Safety and early efficacy outcomes for lentiviral fibroblast gene therapy in adults with recessive dystrophic epidermolysis bullosa (LENTICOL-F)

Su M. Lwin[1], Farhatullah Syed[2], Wei-Li Di[2], Anastasia Petrova[2], Tendai Kadiyirire[1], Jemima E. Mellerio[1], Alain Hovnanian[3], Waseem Qasim[2], John A. McGrath[1].

[1]St John's Institute of Dermatology, King's College London, London, U.K; [2]UCL Institute of Child Health, London, U.K; [3]INSERM UMR 1163, Imagine Institute, University Paris Descartes, Paris, France.

BACKGROUND

Recessive dystrophic epidermolysis bullosa (RDEB) is a debilitating inherited skin fragility disorder with significant morbidity and premature mortality. RDEB is caused by biallelic loss-of-function mutations in *COL7A1,* resulting in absent, deficient or malformed type VII collagen (C7) and consequently anchoring fibrils (AF) that ensure dermal-epidermal adherence. This leads to sub-lamina densa blisters and tissue cleavage.

Treatment options for RDEB are limited, particularly for correcting the underlying lack of basement membrane C7.[1] We developed a self-inactivating (SIN) lentiviral (LV) platform encoding a codon-optimized *COL7A1* cDNA under the control of a human phosphoglycerate kinase (PGK) promoter[2] for a phase I evaluation and present the first-in-man safety outcomes of intradermal injections of *COL7A1*-modified autologous fibroblasts in 4 adults (aged 30-59 years) with RDEB over 12 months.

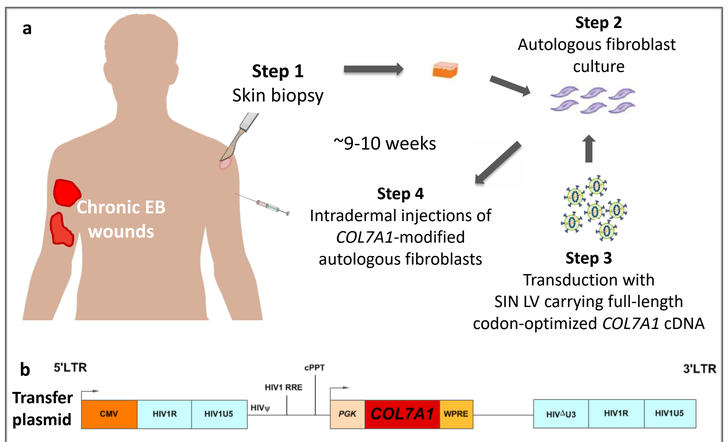

Fig. 1.(a) Illustration of the production of *ex vivo* transduced *COL7A1*-modified autologous fibroblasts for the LENTICOL-F trial. (b) Third generation self-inactivating lentiviral vector carrying full-length *COL7A1* cDNA (SIN LV-*COL7A1*).

OBJECTIVES & ENDPOINTS

Objectives	PRIMARY: SAFETY	SECONDARY: EFFICACY
Endpoints	1. Serious adverse events (SAE) and adverse reactions (AR) 2. Autoimmune reactions against recombinant C7 as measured by ELISA, IIF and ELISPOT from serum	1. C7 expression by IF from skin 2. AF morphology by TEM from skin 3. Vector copy number (VCN) per cell by RT-qPCR from skin

Table 1. Primary and secondary objectives and endpoints of the LENTICOL-F trial. *ELISA: enzyme-linked immunosorbent assay; ELISPOT: enzyme-linked immunosorbent spot assay; IF: immunofluorescence microscopy IIF: indirect immunofluorescence; TEM: transmission electron microscopy.*

TRIAL DESIGN

This single-centre, open-label phase I clinical trial (ClinicalTrials.gov: NCT02493816) was approved by the Medicines and Healthcare products Regulatory Agency and Gene Therapy Advisory Committee in the UK in 2015. Patients were selected from and the trial was conducted at Guy's and St Thomas' Hospitals, London, UK. The *COL7A1*-modified autologous fibroblasts were manufactured at the Good Manufacturing Practice (GMP) facilities at Great Ormond Street Hospital, London, UK.

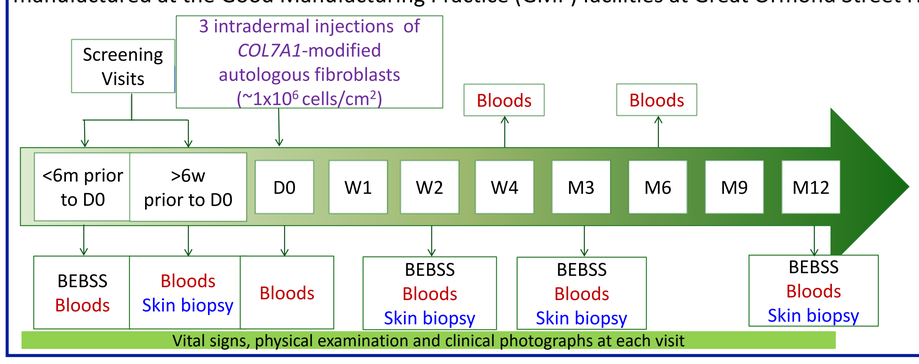

Fig.2. LENTICOL-F trial timeline. Pregnancy test for female subjects of child-bearing age was performed at screening visits and D0. *BEBSS: Birmingham Epidermolysis Bullosa Severity Score; D: Day, W: Week, M: Month.*

SUBJECTS' BASELINE CHARACTERISTICS

Subject No.	1/2	2	3	4
Age (years)	30/31	51	59	30
Sex	Male	Male	Female	Female
Type of RDEB	Generalised intermediate	Pretibial	Inversa	Generalised severe
COL7A1 mutations	+/+ c.8506insC, p.Val2836Argfs*13, exon 115	+/- c.6996C>T, p.Arg2322*, exon 90; +/- c.7344G>A, exon 95	+/- IVS39-1G>T; +/- c.6395G>A, p.Gly2132Asp, exon 78	+/-c.2044C>T, p.Arg682*, exon 15; +/-IVS87+4A>G
C7 IF (NC1)	Trace	Near normal	Near normal	Trace
Anchoring Fibrils (AF)	Rudimentary	Rudimentary	Rudimentary	Rudimentary
BEBSS	27	15	8	54.5
BSA (%)	12	10	0.5	37
History of malignancies	No	No	No	No
C7 ELISA	+	–	+	+
C7 IIF	–	–	–	–
C7 ELISPOT	–	–	–	–
Injection site of intact skin	Left upper arm	Left upper arm	Left upper arm	Left upper arm
Significant co-morbidities	Osteoporosis, pseudosyndactyly	Previous recurrent cellulitis, oesophageal strictures and dilatations, keratitis, corneal erosions, ophthalmic epithelial cysts	Recurrent oesophageal ulceration, strictures and dilatations, haemorrhoids, acute angle glaucoma	Recurrent skin infections, oesophageal strictures and dilatations, gastrostomy, heart failure, chronic liver and kidney disease, recurrent acute kidney injury, haematuria, keratitis

Table 2. Baseline characteristics of 4 adults with RDEB (enrolled as 5 subjects) who received *COL7A1*-modified autologous fibroblasts.

SUMMARY CHARACTERISTICS OF GMP-GRADE *COL7A1*-MODIFIED RDEB AUTOLOGOUS FIBROBLASTS

Subject No.	1	2	3	4
Vector copy number per cell (RT-qPCR)	0.19	0.17	0.56	0.44
Transduction efficiency % C7+ cells (flow cytometry)	3.50	6.56	11.78	9.00
C7 expression (Immunostaining)	Detected	Detected	Detected	Detected
C7 expression (Western blot)	Detected	Detected	Detected	Detected

Table 3. Molecular characteristics of autologous fibroblasts of 4 adults with RDEB transduced with SIN LV-*COL7A1* at multiplicity of infection (MOI)-5.

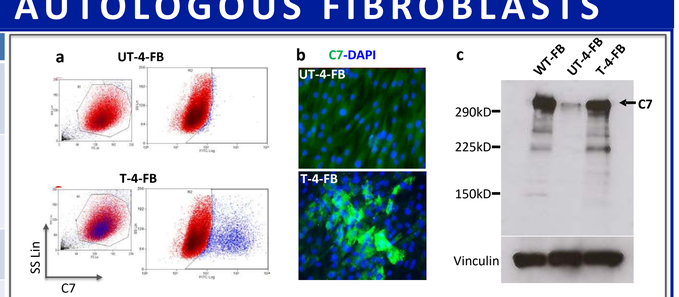

Fig 3. Molecular characteristics of Subject 4's autologous fibroblasts transduced with SIN LV-*COL7A1* at MOI-5 assessed using (a) flow cytometry (b) *in situ* immunostaining for C7 and (c) Western blot. UT: Untransduced; 4-FB: Subject 4's autologous fibroblasts; T: Transduced; WT-FB: Wild-type normal primary fibroblasts.

PRIMARY SAFETY OUTCOMES

- There were no serious adverse reactions.
- Injection site erythema (n=4), injection site bruising (n=1) and injection site pruritus (n=1) were the only adverse reactions (grade 1 severity) related to the administrative procedure.
- No malignancy developed in any of the trial subjects and no significant changes to BEBSS or increase in affected body surface area detected throughout the trial.
- There was no evidence of systemic autoimmune reactions against recombinant C7 post-treatment.

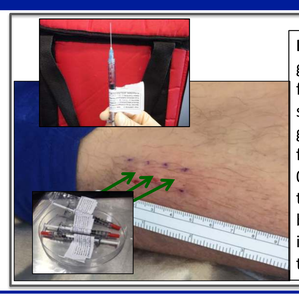

Fig 4. Intradermal injections of gene-modified autologous fibroblasts in Subject 2. Three syringes, each containing ~1x10^6 gene-modified autologous fibroblasts suspended in 0.25ml of 0.9% sodium chloride were transported from the GMP facility to bedside at room temperature and intradermally injected within the tattooed area of the Subject's skin.

SECONDARY EFFICACY OUTCOMES

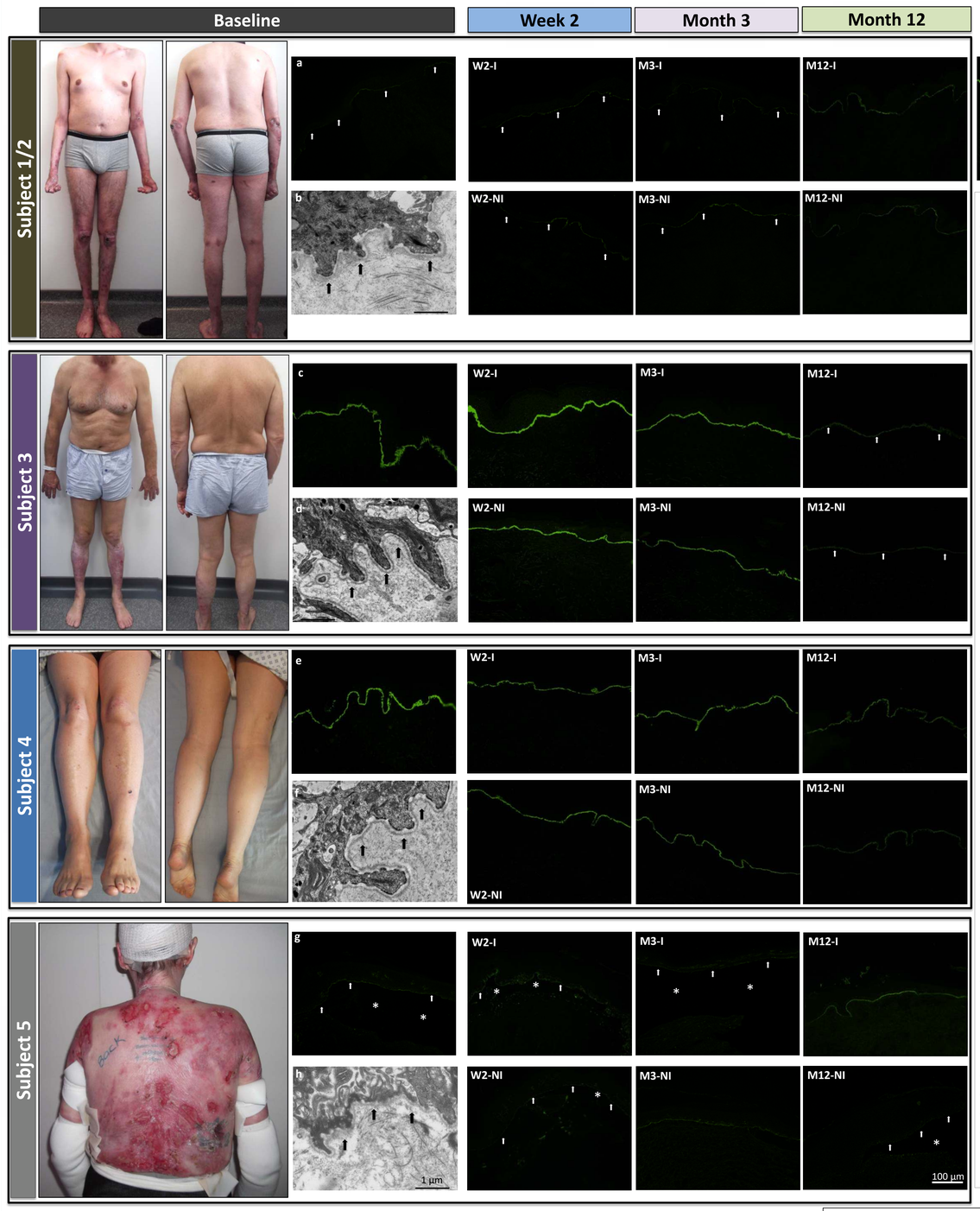

Fig 5. Clinical and molecular features of 4 adults (Subject 1 enrolled twice) treated in the LENTICOL-F Trial.

Baseline IF images (a, c, e, g) showed trace C7 (NC1) on IF staining with LH7.2.

Baseline EM images (b, d, f, h) showed rudimentary (rather than mature fan-shaped) anchoring fibrils along the sub-lamina densa in all 4 adults.

Each IF image at baseline, Week 2, Month 3 and Month 12 correspond to the average mean fluorescence intensity shown in Figure 5 below.

W: Week; M: Month; I: injected skin; NI: non-injected site.

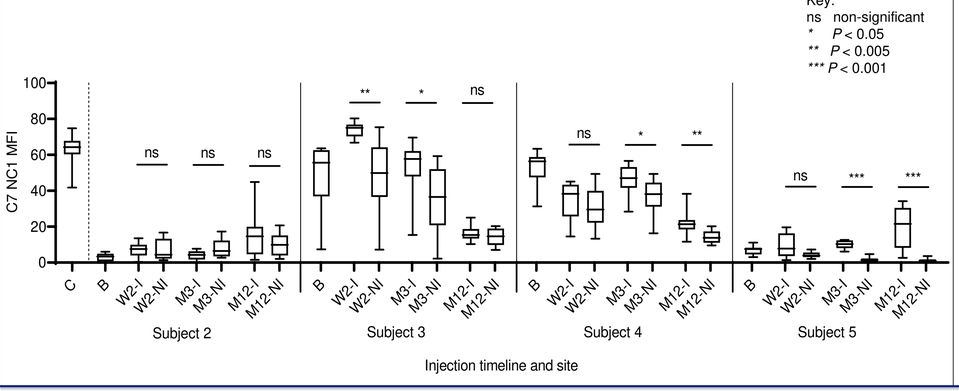

Figure 6. Average MFI of Subjects 2-5 pre- and post-gene therapy over 12 months. MFI was calculated for each sample using Image J (http://rsb.info.nih.gov/ij/)[3]. Briefly, 10 measurements were taken at regular intervals using 8x8pixels every 100 pixel along the dermal-epidermal junction. Each bar corresponds to the images from Figure 5 above. The Box and Whiskers plots showed a significant increase in IF staining in Subject 3 at Week 2 and Month 3, and Subjects 4 and 5 at Months 3 and 12.

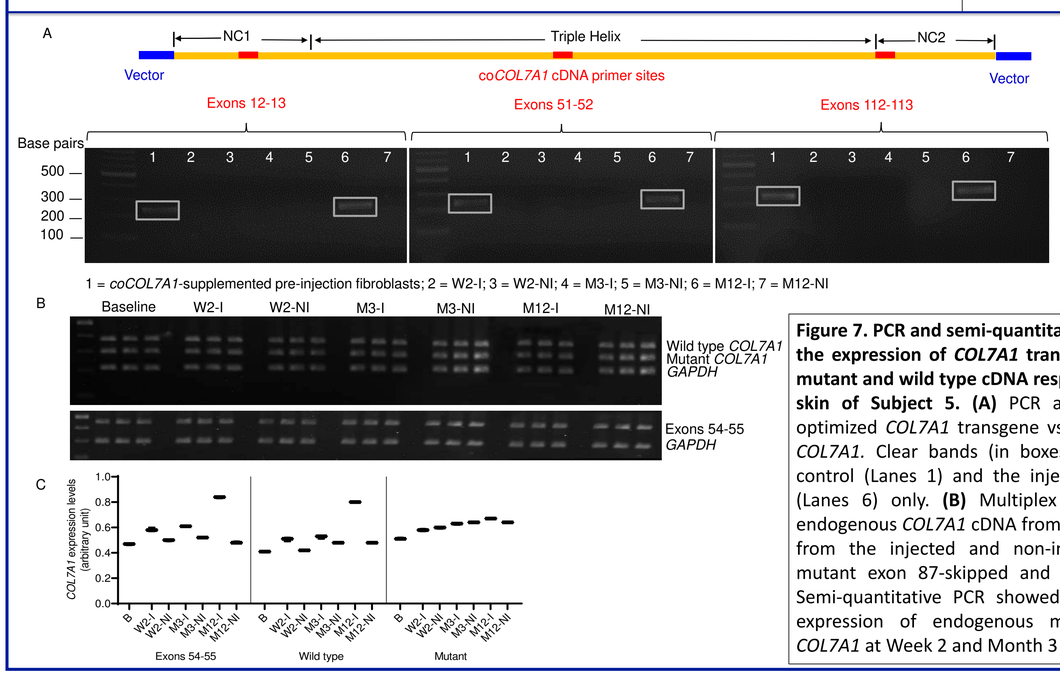

Figure 7. PCR and semi-quantitative PCR demonstrating the expression of *COL7A1* transgene and endogenous mutant and wild type cDNA respectively in the injected skin of Subject 5. (A) PCR amplifications of codon optimized *COL7A1* transgene vs endogenous wild type *COL7A1*. Clear bands (in boxes) are seen in positive control (Lanes 1) and the injected skin at Month 12 (Lanes 6) only. **(B)** Multiplex PCR amplifications of endogenous *COL7A1* cDNA from the total RNA extracted from the injected and non-injected skin to detect mutant exon 87-skipped and wild type *COL7A1*. **(C)** Semi-quantitative PCR showed a relative increase in expression of endogenous mutant and wild type *COL7A1* at Week 2 and Month 3 post-gene therapy.

SUMMARY & CONCLUSION

- In this single-centre, open-label phase I clinical trial, each of 4 subjects with RDEB, aged 30-59 years (2 males and 2 females) with baseline affected body surface areas of ~0.5-37%, received 3 intradermal injections (~1x10^6 cells/cm^2 of intact skin) of *COL7A1*-modified autologous fibroblasts with 0.17-0.56 vector copies/cell and C7 expression on flow analysis of 3.5-11.8%.
- All 4 subjects tolerated the injections without serious adverse reactions or autoimmune reactions against recombinant C7. There were mild adverse reactions related to administrative procedures (n=6) over the 12 months. No one developed malignancy post-injections.
- Regarding efficacy, there was a significant (*P<0.05)* 1.26-fold to 26.10-fold increase in C7 MFI in the injected skin compared to non-injected skin in 3 of 4 subjects with sustained increase up to 12 months in 2 of 4 subjects. The presence of transgene was demonstrated in the injected skin at Month 12 in one subject but no new mature AFs were detected.
- The safety and early efficacy data generated provide a rationale for phase II controlled dose escalation studies to further evaluate this form of *ex vivo* gene therapy in adults and children with RDEB.

REFERENCES

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3. Wong T *et al.* J Invest Dermatol 2008; 128:2179-89.

ACKNOWLEDGEMENT

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