new classification system: epidermolysis bullosa simplex (EBS), junctional epidermolysis bullosa (JEB), dystrophic epidermolysis bullosa (DEB), and Kindler syndrome although the full classification extends to ~20 different clinicopathological variants (Fine et al. 2008, and 2014). The new classification system adopts the 'onion skin approach' that takes into account the EB type, mode of inheritance, phenotype, immunofluorescence antigen mapping findings, and mutation(s) present in each patient (Fine et al. 2014). The main forms of EB are distinguished by different levels of cleavage underlying the blister formation as well as the adhesion proteins that are genetically mutated. In EBS, the level of separation occurs is within the epidermis, in JEB cleavage is within the lamina lucida, and in DEB blisters develop below the lamina densa within the upper papillary dermis. In Kindler syndrome, the plane of cleavage is variable.

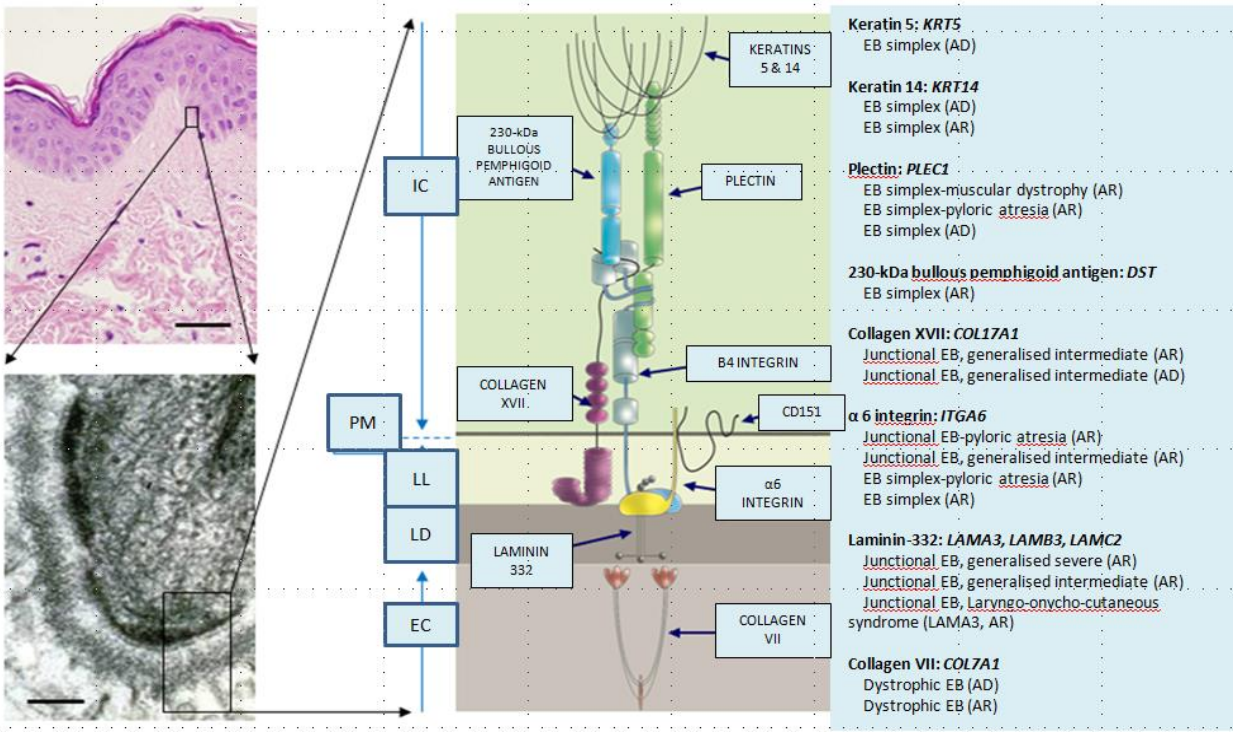


Figure 1: The molecular basis of the major forms of EB. Top left is a light microscopy image

of the skin; the boxed area indicates the dermal-epidermal junction: the section is stained with haematoxylin & eosin (scale bar=50 μm). Bottom left is a transmission electron micrograph of the dermal-epidermal junction; hemidesmosome attachment complexes are boxed (scale bar=0.1 μm). Right side is a schematic representation of the protein organisation of dermal-epidermal attachment complexes, the intrinsic proteins and the genes encoding them, and the associated forms of EBS, JEB and DEB. (IC=intracellular; PM=plasma membrane; LL=lamina lucida; LD=lamina densa; EC=extracellular).

3.2 Recessive dystrophic epidermolysis bullosa (RDEB)

One of the most severe forms of EB is recessive DEB (RDEB) that affects ~800 people in the UK (source: www.debra.org.uk). RDEB is caused by bi-allelic loss-of-function mutations in the type VII collagen gene (*COL7A1*), leading to reduced or absent basement membrane type VII collagen (C7) and poorly formed or absent anchoring fibrils at the dermal-epidermal junction (DEJ) (Hilal et al. 1993). Poor anchoring fibril function leads to lifelong severe skin blistering and erosions following minor mechanical trauma from birth or early infancy. Wound healing is often slow, leading to chronic erosions, secondary infection and progressing to widespread, mutilating scars and contractures. Pseudosyndactyly (mitten deformities) occurs in nearly every patient with the most severe subtype of RDEB (Fine et al. 2005).

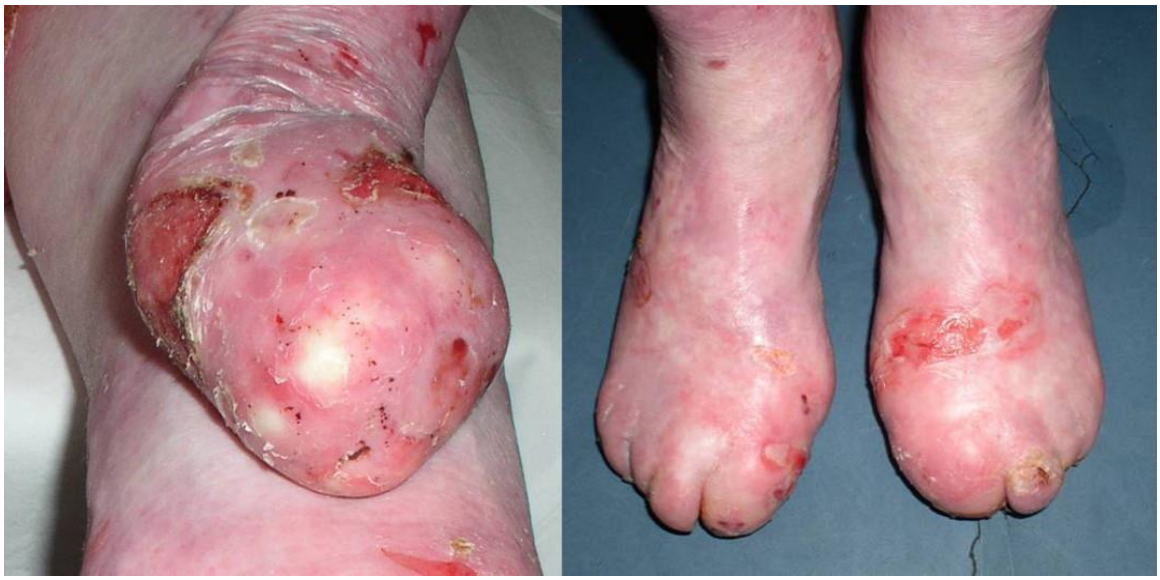


Figure 2: "Mitten-like" appearance of the left hand and feet (pseudosyndactyly) in the patient with severe generalized RDEB.

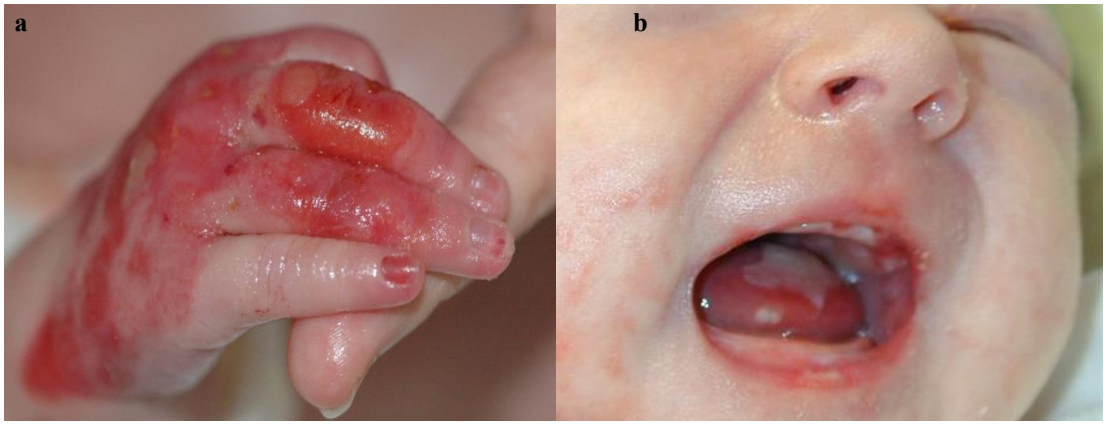


Figure 3a: Erosions and fragile blisters on the dorsum of the right hand of a child with severe RDEB. Figure 3b: Extensive oral ulceration in a child with RDEB–generalised other (Figures courtesy of Dr Jemima Mellerio, co-investigator).

Although cutaneous involvement is the most obvious clinical feature, extracutaneous features of children with severe RDEB include profound anaemia, failure to thrive, growth retardation, severe mucosal involvement, ocular involvement (corneal erosions and scarring) and oesophageal strictures (Pillay et al. 2008; Fine et al. 2009).

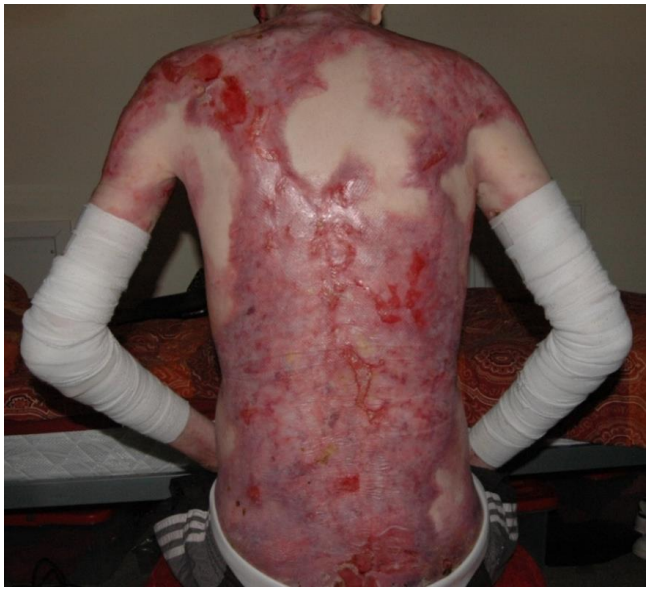


Figure 4: Erosions on the background of extensive scarring on the back of a young adult with severe generalised RDEB (Figure courtesy of Dr Gabriela Petrof).

Currently, there is no effective treatment for RDEB and many individuals develop life-shortening squamous cell carcinomas by the age of 40 years (Martins et al. 2009).

3.3 *COL7A1* mutations leading to RDEB

C7 is synthesised mainly by epidermal keratinocytes and, to a lesser extent by dermal fibroblasts (Bruckner-Tuderman et al. 1987), Over 250 mutations in the *COL7A1* gene have been so far identified. Jarvikallio et al (1997) has described that to some extent the severity of blistering and epidermal involvement can be correlated with the mutation profile in *COL7A1*, with more severe forms of the disease having nonsense or frameshift mutations on both alleles with less severe forms of RDEB harbouring missense or in-frame splice site mutations, at least on one *COL7A1* allele (Jarvikallio et al. 1997).

3.4 Management of RDEB

Best practice treatment involves a multi-disciplinary team of healthcare workers, with daily dressings often taking 1–4 hours to perform (Grocott et al. 2013). Total healthcare costs for individuals with severe RDEB living in the UK are estimated to be in excess of £60,000 per year (source www.debra.org.uk), with repeated applications of dressings to large wounds accounting for much of the overall expense. Currently, there is no effective treatment for RDEB and the management is mainly palliative and supportive. RDEB has a significant medical and socio-economic impact for all patients and their families (Tabolli et al. 2009). Chronic skin blistering affects the personal, physical, emotional and professional aspects of the patient and family (Abercrombie et al. 2008).

3.5 Gene therapy for inherited skin disorders

Gene therapies aim to provide therapeutic benefit through manipulation of DNA or RNA. Single gene disorders can be corrected by gene addition or augmentation, sequence correction, gene knockdown or exon skipping. To date, gene therapies using viral vectors have largely been conducted *ex vivo*, on cells harvested, engineered and then returned to recipients (Appendix 1). This has allowed issues of toxicity, including immune responses against viral antigens and worries about bio-distribution to be circumvented.

Monogenic recessive disorders with a clinically severe or life-threatening phenotype provide the best candidate diseases for the introduction of a single normal copy of the

gene into the target cell. Pre-clinical studies have shown remarkable results in terms of gene correction using both *in vivo* and *ex vivo* approaches. These include JEB (Ferrari et al. 2006; Mavilio et al. 2006), RDEB (Titeux et al. 2010; Baldeschi et al. 2003; Woodley et al. 2003; Chen et al. 2002), lamellar ichthyosis (Choate et al. 1996), X-linked ichthyosis (Freiberg et al. 1997; Choate et al. 1997), xeroderma pigmentosum (XP) (Marchetto et al. 2006; Magnaldo et al. 2004), and Netherton syndrome (Di et al. 2011 and 2013).

With regard to skin studies relevant to EB, target cells have included both fibroblasts and keratinocyte stem cells. Of note, fibroblasts have been transduced and injected into recipient immunodeficient mice and shown to secrete C7 (Chen et al. 2002; Ortiz-Urda et al. 2003; Goto et al. 2006; Ito et al. 2009; Titeux et al. 2010). Moreover, correction of keratinocyte stem cells using integrating viral vectors has been shown in small and large animal models (Chen et al. 2002; Ortiz-Urda et al. 2003; Ito et al. 2009; Titeux et al. 2010; Siprashvili et al. 2010; Gache et al. 2011).

Recently, the Stanford group has successfully begun grafting *ex vivo* autologous epidermal sheets in an adult with RDEB in a phase I clinical trial (ClinicalTrials.gov Identifier: NCT01263379). In this study, autologous keratinocytes were transduced with GMP grade gamma-retrovirus carrying the full-length *COL7A1*. Six ~35cm^2 autologous epidermal sheets were grafted in an adult with RDEB with complete epidermal regeneration 30 days post grafting with clinically normal skin appearance and no signs of immunological rejection (Siprashvili *et al.* 2014).

3.6 Rationale for using lentiviral vectors for gene delivery

RVs are the most widely used vehicles in human gene trials (Khavari et al. 2002; Gache et al. 2004; Gache et al. 2011), and have been used to transduce keratinocytes with *COL17A1* (Seitz et al. 1999)*, LAMB3* (Vailly et al. 1998), *ITGB4* (Dellambra et al. 2001) as well as *COL7A1* (Titeux et al. 2010; Baldeschi et al. 2003; Woodley et al. 2003; Chen et al. 2002; Siprashvili *et al.* 2014). The use of these vectors in RDEB has been limited by the large size (9.2kb) of the *COL7A1* cDNA (Gotto et al. 2006). One study comparing

adenoviral vectors, adenovirus-associated virus, RVs, and LVs concluded that LVs were the most suitable for *ex vivo* gene therapy due to their ability to transduce clonogenic primary keratinocytes (Gagnoux-Palacios et al. 2005). Keratinocytes have been successfully transduced *in vivo* with a C7 LV-containing full-length *COL7A1* transgene (Chen et al. 2002; Baldeschi et al. 2003; Ortiz-Urda et al. 2002). Limitations of oncoretroviral systems include poor translocation across intact nuclear membranes and a tendency for transgene 'silencing' over time (Fenjves et al. 1996). In addition, conventional murine RVs preferentially integrate close to transcriptional start sites and have been shown capable of mediating insertional mutagenesis in clinical gene therapy studies (Hacein-Bey-Abina et al. 2003). In contrast, LVs can transduce quiescent or minimally stimulated cells. LVs are less likely to mediate insertional mutagenesis if suitable internal promoter elements are used (Montini et al. 2006). The investigators have considerable experience in lentiviral technology and have the ability to generate bespoke vectors suitable for fibroblast modification. The vectors are replication-defective, self-inactivating (SIN) and incorporate a central polypurine tract sequence and post-transcriptional regulatory element (Demaison et al. 2002; Qasim et al. 2007).

The investigators are also conducting a Phase I clinical study of *ex vivo* lentiviral modified skin grafts in Netherton Syndrome (ClinicalTrials.gov Identifier: NCT01545323). Vector titre and gene expression levels have remained within target ranges during scaled up production, providing an important precedent for the work for this study. Our preclinical data for expression of *COL7A1* from this gene therapy platform is detailed in the Investigational Medicinal Product Dossier (IMPD) accompanying this protocol, and crucially we have modified cryptic splice donor and acceptor sites within the transgene, eliminating splice variants reported by other groups and affording high levels of gene expression in keratinocytes and fibroblasts.

3.7 Rationale for targeting fibroblasts for gene therapy in RDEB

Skin wounds in RDEB are typically very inflamed and often colonised or infected with numerous bacteria or yeasts and thus the application of any topical bio-material (e.g. keratinocytes or skin bioequivalents) runs a substantial risk of partial or complete loss of

the grafted material and failure to generate adequate safety or feasibility data. By contrast, intradermal injections of fibroblasts into intact, unwounded RDEB skin, offers a “closed” system, one that is not influenced by external organisms or other factors that may damage or destroy the fragile graft. The choice of fibroblasts for gene correction is also supported by transgenic *COL7A1* murine data that found gene-corrected fibroblasts alone were able to restore C7 expression to the basement membrane, i.e. without the need for gene-correction of keratinocytes (Ito *et al*. 2009). Fibroblast targeted gene therapy also has potential clinical extrapolation: some patients with RDEB have localised disease for which localised therapies (rather than systemic measures) may be relevant. Moreover, the data obtained from studies of gene therapy in fibroblasts may have pertinent implications for gene therapy targeting of MSCs and clinical trials of systemic gene therapy for RDEB.

Fibroblast cell therapies have been used to treat a multitude of skin disorders (Appendix 2).The rationale for the use of fibroblasts in RDEB is based on the earlier work in murine models evaluating skin integrity following intradermal fibroblast administration. Fibroblasts are able to secrete C7, and therefore the hypothesis was that intradermal administration of exogenous allogeneic fibroblasts would lead to increased levels of C7 at the DEJ, resulting in new anchoring fibrils. Ortiz Urda *et al* (2002) first showed that intradermally administered genetically engineered human fibroblasts can home to murine skin and generate new C7 at the DEJ. This group used a φC31 based integrase vector (non-viral vector approach) to transduce RDEB fibroblasts. These were administered alongside RDEB(-) fibroblasts and wild type fibroblasts into RDEB human skin tissue regenerated on immune-deficient *scid/scid* mice. Both RDEB(-) and normal fibroblasts failed to restore C7 at the cutaneous BMZ or correct sub-epidermal blistering in any of the injected RDEB(-) grafts. Injected RDEB+ cells, however, achieved both goals with results for the 4-month duration of the experiment in all patient tissue studied. Chen *et al* (2002) used a LV (SIN-LV expressing *COL7A1* cDNA under the control of retroviral-MND promoter) to express C7 in RDEB keratinocytes and fibroblasts (in which C7 was absent) and demonstrated a reversion of the RDEB cellular phenotype *in vitro* (Chen et al. 2002). These gene-corrected cells were then used to

create a composite human skin equivalent transplanted onto *scid/scid* mice. The transplanted human skin exhibited a restoration of type VII collagen and anchoring fibrils at the DEJ (Chen et al. 2002). The same group then went onto investigate whether gene-corrected fibroblasts alone would be able to generate sufficient C7 to correct the phenotype (Woodley et al. 2003). Gene-corrected RDEB fibroblasts, parental RDEB fibroblasts, and normal human dermal fibroblasts were used. 5 x 10^6 fibroblast cells were suspended in Phosphate Buffer Solution (PBS) were injected into the dorsal back skin of 6 week old athymic nude mice. This study demonstrated that gene-corrected fibroblasts alone are able to produce more C7 at the DEJ than wild type *in vivo*. The expression of human type VII collagen by gene-corrected RDEB fibroblasts was sustained for at least 4 months in the mouse DEJ after a single intradermal injection of gene-corrected RDEB fibroblasts (**Figure 5**).

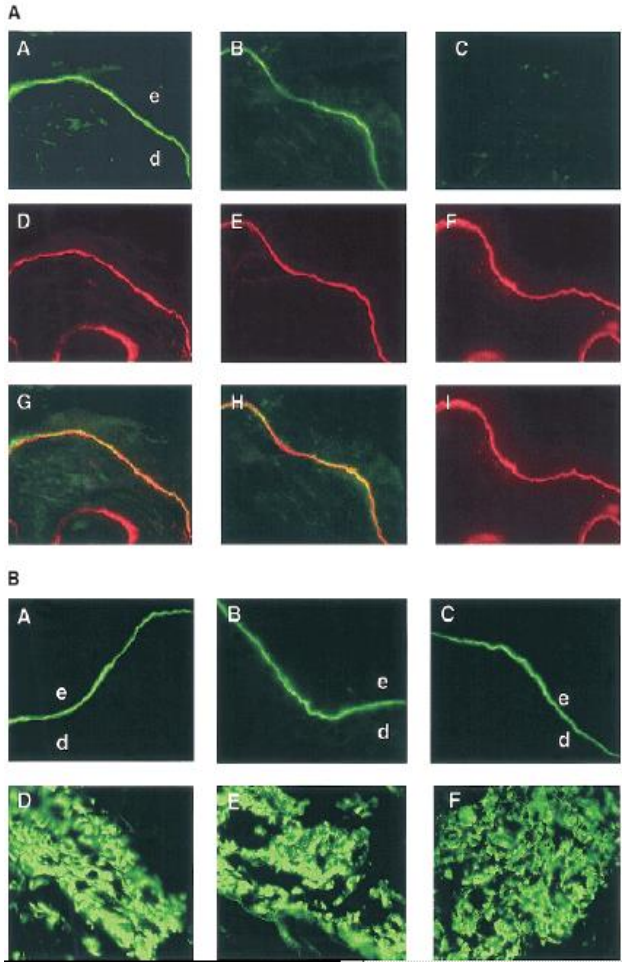


Figure 5: Images of immunofluorescence staining showing deposition of human type VII collagen at the mouse dermal-epidermal junction after a single intradermal injection of fibroblasts (figures courtesy of Woodley et al. 2003)

(*A*) Immunofluorescence staining of mouse skin intradermally injected with fibroblasts 2 wk after injection was performed with either a monoclonal antibody specific for human type VII collagen (*green*, *panels A–C*) or a rabbit polyclonal antibody recognizing both mouse and human type VII collagen (*red*, *panels D-F*). (*A*,*D*,*G*) Skin injected with gene corrected RDEB fibroblasts. (*B*,*E*,*H*) Skin injected with normal human fibroblasts. (*C*,*F*,*I*) Skin injected with parental RDEB fibroblasts. Merged images (*panels G-I*) demonstrate the colocalization of human type VII collagen with mouse type VII collagen in the mouse's BMZ. (*B*) Skin from mice injected intradermally with gene-corrected RDEB fibroblasts was stained with a monoclonal antibody specific for human type VII collagen. (*A*,*D*) Biopsies taken 2 mo after injection; (*B*,*E*) biopsies taken 3 mo after injection; (*C*,*F*) biopsies taken 4 mo after injection. *e*, epidermis; *d*, dermis.

3.8 Previous studies supporting the fibroblast therapy

The **McGrath** group has undertaken first-in-man and early phase studies investigating the use of intradermal allogeneic fibroblasts in patients with RDEB (Wong *et al. 2008;* Petrof *et al. 2013*). The first administration of allogeneic intradermal fibroblasts in patients with RDEB was performed by Wong et al. in 2008. Five adult RDEB subjects (3 with severe generalised RDEB and 2 with a milder phenotype) received six injections: 2 of autologous cultured fibroblasts, 2 of cultured fibroblasts from 1 parent, and 2 injections of cultured fibroblasts from an unrelated donor (Wong et al. 2008) (**Figure 6**). Biopsies were carried out at 2 weeks and 3 months. All five subjects demonstrated an increase in C7 immunolabeling at the DEJ at 2 weeks after allogeneic fibroblast injection, compared with baseline. Importantly, the subjects in whom these data were obtained tended to have some C7 expression at baseline, in keeping with upregulation of mutant but partially functional endogenous C7.

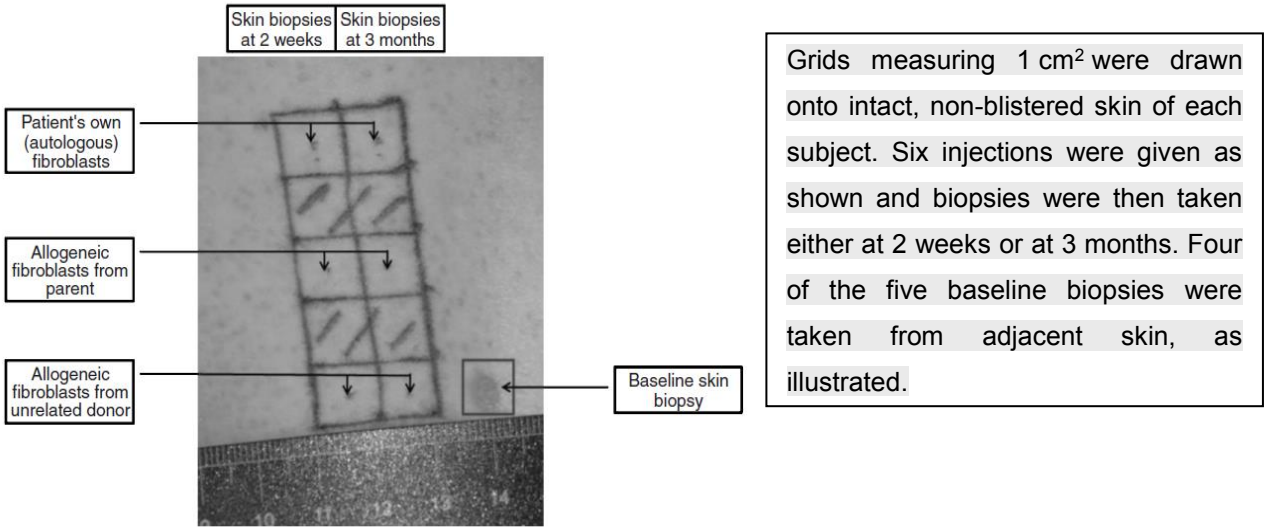


Figure 6: Fibroblast injection sites and biopsy time points (Figure courtesy of Wong et al. 2008)

Subsequently, a phase II clinical trial demonstrated that a single intradermal injection of allogeneic fibroblasts increases the initial rate of erosion healing in subjects with RDEB within the first 28 days but not thereafter (Petrof et al. *2013*), although that trial did not involve further injections of fibroblasts or optimisation of cell dose or dosing

regimens. In this study, allogeneic fibroblasts increased the *COL7A1* gene expression for up to 3 months and the protein levels for up to 9 months (Petrof et al. 2013). In some individuals, injection of allogeneic fibroblasts into intact skin led to increased C7 at the DEJ within two weeks of a solitary injection, and increased (though not normalised) anchoring fibrils were visible on transmission electron microscopy (TEM). Despite this beneficial effect, donor fibroblasts were not detected in the patient skin biopsies at 2 weeks and 3 months, therefore suggesting that the allogeneic fibroblasts were cleared relatively quickly by the hosts' immune response. It is likely that the injection of allogeneic fibroblasts elicit an immune reaction that leads to synthesis of heparin binding–EGF-like growth factor (HB-EGF), which upregulates the synthesis and assembly of the patient's own mutated C7 (Nagy *et al*. 2011). Importantly, this immune response however did not result in any adverse events or pathogenic consequences.

In all murine studies of gene, cell and protein therapy for RDEB, although circulating anti-C7 antibodies have been identified, none of the positive sera have shown binding to the dermal-epidermal junction (DEJ) and therefore their pathogenic significance (if any) is unknown. Clinically, thus far, there are no reports of any patients with RDEB developing EB acquisita (EBA) as a consequence of an interventional therapy or a natural event such as revertant mosaicism. In Wong *et al.* (2008) study,the immunostaining for anti-C7 antibodies at three months showed that none of the five subjects with RDEB receiving intradermal injections of autologous, related donor allogeneic and unrelated donor allogeneic fibroblasts, developed circulating anti-C7 antibodies at three months.

3.9 Experience from clinical gene therapy studies

Qasim is leading clinical trials of T cell therapies after haematopoietic stem cell transplant (HSCT), and has completed a Phase I study of gene-modified T cells, which required production of good manufacturing practice (GMP) grade vector in London (Qasim et al. 2007; Qasim et al. 2009). The experience has been applied to the production of T cell receptor modified cells for two additional studies which are now

open, and forthcoming trials of lentiviral engineered T cells for malignancy. **Thrasher** has undertaken successful gene therapy studies using gamma-retroviral vectors for inherited immune deficiency and is leading next generation trials including lentiviral gene therapy for Wiskott Aldrich syndrome (Galy et al. 2011), chronic granulomatous disease (Grez et al. 2010) and SCID-X1 (Gaspar et al. 2004). There is now an established core of expertise in vector production, cell transduction, regulatory issues and trial management.

Di and **Qasim** have taken a novel gene-based therapy for NS from bench to bedside. Initial studies using second generation lentiviral vectors showed that it was possible to transduce and correct keratinocyte stem cells from patients with NS. **Di** has produced *in vitro* organotypic cultures showed restoration of defective LEKTI (the protein encoded by *SPINK5*, the NS gene) production and correction of abnormal NS skin architecture. *In vivo* modelling using human:murine skin graft chimeras confirmed durability of gene correction (Di et al. 2011). Limitations associated with methylation-mediated repression of gene expression were successfully addressed by incorporating the human involucrin promoter, which mediated compartment specific and high level expression of transgene in human keratinocytes (Di et al. 2011). A Phase I study of *ex vivo* lentiviral gene therapy for the NS is currently underway (ClinicalTrials.gov Identifier: NCT01545323). This study uses a third generation lentiviral configuration, identical to the vector for this study, to generate *SPINK5* gene-modified epidermal skin sheets which will then be grafted on the individuals with NS.

3.10 GMP grade lentiviral vector expressing C7 protein

Using similar processes, we have generated a third generation lentiviral vector (detailed in the accompanying Investigational Medicinal Product Dossier) expressing a modified *COL7A1* transgene, devoid of cryptic splice sites and repressive sequences, but including a Kozak consensus sequence. Expression is driven by the human PGK promoter. The vector has been used to transduce both fibroblasts and keratinocytes and mediates restoration of migratory function *in vitro*. In a human:murine chimeric skin model the vector supports reconstitution of basement membrane C7 expression,

and recovery of anchoring fibrils (seen by TEM). Previous reports had indicated that retroviral vectors expressing *COL7A1* were prone to a high frequency of re-arrangements in KC. We isolated and expanded clonal populations of keratinotyes that had been transduced with pCCL-*COL7A1* and found 3/49 clones expressed truncated protein bands as a result of deleted sequences within the transgene. It is uncertain whether the occurrence of such deletions can be completely eradicated from vectors which undergo reverse transcription, but functional studies in these clones found no additional aberrant effects. Subsequently a batch of GMP compliant lentiviral vector expressing *COL7A1* has been generated with a titre of $1.16x10^7$ TU/ml, sufficient for transduction of fibroblasts. Release testing has been completed, including the exclusion of adventitious pathogens and replication-competent lentivirus (RCL).

3.11 Fibroblast harvest, transduction, expansion and cryopreservation

In our GMP validation studies, a 6mm fresh skin biopsy from subjects with RDEB was treated with collagenase to extract fibroblasts which were then expanded until sufficient to fill a T25 flask. Approximately one million autologous fibroblasts were then transduced by exposure to pCCL-PGK-COL7A1 vector, before further expansion over 3-4 weeks to reach $>8x10^6$ cells. Samples were tested for vector copy number and collagen expression by western blot, flow cytometry and *in situ* staining, and archived for RCL and integration site analysis (ISA). Immediate recovery after a freezing-thawing procedure was evaluated to range from 45% to 75%, rising to 100% viability after one week of subsequent culture. We anticipate similar recovery after *in vivo* inoculation. Cells thawed and re-suspended in saline remained viable for over 8 hours in a 1ml syringe, and the plan for the clinical trial is to inject within this time window.

3.12 Restoration of C7 expression in gene-modified fibroblasts

C7 restoration in genetically modified RDEB primary fibroblasts was further confirmed by Western blot and *in situ* immunohistochemistry **(Figure 7a & b)**.

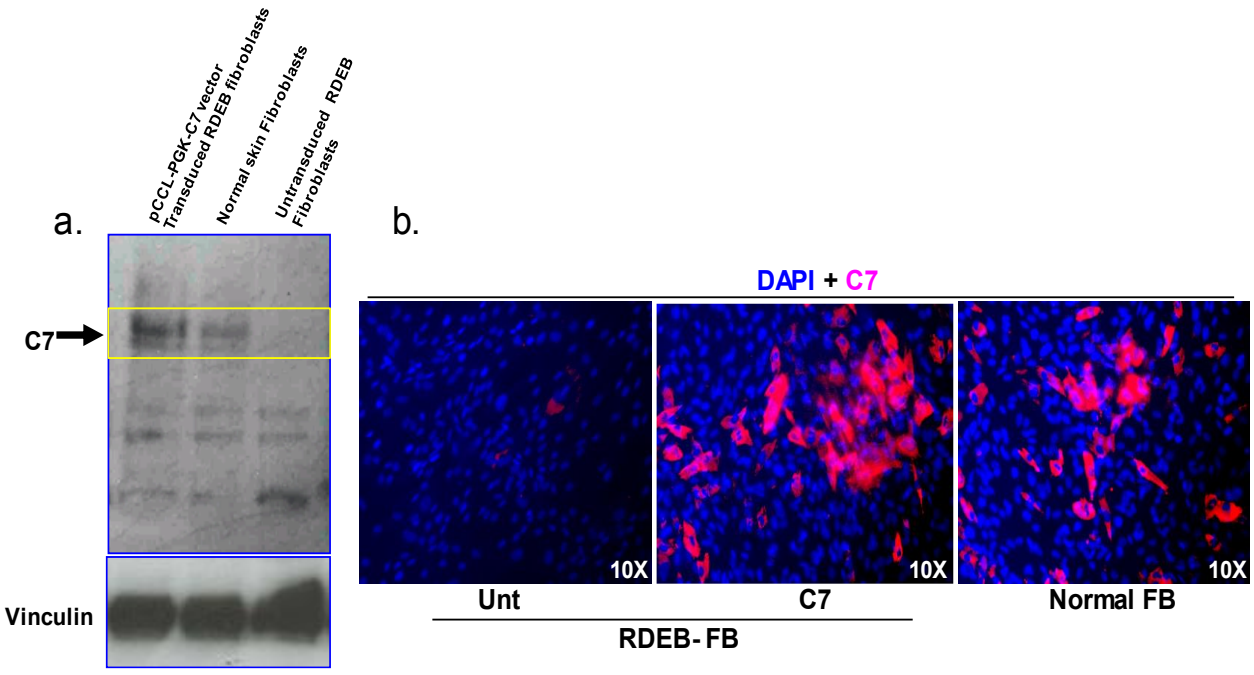


Figure 7: Fibroblasts isolated from RDEB patient were transduced with pCCL-PGK-COL7A1 vector. a. The Collagen VII expression was detected by Western blot with specific polyclonal anti-Collagen VII antibody. Full-length type VII collagen at the size of 290Kda was detected in cells transduced with therapeutic vector pCCL-PGK-COL7A1(line 1). A similar detection pattern was also seen in normal fibroblasts from normal skin (Lane 2). In contrast, no Collagen VII was detected in untransduced RDEB fibroblasts (Lane 3). **b.** Primary fibroblasts were stained with COL7A1 antibody (Red) and cell nuclei were stained with DAPI (blue). There was C7 protein expression (red) in transduced cells (C7) and normal fibroblasts (FB) compared to untransduced cells (Unt).

3.13 *In vitro* function tests for gene-modified fibroblasts

Primary fibroblasts from RDEB modified for C7 showed comparable viability/metabolic activity assessed using WST-1 kit (**Figure 8**). Importantly, migration assays show gene modified RDEB cells efficiently closed the wound compared to unmodified RDEB cells, indicating functional restoration in transduced cells (**Figure 9**).

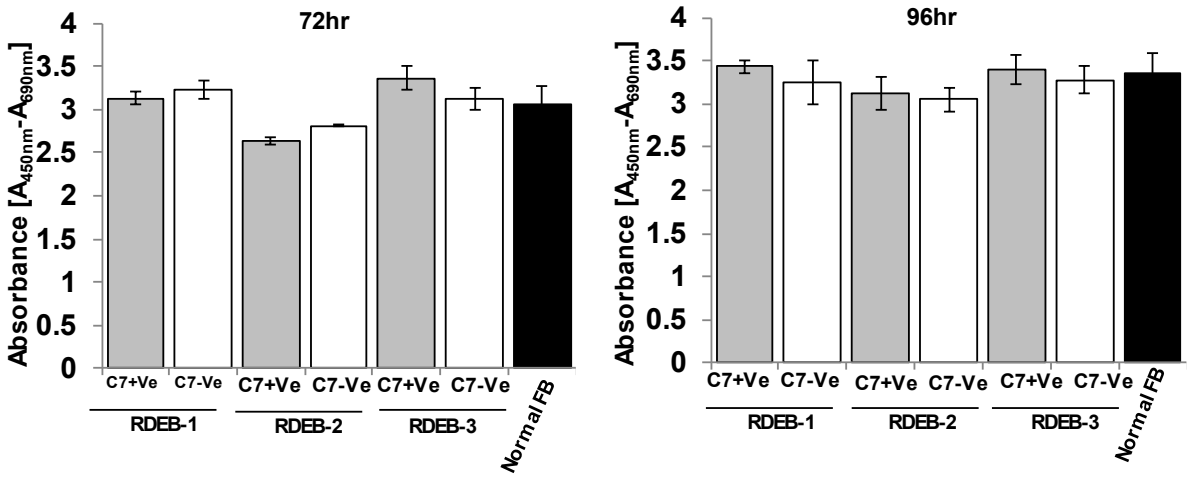
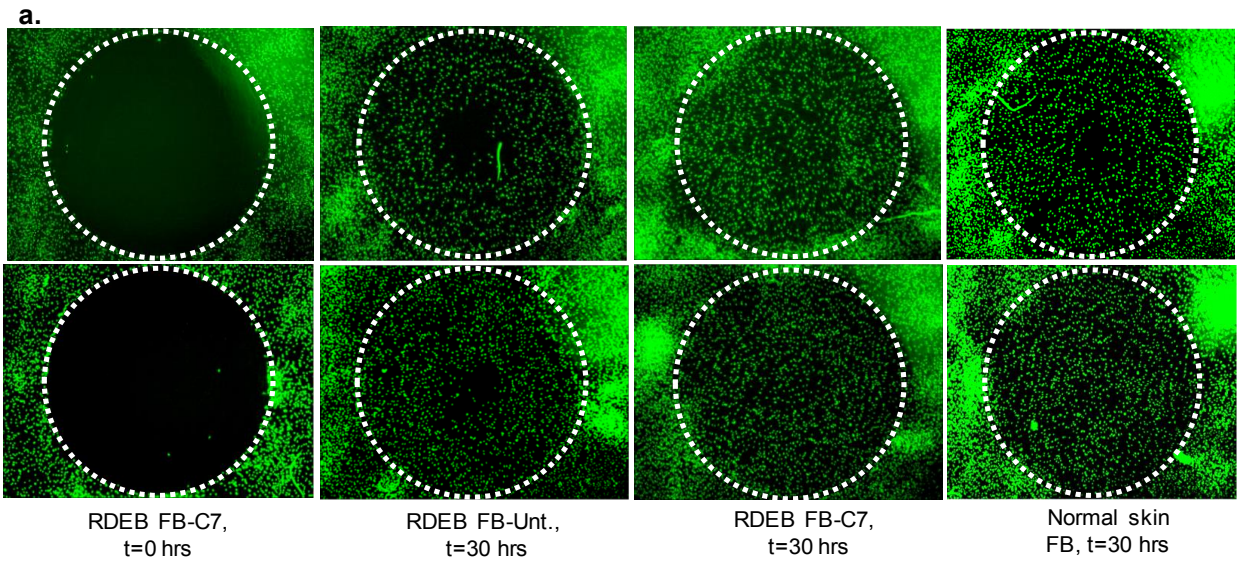


Figure 8: Metabolic activity and cell viability in cells transduced with pCCL-PGK-COL7A1 vector. Transduced and untranduced RDEB fribroblasts from three patients were plated in 96 well plate (1X10^4 cells/well) and cultured for 72 and 96 hours. Cell viability and metabolic activity using WST-1 assay was performed and results showed there were no differences between transduced and untransduced cells.



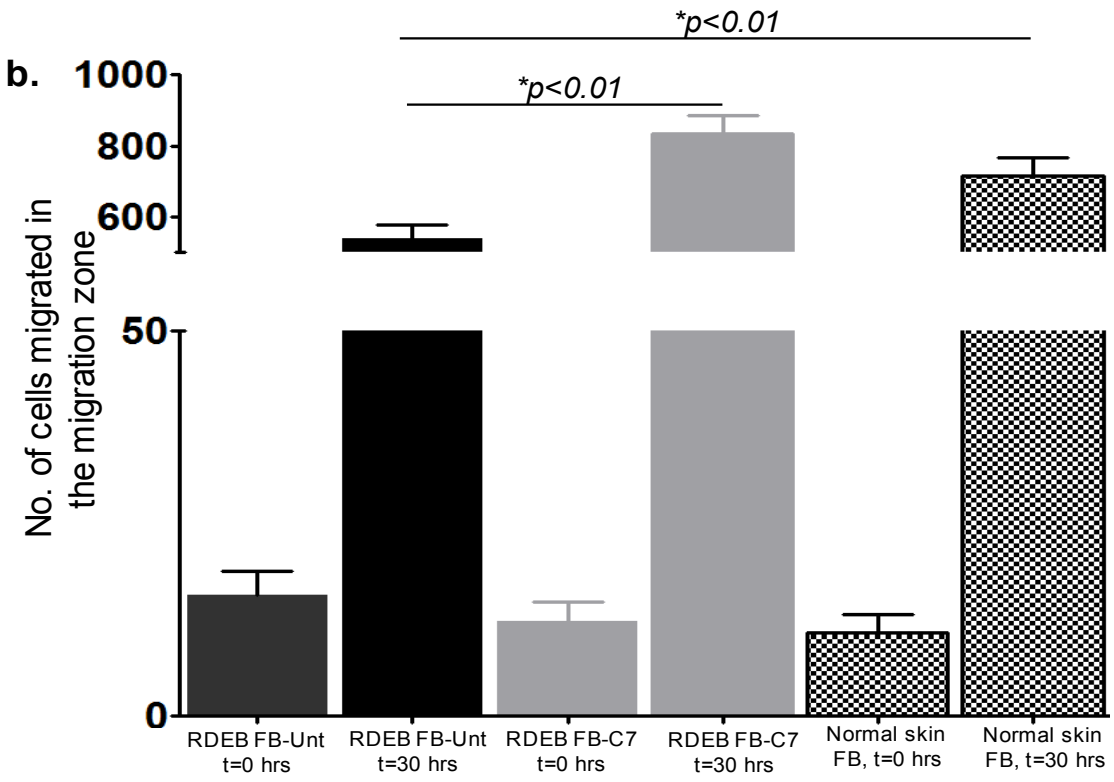


Figure 9: Normalised migration and wound closure in cells transduced with pCCL-PGK-COL7A1. Panel a. shows migration pattern in 2mm migration zone at 30 hrs which were taken from example micrographs of untransduced and transduced RDEB fibroblasts. The migration speed was analyzed by in three independent experiments and results showed increase in migration of C7 modified RDEB primary fibroblasts towards normal compared to unmodified RDEB primary fibroblasts at 30 hrs (**Panel b**).

3.14 *In vivo* skin graft mouse model to test gene-modified fibroblasts

A human-murine chimeric mouse model (Larcher et al. 2007; Di et al. 2010; Di et al. 2011) was used to evaluate the efficacy of the C7 lentiviral vector. NOD/SCID/□c knockout mice which are immunodeficient and tolerate human skin organotypic grafts were grafted with human transduced or untransduced RDEB fibroblasts and keratinocytes to allow C7 expression at dermal-epidermal junction to be examined in conditions that resemble skin development *in vivo*.

The results showed that in the skin grafts generated by gene modified cells, the epidermis tightly attached to the dermis, whereas, in the skin grafts generated by

unmodified cells, there was detached epidermis in the grafts and there was restoration of C7 expression in dermal-epidermal junction in the skin graft generated by gene modified RDEB fibroblasts (**Figure 10**).

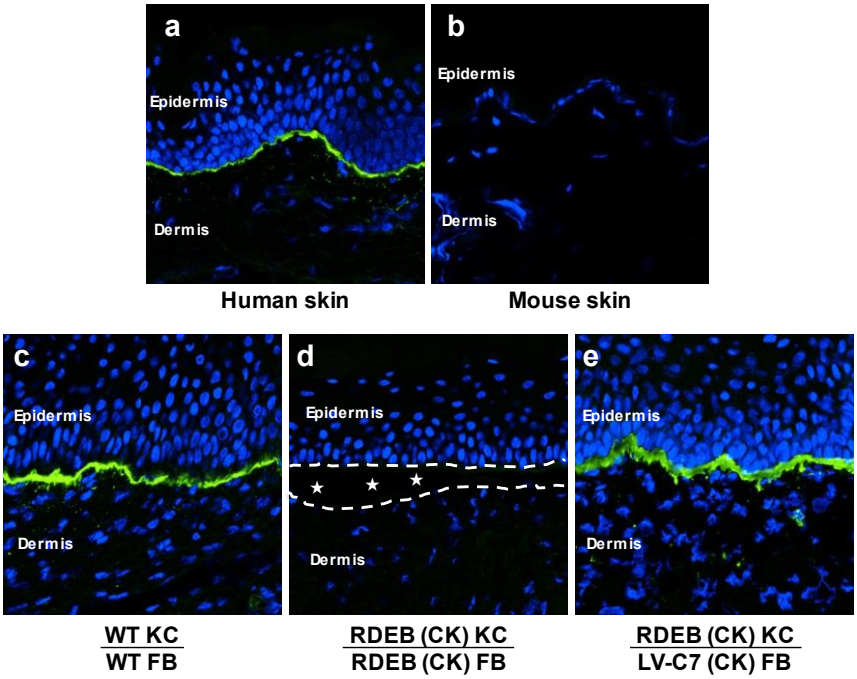


Figure 10: Restoration of C7 expression at DEJ in the grafts generated by gene modified RDEB cells. Bio-engineered skin sheets generated from normal, non-gene modified or gene modified RDEB keratinocytes and fibroblast cells were grafted onto NSG mice. Grafts were harvested 8 weeks post grafting. The morphology showed a splitting phenotype between epidermis and dermis in the graft generated by non-modified RDEB keratinocytes/fibroblasts (marked as dotted line on middle image, lower panel), whereas, there was no such phenotype in grafts generated by normal keratinocytes/ fibroblasts (left lower panel) or unmodified RDEB keratinocytes/modified fibroblasts (right lower panel). Immunofluorescent staining using human specific anti-C7 antibody showed C7 to be expressed at the DEJ in normal skin (left upper panel) and graft generated by normal cells (left lower panel) but not in the mouse skin (right upper panel). The staining also confirmed that the expression of C7 in the graft generated by modified fibroblasts (right lower panel). In contrast, there was no C7 expression in the graft generated by unmodified RDEB cells (left lower panel). These results indicate the restoration of C7 in the gene modified skin grafts.

To evaluate whether there was the formation of anchoring fibrils which a sign of functional correction of C7 protein in the skin graft generated by RDEB transduced keratinocytes and transduce fibroblasts, ultrathin tissue sections from the graft were analysed by transmission electron microscopy. The results clearly demonstrate an abundance of rope-like structures of type VII collagen anchoring fibrils throughout the DEJ. Similar to the healthy control graft they exhibit normal cross-banding with the NC-2 domain extending to 200nm into the dermis and looping around type I and III collagen fibres (**Figure 11**).

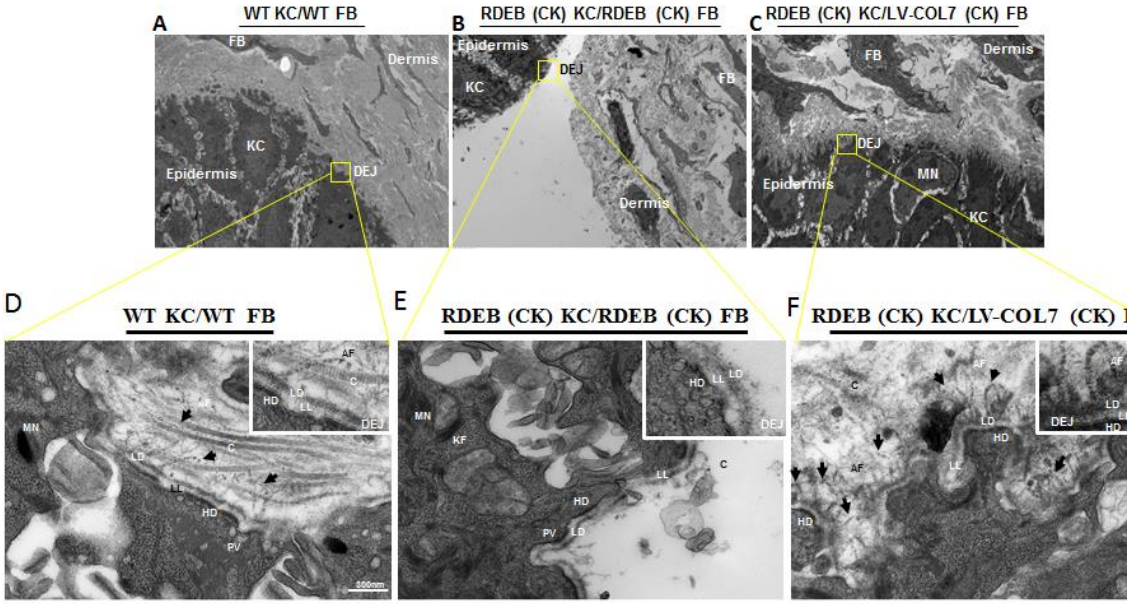


Figure 11: Restoration of type VII collagen anchoring fibrils in the skin graft generated by gene-modified RDEB keratinocytes and fibroblasts. Bioengineered human skin equivalents grafted onto NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mice and harvested after 8-weeks. **A-C:** low magnification (1200x) EM micrographs and **D)** WT human keratinocyte and fibroblast combination showing well formed DEJ with numerous large hemidesmosomes. Thick, cross-banded type VII anchoring fibrils (arrows) extend ~200nm into the dermis looping round type I and III collagen. High proportion of C7 protein depositing plasmalemmal vesicles; **E)** Untransduced RDEB patient (CK) keratinocyte and fibroblast combination showing extensive tissue cleavage at the DEJ with lamina densa reduplication. Complete absence of type VII collagen fibrils resulting in separation of epidermis from underlying dermis; **F)** Untransduced

RDEB patient (CK) keratinocyte and LV-COL7A1 transduced fibroblast combination showing restoration of dermal-epidermal adhesion after LV-COL7A1 mediated correction of RDEB fibroblasts. Several thick, looping, arching and cross-banded type VII collagen anchoring fibrils shown providing attachment at the DEJ. Anchoring fibril (AF), collagen type I and III (C), dermal–epidermal junction (DEJ), hemidesmosome (HD), keratin filament (KF), lamina densa (LD), lamina lucida (LL), melanin (MN), plasmalemmal vesicle (PV). Images taken at x13,000 and x30,000 magnification. Scale bar =300nm.

3.15 Detection of shortened C7 protein in gene-modified keratinocytes

Abnormal, shorted C7 protein bands have previously been reported in keratinocytes following viral vector transduction at a frequency of 25-33% (Titeux et al. 2010; Chen et al. 2000). There is a known risk of variant splicing or rearrangements of repetitive sequences during reverse transcription or other aberrations.

We used a codon optimisation *COL7A1* gene and had anticipated that *coCOL7A1* would minimize aberrant splicing and may reduce the frequency of shortened C7 protein in gene modified cells. We assessed the frequency of fragmented C7 protein in RDEB keratinocytes rather than fibroblasts, as this allowed clonal populations to be efficiently derived and studied. Cells were transduced with pCCL-PGK-COL7A1 and a small proportion found to express a shortened form of C7 protein assessed by western blot (**Figure 12a**). Following single cell cloning we found the majority of transduced clones expressed full length C7 but about ~6% (3/49; Clone-34, Clone-E8 and Clone-33) of clones had a shortened form of the protein (**Figure 12b**). Direct Sanger sequencing on these clones showed that there were deletions in these clones, for example, 5280-6446bp (∆1166bp) deletion in clone-34; 5516-6681 (∆1165bp) and 7329-7827bp (∆498bp) deletion in clone-E8; 5532-5654bp (∆122bp) and a further deletion between 6139-6628bp (∆498bp) in clone-C33 (**Figure 13**). Interestingly, all three deletions occurred within between 5200bp to 7900bp. Further studies will be carried out to understand the mechanism underlying these deletions but we suspect they arise during reverse transcription due to repeat sequences within the C7 gene.

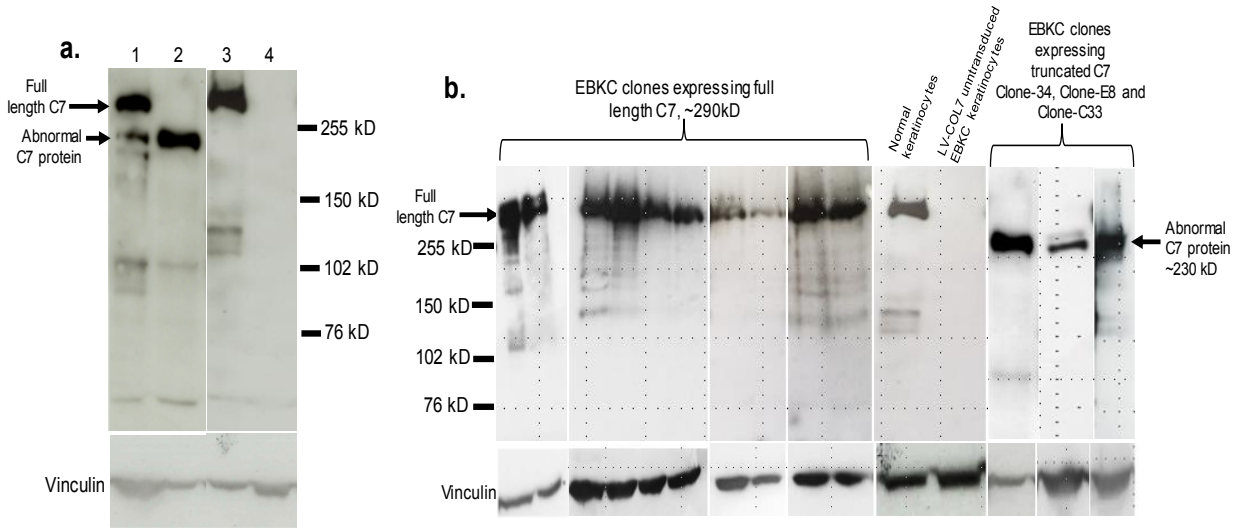


Figure 12: Normal and abnormal expression of C7 in RDEB keratinocytes transduced with LV-COL7A1. a. Lane 1: Heterogeneous populations of transduced EBKC keratinocytes showing both full length and shortened C7 expression; Lane 2: EBKC Clone C34 expressing shortened C7; Lane 3: human normal skin keratinocytes showing expression of full length C7; Lane 4: untransduced EBKC keratinocytes with no expressed C7. **b.** EBKC clones, expressing full length or shortened C7 protein (Clone C34, E8 and C33).

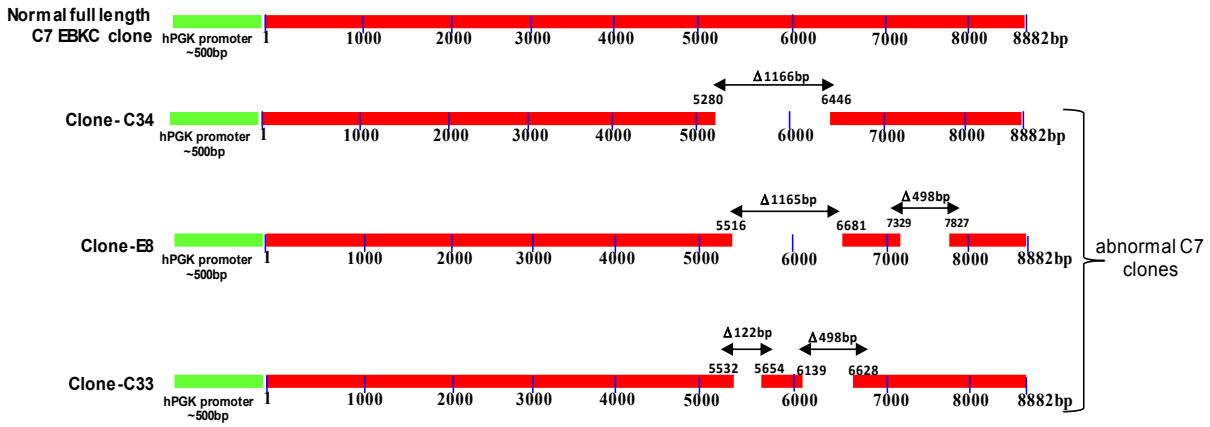


Figure 13: Direct sequencing of normal and abnormal clones detected on western blotting. Direct Sanger sequencing of entire C7 showed deletions in three clones expressing shortened C7 protein, whereas the majority had the expected full length COL7A1 sequences (uppermost readout).

In vitro functional assessment for these shortened C7 protein clones included viability and metabolic activity using a WST-1 assay and xCELLigence Real-Time Cell

Attachment (RTCA) assay. Results showed increased viability/metabolic activity in C7 transduced RDEB Keratinocytes clones with normal protein compared to unmodified RDEB keratinocyte clones or clones transduced and expressing shortened C7 protein. Better cell attachment, spreading and proliferation were also observed in appropriately modified RDEB keratinocyte clones compared to untrasduced RDEB keratinocytes or transduced clones expressing abnormal C7 (**Figure 14 and 15**).

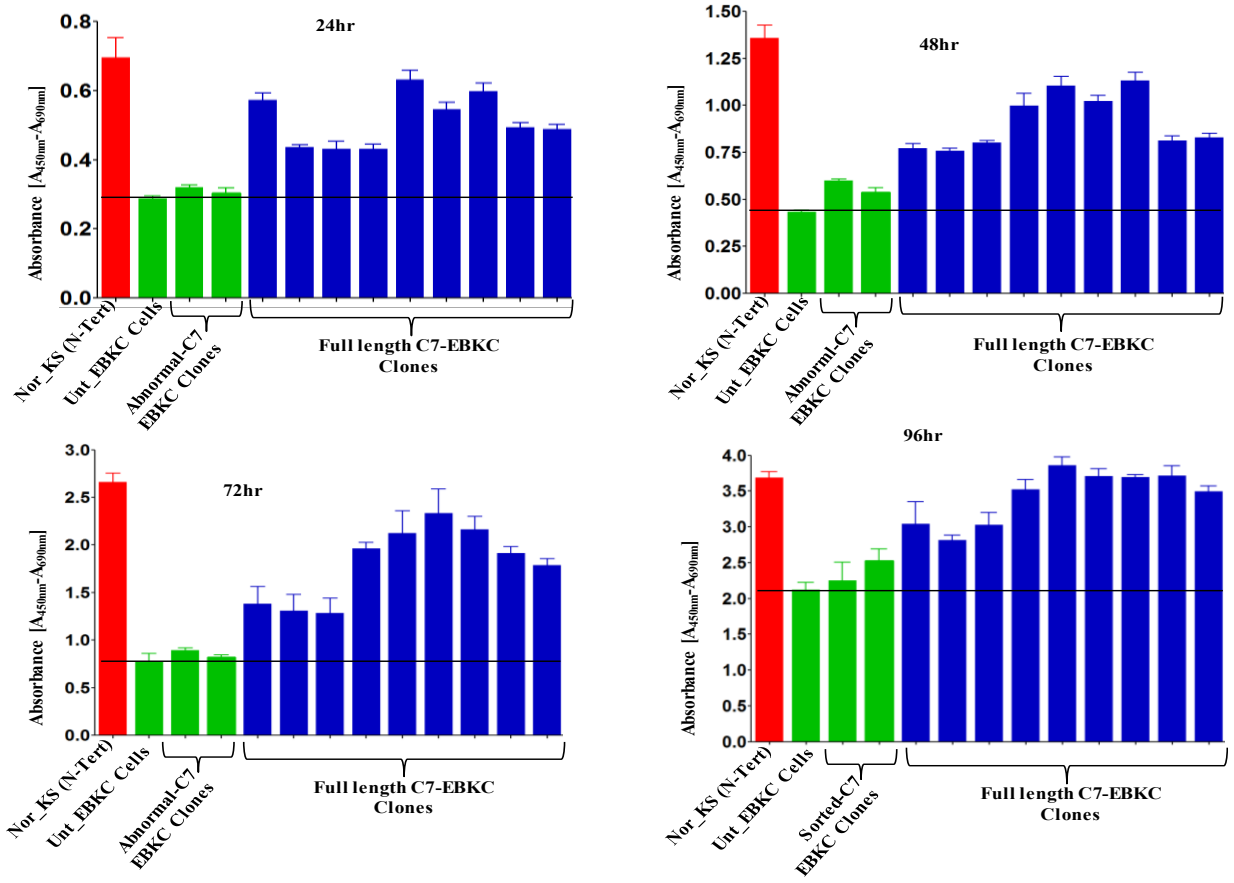


Figure 14: Viability/metabolic activity of normal and abnormal C7 expressing RDEB keratinocytes. Cells expressing shortenedC7 protein showed a decreased viability/metabolic activity compared to cells expressing full length C7 protein.

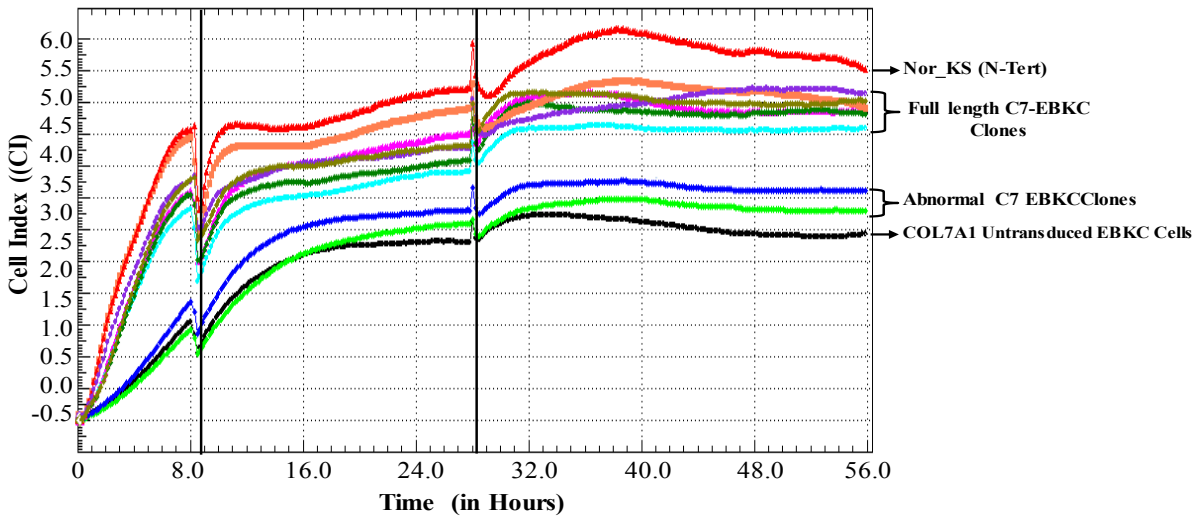


Figure 15: Assessment of cell attachment, spreading and proliferation on Real-Time Cell Analyzer. Clones expressing shortened C7 protein and untransduced RDEB keratinocytes showed decreased cell attachment, spreading and proliferation compared to normal cells assessed by Real-Time Cell Analyzer (RTCA).

Although there were about 6% shortened C7 protein expressed in transduced RDEB keratinocyte, shortened C7 protein has not been detected in transduced RDEB fibroblasts in our experiments to date. Studies by others also reported the frequency of shortened C7 proteins in transduced fibroblasts is much lower compared to around 25% keratinocytes (personal communication with Prof A Hovnanian).

In summary, the codon optimised COL7A1 vector is susceptible to residual aberrant protein expression effects, but at a notably reduced frequency compared to previously described, non-optimised configurations. This is likely to be related to regions with clusters of repeat sequences, but we found no evidence that the consequential truncated proteins caused any further deterioration in function.

4. Trial Objectives and Design

4.1 Trial objectives

4.1.1 Primary objective

To evaluate the safety of intradermal injections of SIN LV-mediated *COL7A1* gene-modified autologous fibroblasts in adults with RDEB.

4.1.2 Secondary objectives

I. To evaluate the potential efficacy of intradermal injections of SIN LV-mediated *COL7A1* gene-modified autologous fibroblasts in adults with RDEB at week (W) 2, month (M) 3 and M12 after the IMP injections.

II. To screen for immune response against the recombinant C7 at W2, W4, M3, M6 and M12 after the IMP injections compared to baseline.

4.1.3 Primary endpoint

- Adverse events (AEs), Serious Adverse Events (SAEs), Adverse Reactions (ARs) and Serious Adverse Reactions (SARs) at each visit after screening over a 12-month follow-up period.

4.1.4 Secondary endpoints

- Skin biopsy analysis of treated skin at W2, M3 and M12 compared to untreated skin:
  I. C7 protein expression by immunofluorescence microscopy (IF)
  II. Morphology of anchoring fibrils at the dermal-epidermal junction (DEJ) by transmission electron microscopy (TEM)
  III. Vector copy number by quantitative polymerase chain reaction (qPCR)
- Serum analysis for:
  IV. Detection of anti-C7 antibodies by enzyme-linked immunosorbent assay (ELISA) (against NC1 and NC2 domains of C7) and indirect immunofluorescence (IIF) at W2, W4, M3, M6 and M12 post-injections
  V. Detection of T-cell responses to the full length C7 by enzyme-linked

immunosorbent spot (ELISPOT) assay at W4, M6 and M12 post-injections.

4.2 Trial design

This is a prospective phase 1, non-randomised, open label, single-centre, proof-of-concept study. Each study participant will receive three intradermal injections of *ex vivo* transduced autologous fibroblasts expressing codon-optimised *COL7A1* as the IMP on Day 0 only. Each injection of the IMP containing 0.8–1.2x10^6 cells suspended in 0.25ml of 0.9% saline, will be administered intradermally into 1cm^2 area of intact skin (x3). Participants will be followed up with study interventions for a 12-month period at various time points as outlined in the trial timeline (**Figure 16**). All follow-ups, where possible, will be co-ordinated to try to coincide with the individuals' routine clinic reviews.

Each subject will undergo an initial screening including a physical examination and assessment of disease severity. Blood analyses and skin biopsies will be performed at various time points as per the monitoring schedule (**Table 2 and Figure 16**). The second participant will be treated only if there is no safety concern 4 weeks after the first participant's IMP injections. All patients with RDEB are followed up on a lifelong basis, and we will therefore be able to capture any long-term possible adverse effects related to the IMP.

4.3 Trial timeline

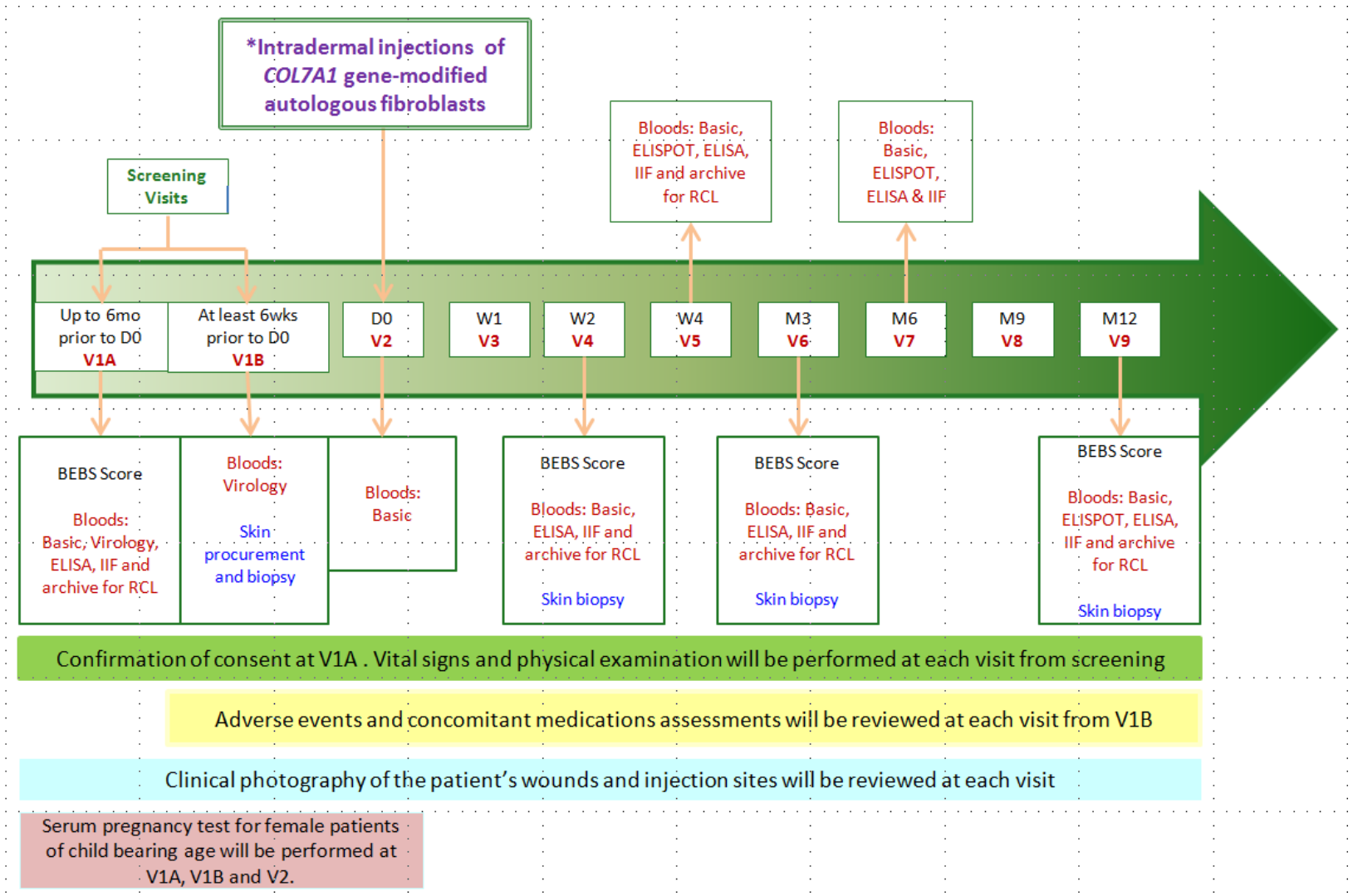


Figure 16: LENTICOL-F trial timeline. *Each injection consists of 0.8–1.2 million cells suspended in 0.25ml of 0.9% saline. Each of three syringes containing the IMP will be administered over 1 cm^2 area of intact skin. . Virology tests done at V1A & V1B = HIV, HepBsAg, HepBcAb, HepCIgG, HTLV 1&2 and Treponema pallidum serology; Skin procurement and biopsy at V1B = 2 to 3 biopsies

(one 6mm procurement for tissue culture for IMP production; and one to two 4mm for C7IF, TEM, qPCR and histology); Skin biopsy @ W2, M3 and M12 = Two 6-8mm biopsies for C7 IF, TEM, qPCR and histology (one from injected site and one from non-injected site). All skin biopsies will be archived for integration site analyses in the rare event of clinically indicated serious adverse events. Pregnancy tests at V1A, V1B and V2: if serum is not obtainable, urine pregnancy test may be performed instead. Historical samples/ results may be used for C7 ELISA & IIF, ELISPOT, C7 IF microscopy & TEM and virology tests.

NB: If W2 and M3 skin biopsy results are negative then M12 biopsy may not be performed.*Abbreviations: BEBS score=Birmingham Epidermolysis Bullosa Severity Score; IMP=Investigational medicinal product.*

5 Trial Medication

5.1 Investigational Medicinal Product

The patient specific gene-modified autologous fibroblasts are classified as an Investigational Medicinal Product (IMP) that comes under the European Union Directives (EUDs) on medicinal products and are subject to advanced therapy medicinal product (ATMP) guidelines and will be manufactured and expanded according to GMP regulations. Each participant will be screened for infectious diseases in accordance with the EU directive 2006/17 (Commission Directive 2006/17/EC of 8 February 2006) prior to skin biopsy for cell culture to produce autologous fibroblasts under GMP conditions. The pCCL-PGK-COL7 lentiviral vector gene modified autologous fibroblasts which are suspended in saline will be produced under MIA(IMP) 17328 at Cellular Therapies, Great Ormond Street Hospital for Children NHS Foundation Trust (GOSH), Great Ormond Street, London, WC1N 3JH. The IMP manufacturing site is non-commercial and part of an academic institution with a certified manufacturer/ importer's licence as well as GMP and QP certification. The unit also has a HTA licence and JACIE accreditation for transplantation.

Release of the IMP will require qualified person (QP) examination of the manufacturing record, liquid nitrogen temperature monitor recorder and other tests undertaken on cells to confirm transduction (copy number). Assays for information include evidence of reconstitution of C7 expression by flow cytometry, immunostaing and immunoblot **(Table 1).**

If the genetically modified autologous RDEB primary fibroblasts do not meet specification, the transduced cells will not be released. Efficacy and safety assessments will be carried out and data will be recorded in the Case Report Forms (CRFs).

Test	Day/week during manufacturing	Specification
BACTalert	Weekly	No organisms detected
Cell number	Before release	2.4-3.6X10^6 viable cells for gene modified fibroblasts
Stable lentiviral transduction	specific qPCR	Copy number = 0.01-1.0 copy/cell
C7 protein expression*	Flow cytometry, immunostaining and immunoblot	restoration of C7 expression compared to baseline C7
Cell storage temperature	Daily record	Less or equal to -70^0C

**depending on the mutation, some subjects may have residual protein expression*

Table 1: Specification for releasing transduced RDEB autologous fibroblasts.

5.2 Dosing regimen

Each trial participant will receive three intradermal injections of gene-modified autologous fibroblasts on Day 0. Each 1cm^2 of intact skin will be injected with 0.8–1.2x10^6 cells/ 0.25ml of normal saline. Once all 3 syringes are received, they will be injected as described in Section 5.7. The person handling and injecting the IMP will have appropriate training. Trial subjects will be monitored closely for any immediate allergic reactions and their vitals monitored every 15mins for half an hour. Vital signs will include blood pressure, respiratory rate, heart rate, pulse oximetry and temperature.

5.3 Description of IMP

The patient-specific gene modified autologous fibroblasts (IMP) are generated from primary RDEB fibroblasts using pCCL-PGK-COL7 in its final formulation which is intended for injection.

The pCCL-PGK-COL7A1 vector is a third generation lentiviral vector encoding the gene codon optimised *(co)COL7A1* (**Figure 17**). It is replication defective, does not encode pathogenic genes and is only packaged in the presence of minimal viral proteins provided in *trans* by transient transfection. It is self-inactivating (SIN), which restricts transcriptional activity to the internal expression cassette incorporating a human promoter PGK. The whole production phase for pCCL-PGK-*COL7* involved one production run from which ~20 litres of virus supernatant were produced and then concentrated.

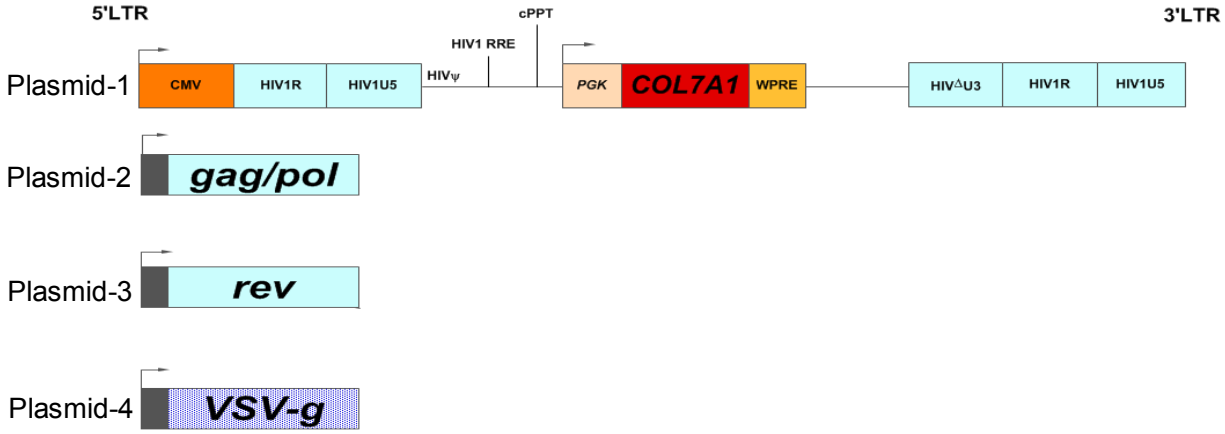


Figure 17: Structure of the pCCL-PGK-COL7A1 lentiviral vector delivery system.

The appearance of the final medicinal product is cell/saline suspension of 0.25ml in 1ml luer-screw capped syringes. The product is labelled with a completed label corresponding to that in the batch manufacturing records (BMR) and further kept in a clean container sealed with clinical tape Tagaderm (from 3M) and then placed in a transport container for release. GMP compliant manufacturing and quality control procedures used to produce the IMP at the Cellular Therapies at GOSH are detailed in the accompanying IMPD.

5.4 Packaging and labelling

Gene-modified fibroblasts are thawed, washed, counted and then packed into syringes capped with luer-screw. A total of 3 labelled syringes containing gene-modified autologous fibroblasts are then put into a petri dish, and sealed with clinical tape. Both syringes and petri dishes are clearly labelled with the study ID and IMP information. The product is finally placed into a medical cooler bag to maintain the product at ambient temperature.

5.5 Distribution and shipment

The IMP will be transported from the Cellular Therapies at GOSH to the trial site – clinical research facility at 15th Floor, Tower Wing, Guy's Hospital by a specialised courier at the ambient temperature with a temperature monitor within 8 hrs of the IMP release.

5.6 Storage, dispensing and return of the IMP

Cultured transduced autologous fibroblasts are cryopreserved and store in a liquid nitrogen vessel with vapour phase. On the day of therapy, frozen cells will be removed from liquid nitrogen and quickly thawed in the Cellular Therapies at ICH/GOSH. Thawed cells are recovered and counted. After counting, cells are re-pelleted, and resuspended in 0.9% saline with an approximate concentration of 2-4x10^6 viable cells/ml. 0.25ml cell suspensions containing approximately 0.8-1.2x10^6 viable cells are packed into syringes capped with luer-screw. There will be three syringes of transduced cells provided. All syringes are clearly labelled with patient specific identifiers and IMP information and put into a transfer container for transport.

A Proof of Receipt form will be completed to confirm receipt of the product and faxed back to the sponsor and manufacturer. A copy of the form will be filed in the Investigator Site File. If it is found that the packaging has been tampered with or is

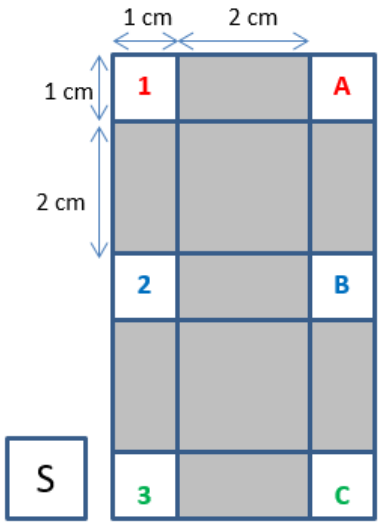
damaged, the sponsor will be notified immediately. The product(s) affected will not be used. Each dispensing will be recorded on the IMP Accountability Record. Any unused IMP may be returned to GOSH and stored for research use only.

5.7 IMP administration

The selected area of intact skin of each trial participant will be marked with an ink tattoo marker that is normally used for to mark the sites of radiotherapy on cancer patients. No radiotherapy or irradiation will be involved here. The schema of the tattoo sites may vary depending on the individuals but the investigator will aim to provide the minimal number of tattoos required in order to aid the accurate identification of the injection sites at future visits. Prior to injections, the intact skin will be cleaned with Chlorhexidine solution and the IMP administered by the trained investigator. Each injection of the IMP containing 0.8–1.2x10^6 cells suspended in 0.25ml of 0.9% saline, will be administered intradermally into 1cm^2 area of intact skin (x3). Trial subjects will be monitored closely for any immediate allergic reactions and their vitals monitored every 15mins for half an hour.

5.8 Drug accountability

Each IMP dispensing will be documented on the IMP accountability form, along with unused returned study treatments. The study monitor will check all dispensing against the study schedule and record all administrations to the trial participants. A full review of the actual dispensed, administered and returned study treatments will be performed.



Screening
Site S (at screening visit V1B): 2-3 skin samples will be taken. One the size of an adult's little finger nail (6mm) and the other(s) slightly smaller (4mm) for production of the gene-modified fibroblasts and for baseline tests respectively.

Injections
Squares 1, 2 and 3 will be injected with your own gene-modified fibroblasts on Day 0 at approximately 1 million cells in each square. Squares A, B and C will not receive any treatment.

Follow up skin biopsies
2 skin samples (4mm each) from each square will be taken at the following timelines:
Squares 1 and A at Week 2

Squares 2 and B at Month 3

Squares 3 and C at Month 12

No procedures will take place over the grey-shaded areas.

Figure 18: Map showing the sites of IMP injection and the timeline of the biopsies to be taken.

5.9 Subject compliance

The IMP will be administered to the trial participants attending St John's Institute of Dermatology at GSTT in the designated Clinical Research Facilities, 15th floor, Guy's Tower. Non compliance issues are not expected as the treatment is administered in hospital setting by a member of the research team. Skin biopsies and blood tests will also be performed by a member or members of the clinical team. However, because the study population involves adults with a severe blistering skin disease, if a blood test is unobtainable at a particular time, the investigators may use a blood sample obtained at a more convenient time (but chronologically related). Participants will be assessed for any adverse events and concomitant medications at each visit from V1Bbut otherwise, no assessments are scheduled to be performed by study subjects

at home so no subject compliance issues are expected. Support and guidance will be provided by members of the clinical research team as required.

5.10 Concomitant medication

For management of concomitant therapies, please refer to the Investigators Brochure (IB). The study subjects can continue to receive their regular medication(s). A complete listing of all concomitant medication received during the treatment phase will be recorded in the relevant CRF.

6 Selection and withdrawal of subjects

6.1 Inclusion criteria

I. Clinical and genetic diagnosis of RDEB with confirmed bi-allelic *COL7A1* mutations.
II. A reduced number or morphologically abnormal anchoring fibrils confirmed by TEM.
III. At least 5x3cm of intact skin on the trunk and/or extremities that is suitable for cell injections.
IV. Able to undergo local anaesthesia.
V. Subjects aged ≥ 17 years and able to give informed consent prior to the first study intervention.

6.2 Exclusion criteria

I. Subjects who received other investigational medicinal products within 6 months prior to enrolment into this study.
II. Past medical history of biopsy proven skin malignancy.
III. Subjects who have received immunotherapy including oral corticosteroids (Prednisolone >1mg/kg) for more than one week (intranasal and topical preparations are permitted) or chemotherapy within 60 days of enrolment into this study.
IV. Known allergy to any of the constituents of the investigational medicinal product (IMP).
V. Subjects with **BOTH**:
- positive serum antibodies to C7 confirmed by ELISA **and**
- positive IIF with binding to the base of salt split skin.

VI. Subjects with positive results for HIV, Hepatitis B, Hepatitis C, HTLV or Syphilis.

VII. Subjects who are pregnant or of child-bearing potential who are neither abstinent nor practising an acceptable means of contraception when this is in line with the usual and preferred lifestyle of the subject, as determined by the Investigator, for 12 months after the cell injections.

6.3 Selection of participants

The patient population is under the care of the specialist EB team at St John's Institute of Dermatology, GSTT. As a tertiary care centre, patients may have been referred from other regional district hospitals in the first instance. Potential study subjects will be identified from the National EB database that includes patients from both GSTT and nationally and an initial approach made by members of the clinical team. Potential participants may also be recruited from Patient Identification Centres. Study information sheets will be provided and individuals interested will be invited for further consultation and screening, assessed for eligibility, and consented as necessary. All individuals invited to participate in the study will have a minimum of 24 hours to make their decision.

6.4 Randomisation procedure / code break

This is a phase 1, non-randomised, open-label, single-centre, proof-of-concept study, therefore randomisation procedure/code break is not applicable.

6.5 Withdrawal of subjects

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study drug in the event of inter-current illness, AEs, SAEs, SUSARs, protocol violations, cure, administrative reasons or other reasons. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable, therefore unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible.

Should a participant withdraw from the study after the injection of the IMP, efforts will be made to continue to obtain follow-up data, with the permission of the participant. If a participant withdraw before the IMP injection, the participant will be replaced.

6.6 Expected duration of trial

The planned duration of the study will be 32 months. Each trial participant will be followed up for 12 months after the last intradermal injections of the IMP: gene-modified autologous fibroblasts. We may recruit and produce the IMP for more than one participant at a time, though only administer the IMP with minimal intervals between individuals.

Each participant will have a screening visit up to 4 months prior to Day (D) 0 when the IMP will be administered. There will be 7 scheduled follow-up visits over 12 months from Day 0. Safety and efficacy will be assessed initially using the first 12 month data. Participants will then be reviewed every 4 months for the second year and then every 6 months for the third year by the consultant dermatologist responsible for their clinical care (Professor John McGrath or Dr Jemima Mellerio who are the tenured senior clinical investigators for this trial) or an experienced EB specialist nurse. If a participant does not wish to attend for a 4-month or 6-month hospital visit, we will offer them the option of a telephone call to enquire about adverse events which will be recorded in the eCRF. All trial participants also have direct access to the EB specialist nurses should they develop any untoward symptoms requiring urgent clinical review, or wish to discuss any additional aspects of the clinical trial, in the interim between these time points. All adult subjects (aged ≥ 17 years) within the EB service are regularly seen at St John's Institute of Dermatology at GSTT. Dr Jemima Mellerio and Professor John McGrath provide consultant care dedicated to this service.

6.7 End of trial definition

The point will be defined as the final study participant's last scheduled follow-up visit according to the protocol, which will be the 12 month follow-up visit after the intradermal injections of the IMP of the last subject entering the trial.

7 Trial Procedures

7.1 Trial course

This clinical trial will be conducted within the clinical research facility (CRF), 15th floor, Tower Wing, Guy's Hospital, London whilst some of the screening and follow up visits may be carried out in the Dermatology outpatient at GSTT. Subjects will be required to visit the clinic 10 times in 12 months. The screening assessment – visit (V1A) will be conducted up to 6 months prior to D0 – the day of the IMP injections. The timings of the subsequent visits will be relative to D0 (V2) **(Table 3)**. Timing of screening, treatment and follow-up visits are summarised in the trial flowchart and monitoring schedule.

7.2 Long-term follow-up

Patients with RDEB are regularly reviewed, often for life, by the consultant Dermatologists in the multi-disciplinary setting at the regular EB clinics at GSTT, providing opportunities for detection of late adverse events. Once these studies are officially closed we will continue to report serious events related to the IMP (SARs and SUSARs) to the MHRA and London – West London & Gene Therapy Advisory Committee (GTAC) in the same way as during the study.

7.3 Informed consent

Once the Investigator has determined the subject's potential eligibility for the study, the background of the proposed study, the benefits and risks of the procedures and the study, will be explained to the potential study participant both during the consultation and through the participant information sheet. Potential participants will be given a minimum of 24 hours to review the information received and may take the information

away to consider his/her decision to participate in the study. Written informed consent will be obtained from the subject by a physician listed on the delegation of duties log before any study related procedures, including screening tests, are performed. Prior to screening, the subject must first sign the institution's Independent Ethics Committee (IEC) approved informed consent form (ICF). Failure to provide informed consent renders the subject ineligible for the study. Study participants will be instructed that further information can be obtained at any time from the Investigator, and that they are free to withdraw their consent and to discontinue participation in the study at any time without any impact on the standard of their routine clinical care.

7.4. Procedures by visit

Visit number	V1A	V1B	V2	V3	V4	V5	V6	V7	V8	V9
Timeline of each visit relative to the day of gene-modified fibroblast injections (D0)	Up to 6M prior to D0	At least 6W prior to D0	**D0**	**W1** (±3D)	**W2** (±3D)	**W4** (±14D)	**M3** (±1M)	**M6** (±1M)	**M9** (±1M)	**M12** (±1M)
Approximate duration of each visit (hours)	**2**	**2**	**1.5**	**1.5**	**2**	**1.5**	**2**	**1.5**	**1.5**	**2**
Informed consent	X									
Inclusion / exclusion	X	X								
Demographics	X									
Medical history	X									
Vital signs	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X
Serum pregnancy test for female participants with child-bearing potential (if serum is not obtainable, urine pregnancy test may be used)	X	X	X							
[a]BEBS Score	X				X		X			X

Visit number		**V1A**	**V1B**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**
Timeline of each visit relative to the day of gene-modified fibroblast injections (D0)		Up to 6M prior to D0	At least 6W prior to D0	**D0**	**W1** (±3D)	**W2** (±3D)	**W4** (±14D)	**M3** (±1M)	**M6** (±1M)	**M9** (±1M)	**M12** (±1M)
[b]Clinical photography		X	X	X	X	X	X	X	X	X	X
Blood samples	[c]Basic bloods	X		X		X	X	X	X		X
	[d]Virology	X	X								
	C7 ELISA & IIF	X				X	X	X	X		X
	ELISPOT			X			X		X		X
	[e]RCL	X				X	X	X			X
Skin biopsies	C7 IF microscopy & TEM		X			X		X			X
	[f]qPCR		X			X		X			X
	[g]Integration site analysis		X			X		X			X
Skin procurement (1x6mm)	Tissue culture for IMP production		X								
[h]Intradermal injections of IMP				X							
Adverse event(s) assessment			X	X	X	X	X	X	X	X	X
Concomitant medications assessment			X	X	X	X	X	X	X	X	X

Table 2: LENTICOL-F trial flowchart with details of interventions at each visit. *Abbreviations: V=Visit; D=Day; W=Week; M=Month.* Consent will be obtained at each visit. On Day 0, your vital signs will be monitored every 15mins for 30mins after the gene-modified fibroblast injections. There will be one-off readings of the vital signs on the rest of the other visits during the study. Historical samples/ results may be used for C7 ELISA & IIF, ELISPOT, C7 IF microscopy & TEM. Historical virology results performed within the last 3 months may be used. NB: If W2 and M3 skin biopsy results are negative then M12 biopsy may not be performed.

[a]Birmingham Epidermolysis Bullosa Severity Score;

[b]Clinical photography of potential injection sites over an area of the intact skin will be taken at screening on visit 1, post-IMP injections on visit 2 and at each visit thereafter;

[c]Includes FBC, U&Es, LFTs, ESR and CRP; [d]Includes HIV, HepBcAb, HepBsAg, HepCIgG, HTLV 1&2 and Treponema pallidum serology; [e]Blood samples will be archived for replication-competent lentivirus (RCL) - may be archived for at least 15 years and analysed in the event of relevant serious adverse reactions (SAEs) as clinically indicated;

[f]Vector copy number and *COL7A1* gene expression will be measured using quantitative polymerase chain reaction (qPCR) and some skin samples retained for integration site analysis in the event of clinically indicated SAEs;

[g]DNA will be extracted from part of the skin biopsy and archived for integration site analysis in the event of relevant adverse reactions as clinically indicated;

[h]Each of 3 intradermal injections of IMP (*COL7A1* gene-modified autologous fibroblasts) will be administered to 1cm^2 of intact skin

7.5 Laboratory tests

Blood samples (approximately 15-20ml) will be taken by venepuncture. At screening, trial subjects will be tested for HIV, hepatitis B surface antigen (HepBsAg), hepatitis B core antibody (HepBcAb) and hepatitis C IgG (HepCIgG), HTLV1&2 and *Treponema pallidum* serology. The subjects will also have a blood DNA sample sent for mutation analysis for the *COL7A1* gene if this result is not already available. All of the blood tests will be analysed at the Viapath laboratories apart from the DNA mutation analysis, and ELISA and IIF which will be performed at The National Diagnostic EB laboratory and Viapath Immunofluorescence Laboratory, both at St Thomas' Hospital, respectively. Blood samples for RCL analysis will be archived. Blood samples for ELISPOT will be couriered to Institut Imagine in Paris as shown below. qPCR for the *COL7A1* expression on the skin biopsy will be performed at Cellular Therapies at GOSH **(Table 3 and 4)**. The following blood analyses will be performed at screening and at each subsequent review as outlined in the trial flowchart in section 4.3.

Type of Blood Analysis	**Tests**	**Laboratory**
Haematology	FBC ESR	Viapath
Biochemistry	U&Es LFTs CRP Beta-HCG	Viapath
Infection screen (at baseline	HIV HepBsAg	Viapath

only)	HepBcAb HepCIgG HTLV 1&2 *Treponema pallidum*	
Serum	ELISA and IIF for C7 antibodies	Immunodermatology Laboratory (Viapath)
	ELISPOT for detection of T-cell responses to the full length C7	Institut Imagine, INSERM UMR1163 Équipe du Pr. Hovnanian 2ème étage, pièce 221-B2 ou 220-B1 142 rue du Cherche-Midi 75015 PARIS
	Replication competent lentivirus	Archived at UCL ICH/Guy's
Blood for DNA analysis (screening only if not available)	Mutational analysis	The Robin Eady National Diagnostic EB laboratory

Table 3: Types of blood analysis and where tests are performed for the trial.

Skin biopsies will be taken at three time points – from injected site and approximately 3cm away from the injected site (non-injected site); at W2, M3 and M12. NB: If W2 and M3 skin biopsy results are negative then M12 biopsy may not be performed. The samples will be analysed for the following:

Type of Analysis	**Tests**	**Laboratory**
Skin biopsies	1) C7 IF microscopy (stored in Michel's medium) 2) TEM of DEJ for anchoring fibrils	**The Robin Eady National Diagnostic EB laboratory**
	3) qPCR for vector copy number and *COL7A1* gene expression	**Cellular Therapies at GOSH/Guy's/ The Robin Eady National Diagnostic EB**

		laboratory
	5) Integration site analysis	**Archived at UCL ICH/Guy's**

Table 4: Table showing where skin biopsy analyses are performed for the trial.

8 Assessment of Efficacy

8.1 Primary efficacy parameters

The primary efficacy of this phase 1 trial will be based on the safety of the administration of IMP in adults with RDEB, as analysed according to the incidence of Adverse events (AEs), Serious Adverse Events (SAEs), Adverse Reactions (ARs) and Serious Adverse Reactions (SARs) at each visit after screening over a 12-month follow-up period.

8.2 Secondary efficacy parameters

- Skin biopsy analysis of treated skin at W2, M3 and M12 compared to untreated skin:
  - I. C7 protein expression by IF microscopy
  - II. Morphology of anchoring fibrils at the DEJ by TEM
  - III. Vector copy number by qPCR

- Serum analysis for:
  - IV. Detection of anti-C7 antibodies by ELISA (against NC1 and NC2 domains of C7) and IIF at W2, W4, M3, M6 and M12 post-injections
  - V. Detection of T-cell responses to the full length C7 by ELISPOT assay at W4, M6 and M12 post-injections.

8.3 Procedures for assessing efficacy parameters

Clinical assessment: Review of adverse events and concomitant medications will be performed at each visit from V1B. Physical examination, vital signs and clinical

photographyof the injection sites will be performed at each visit. BEBS score will be documented at screening, W2, M3 and M12. Efforts will be made for the clinical assessment to be performed by the same assessor for each participant throughout the trial to minimise inter-observer variations.

Biochemical analysis: Routine blood analysis including FBC, U&Es, LFTs, ESR and CRP will be measured at screening, D0, W2, W4, M3, M6 and M12 to monitor any potential systemic adverse effects. At the screening visits, virology tests for HIV, HepBcAb, HepBsAg, HepCIgG, HTLV1&2 and *Treponema pallidum* serology will be performed to screen for blood borne viral infections and treated as per GSTT guidelines. Analysis of antibodies for C7 and IIF will be performed both prior to and after IMP administration (screening, W2, W4, M3, M6 and M12) in order to detect any potential development of EB acquisita in participants with RDEB as a potential adverse effect of introducing new C7 expression from the gene-modified autologous fibroblasts. ELISPOT assay for detection of T-cell responses to the full length C7 will be performed at screening, W4, M6 and M12.

Skin Ultrastructural Analysis: Skin biopsies will be taken at four time points (screening, W2, M3 and M12) for C7 IF microscopy to assess a change in the level of C7 expression. This will be correlated with the presence and morphology of anchoring fibrils at the DEJ as assessed by TEM.

Vector copy number (VCN) and *COL7A1* gene expression: Real-time polymerase chain reaction (qPCR) will be used to analyse vector copy in the skin biopsy of the cell injection sites at W2, M3 and M12.

Histology: Skin histology will be used to analyse the difference in the morphology of DEJ between treated and untreated skin at W2, M3 and M12.

9 Assessment of Safety

Adverse events and concomitant medications will be reviewed at every visit after V1A. Clinical photographs will be taken at each visit in order to monitor changes in the injection sites especially with regards to any suspicious lesions or adverse reactions locally. If suspicious lesions are detected then, the participant will be immediately assessed and managed by a consultant dermatologist and if found malignant then, the lesion will be excised as per GSTT guidelines. Physical examination and vital signs will be performed at every visit in order to assess any potential systemic adverse effects and treated as appropriate as per GSTT guidelines.

9.1 Assessment of benefits and risks

Potential benefits

The injection of fibroblasts expressing *COL7A1* may allow localised recovery of anchoring fibril production and assembly, and restoration of basement membrane integrity. This study is designed to test the safety of injecting autologous gene-modified fibroblasts, and the three injection sites will allow serial histological assessment of skin architecture in direct comparison to a paired site injected with non-engineered autologous fibroblasts. Whilst no direct benefit is expected for the subjects at this stage, establishing safety would allow more extensive therapies to be offered.

Potential risks

Insertional mutagenesis

Gamma-retroviral vector mediated gene therapy for another skin disease, JEB, has shown impressive therapeutic efficacy (Mavilio et al. 2006) and long-term safety (>6 years after skin grafting) (Dr F.Mavilio, personal communication). Of note in RDEB, previous modelling suggests that deficiency off *COL7A1* is associated with a carcinogenic phenotype. Fibroblasts collected form RDEB patients with down-regulated *COL7A1* or null C7 expression exhibit promotion of substrate adhesion and invasion of tumour keratinocytes (Ng et al. 2012). Correction of this deficiency is expected to reduce transformational risk.

Concern regarding the insertional mutagenesis using gamma-RVs has prompted the development of safer delivery mechanisms using lentiviral vectors. Although there is no published date indicating the safety and efficacy of LVs configured with internal promoter elements in fibroblast transduction, LVs encoding the human internal promoters driving expression of various transgenes have been used to treat Wiskott-Aldrich Syndrome (Galy et al. 2011; Scaramuzza et al. 2012), ADA-SCID (Gasper et al. 2004) and chronic granulomatous disease (Grez et al. 2010). Older configurations using viral internal LTRs have been reported safe in children treated for adrenoleukodystrophy (Cartier et al. 2009; Biffi et al. 2011).

The pCCL-PGK-COL7A1 vector is a GMP grade recombinant LV for *ex vivo* gene therapy. This third generation SIN lentiviral vector lacks viral coding sequences that could give rise to the formation of replication competent lentivirus or immunogenic peptides. It is also devoid of viral enhancer-promoter sequences that are known to be involved in insertional mutagenesis by retroviruses and derived vectors. The internal promoter is the 516bp sequence of human PGK gene promoter (Ginn et al. 2010; Huston et al. 2011). A mutated woodchuck post-transcriptional regulatory element (WPRE) sequence is incorporated in the vector to enhance transgene expression.

Replication-competent lentivirus (RCL)

Emergence or transfer of RCL is highly unlikely in this SIN configured vector with a four plasmid packaging system. The vector has been found to be free of RCL and has not been detected in any preclinical study with similar vectors. Culture supernatant, transduced cells and patient samples will however be archived for RCL testing should the need to analyse these arise. Blood samples will be taken from the participating subjects at the screening visit, week 2, month 3 and month 12. These samples will be archived at the research laboratories, 9th floor Tower Wing at Guy's Hospital for at least 15 years and will be analysed for RCL in the event of relevant adverse reactions as clinically indicated.

Germ-line transmission of vector sequences

This is an *ex vivo* modification procedure with extended culture periods, therefore the risk of germ line transmission is negligible.

Allergic/immunological responses

Whilst the risk of immune responses against excipients is extremely low, immediate or delayed host-mediated immune responses against recombinant C7 are possible. All injections and monitoring will be given in a clinical area supported by resuscitation facilities (Clinical Research Facility). Participants will be monitored regularly as part of the study protocol and asked to report any systemic symptoms to the research team. Blood analysis for ELISA and IIF to C7 will be undertaken at baseline, W2, M1, M3, M6 and M12 to monitor for a delayed immunological response to the IMP, as well as ELISPOT assay for detection of T-cell responses to the full length C7.

Expected Adverse Reactions (ARs) following IMP administration

The following ARs, such as any untoward and unintended response in a subject to an IMP which is related to any dose administered to that subject, are considered expected reactions that could occur following administration of the IMP:

i) Localised mild pain during administration

ii) Mild bruising at the site of the administration, localized erythema, pruritus

iii) Scarring and dyspigmentation at the site of injection

Recognition and treatment of possible overdose

The intradermal injections will be performed by the Chief Investigator or delegated clinician who will have been appropriately trained in this procedure. Only the aliquoted amount of fibroblasts in normal saline solution (0.25ml) will be given at each site. The IMP volume will be labelled and crosschecked by the clinical research nurse/research team member at the time of administration and documented in the participants' drug charts, as per standard SOPs for drug administration used at the clinical site.

Other adverse clinical consequences

As with any new form of therapy, there may be risks which are unknown or not anticipated.

9.2 Specification, timing and recording of safety parameters

Adverse events (AEs): All researchers involved in this trial must be familiar with the assessment of safety for the trial. Researchers are defined as any clinical researcher, investigator, research nurse or other personnel member conducting trial related clinical research activity. It is imperative that all investigators have a thorough understanding of anticipated AEs and the reporting process of these events. All AEs will be recorded in accordance with ICH GCP, EU directives and King's Health Partners Clinical Trials Office (KHP-CTO) SOPs.

Medical history: A full medical history will be taken from the patient by a member of the clinical team during the screening visit. This ensures that the study participants satisfy the eligibility criteria and do not have any medical conditions that would make it

inappropriate for them to participate in the study. All known medical history since birth, including ongoing conditions, will be recorded in the CRF, together with any other clinically important findings that the Investigator considers relevant to the study.

Physical examination: A physical examination of each subject will be conducted by the Investigator at all trial visits. All abnormal findings (including gastrostomies and surgical scars) will be documented at screening. Any changes that occur during the study will be documented.

Vital signs: Vital signs (heart rate, blood pressure, respiratory rate and temperature) will be measured at screening, day 0 (treatment day), and at every follow-up visit.

Serum analyses: Routine blood analysis including FBC, U&Es, LFTs, ESR and CRP will be measured at screening, D0, W2, W4, M3, M6 and M12 to monitor any potential systemic adverse effects. Analysis of antibodies for C7 and IIF will be performed both prior to and after IMP administration (screening, W2, W4, M3, M6 and M12) in order to detect any potential development of EB acquisita in participants with RDEB as a potential adverse effect of introducing new C7 expression from the gene-modified autologous fibroblasts. ELISPOT assay for detection of T-cell responses to the full length C7 will be performed at screening, W4, M6 and M12.

9.3 Procedures for recording and reporting Adverse Events

All researchers involved in this trial must be familiar with the assessment of safety for the trial. Researchers are defined as any clinical researcher, investigator, research nurse or other personnel member conducting trial-related clinical research activity. It is imperative that all investigators have a thorough understanding of anticipated adverse events (AEs) and the reporting process of these events. All AEs will be recorded in accordance with GCP, EU directives and KHP-CTO SOPs.

The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 gives the following definitions:

Adverse Event (AE): Any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction (AR): Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

Unexpected Adverse Reaction (UAR): An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the Investigator's Brochure (IB) relating to the trial in question (for any other investigational product).

Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR): Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that

- Results in death;
- Is life-threatening;
- Required hospitalisation or prolongation of existing hospitalisation;
- Results in persistent or significant disability or incapacity;
- Consists of a congenital anomaly or birth defect.

Suspected Unexpected Serious Adverse Reaction (SUSAR): Is defined as a serious adverse drug reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the IB relating to the trial in question, and which results in any of the outcomes set above.

Important Medical Events (IME) & Pregnancy: Although not a serious adverse event, any unplanned pregnancy will also be reported via the SAE reporting system as stated below.

Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

9.4 Reporting Responsibilities

All AEs, SAEs, SARs and SUSARs will be documented in the patients' notes. Events defined as serious will be reported in as much detail as possible to the sponsor as soon as possible (or immediately) within 24 hours after the Investigator has been notified. King's College London (KCL) has delegated the delivery of the Sponsor's responsibility for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004 to the King's Health Partners Clinical Trials Office (KHP-CTO). The latest version of the SAE reporting form is available on the KHP-CTO website.

The **Chief Investigator (CI)** has overall responsibility for the conduct of a study/trial. The CI has coordinating responsibility for reporting SAEs to the study/trial Sponsor.

The **Principal Investigator (PI)** has overall responsibility for the study/trial being conducted in the site. The PI is responsible for informing the CI, or the organising research team, of all SAEs that occur.

The **Sponsor** of the study/trial is responsible for ensuring that all relevant information about a SUSAR is reported to the Competent Authority (the Medicines and Healthcare products Regulatory Agency).

All SAEs, SARs and SUSARs (except those specified in this protocol as not requiring reporting) will be reported immediately (and certainly no later than 24hrs) by the Investigator to the KHP-CTO and CI for review in accordance with the current Pharmacovigilance Policy. All medical emergencies including severe allergic reaction, anaphylaxis, pulmonary embolism, pneumonia and myocardial infarction will be managed according to GSTT SOPs.

Death as a result of disease progression and other events that are primary or secondary

outcome measures are not considered to be SAEs and should be reported in the normal way, on the appropriate CRF.

The KHP-CTO will report SUSARs to the regulatory authorities (MHRA, competent authorities of other EEA (European Economic Area) states in which the trial is taking place. The CI will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.
- The CI and KHP-CTO (on behalf of the co-sponsors), will submit a Development Safety Update Report (DSUR) relating to this trial IMP, to the MHRA and REC annually.

9.5 Adverse Events (AEs) and Serious Adverse Events (SAEs) that do not require reporting

The following will not require reporting although will be documented on the eCRF.

Adverse event as a result of venesection and cannulation include:

i) Mild bruising at site of needle puncture

Adverse event as a result of the skin biopsy include:

i) Mild bruising at the site of the skin biopsy
ii) Cutaneous skin infection requiring oral course of antibiotics
A small scar will result after each skin biopsy, resembling an old chickenpox scar.

All hospitalisations which are expected to take place as a result of disease progression including any planned elective surgeries will not be reported at the interim and the

end-of-trial safety data analysis but will be documented in the patient notes. This may include but not limited to:

- Skin
    - Skin infection
    - Review of a wound
    - Skin blisters/ erosions as part of EB wound
- Teeth
    - Dental extractions/ abscess
- Hand
    - Hand surgery
    - De-gloving injury
    - Occupational Therapy review and splints
- Transfusions
- Overnight hospital stay for reviews
- Blood monitoring, routine blood tests
- Corneal abrasions
- Eye Infections
- Chronic eye problems due to EB
- Gastrointestinal problems
    - Dysphagia, oesohageal stricture and dilation
    - Gastrostomy insertion, leakage or blockage/ jejunal tube insertion leakage/ blockage
    - Regurgitation or vomiting as a result of oesophageal blisters/ stricture
    - NG tube insertion
    - Constipation
- Vertebral or other fractures
- IV bisphosphonates

- IV iron infusion
- Contractures requiring physiotherapy
- Hydrotherapy
- ENT
    - Tonsillitis
    - Otitis externa
    - Otitis media
- Pain
    - pain assessment for acute or chronic pain
    - pain from ongoing EB wounds
- Accidental injuries causing minor skin wounds

Thus, any hospitalisation that is not associated with the IMP will not be reported, unless the IMP results in a prolongation of existing hospitalisation. Unscheduled and/or emergency hospitalisations that are not expected due to the natural course of the disease will be reported via the sponsor's normal serious adverse event (SAE) reporting practice. AEs will be reviewed at each visit both from the patient's report as well as documentation from medical notes.

9.6 Treatment stopping rules

The trial may be prematurely discontinued by the Sponsor, Chief Investigator or Regulatory Authority on the basis of new safety information or for other reasons given by the Data Monitoring & Ethics Committee/ Trial Steering Committee regulatory authority or ethics committee concerned.

The trial may also be prematurely discontinued due to lack of recruitment or upon advice from a Trial Steering Committee, who will advise on whether to continue or discontinue the study and make a recommendation to the sponsor. If the trial is prematurely discontinued, active participants will be informed and no further participant

data will be collected. The Competent Authority and Research Ethics Committee will be informed and no further participant data will be collected.

Treatment stopping rules for individual patients will include voluntary discontinuation by the patient, a change in inclusion or exclusion criteria of the clinical trial or the development of a SUSAR/SAE/SAR.

10 Statistics

10.1 Sample size

The aim of this phase 1 trial will be to recruit approximately 5–10 patients from GSTT EB clinical database. As described previously this is a rare disease with an incidence of 1 in 17,000 live births. As per our statistician's advice, even if no subjects experienced adverse reactions (i.e. 0% AR), a sample size of 10 would give a 95% confidence interval of 0% to 31% (exact CI from Stata®). A sample of 10 could still potentially provide reassurance that no more than 3 in 10 subjects should experience a given side-effect.

10.2 Analysis

All study participants will be included in all analyses unless they have withdrawn consent. For subjects who drop-out rather than withdrawn consent, a request will be made to use data collected prior to drop-out. Descriptive statistics such as means, standard deviations (SDs), minimum and maximum frequencies and proportions, as appropriate will be presented. No hypothesis tests will be performed. When all participants complete Visit 7 (Month 6), an interim analysis of the collected data will be performed and the results will be published in a peer-reviewed journal and there will be another analysis at the end of the trial (12 months after the last participant's cell injections).

11 Trial Steering Committee

The Trial Steering Committee (TSC) consists of a panel of scientists specialised in the field of EB who will provide constructive criticism and suggestions to improve both the clinical trial implementation and data evaluation.

The Steering Committee shall be responsible for:

- Providing feedback and routine assessment of progress of LENTICOL-F as a whole.
- Ensuring all the work meets the functional requirements and is in accordance with the project deliverables and where necessary GCP and/or GMP compliant.
- Maintaining a complete overview of the scientific and process development progress of the project in relation to external progress in the various fields related to the project.
- Collecting and reviewing aggregate safety data, with the authority to recommend premature termination of the trial if felt necessary.

12 Direct Access to Source Data and Documents

The Investigator(s) will permit trial-related monitoring, audits, IREC review, and

regulatory inspections by providing the Sponsors, Regulators and REC direct access to source data and other documents (e.g. patients' case sheets, blood test reports, X-ray reports, histology reports etc.). The Investigators will also permit authorised inspectors to inspect all facilities and records relating to the study and aid the inspector(s) to perform the audit in a timely manner.

13 Ethics & Regulatory Approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

Prior to the initiation of the trial, the Investigator will submit the protocol, subject information sheet, subject consent form, letter to the GP and any other related documents to the appropriate Independent Research Ethics Committee (IREC), London – West London & GTAC, and to the MHRA for Clinical Trial Authorisation. The Chief Investigator will submit a final report at conclusion of the trial to the KHP-CTO (on behalf of the Sponsor), the REC and the MHRA within the timelines defined in the Regulations.

14 Quality Assurance

Monitoring of this trial will be to ensure compliance with Good Clinical Practice and scientific integrity will be managed and oversight retained by the KHP-CTO Quality Team. The relevant regulatory authorities also have the right to conduct an inspection of the study, that the study was, in fact, performed at stated sites and that the data reported to the authorities reflects the data in the records of the Investigator. The authorities also inspect such studies to verify that the studies were conducted in accordance with government regulations relating to the IEC and informed consent. It is

the joint responsibility of the sponsor, co-sponsor and the Investigators to ensure that the study has been conducted in line with all relevant regulations and KCL standard operating procedures.

15 Data Handling

The Chief Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

- Patient data will be anonymised and coded.
- This clinical trial will use the Biomedical Research Centre's (BRCs) industry-standard secure database called MedSciNet. All access to the MedSciNet data system is controlled using a Username/Password login. Passwords are encrypted before storing to database, using SHA-1 hash (MS .NET SHA1CryptoServiceProvider). These are created and controlled by Administrative users of the system as identified by the Chief Investigator. The data is stored in the BRC eCRF servers which are located in Holland and meet or exceed all MHRA requirements for CTIMP data storage. Only the server administrator has access to this server and the core database, via remote connection. The back up process is twofold: every 24hrs the database is backed-up to the server and every 7 days the entire server is backed up to an archival tape system.
- The final database will be stored in a read-only format for 5 years before being archived onto the server for a further 2 years. At this point the archived version will be compressed and will remain in the server and a copy will be sent to the CI. The documents that relate to ATIMP traceability will be retained for a minimum of 30 years after the expiry date of the IMP. In order to meet regulatory requirement, at GSTT, hard copies of Care Report Forms will be kept in a locked filing cabinet in a locked office with the Trial Site File as per King's Health Partners-Clinical Trial Office (KHP-CTO) Standard Operating Procedure (SOP) – The Creation and Maintenance of Trial Master Files and Essential Documentation (available on the KHP-CTO website). The Trial Master File will be archived as per current KHP-CTO SOPs. All trial data will be stored in line with the Medicines for Human Use (Clinical

Trials) Amended Regulations 2006 and the Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the KHP-CTO Archiving SOP.

16 Data Management

Electronic Case Report Forms (eCRFs) will used to capture the data of each subject enrolled in the study. These will be standardised and protocol specific and create a time stamped electronic record of any amendments or additions to the document. eCRF pages will be monitored on an on-going basis by the study monitors.

17 Publication Policy

It is intended that the results of the study will be reported and disseminated through posters and oral presentations at international conferences, scientific meetings and in high impact peer-reviewed scientific journals. Publicity will be performed through press releases at the local, national and international levels. Press releases will be co-ordinated through King's College London, Sohana Research Fund (SRF) and DEBRA UK. SRF and DEBRA UK will also inform patients and potential participants through their website and newsletters.

18 Insurance / Indemnity

The trial is co-sponsored by King's College London and Guy's and St. Thomas' NHS Foundation Trust. The co-sponsors will at all times maintain adequate insurance in relation to the study independently. King's College London, through its own professional indemnity (Clinical Trials) and no fault compensation and the Trust having duty of care to patients via NHS indemnity cover, in respect of any claims arising as a result of clinical negligence by its employees, brought by or on behalf of a study patient.

19 Financial Aspects

Funding to conduct this trial is provided by Sohana Research Fund (SRF) and registered charity DEBRA UK.

20 Signatures

Chief Investigator: Professor John McGrath

Signature: **Date:**

21 References

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Appendix 1: Development of gene therapy for inherited skin diseases

Method	Target gene	Condition	Target cell	Comments	Reference
Self-inactivating lentiviral vector	*COL7A1*	Recessive dystrophic EB	In vivo	In vivo inoculation of skin equivalents grafted onto mice, C7 expression up to 3 months	1
Self-inactivating lentiviral vector	*COL7A1*	Recessive dystrophic EB	Fibroblast	Gene corrected fibroblasts injected into murine skin able to produce C7 at basement membrane zone	2
Gamma retroviral vector	*COL7A1*	Recessive dystrophic EB	Keratinocyte	Construction of skin equivalents using classical retroviral gene transfer; transduction efficiency 40%	3
Self-inactivating gamma retroviral vector	*COL7A1*	Recessive dystrophic EB	Keratinocyte + Fibroblast	Generation of human skin equivalents showing in vivo functional correction of phenotype; C7 expression of recombinant C7 ranging between 30-80% keratinocytes and 40-70% fibroblasts	4
Retroviral vector	*COL7A1*	Recessive dystrophic EB	Keratinocyte	Genetic correction of both dog RDEB and human primary RDEB cells	5
φC31 integrase based plasmid	*COL7A1*	Recessive dystrophic EB	Keratinocyte	Nonviral approach into primary RDEB cells; regenerated skin (40% efficiency); stable correction required short-term drug selection and enrichment	6
3' pre-transplicing molecule	*COL7A1*	Recessive dystrophic EB	Keratinocyte	Trans-splicing used to reduce size of *COL7A1* transcript and therefore risk of genetic rearrangement	7
φC31 integrase based	*LAMB3*	Junctional EB	Keratinocyte	Nonviral approach showing corrected hemidesmosome formation in JEB primary cells	8

plasmid					
Transposase (sleeping beauty)	*LAMB3*	Junctional EB	Keratinocyte	Sleeping beauty transposable element used to integrate *LAMB3* cDNA into epidermal cells in 6 JEB patient primary cells; transfected cells enriched using drug selection.	9
PAC (P1-based artificial chromosome clone)	*COL7A1*	Recessive dystrophic EB	Keratinocyte	Gene transfer using micro-injection of entire human locus *COL7A1* in a P1 derived artificial chromosome	10
TALEN gene editing	*COL7A1*	Recessive dystrophic EB	Fibroblasts	Transciption activator-like endonucleases (TALEN) gene editing of RDEB fibroblasts subsequently reprogrammed into iPS cells (induced pluripotent stem cells)	11
Gamma retroviral vector	*COL17A1*	Junctional EB	Keratinocyte	Restoration of full-length BP180 protein expression in primary keratinocytes then used to regenerate human skin on immunodeficient mice	12
Gamma retroviral vector	*LAMB3*	Junctional EB	Keratinocyte	Only case of successful gene transfer in an inherited skin disorder in an adult patient with non-herlitz junctional EB; restored phenotype and laminin 332 expression 7 years post graft	13
Self-inactivating lentiviral vector	*LAMB3*	Junctional EB	Keratinocyte	Pre-clinical study using potentially safer LV	14
Gamma retroviral vector	*XPC*	Xeroderma pigmentosum	Keratinocyte + Fibroblast	Functional in vitro correction of *XPC* keratinocytes	15
Adenoviral vector	*XPA*	Xeroderma pigmentosum	In vivo	In vivo inoculation of recombinant AV vector in UV irradiated murine skin	16
Self-inactivating lentiviral vector	*XPA*/ *XPC*/ *XPD*	Xeroderma pigmentosum	Fibroblast	Efficient SIN LV vector able to correct cellular phenotype in XP	17
Gamma retroviral vector	*XPC*	Xeroderma pigmentosum	Keratinocyte	MLV derived vector able to transduce primary cells and maintain *XPC* expression in up to 130 population doublings	18

TALEN gene editing	*XPC*	Xeroderma pigmentosum	Fibroblast cell line	Successful targeted gene correction of *XPC* cell line	19
Self-inactivating lentiviral vector	*SPINK5*	Netherton syndrome	Keratinocyte	Pre-clinical work demonstrating full length LEKTI expression in organotypic cultures grafted onto mice. Currently recruiting for phase I clinical trial	20
Retroviral vector	*STS*	X-linked ichthyosis	Keratinocyte	Restoration of enzymatic activity in cultured primary keratinocytes deficient in steroid sulfatase	21
siRNA	*KRT5*	EB Simplex	Keratinocyte	Pre-clinical study, specific knockdown of mutant keratin 5	22
siRNA	*KRT6A*	Pachyonychia congenital	Keratinocyte	Phase 1b trial injecting TD101 siRNA locally for plantar keratoderma in PC	23
Direct injection of plasmid	*TGM1*	Lamellar Ichthyosis	Keratinocyte	Non-uniform restoration of gene expression on regenerated LI skin	24

ABBREVIATIONS: AV, adenoviral; C7, type VII collagen; EB, epidermolysis bullosa; LI, lamellar ichthyosis; LV, lentiviral; PC, pachyonychia congenita; RDEB,recessive dystrophic epidermolysis bullosa; SIN, self-inactivating vectors.

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Appendix 2: Summary of published clinical studies of allogeneic and autologous fibroblasts used to treat skin disorders

Cell	Study type	Clinical indication	No. Of patients	Cell type	Outcome	Reference
Fibroblast	Prospective pilot study	Prominent facial scar	10	Intradermal cultured autologous fibroblasts	Size reduction between 10%-85%; histologically ñ dermal thickness	1
Fibroblast	Prospective placebo controlled Phase III trial	Facial Contour deformities	215	Intradermal cultured autologous fibroblasts	Most significant difference evident in acne scars; continued response rates up to 12 months post treatment	2
Fibroblast	Interventional non-blinded prospective study	Adult RDEB	5	Intradermal injection allogeneic cultured fibroblasts; intact skin	↑*COL7A1* and C7 for 3 months; can remain raised for up to 9 months	3, 4
Fibroblast	Phase II placebo controlled double blind RCT	Adult RDEB	5	Intradermal cultured allogeneic fibroblasts	No significant difference between placebo; improvement in QOL	5
Fibroblast	Interventional	Ageing skin	5	Intradermal cultured	Benefits limited to slight reduction	6

	non-blinded study			autologous fibroblasts	in skin fragility	
Fibroblast	Phase II open label dose escalation pilot study	Ageing skin	10	Intradermal cultured allogeneic fibroblasts	Slight reduction in nasolabial crease	7
Fibroblast	Single centre interventional study	Ageing skin and scars	20	Intradermal cultured autologous fibroblasts	Variable improvement at 6 months	8
Keratinocyt	Phase II placebo controlled double blind RCT	Chronic Venous Ulcers	228	Spray allogeneic neonatal keratinocyte and fibroblast cell applied therapy	Greater mean reduction of wound size compared with placebo	9
Fibroblast	Prospective interventional study	Burns (third degree)	14	Allogeniec fibroblasts on meshed split thickness skin grafts	Improved healing time and hypertrophic scar formation compared with conventional method	10
Fibroblast	Multicentre double blind placebo controlled Phase II RCT	Ageing skin	372	Intradermal cultured autologous fibroblasts	Moderate improvement in nasolabial fold wrinkles compared to placebo, only 1 point subjective difference	11
Cultured dermal substitute						
CDS	Prospective interventional study	RDEB	3	Allogeneic cultured dermal substitute using cryopreserved normal human	++Granulation found on wound surface within 1 week; epithelialisation at margins of ulcer at 4 weeks	12

				fibroblasts on matrix of hyaluronic acid and Atelo-collagen		
CDS	Case report	Digital gangrene associated with hypereosinophilic syndrome	1	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen; replaced every 3 days for 5 weeks	Pain reduction at site; healthy granulation tissue during treatment and so replaced with split skin graft	13
CDS	Prospective interventional study	Full thickness skin defects following tumour resection	12	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	9/10 patients developed healthy granulation tissue allowing split skin graft; Others showed rapid re- epithelialisation	14
CDS	Case report	Chronic venous ulcer	1	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	Granulation tissue within 2 weeks; completely healed by 9 weeks	15

CDS	Multi-centre prospective interventional study	Chronic venous and arterial ulcer	13	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	9/13 cases (arterial + venous) healed well, one complicated by local infection; larger ulcers still required grafting	16
CDS	Case report	Ulcer associated with Anti-Phospholipid syndrome + stasis dermatitis	1	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen; replaced every week	All ulcers healed within 4 weeks	17
CDS	Prospective interventional study	Intractable ulcers lower leg	5	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	Wound size decreased in all cases (33% reduction in 7 ulcers, complete resurfacing in 2 cases); no control comparator	18
CDS	Prospective interventional study	Wound donor site following skin graft	14	Comparitive between fresh and cryopreserved human fiboblasts in allogeneic cultured dermal	No difference noted in re-epithelialisation between the preparation methods	19

				substitute		
CDS	Prospective, open label proof of concept clinical trial	Diabetic foot ulcer	5	Autologous fibroblast seeded artificial dermis using animal product free serum	3/5 complete wound healing within 12 weeks; no deleterious effects using animal free serum	20

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