

Phase I Study Protocol for *Ex Vivo* Lentiviral Gene Therapy for the Inherited Skin Disease, Netherton Syndrome

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Abstract

Netherton syndrome (NS) is a serious inherited skin disorder caused by mutations in the serine protease inhibitor Kazal type 5 gene (*SPINK5*), which encodes for a serine protease inhibitor lymphoepithelial Kazal type-related inhibitor (LEKTI). Patients with NS have defective keratinization, hair shaft defects, recurrent infections, atopy, and a predisposition to skin malignancies. Historically, 1 in 10 infants has died before their first birthday. Currently, there are no proven treatments to cure this condition.

A SIN-lentiviral vector encoding the codon-optimized *SPINK5* gene under the control of a 572 bp element derived from the human involucrin promoter can confer compartment-specific LEKTI expression in NS keratinocytes with restoration of normal skin architecture. Here we detail a study protocol for a phase I trial for feasibility and safety evaluations of autologous epidermal sheets generated from *ex vivo* gene-corrected keratinocyte stem cells, which will be grafted onto patients with mutation-proven NS.

Introduction

NETHERTON SYNDROME (NS) is a rare but debilitating autosomal recessive skin disorder condition caused by mutations in the serine protease inhibitor Kazal type 5 gene (*SPINK5*). Clinically, it is characterized by defective keratinization, hair shaft defects, recurrent infections, atopy, and a predisposition to skin malignancies (Chavanas *et al.*, 2000; Bitoun *et al.*, 2003). Prognosis is often poor, with high risk of life-threatening complications such as bronchopneumonia, sepsis, and hypernatremic dehydration secondary to severe water loss through the defective skin barrier. Currently, there is no curative treatment for this condition.

SPINK5 encodes a 1094 amino acid protein known as lymphoepithelial Kazal type-related inhibitor (LEKTI). This serine protease inhibitor has 15 Kazal type-related inhibitory domains. It is expressed in the thymus, epithelial tissues, oral mucosa, tonsils, parathyroids, bartholin glands, and skin (Magert *et al.*, 1999; Chavanas *et al.*, 2000; Komatsu *et al.*, 2002). In the skin, the expression of LEKTI is localized to the uppermost spinous and granular layers of normal human epidermis (Chavanas *et al.*, 2000; Bitoun *et al.*, 2003). Pro-LEKTI is synthesized in keratinocytes and is rapidly pro-

cessed into proteolytic fragments and secreted into the extracellular compartment. *In vitro* studies have confirmed that full-size LEKTI and its fragments are able to inhibit various serine proteases such as plasmin, trypsin, elastase, and tissue kallikreins (KLKs), in particular KLK5 and 7 (Mitsudo *et al.*, 2003; Jayakumar *et al.*, 2004; Egelrud *et al.*, 2005; Deraison *et al.*, 2007). KLKs have a typical serine protease catalytic domain. They play a major biological role in the epidermis by controlling desquamation by degrading the adhesive ectodomain of desmoglein I and maintaining the integrity of epidermis. LEKTI controls the activity of KLK5 by forming an inhibitory complex with KLK5 in a pH-dependent manner (Deraison *et al.*, 2007). Under acidic pH, the LEKTI-KLK5 inhibitory complex is dissociated and inhibitor-free KLK5 can be activated by matriptase, a transmembrane serine protease with the ability to upregulate inhibitor-free KLK5, resulting in KLK5-mediated degradation of corneodesmosomes (Sales *et al.*, 2010). In patients with NS, deficient LEKTI expression causes increased inhibitor-free KLK5 that can be activated by matriptase and cause overdegradation of corneodesmosomes. Activated KLK5 also stimulates an inflammatory cascade via the protease-activated receptor-NF κ B pathway and further impairs skin

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barrier formation (Briot *et al.*, 2009; Sales *et al.*, 2010). In addition, KLK5 can upregulate elastase 2 activity, resulting in excessive degradation of (pro-) filaggrin and alteration of intercorneocyte lipid formation in the skin barrier (Bonnart *et al.*, 2010) (Fig. 1).

As the molecular basis of inherited skin diseases becomes characterized, gene-based therapies are being developed for the most serious and debilitating conditions. This includes the blistering disorder epidermolysis bullosa (Cavazza and Mavilio, 2012; Uitto *et al.*, 2012), and defective keratinization disorders such as xeroderma pigmentosum (Marchetto *et al.*, 2006). These conditions are attractive targets for gene therapy as the epidermis from patients can be readily harvested and manipulated *ex vivo*. Advances in culture techniques have allowed keratinocytes including keratinocyte stem cells (KSCs) or holoclones to be cultured *in vitro* (Rheinwald, 1989; Watt, 2002). Sheets of epithelium can be generated and are now widely used as autologous skin grafts in patients with severe burns, skin ulcers, or impaired wound healing (MacNeil, 2007). Similar grafts have been used for genetic correction of laminin 5 expression, a defective protein in junctional epidermolysis bullosa. After *ex vivo* retroviral transduction of epidermal stem cells, corrected epidermal sheets generated from transduced cells were subsequently used to treat areas of chronically ulcerated skin (Mavilio *et al.*, 2006).

Successful skin gene therapy must target KSCs and mediate durable high-level gene expression in cell populations

undergoing continuous turnover. As true, long-lived KSCs lack identifiable markers, heterogeneous keratinocyte populations isolated from skin biopsy samples must be targeted. Various viral vector systems have been evaluated and oncoretroviral vectors have proven to be well-suited for the stable integration of transgenes into the genome of target cells (Gerrard *et al.*, 1993; Choate *et al.*, 1996; Mavilio *et al.*, 2006). 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 retroviral vectors 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, lentiviral vectors can transduce quiescent, or minimally stimulated cells and exhibit improved transduction of KSC *in vitro* and *in vivo* and may be better suited to preserving stem cell potential (Kuhn *et al.*, 2002; Gagnoux-Palacios *et al.*, 2005). These vectors are less likely to mediate insertional mutagenesis if suitable internal promoter elements are used (Montini *et al.*, 2002, 2006).

The vector (pCCL-SIN-cPPT/CTS) encodes codon-optimized cDNA for *SPINK5* under the control of a 572 bp enhancer derived from the human involucrin promoter (INVO) (Ghazizadeh *et al.*, 2002) (Fig. 2), which can drive compartment-specific gene expression restricted to the uppermost epidermis, the predominant site of endogenous LEKTI activity. We have

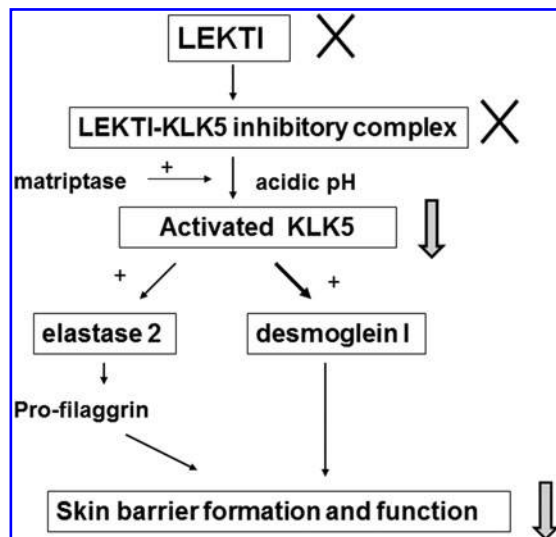


FIG. 1. A schematic of LEKTI function. In normal epidermis, LEKTI inhibits the activity of KLK5 by forming an inhibitory complex with KLK5. Under acidic pH, this complex is dissociated and free KLK5 can be activated by matriptase, resulting in KLK5-mediated degradation of desmoglein 1. Activated KLK5 can also upregulate the serine protease elastase 2, resulting in degradation of (pro-)filaggrin. Both desmoglein 1 and filaggrin are important molecules involved in skin barrier formation and function. In NS, deficient LEKTI expression (shown by X) causes increased KLK5 activity and alteration of the downstream molecules desmoglein 1 and elastase 2 activity/function (shown as nonfilled arrows), leading to defective skin barrier formation and function. KLK5, kallikrein 5; LEKTI, lymphoepithelial Kazal type-related inhibitor; NS, Netherton syndrome.

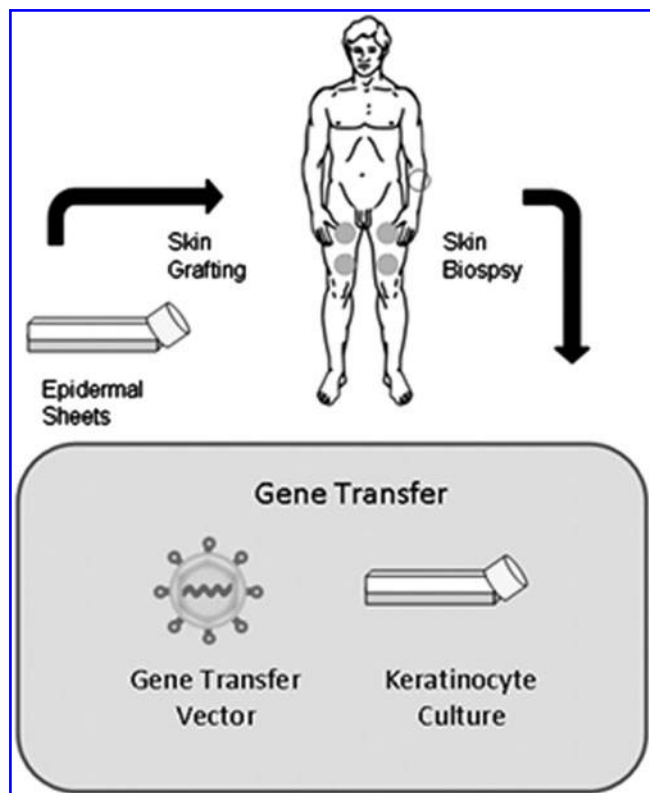


FIG. 2. Schematic of *ex vivo* gene therapy for NS. A small skin biopsy is taken from the patient with NS. Keratinocytes including keratinocyte stem cells are isolated, cultured, and transduced with lentiviral vector pCCL-INVO-SP. After transduction, cells are continuously cultured to form an epithelial sheet and then grafted back onto the patient after preparation of a de-epidermized bed.

demonstrated that INVO mediates enhanced expression of a codon-optimized transgene *SPINK5* and reduced silencing effects in skin (Di *et al.*, 2012). *In vitro* 3D organotypic cultures derived from NS cells transduced with *SPINK5* recovered morphology of epidermal structures and exhibited restored architecture (Di *et al.*, 2011). As *SPINK5* null mice die in the neonatal period, we developed a human murine chimeric model whereby nude mice received human skin grafts generated from human keratinocytes. Staining for human involucrin allowed areas of human and murine epidermis to be clearly delineated. NS grafts transduced with control vector were found to have histological features of NS, including psoriasiform changes and hypogranulosis with a parakeratotic stratum corneum and exfoliated corneocytes. In contrast, in grafts generated from cells transduced with vector encoding *SPINK5*, the epidermis exhibited corrected morphology with reduced rete ridges and almost complete restoration of normal epidermal architecture. In addition, the localization of suprabasal LEKTI in NS grafts driven by the INVO promoter closely mirrored the compartmentalization of native LEKTI expression (Di *et al.*, 2011, 2012).

Study Objectives

This study is a phase I clinical study of autologous gene-modified skin grafts in NS using a SIN lentiviral vector (Figs. 2 and 3). The study aims to determine the feasibility and safety of lentivirus-modified autologous skin sheets, and will assess whether *SPINK5* gene transfer can mediate localized correction of skin architecture inside and outside of the grafted area.

Study Overview

This is an open-label, nonrandomized, single-armed, phase I safety study. We describe our clinical protocol for first-in-man evaluation of lentiviral-mediated gene transfer of codon-optimized *SPINK5* to KSCs collected from subjects with NS, and the production and application of autologous gene-modified epidermal sheets. Previous correction of inherited skin disease has been undertaken for epidermolysis bullosa by *ex vivo* transduction of KSCs and skin sheet production using an LTR-intact gamma retroviral vector. Here we will attempt lentiviral-based correction using a compartment-specific INVO and aim, in the first instance, to generate safety and feasibility data by correcting a small area of epidermis. Successful correction of skin architecture within the graft area is anticipated, and there is a possibility of benefit in areas surrounding the graft as LEKTI is secreted and disseminates.

Such effects would provide supportive evidence for larger areas of skin grafting, which could provide particular benefit in infants at highest risk of systemic complications.

Monitoring in this study will include regular inspection of graft areas, serial assessment of barrier function inside and outside the graft, tracking of total serum immunoglobulin E (IgE) levels, and screening for cellular immune responses against LEKTI. The latter will rely on enzyme-linked immunosorbent spot (ELISPOT) assays to detect interferon- γ response in lymphocytes cocultured with autologous peripheral blood mononuclear cells and recombinant LEKTI. The study is not employing any preconditioning or immunosuppression regimens, and while patients with NS may have impaired immunity because of impaired skin barrier function, cellular immunity is intact. In the absence of preexisting data of such responses in NS patients, serial samples will be analyzed for evidence of emerging T cell responses after grafting. Consideration has been given to adverse effects relating both to the grafting procedure and the integrating vector technology being applied. Subjects may have preexisting bacterial skin colonization, which has necessitated the inclusion of antibiotics in the KSC culture medium, and the stipulation of exclusion criteria based on the detection of pathogenic bacteria with antibiotic resistance. As with other surgical skin procedures, subjects will be advised that there may be risks of localized bleeding, bruising, and discoloration. Risks related to the lentiviral vector are more difficult to quantify, though are expected to be minimal, both in terms of unregulated though compartment-specific (INVO-mediated) LEKTI expression and the risk of insertion-related adverse events. To date, insertional mutagenesis has been reported only after hematopoietic stem cell transduction with LTR-intact gamma-retroviral vectors. There has been a single report of clonal expansion (though not mutagenesis) after lentiviral-mediated HSC modification (Cavazzana-Calvo *et al.*, 2010), and a number of other hematopoietic stem cell modification trials underway without any similar phenomena to date (Aiuti *et al.*, 2013; Biffi *et al.*, 2013). In the absence of robust models of vector-mediated insertional mutagenesis in keratinocytes, the relative safety of SIN lentiviral vector or LTR-intact gamma retroviral vectors in skin studies is difficult to quantify. We anticipate that the risk of such effects will be extremely low after KSC modification, and in the unlikely event of transformational change, graft excision is a feasible option if required. Finally, integration site analysis of serial biopsy samples may allow clonally based assessment of KSCs contributing to epidermal regeneration in NS and in skin grafts generally.



FIG. 3. Lentiviral vector configuration. The pCCL-INVO-SP lentiviral vector containing the pCCL SIN lentiviral vector is derived from HIV-1. Open-reading frames of HIV-1 genes have been deleted from the vector, leaving SIN LTRs, elements of packaging sequences (Ψ), an RRE, and a cPPT. INVO represents short 572 bp sequence adapted from human involucrin promoter. *SPINK5* represents codon-optimized *SPINK5* cDNA sequences. cPPT, central polypurine tract; INVO, the human involucrin promoter; LTRs, long terminal repeats; RRE, rev-responsive element; SIN, self-inactivating; *SPINK5*, the serine protease inhibitor Kazal type 5 gene.

The trial has been approved by Gene Therapy Advisory Committee (GTAC) (Ref No. GTAC188) and Medicines and Healthcare products Regulatory Agency (MHRA; Ref No. EUDRACT 2011-003212-22). It has also been registered with ClinicalTrials.gov (<http://clinicaltrials.gov/number>) under identifier number NCT01545323.

Subject Selection

Inclusion criteria

1. Confirmed biallelic *SPINK5* mutations by direct DNA sequencing
2. Absence of LEKTI protein expression in the skin by immunostaining
3. Patient informed consent, or parental/guardian consent in the case of children

Exclusion criteria

1. Current or previous malignant skin disease
2. Pregnancy
3. Hepatitis B or C, or HIV infection
4. Current colonization with antibiotic-resistant pathogens
5. Known hypersensitivity to penicillin or streptomycin antibiotics

Recruitment and consent process

Patients will be identified in specialist skin clinics and an initial approach made by the treating consultant. Information sheets will be provided, and patients interested in participating will be invited for follow-up consultation, assessed for eligibility, including screening for blood-borne pathogens, and consented as necessary. Examples of an information sheet and consent form are included in Supplementary Data (Supplementary Data are available online at www.liebertonline.com/humc).

Production of the Investigation Medicinal Product

Good Manufacturing Practice production of lentiviral vector stock

293T cells from a characterized cell bank previously produced at King's College London were expanded in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS; Good Manufacturing Practice grade [GMP]; Life Technologies, Paisley, United Kingdom) for 14 days ahead of seeding into Millicell HY T-1000 multilayer flasks (Millipore, Middlesex, United Kingdom). Subconfluent cells were then transfected with high-grade accessory plasmid stocks encoding Rev, gag/pol, and envelope (VSV-g) components, along with the lentiviral vector plasmid encoding codon-optimized *SPINK5* cDNA (pCCL-INVO-SP) (Plasmid Factory, Bielefeld, Germany), by calcium/phosphate coprecipitation. The medium was changed after 16 hr, and 10 units/ml benzonase (Millipore) was added to reduce plasmid DNA carriage. After a further 24 and 48 hr, supernatant was harvested, filtered (0.45 μ m), and concentrated 400-fold. The concentrated vector was resuspended and pooled in Optimem medium (GMP grade; Life Technologies) for rapid freezing. Vector stocks underwent detailed characterization for sterility, adventitious pathogens, contamination screening, and exclusion of replication-competent lentivirus (Table 1).

TABLE 1. SUMMARY OF VECTOR CHARACTERIZATION AND RELEASE TESTING

Description	Assay
Quantification of residual host cell proteins	Enzyme immunoassay for total immunoreactive HEK293 host cell protein
Quantification of residual host cell DNA	qPCR quantification for residual 293 DNA in biological sample
Quantitation of residual benzonase	Enzyme immunoassay confirming
Absence of SV40 DNA replication	qPCR detecting presence of lentiviral plasmid and serial sampling to confirm signal dilution in absence of replication
Endotoxin	LAL assay for endotoxin
Screen for adventitious viral contaminants	Extended <i>in vitro</i> assay for the detection of viral contaminants in six-well plates using 3 detector cell lines
Detection of mycoplasma	Direct inoculation onto agar plates screen for inhibitory substances, cultivation assay, and DNA fluorochrome
Sterility testing	Direct inoculation
Detection of replication-competent lentivirus	Serial passage in C8166 detector cell with microscopical examination and FPRT analysis

qPCR, quantitative polymerase chain reaction; FPRT, fluorescent product enhanced reverse transcriptase.

Isolation and transduction of keratinocytes

Clinical-grade culture media, approved source FBS, and additional supplementary reagents for keratinocyte culture are provided by a U.K.-based specialist supplier (Altrika, Sheffield, United Kingdom), along with irradiated 3T3 feeder cells from a characterized 3T3 master cell bank produced under GMP conditions.

In the study, primary keratinocytes will be obtained from patients with NS by a 6 mm punch skin biopsy. After digestion with 0.25% trypsin-EDTA (Altrika) at 37°C for approximately 3–4 hr, heterogeneous cell populations including KSCs and progenitor keratinocytes will be filtered through a sterile cell strainer (100 μ m; Becton Dickinson, Oxford, United Kingdom) to obtain single-cell suspensions. Cells will be centrifuged and re-suspended in a complete keratinocyte culture medium containing DMEM and Ham/F12 medium (3:1) with 10% FBS and supplemented with 0.4 μ g/ml hydrocortisone, 10 ng/ml epidermal growth factor, 5 μ g/ml transferrin, 5 μ g/ml insulin, 1×10^{-10} M cholera toxin, 2×10^{-11} M liothyronine, and 100 unit/100 μ g/ml penicillin/streptomycin. Suspended cells will be seeded into culture flasks with 60 Gy-irradiated 3T3 feeder cells from Altrika, and cultured at 37°C with 10% CO₂. About $1\text{--}2 \times 10^6$ keratinocytes will be subcultured in two T25 flasks, one for generating the gene-modified graft and another flask for generating transduced cells for characterization studies and

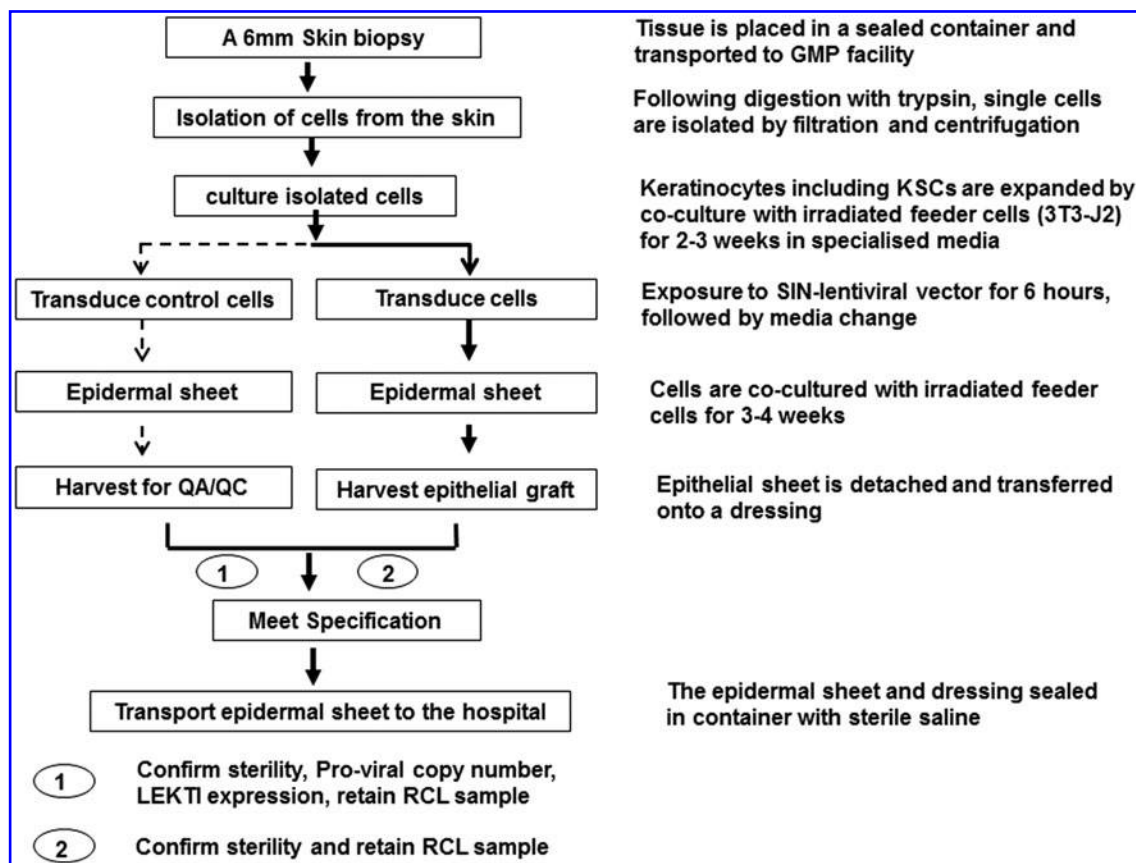


FIG. 4 Generation and release of investigational medicinal product. Keratinocytes isolated from a 6 mm skin biopsy obtained from the NS patient will be cultured and transduced with the lentiviral vector encoding codon-optimized *SPINK5* gene. Transduced keratinocytes will then be cultured as two epithelial sheets, one for grafting and another one for characterizing for quality assurance/quality control. Matured sheets will be harvested, transferred to a nonadherent dressing, and sealed in a sterile container. The gene-modified autologous epithelial sheet will be transported to the hospital for grafting within 12 hr.

quality control. Around 5–7 days after seeding, cells will be transduced with pCCL-INVO-SP virus at a multiplicity of infection of ~15. After 6 hr, the medium will be replaced with a fresh keratinocyte medium. Cells will then be cultured until 80% confluence whereupon they will be trypsinized and transferred to 100 mm culture dishes and continuously cultured in a complete keratinocyte medium until they form epithelial sheets (Fig. 4).

Preparation of gene-modified epidermal sheets

About 72 hr before harvesting, the cultured sheet will be trypsinized briefly using TrypLE to remove any residual 3T3 cells. At this point, cultures will be free of cholera toxin and transduced keratinocytes grown to borderline over-confluence before dispase treatment (2 mg/ml in PBS) to detach the sheets. Tricotex (Smith and Nephew, Warwick, United Kingdom), a nonadherent clinical-grade dressing, will be placed on top of the graft, before washing and lifting into a sterile container for transportation. The graft is stable at 2–8°C in sterile saline for 12 hr and will be assessed and scored by the surgeon before preparation of the receiving graft bed (Table 2). In parallel, a second flask of transduced KSCs will be used for analysis of transduction efficiency by quantitative polymerase chain reaction and immunostaining/immunoblot. Samples from both flasks will be assessed

TABLE 2. SCORING AND ASSESSMENT OF GENE-ENGINEERED EPITHELIAL SHEETS

Assessment	Time of release	Time of grafting	Definition
Clarity	1/0	1/0	1 = media transparent 0 = media cloudy
Size	1/0	1/0	1 = sheet size > target size of 20 cm ² for adults/10 cm ² for children 0 = sheet size ≤ 20 cm ² /10 cm ²
Integrity	1/0	1/0	1 = visibly intact with no disintegration 0 = visible disintegration
Color	1/0	1/0	1 = pale white Otherwise scored as 0
Lift ability	1/0	1/0	1 = the sheet attached to Tricotex and can be lifted from the container to graft area 0 = unliftable

The graft must be scored 5/5 both at time of release and at the time of grafting.

for sterility and retained replication-competent lentivirus (RCL) and molecular analyses (Fig. 4).

Grafting Procedure

Before grafting, photographs will be taken of the graft area for baseline recording. Adults will receive a graft of $\sim 20 \text{ cm}^2$ (the size of four U.K. postage stamps), and the graft bed location will be selected on the anterolateral aspect of one thigh. Briefly, after photographing, the wound bed for grafting will be marked with black indelible marker and 20 g of Eutectic Mixture of Local Anesthetics (EMLA) cream will be applied to the marked area and 2–3 cm beyond. After at least 2 hr, the wound bed will be prepared under aseptic technique by a DermaBlade and the genetically modified epithelial sheet that will be grafted. The graft will then be secured by gluing the rim of overlapping margins to the underlying skin, and the area dressed with a semioclusive dressing.

Clinical Monitoring

Graft therapy will initially be performed and evaluated in adult patients, and all patients will be monitored for at least 3 years (Fig. 5). If no safety concerns are identified by independent trial medical advisors 12 months after the first patient has been grafted, recruitment will then be opened to children. A total of five patients will be treated.

There will be at least a 1-month interval between patients to ensure that grafting in the previous subject has been successful, without technical or infectious complications.

Laboratory monitoring

The schedule of sample collection, including skin biopsy and blood sampling, is shown in Figure 5. Two 4 mm punch biopsies will be taken at various time points postgraft, one within the grafted area and the other outside the graft but within 2 cm of the perimeter.

Skin samples will be analyzed as follows:

1. Morphological changes by histochemistry
2. Evidence of LEKTI expression using immunostaining and/or immunoblot

3. Vector copy number and, subsequently, vector integration sites
4. Functional correction by measuring the expression of LEKTI-targeting protein KLK5 and other relevant epidermal proteins, including desmoglein 1 and filaggrin

Blood samples will be taken for

1. Serum total IgE levels
2. ELISPOT tests to detect immune responses against the graft/transgene
3. RCL sample storage

Outcome Measures

The endpoint for each subject will be 36 months after grafting of genetically modified skin grafts.

1. Histological evidence of correction of skin architecture and evidence of LEKTI expression will be sought at sites within the gene-modified graft and at single site outside the graft. The histological changes in the gene-corrected graft will be scored on three key features (Table 3). The pretherapy skin score will provide a base line. Increased scores in the gene-corrected skin graft and/or the skin outside the graft will be used as indicators of possible efficacy.
2. Detection of possible cellular immunological responses against LEKTI by ELISPOT
3. Reduction of elevated serum total IgE levels
4. Assessments of trans-epidermal water loss (TEWL)

TEWL through the skin is an indicator of the integrity of the barrier function of the skin. It can be measured in a standardized fashion ($\text{g of water/m}^2/\text{hr}$) by a validated VapoMeter. We will assess TEWL on grafted and nongrafted areas by a VapoMeter (Delfin, Kuopio, Finland). A mean of three readings inside and outside the graft area will be recorded at the time points indicated.

Safety and Adverse Events

Risks from procedures

Skin biopsies will be performed by a 4 mm punch biopsy under local anesthesia for monitoring and a 6 mm punch biopsy for the initial keratinocyte harvest. Subjects will be

FIG. 5. Patient monitoring schedule after preparation and application of gene-engineered skin graft in NS. The uppermost gray bar indicates the time schedule in weeks (w) and months (m), and the ticks (✓) represent the monitoring schedule. Visual inspection, blood pressure, and photographs will be taken before and after grafting and will be measured at all scheduled follow-up visits. During follow-up visits, blood and skin biopsies will be taken for monitoring total immunoglobulin E levels, cellular immune responses against recombinant LEKTI (ELISPOT), skin architecture, and LEKTI expression in the grafts as well as archiving samples for replication-competent lentivirus (RCL) screening. In addition, trans-epidermal water loss will be measured using a VapoMeter device.

Skin biopsy		Sheet graft																	
				-2	0	1	2	1	2	3	6	12	15	18	21	24	30	36	
				m ^a	w ^b	w	w	m	m	m	m	m	m	m	m	m	m	m	
Visual inspection	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Blood pressure	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
IgE		✓						✓				✓				✓		✓	
Punch biopsy	✓							✓		✓	✓	✓				✓		✓	
ELISPOT		✓						✓			✓	✓				✓		✓	
RCL sample		✓						✓		✓	✓	✓				✓		✓	
TEWL ^c	✓									✓	✓	✓				✓		✓	
Photograph	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	

a=month; b=week; c= trans-epidermal water loss

TABLE 3. OUTCOME MEASUREMENTS BY HISTOLOGICAL CHANGES

	Score			Definition
	Gene-corrected skin	Skin outside the graft	Original skin	
LEKTI expression	0–2	0–2	0	0 = absent 1 = reduced Reduced compared with normal skin 2 = normal Similar to normal skin
Epidermis thickness hypergranulosis and psoriasiform	0–2	0–2	0–1	0 = severe Unchanged compared with original skin 1 = mild Improved compared with original skin 2 = normal Similar to normal skin
Cornification integrity of the outermost layer of the skin (cornified layer), parakeratosis, and exfoliated corneocytes	0–2	0–2	0–1	0 = severe Unchanged compared with original skin 1 = mild Improved compared with original skin 2 = normal Similar to normal skin

LEKTI, lymphoepithelial Kazal type-related inhibitor.

advised that the risk of bleeding or infection is expected to be small. Discomfort, pain, or itch may be encountered and will be managed with simple analgesics or antipruritics, and infection may require topical or systemic antibiotic therapy. Patients with known skin colonization may be treated with prophylactic antiseptics and/or antibiotics. We anticipate a small risk of bleeding, which should be manageable with the application of a pressure dressing. There may also be a small risk of scarring at the graft edges and subjects will be advised that there is a possibility that the graft may fail, degrade, or be immunologically rejected. After healing, skin may be scarred or there may be dyspigmentation, whereby some or all of skin color at the graft site may change or become patchy.

Vector-related risks

While trials of gene-modified hematopoietic stem cells have uncovered serious adverse events related to vector insertion (Hacein-Bey-Abina *et al.*, 2003, 2008; Ott *et al.*, 2006; Howe *et al.*, 2008), there have been no reports of such phenomena in skin cells, either with gamma retroviral or lentiviral vectors. In the event of transformation of cells within the gene-modified graft, the graft could potentially be excised. Similarly, generation of RCL is highly unlikely because of the SIN configuration and split plasmid system. Blood and/or skin samples will be stored at time points of 1, 3, 6, and 12 months after grafting and analyzed for RCL if complications arise.

Discussion

This protocol for first-in-man testing of an *ex vivo* gene-modified skin graft for NS has recently been approved in the United Kingdom, and a phase I study in adult patients is now open for recruitment. Consideration has been given to important issues raised during regulatory and ethical review process, as well as feedback from patients with the condition. Clearly, skin grafting with gene-modified epidermal sheets

has the potential for direct correction of a limited surface area, but in the case of NS, it is possible that secreted LEKTI may provide wider benefits, beyond the graft area. In the context of phase I testing, the primary aim of this study is to establish the safety and feasibility of applying lentiviral-modified autologous epidermal sheets, and this study will initially evaluate relatively small (20 cm²) grafts, in a limited number of initial subjects. Nonetheless, we anticipate that these grafts will be of sufficient size to allow serial monitoring, including periodic biopsies and TEWL assessments. Serial comparisons within and outside the graft area should provide valuable information regarding efficacy. Blood monitoring of total IgE levels was recommended at regulator review for tracking of possible systemic effects, and samples will be retained for evaluation of possible T cell-mediated interferon immunological responses against the gene-modified grafts. Patients will also undergo monitoring of blood pressure in case of systemic effects on KLK pathways involved in blood pressure control. In combination, the data will provide valuable information ahead of using larger *ex vivo*-modified grafts or possible *in vivo* local or systemic gene therapies.

The vector configuration used in this study is already in phase I testing for *ex vivo* correction of HSC in inherited immune and metabolic disorders, and to date there have been no vector-related adverse effects. The vector includes a tissue-specific, compartment-specific INVO promoter, in combination with a codon-optimized transgene. Vector stocks were produced under GMP and have been extensively characterized before release.

The production of gene-engineered grafts under GMP conditions requires considerable infrastructure and expertise. Aspects of the manufacturing process requiring specialist media and xenogeneic cells for the generation of epithelial sheets have been adapted from a commercial provider (Altrika) of skin graft products. The company has kindly supplied supplemented media and 3T3 feeder cells from a

characterized master cell bank. The latter have been approved by the MHRA and used in previous studies (Moustafa *et al.*, 2004; Hernon *et al.*, 2006). The cells are irradiated and are thought to carry negligible risk of engraftment in immunocompetent hosts. All bovine or fetal calf serum used during vector production or skin sheet generation is from certified approved sources. In this study, the skin grafts comprise an investigational medicinal product and will be subject to release testing ahead of use. We have designed a bespoke scoring system to confirm the integrity and viability of the graft, and this will be combined with additional laboratory data available for a second gene-modified sheet produced in parallel. There is a notable concern that bacterial skin colonization in NS patients may be problematic, in terms of both contamination of the skin cultures and after the graft procedures. We will exclude patients known to be colonized with antibiotic-resistant pathogens, and antibiotics will be used during *ex vivo* culture. Sterility of the culture supernatant will be confirmed by gram staining before release. Additional material will be subjected to extended culture, and samples archived for RCL testing. The risk of RCL is considered negligible given the detailed characterization of the vector stock, but serial blood and skin samples will be retained.

NS is a rare disorder, of limited commercial interest, but provides an important model as an orphan disorder where emerging gene-based therapies may have a notable impact and experience developed during this study will be applicable across a wider range of inherited skin disorders.

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Author Disclosure Statement

No competing financial interests exist.

References

- Aiuti, A., *et al.* (2013). Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. *Science* 341, 1233151.
- Biffi, A., *et al.* (2013). Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science* 341, 1233158.
- Bitoun, E., *et al.* (2003). LEKTI proteolytic processing in human primary keratinocytes, tissue distribution and defective expression in Netherton syndrome. *Hum. Mol. Genet.* 12, 2417–2430.
- Bonnart, C., *et al.* (2010). Elastase 2 is expressed in human and mouse epidermis and impairs skin barrier function in Netherton syndrome through filaggrin and lipid misprocessing. *J. Clin. Invest.* 120, 871–882.
- Briot, A., *et al.* (2009). Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. *J. Exp. Med.* 206, 1135–1147.
- Cavazza, A., and Mavilio, F. (2012). Gene therapy of skin adhesion disorders (mini review). *Curr. Pharm. Biotechnol.* 13, 1868–1876.
- Cavazzana-Calvo, M., *et al.* (2010). Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature* 467, 318–322.
- Chavanas, S., *et al.* (2000). Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat. Genet.* 25, 141–142.
- Choate, K.A., Medalie, D.A., Morgan, J.R., and Khavari, P.A. (1996). Corrective gene transfer in the human skin disorder lamellar ichthyosis. *Nat. Med.* 2, 1263–1267.
- Deraison, C., *et al.* (2007). LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. *Mol. Biol. Cell* 18, 3607–3619.
- Di, W.L., *et al.* (2011). *Ex-vivo* gene therapy restores LEKTI activity and corrects the architecture of Netherton syndrome-derived skin grafts. *Mol. Ther.* 19, 408–416.
- Di, W.L., *et al.* (2012). Human involucrin promoter mediates repression-resistant and compartment-specific LEKTI expression. *Hum. Gene Ther.* 23, 83–90.
- Egelrud, T., *et al.* (2005). hK5 and hK7, two serine proteinases abundant in human skin, are inhibited by LEKTI domain 6. *Br. J. Dermatol.* 153, 1200–1203.
- Fenjves, E.S., Yao, S.N., Kurachi, K., and Taichman, L.B. (1996). Loss of expression of a retrovirus-transduced gene in human keratinocytes. *J. Invest. Dermatol.* 106, 576–578.
- Gagnoux-Palacios, L., *et al.* (2005). Assessment of optimal transduction of primary human skin keratinocytes by viral vectors. *J. Gene Med.* 7, 1178–1186.
- Gerrard, A.J., Hudson, D.L., Brownlee, G.G., and Watt, F.M. (1993). Towards gene therapy for haemophilia B using primary human keratinocytes. *Nat. Genet.* 3, 180–183.
- Ghazizadeh, S., Doumeng, C., and Taichman, L.B. (2002). Durable and stratum-specific gene expression in epidermis. *Gene Ther.* 9, 1278–1285.
- Hacein-Bey-Abina, S., *et al.* (2003). LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 302, 415–419.
- Hacein-Bey-Abina, S., *et al.* (2008). Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Invest.* 118, 3132–3142.
- Hernon, C.A., *et al.* (2006). Clinical experience using cultured epithelial autografts leads to an alternative methodology for transferring skin cells from the laboratory to the patient. *Regen. Med.* 1, 809–821.
- Howe, S.J., *et al.* (2008). Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J. Clin. Invest.* 118, 3143–3150.
- Jayakumar, A., *et al.* (2004). Expression of LEKTI domains 6–9' in the baculovirus expression system: recombinant LEKTI domains 6–9' inhibit trypsin and subtilisin A. *Protein Expr. Purif.* 35, 93–101.
- Komatsu, N., *et al.* (2002). Elevated stratum corneum hydrolytic activity in Netherton syndrome suggests an inhibitory regulation of desquamation by SPINK5-derived peptides. *J. Invest. Dermatol.* 118, 436–443.
- Kuhn, U., *et al.* (2002). *In vivo* assessment of gene delivery to keratinocytes by lentiviral vectors. *J. Virol.* 76, 1496–1504.

- MacNeil, S. (2007). Progress and opportunities for tissue-engineered skin. *Nature* 445, 874–880.
- Magert, H.J., *et al.* (1999). LEKTI, a novel 15-domain type of human serine proteinase inhibitor. *J. Biol. Chem.* 274, 21499–21502.
- Marchetto, M.C., Correa, R.G., Menck, C.F., and Muotri, A.R. (2006). Functional lentiviral vectors for xeroderma pigmentosum gene therapy. *J. Biotechnol.* 126, 424–430.
- Mavilio, F., *et al.* (2006). Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. *Nat. Med.* 12, 1397–1402.
- Mitsudo, K., *et al.* (2003). Inhibition of serine proteinases plasmin, trypsin, subtilisin A, cathepsin G, and elastase by LEKTI: a kinetic analysis. *Biochemistry* 42, 3874–3881.
- Montini, E., *et al.* (2002). *In vivo* correction of murine tyrosinemia type I by DNA-mediated transposition. *Mol. Ther.* 6, 759–769.
- Montini, E., *et al.* (2006). Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. *Nat. Biotechnol.* 24, 687–696.
- Moustafa, M., *et al.* (2004). A new autologous keratinocyte dressing treatment for non-healing diabetic neuropathic foot ulcers. *Diabet. Med.* 21, 786–789.
- Ott, M.G., *et al.* (2006). Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nat. Med.* 12, 401–409.
- Rheinwald, J.G. (1989). Methods for clonal growth and serial cultivation of normal human epidermal keratinocytes and mesothelial cells. In *A Practical Approach*. R. Baserga, ed. (IRL Press, Oxford), pp. 81–94.
- Sales, K.U., *et al.* (2010). Matriptase initiates activation of epidermal pro-kallikrein and disease onset in a mouse model of Netherton syndrome. *Nat. Genet.* 42, 676–683.
- Uitto, J., Christiano, A.M., McLean, W.H., and McGrath, J.A. (2012). Novel molecular therapies for heritable skin disorders. *J. Invest. Dermatol.* 132, 820–828.
- Watt, F.M. (2002). The stem cell compartment in human inter-follicular epidermis. *J. Dermatol. Sci.* 28, 173–180.

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Supplementary Data

Information Sheet for Young Adults Aged 16 Years and Over (version xxxx, dated xxxx)

Part 1

1.1 Study title

Phase I Study Protocol for *Ex Vivo* Lentiviral Gene Therapy for the Inherited Skin Disease, Netherton Syndrome.

1.2 Invitation paragraph

You are being asked for your consent to participate in a study of skin stem cell gene therapy for Netherton syndrome (NS). Skin cells are also called keratinocytes.

Before you decide whether you wish to participate, you need to understand why the research is being done and what it would involve for you. This study is entirely voluntary. Please take time to read the following information carefully. You will be given the opportunity to discuss the study in more detail afterward.

Part 1 of this information sheet tells you the purpose of this study and what will happen to you if you decide to take part. Part 2 gives you more detailed information about the conduct of the study.

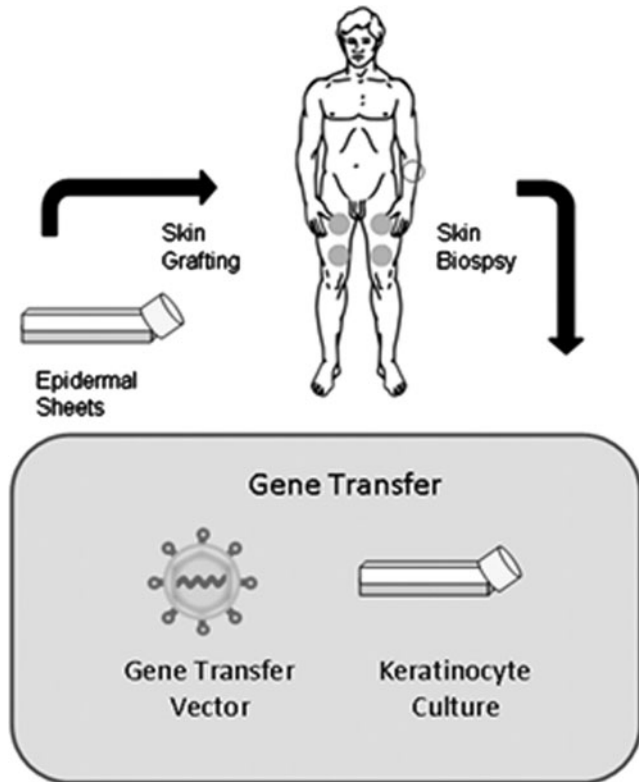
1.3 What is the purpose of the study?

NS is a profound skin disorder caused by damage in a gene called *SPINK5*. This gene controls production of a protein called LEKTI, which is involved in the control of skin barrier formation and function. The absence of this protein leads to defects in the outermost part of the skin known as the epidermis.

At the moment, there are no treatments known to cure NS and we would like to see if gene therapy can improve the condition. In the laboratory, we have used modified viruses to insert a corrected copy of the gene responsible for NS into skin stem cells. We have been able to grow sheets of gene-corrected skin cells taken from patients with NS. Now we would like to see if a small skin graft made in this way can help improve the condition of NS patients. We hope that the corrected skin will make and release the LEKTI protein into the wider body. In the first instance, the trial will consider if the procedure is safe and is set up to look for side effects.

1.4 Why are viruses needed for gene therapy?

Skin cells divide quickly, each time replicating their genes, including any that are not working properly. Gene therapy works by adding an extra, working copy of the defective gene into the DNA of the cell. New technologies have made it possible to use virus particles to carry and permanently insert a corrected gene into the cell. The virus is disabled and cannot divide or cause disease, but is a very useful delivery vehicle.



1.5 Why have I been chosen and do I have to take part?

You are suffering from the genetic disorder NS, and gene therapy could help treat your condition. It is up to you to decide whether or not to take part. If you do decide to participate in the trial, we will ask you to sign a consent form to show you have agreed to take part. You are free to withdraw from the research at any time and without giving a reason. This study is entirely voluntary. If you choose not to do so, your treatment will not be compromised in any way.

1.6 What will happen to me if I agree to take part?

You will be selected for the study on the basis of a diagnosis of NS. If selected, two 5 ml blood samples (around two teaspoons) will be taken and a 6 mm skin biopsy (removal of skin cells) will be performed. The skin biopsy procedure will be performed under local anesthetic. The skin biopsy will be taken to a special gene therapy laboratory at Great Ormond Street Hospital, where the new gene will be introduced into skin cells obtained from the biopsy. The gene-corrected skin cells will then be cultured as a skin sheet. This will take 4–6 weeks.

Once the gene-corrected skin sheet is ready, one 20 cm² area (about the size of four postage stamps) on the top of one of your thighs will be marked and cleaned with a solution. The epidermis (the top layer of the skin) will be removed from the thigh under local anesthetic. We would like to request excess samples to the clinical procedure. The layer underneath will be rinsed with a sterile solution and covered with the new skin sheet. The genetically modified skin sheet will be held in place with a dressing, consisting of one layer of Vaseline gauze, followed by several layers of dry gauze and traditional bandages. The procedure will take approximately 1–2 hr. After 1 week, the dressings will be changed and silicon-gauze bandages will then be used for a further 2 weeks. You will be advised not to expose these areas to detergents, creams, or perfumes for 1 month.

After you have received the graft, we will need to take routine blood samples and small skin biopsies to monitor the gene therapy procedure. For this purpose, up to two 5 ml blood samples (around two teaspoons) and two skin biopsies (4 mm across) will be taken at 1 week (only blood sample) and 1, 3, 6, 12, 24, and 36 months after grafting. We will also be taking photographs of the graft area before the gene therapy procedure and at all subsequent visits. Your blood pressure will also be monitored before the graft and at all follow-up visits, and any changes will be followed up as clinically required.

1.7 What will I have to do if I agree to take part?

If you are happy to take part and are satisfied with the explanations from your research team, you will be asked to sign a consent form. You will be given a copy of this information sheet and signed consent form to keep for your records. It could take several months for you to recover after treatment, although it is likely to be much less. If you are coming from overseas, you will need to be prepared to stay in the United Kingdom during this recovery period. It is very important that you attend all scheduled follow-up visits to the hospital once you have returned home, so that your progress can be closely monitored. If you are coming from overseas, you will need to return to Guy's and St. Thomas' Hospital for these follow-up visits.

1.8 What if I do not wish to take part in the study?

If you do not wish to take part in the study, you will continue to be treated by the medical and nursing staff in the usual way.

1.9 What happens after the procedure is completed?

You will not have to remain in hospital if you are well, but if treatment is required for infections or other health aspects, you will stay on the ward until well enough to go home. This may take up to a few weeks, but is likely to be much less. Because of the nature of this procedure, we will also need to take routine blood samples, blood pressure readings, and skin biopsies at regular intervals at the hospital, even after discharge, to check on progress and to monitor the safety and effectiveness of the gene therapy procedure. These visits will be 1 and 2 weeks and 1, 2, 3, 6, 12, 15, 18, 21, 24, 30, and 36 months after grafting. Afterward, you will be monitored at least annually.

1.10 What are the possible disadvantages and risks of taking part?

1. The virus carrying the new gene has been prepared in a way comparable to all drugs that are administered to humans. This means that it undergoes extensive testing for contamination and safety. The skin cells will be grown and exposed to the virus in a special laboratory using specially provided reagents in a sterile environment. The skin sheets take around 3–4 weeks to grow, and so by the time they are used, there is no virus left. You will not receive any virus particles directly, so there is negligible risk of other cell types being infected.

2. When the virus enters the skin cell, the new gene is spliced (inserted) into the existing chromosomes so that it can be passed on to daughter cells. Because we cannot control where in the DNA the new gene will be placed, we have to warn you that unexpected side effects could occur. For example, it is possible that nearby genes could be affected, and this in turn could lead to uncontrolled skin cell growth and in theory this could lead to the development of skin cancer. This has never been reported in research studies where skin cells have been infected with this type of virus in the lab. However, in the previous trials of genetically modified bone marrow stem cells, serious adverse events have been linked to the use of viral vectors. The virus used in this trial has been designed to minimize the risk of side effects, but we cannot be fully sure until it is tested in people. In the event of uncontrolled skin cell growth within the gene-modified graft, the graft may be surgically removed. The ethics and scientific committees that regulate the conduct of gene therapy studies in the United Kingdom have agreed that research into the treatment of NS by gene therapy can go ahead.

3. There is a small risk of bleeding and infection associated with the skin biopsy. You will have a local anesthetic for the skin biopsy. Any discomfort will be managed by pain-relieving medicines (simple analgesics). A small mark may be left behind similar to a chickenpox scar.

4. A graft bed will be prepared by removing the outer layer of skin (the epidermis) from the thigh using local anaesthesia. Any discomfort or pain will be managed with simple pain killers such as paracetamol. There is a risk of infection that may require antibiotic therapy, especially if the skin is already colonized with bacteria. There is a risk of scarring, especially at the edges of the graft. There is a small risk of bleeding, which will be controlled with the application of a pressure dressing.

5. The graft may not “take” and could fall off. We think this is unlikely, but could happen especially if there is an infection. The area would heal over time and we may give antibiotics. There is a risk of dyspigmentation, whereby some or all of the skin color at the graft site changes or becomes patchy.

1.11 What are the possible benefits of taking part?

If the treatment works, the skin cells with the new gene will produce the missing LEKTI protein and release it into surrounding areas. We hope that even a small patch of gene-corrected skin could provide benefits across the body.

1.12 What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will

be addressed. In the first instance, please contact the chief investigator Dr. Waseem Qasim:

Dr. XXXX
Consultant in Immunology
Molecular Immunology Unit
Institute of Child Health
30 Guilford Street
London WC1N 1EH

☎: XXXX 📠: XXXX 📞: XXXX

You may also contact Guy's & St. Thomas' Complaints Department:

☎: XXXX 📞: XXXX

1.13 What if I have further questions?

The procedures will be discussed with you in detail by the study team listed below. You are encouraged to ask as many questions as you like.

Dr. XXXX
Consultant in Dermatologist
St. John's Institute of Dermatology
St. Thomas' Hospital
London

☎: XXXX 📞: XXXX

Dr. XXXX
Dermatology Specialist Registrar
St. John's Institute of Dermatology
9th Floor, Tower Wing
Guy's Hospital, Great Maze Pond
London, SE1 9RT

☎: XXXX 📞: XXXX

Prof. xxxx
Consultant in Dermatology
Department of Dermatology
Great Ormond Street Hospital
London WC1N 3JH

☎: XXXX 📠: XXXX 📞: XXXX

Prof. xxxx
Consultant in Immunology
Molecular Immunology Unit
30 Guilford Street
London WC1N 1EH

☎: XXXX 📠: XXXX 📞: XXXX

Dr. XXXX
Plastic Surgeon
St. John's Institute of Dermatology
St. Thomas' Hospital
London

The study team above can be contacted during the hours of 9 am–5 pm Monday to Friday. In case you need to speak urgently to a doctor outside of these hours, please telephone Guy's and St. Thomas' Hospital on xxxx and ask for the on-call Dermatology Registrar.

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

2.1 What if relevant new information becomes available?

Should any relevant new information regarding your participation in the project become available, we will discuss it with you.

2.2 What will happen if I do not want to carry on with the research?

You can withdraw from study treatment at any time, up to the point where the gene-modified cells are given back to you. However, you are free to withdraw your consent for the study at anytime.

2.3 What if there is a problem?

In the first instance, you should contact the chief investigator Dr. Waseem Qasim:

Dr. XXXX
Consultant in Immunology
Molecular Immunology Unit
Institute of Child Health
30 Guilford Street
London WC1N 1EH

☎: XXXX 📠: XXXX 📞: XXXX

2.4 What are the arrangements for compensation?

This project has been checked and approved by an independent research ethics committee (Gene Therapy Advisory Committee) who believe that it is likely to be of very little harm to you. However, there are some risks that we cannot predict and we want you to be informed of your rights just in case any harm should occur as a result of taking part in this study.

This research is covered by the National Health Service (NHS) Indemnity (Arrangements for Clinical Negligence Claims in the NHS). If something does go wrong and you are harmed during the research, and if this is caused by someone's negligence, then you may have grounds for a legal action for compensation against Great Ormond Street Hospital as the sponsor and the NHS trust, but you may have to pay your legal costs. The normal NHS complaint mechanisms will still be available to you.

2.5 Will my taking part in this study be kept confidential?

The research team will need to have access to your medical records. In addition, monitors, auditors, and inspectors from the regulatory authorities will need to have access to your medical records. However, all information that is collected about you during the course of the research will be

kept strictly confidential, and any information that leaves the hospital will have your name and address removed, so you cannot be recognized. The data collected for this study will be stored in a computer database, with confidentiality maintained in accordance with local data legislation. Passwords will protect access to electronic databases.

2.6 Involvement of the general practitioner/family doctor

Your general practitioner will be informed of your taking part in this project.

2.7 What will happen to any samples I give?

Cells and DNA will be stored at Great Ormond Street Hospital/Guy's and St. Thomas' Hospital indefinitely in order to preserve samples that can be analyzed in case of future adverse events. The excess sample from the clinical procedure and any other research samples could be used for future ethically approved projects. Should it be required, samples will be disposed of in the appropriate manner according to local hospital procedures. Some samples will be transferred to recognized specialist laboratories outside of the United Kingdom to carry out gene therapy safety tests as part of the research. These samples would not contain your

name or address and confidentiality would be strictly maintained.

2.8 Will any genetic tests be done?

In most patients, the diagnosis of NS will be confirmed by DNA sequencing.

2.9 What will happen to the results of the research study?

The results will be published in scientific journals. All data used will be fully anonymized.

2.10 Who is organizing and funding the research?

Great Ormond Street Hospital for Children NHS Trust is organizing the research. The Moulton Charitable Foundation is funding the project.

2.11 Who has reviewed the study?

This study has been reviewed and approved by an independent research ethics committee (Gene Therapy Advisory Committee) and the Medicines and Healthcare products Regulatory Agency.

Consent Form for Young Adults 16 Years and Over (Version xxxx, dated xxxx)

Title of the Research Project: Phase I Study Protocol for *Ex Vivo* Lentiviral Gene Therapy for the Inherited Skin Disease, Netherton Syndrome

Sponsor Protocol No.: xxxx

EudraCT No.: xxxx

Chief Investigator: Dr. xxxx

Contact Details: ☎: XXXX ☒: XXXX

Principal Investigator: Dr. xxxx

Subject Identification No. for This Trial: _____

Please initial box
to indicate agreement:

1	I confirm that I have read and understood the information sheet dated xxxx (version xxxx) for the above study. I have had the opportunity to consider the information and ask questions, and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3	I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by employees from regulatory authorities or from Great Ormond Street Hospital/Guys' & St. Thomas' Hospital, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
4	I agree to my general practitioner being informed of my participation in the study.	
5	I agree to taking part in the above study.	
6	I agree to my cells and DNA being stored at Great Ormond Street Hospital/Guys' & St. Thomas' Hospital.	
7	I agree to my samples being used for future ethically approved research projects.	

_____ Name of patient	_____ Date	_____ Signature
_____ Name of person taking consent (if different from investigator)	_____ Date	_____ Signature
_____ Investigator	_____ Date	_____ Signature

1 copy for parent/guardian, 1 copy to be retained in patient's medical notes, original to be filed in Investigator Site File