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Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells

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The continuous renewal of human epidermis is sustained by stem cells contained in the epidermal basal layer and in hair follicles[1,2]. Cultured keratinocyte stem cells, known as holoclones[3–6], generate sheets of epithelium used to restore severe skin, mucosal and corneal defects[7–9]. Mutations in genes encoding the basement membrane component laminin 5 (LAM5) cause junctional epidermolysis bullosa (JEB), a devastating and often fatal skin adhesion disorder[10]. Epidermal stem cells from an adult patient affected by LAM5-β3–deficient JEB were transduced with a retroviral vector expressing *LAMB3* cDNA (encoding LAM5-β3), and used to prepare genetically corrected cultured epidermal grafts. Nine grafts were transplanted onto surgically prepared regions of the patient's legs. Engraftment was complete after 8 d. Synthesis and proper assembly of normal levels of functional LAM5 were observed, together with the development of a firmly adherent epidermis that remained stable for the duration of the follow-up (1 year) in the absence of blisters, infections, inflammation or immune response. Retroviral integration site analysis indicated that the regenerated epidermis is maintained by a defined repertoire of transduced stem cells. These data show that *ex vivo* gene therapy of JEB is feasible and leads to full functional correction of the disease.

The patient enrolled in this phase I/II clinical trial was a 36-year-old male (referred to as KEP25) affected by nonlethal JEB. He was a double-heterozygous carrier of a null allele and a single point mutation (E210K) in the *LAMB3* gene (encoding LAM5-β3), impairing the normal assembly of LAM5 (ref. 11). Since his birth, he had suffered from blistering of the skin that occurred either spontaneously or after minimal injury and culminated in infected lesions (**Fig. 1a**). Most of his body was covered by large, hard-to-heal blisters or infected crusts, with few moderately affected areas (**Fig. 1a**,**b**). To select a donor site suitable for epidermal stem cell correction, we took skin biopsies from different areas of his body for clonal analyses of keratinocytes. We were unable to obtain clonogenic and holoclone-forming cells from most of the patient's skin (**Fig. 1b**), most probably because of the continuous proliferative stimulus associated with the wound-healing process. Only his palms contained a sufficient number of holoclones.

Primary KEP25 keratinocytes, obtained from two palm biopsies (1.5 cm^2), were transduced by a retroviral vector expressing the full-length *LAMB3* cDNA under the control of the Moloney leukemia virus (MLV) long terminal repeat (LTR) (**Fig. 2a**). Clonogenic cells were transduced at virtually 100% efficiency, as indicated by immunofluorescence analysis of cytoplasmic LAM5-β3 (**Fig. 2b**). Southern blot analysis of genomic DNA indicated the presence of an average of two intact vector copies per genome, with no sign of rearranged proviruses (**Fig. 2c**). Northern analysis of total RNA showed abundant accumulation of a single vector-derived mRNA of the expected size (**Fig. 2d**). A LAM5-β3–specific antibody immunoprecipitated (from total lysates of transduced keratinocytes) an amount of heterotrimeric LAM5 virtually indistinguishable from that of normal keratinocytes (**Fig. 2e**). Untransduced KEP25 palm keratinocytes contained barely detectable amounts of LAM5-β3 protein and *LAMB3* mRNA (**Fig. 2b**,**d**,**e**). Transgene expression persisted at constant levels throughout the lifespan of the culture (>120 cell doublings, data not shown).

The anterior upper regions of the patient's legs, which were covered by a very fragile epidermis and contained several infected nonhealing lesions, were selected for transplantation. We removed LAM5-β3–deficient epidermal remnants by Timedsurgery[12] under local anesthesia (**Supplementary Fig. 1** online). We transplanted four and five genetically modified grafts, each 55 cm^2 (a total of ~500 cm^2), on the right and left legs, respectively. We observed complete epidermal regeneration on both legs at day 8, and a normal-looking epidermis was maintained throughout the 1-year follow-up (**Fig. 3a**,

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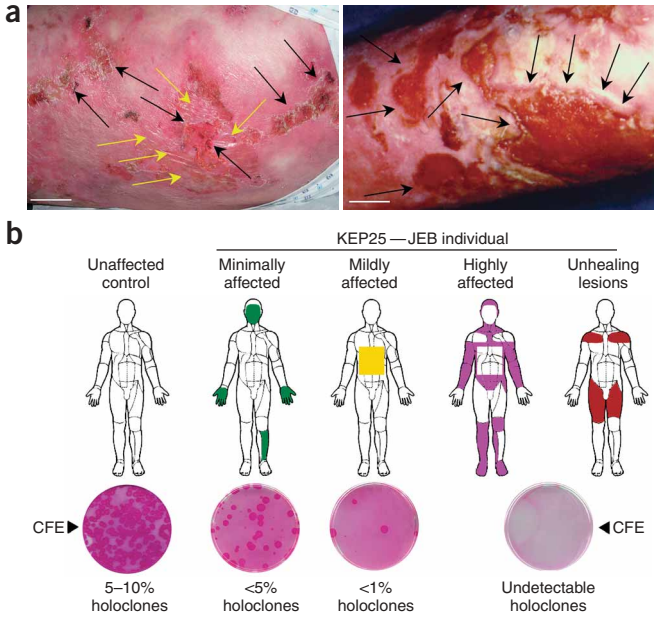

Figure 1 Analysis of the skin of patient KEP25. (**a**) LAM5-β3 deficiency causes continuous skin blistering (left, yellow arrows) that culminate in crusts (left, black arrows) or infected unhealing lesions (right, black arrows). Scale bar, 3.5 cm. (**b**) Keratinocytes were cultured from punch biopsies taken from different areas of the patient's body. Colony-forming efficiency (CFE) was evaluated as described in the **Supplementary Methods**. A progressive decrease of clonogenic keratinocytes and stem cells (holoclones) was detected in minimally (green) and mildly (yellow) affected body sites compared to those in a normal control. Colony-forming cells were undetectable in highly affected (pink) or unhealing (red) areas.

Supplementary Fig. 1). The regenerated epidermis remained robust, did not itch and did not form blisters, even after induced mechanical stress (**Supplementary Fig. 1**), whereas large spontaneous blisters were observed around the transplanted area. The clinical picture remained stable for the entire follow-up, during which the epidermis underwent at least 12 complete renewing cycles. We extracted DNA from a fraction of the right and left leg biopsies at 1 and 4 months, and conducted a semi-quantitative PCR analysis. Vector-specific bands of the expected size were amplified from all biopsies (**Fig. 3b**). Considering that the epidermal layer accounts for only a fraction of the cells present in the (full-thickness) biopsies, the intensity of the PCR signal was compatible with full transduction of the transplanted epidermis. Indeed, *in situ* hybridization, performed on multiple sections of biopsies taken at 4 months, showed that vector-derived LAM5-β3 transcripts were present in virtually all cells of the regenerated epidermis (**Fig. 3c** and **Supplementary Fig. 1**).

On histological analysis, we observed a normal and fully differentiated epidermis, with well-developed stratum spinosum and stratum granulosum and a normal dermal-epidermal junction, on both legs in biopsies taken at 1, 4, 6 and 12 months (**Fig. 3c**, **Supplementary Fig. 1**). The thickness of the stratum corneum, and the synthesis of palm-specific keratin 9 (data not shown), reflected the histological origin of the grafted keratinocytes.

Immunofluorescence analysis showed that the regenerated epidermis contained an amount of LAM5-β3 comparable to that in a normal control, properly located at the epidermal-dermal junction (**Fig. 3d**,**e** and **Supplementary Fig. 1**). The basal lamina also stained positively for LAM5-γ2 and α6β4 integrin, which are usually decreased in the absence of LAM5, indicating that transduced keratinocytes express sufficient β3 chain to assemble an appropriate amount of LAM5 α3β3γ2 heterotrimer at the correct location (**Fig. 3e**, **Supplementary Fig. 1**). Finally, the ΔNp63α transcription factor[13,14], the expression of which has been associated with basal keratinocytes endowed with

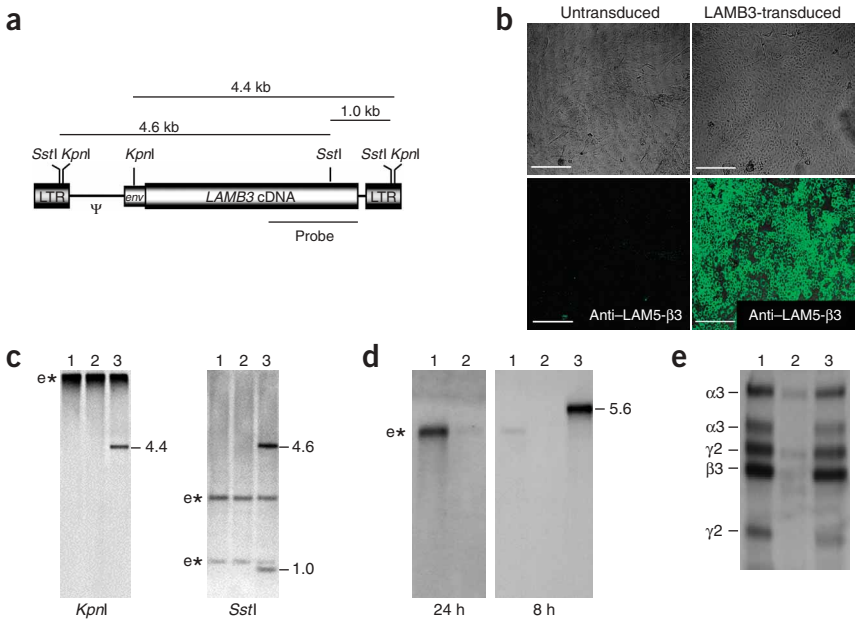

Figure 2 Correction of LAM5-β3 deficiency in epidermal cells of KEP25. (**a**) Schematic map of the MFG-LAMB3 retroviral vector. LTR: long terminal repeats; *env*: leader sequence of the MLV envelope gene; ψ: packaging signal. The LAMB3 fragment used as probe for Southern and northern analysis, and size and position of the bands detected by the probe after restriction with *Kpn*I (4.4 kb) and *Sst*I (1.0 and 4.6 kb) are indicated. (**b**) Immunofluorescence analysis of LAM5-β3 expression (in green, lower panels) in keratinocyte cultures from KEP25 before (left) and after (right) transduction with MFG-LAMB3. Scale bar, 100 μm. (**c**) Southern blot analysis of DNA from unaffected (1) and KEP25-cultured keratinocytes before (2) and after (3) transduction, after digestion with *Kpn*I or *Sst*I and hybridization to the LAMB3-specific probe. The presence of bands of the expected size indicates absence of rearranged proviruses. e*: endogenous LAMB3 fragments. (**d**) Northern blot analysis of RNA from unaffected (1) and KEP25-cultured keratinocytes before (2) and after (3) transduction with MFG-LAMB3. Blots were exposed for 24 h (left panels) or 8 h (right panels). The 5.4-kb vector-specific transcript is indicated. e*: endogenous *LAMB3* mRNA. Vector-derived transcripts were overexpressed approximately fivefold compared to controls, as estimated by Phosphorimager scanning after normalization for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. (**e**) Immunoprecipitation of ^{35}S-labeled LAM5 heterotrimers from culture media of unaffected (1) and KEP25-cultured keratinocytes before (2) and after (3) transduction with MFG-LAMB3, indicating complete reconstitution of LAM5 synthesis in transduced cells.

long-term proliferative potential *in vivo*[15] and with holoclones *in vitro*[16,17], was expressed at similar levels and in a comparable number of cells in the control and transgenic epidermis (**Fig. 3e**, red arrows).

The patient's compound LAMB3 mutations allowed residual synthesis (<5% of normal levels, **Fig. 2b**,**e** and **Fig. 3d**) of a LAM5-β3 carrying a single amino acid substitution (E210K)[11]. We analyzed the binding of all possible 9-mer peptides encompassing the substitution to the patient's class-I HLA molecules (*HLA-A*01/*0301*, *HLA-B*3503/*3801*, *HLA-Cw*0401/*1203*) using a reverse immunology approach based on a peptide-binding prediction algorithm[18], and found a very low potential binding score (<2). Similarly, no potential class II–restricted response was predicted[19] for the patient's class II phenotype (*DRB1*1101/*1301*, *DQB1*0301/*0603*). We searched for antibodies to the vector-derived LAM5-β3 in the patient's serum, using western blot analysis (**Fig. 4a**,**b**), immunoprecipitation and immunohistochemistry (data not shown), and obtained negative results. To test for a class I–restricted cytotoxic immune response, peripheral blood mononuclear cells obtained 1 to 7 months after transplantation were stimulated with autologous, irradiated T cells transduced with the MFG-LAMB3 retroviral vector or, as a control of the patient's immune competence, with allogeneic T cells. We detected neither a cytotoxic nor a IFN-γ response to an autologous, LAMB3-transduced lymphoblastoid cell line target in any of the tested samples in the presence of a normal response to allogeneic targets (**Fig. 4c**,**d**). We obtained no evidence of inflammatory cell infiltration in the skin biopsies throughout the follow-up.

As part of the safety assessment of the clinical trial, we carried out a genome-wide analysis of the retroviral integration sites on DNA extracted from the regenerated epidermis. Libraries of vector-genome junctions were generated by linker-mediated (LM) nested PCR, and sequenced to saturation. By this approach, 36 and 26 independent integrations were unambiguously mapped on human chromosomes from 1- and 4-month biopsies, respectively. Three integrations were found at both time points (a detailed list of integrations is in **Supplementary Table 1** online). Considering an average of two proviral copies per genome (**Fig. 2c**) and an overall cloning efficiency of <50%, we estimate the presence of at least 30 independently transduced stem cells in 0.1 cm^2 of regenerated epidermis. This figure

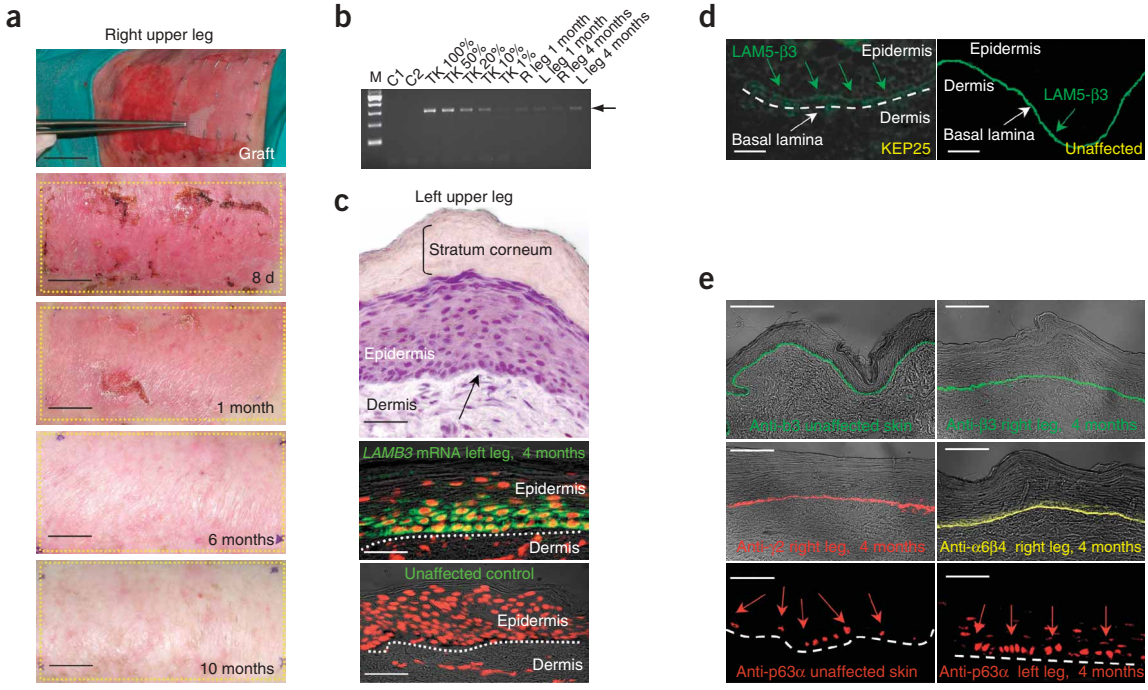

Figure 3 Regeneration of a genetically corrected, functional epidermis. (**a**) Genetically modified cultured epidermal sheets were transplanted on the prepared wound bed of the right upper leg (graft). Follow-up examinations were carried out at 8 d and 1–12 months after transplantation (see also **Supplementary Fig. 1**). Punch biopsies (each of diameter 0.4 cm) were taken 1, 4, 6 and 12 months after transplantation. Scale bars, 3.5 cm. (**b**) Semi-quantitative PCR analysis with two vector-specific primers on DNA extracted from right and left leg biopsies at 1 and 4 months. DNA from transduced keratinocyte (TK) cultures, pure or diluted in a proportion of 50% to 1% with DNA from untransduced cells, was used as a positive control. M: molecular weight marker; C1: reaction mixture; C2: DNA from untransduced keratinocytes. The arrow indicates an amplified band of the expected 315-bp size. (**c**) Hematoxylin/eosin staining of 5-μm-thick skin sections from a left leg biopsy at 4 months shows a fully differentiated epidermis adhering to the underlying dermis (upper panel). The thickness of the stratum corneum (bracket) is typical of palm epidermis. *In situ* hybridization with a vector-specific probe on 5-μm-thick skin sections from the same left leg biopsy shows the presence of LAM-β3 transcripts in all epidermal layers (middle panel), which is consistent with the engraftment of the transduced epidermal cells. The white dotted line indicates the basal lamina. Sections from unaffected skin were used as a control (lower panel). Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI; red). Similar results were obtained at 1 and 6 months. Scale bars, 50 μm. (**d**) Immunofluorescence analysis of LAM5-β3 expression (green arrows) in skin sections from KEP25 (left) and an unaffected control (right). The white dotted line in the left panel indicates the basal lamina. Scale bars, 100 μm. (**e**) Immunofluorescence analysis of skin sections from biopsies taken at 4 months. The expression of LAM5-β3 (in green) observed on the right leg (upper right) was virtually indistinguishable from that of a healthy individual (upper left). Similar results were obtained at 6 and 12 months. The transgenic epidermis showed normal synthesis of LAM5-γ2 (middle left) and α6β4 integrins (middle right), which are usually decreased in the absence of LAM5. The ΔNp63α transcription factor, whose expression is related to the epidermal renewal capacity, was expressed with a comparable pattern in the basal layer of normal (lower left) and transgenic (lower right) epidermis (red arrows). Scale bar: 100 μm in upper left panel; 50 μm in the other panels.

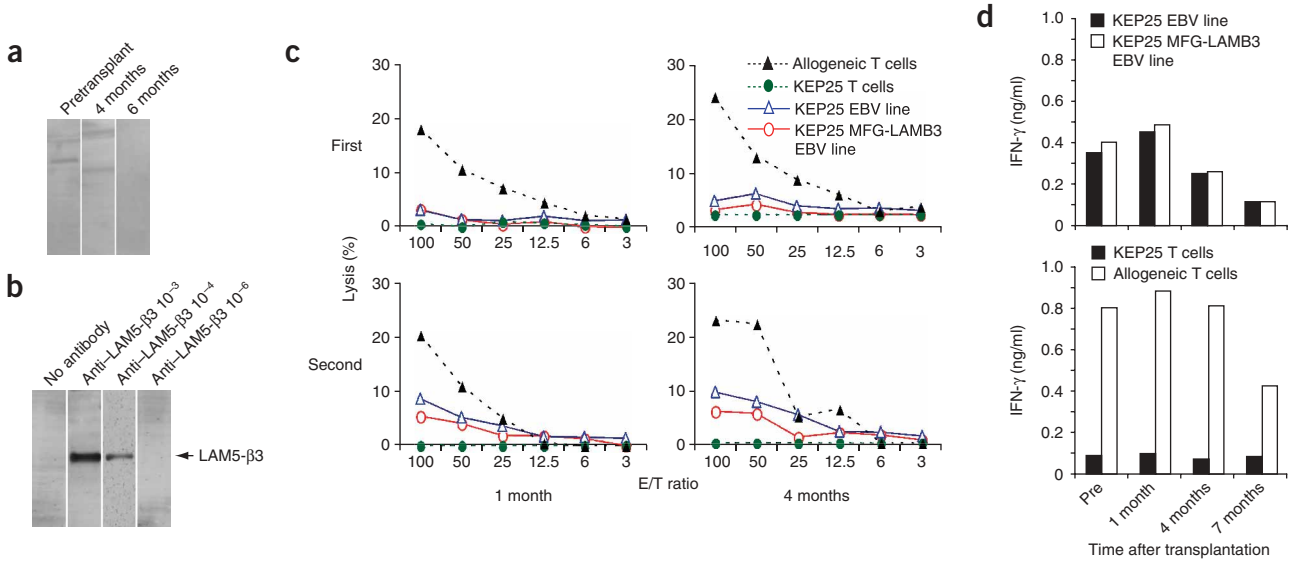

Figure 4 Absence of humoral or cytotoxic immune response to LAM5-β3. (**a**,**b**) Western blot analysis of the presence of antibodies to LAM5-β3 (anti–LAM5-β3) in the patient's serum. (**a**) Normal keratinocyte protein extracts were run on SDS-PAGE gels, blotted onto nylon membranes, and incubated with undiluted serum obtained from KEP25 before or 4 and 6 months after transplantation, followed by a sheep antiserum to human IgG. (**b**) As a positive control, the blots were incubated with a mouse antiserum to LAM5-β3, diluted 1:1,000 to 1:1,000,000 in the patient's serum, followed by a goat antiserum to mouse IgG. (**c**) Cytotoxic T-cell assay. Peripheral blood mononuclear cells (PBMCs) obtained 1 month (left panels) and 4 months (right panels) after transplantation were subjected to two rounds of stimulation with irradiated autologous T cells transduced with the MFG-LAMB3 retroviral vector, or with irradiated allogeneic T cells. Autologous T cells were transduced at 20–30% efficiency, as evaluated by quantitative PCR. Expression of vector-derived *LAMB3* mRNA expression was checked by RT-PCR. Lytic activities of the effectors were evaluated 10 d after the first (upper panels) or second (lower panels) stimulation at increasing effector/target (E/T) ratios. Effectors stimulated with transduced autologous T cells (full lines) were challenged with an autologous, Epstein-Barr virus (EBV)-immortalized cell line transduced with the *LAMB3* retroviral vector (diamonds). The untransduced EBV line (circles) was used as a negative control. Effectors stimulated with allogeneic T cells (dotted lines) were challenged with the same T cells (crosses), and with autologous T cells (squares). (**d**) γ-interferon (IFN-γ) release assay. PBMCs obtained before or 1, 4 and 7 months after transplantation were subjected to three rounds of stimulation with irradiated, autologous, MFG-*LAMB3*–transduced T cells or with irradiated, allogeneic T cells. Effectors stimulated with transduced autologous T cells (left panel) were challenged with an autologous, MFG-*LAMB3*–transduced EBV cell line (white columns) or an untransduced EBV line (black columns) as a negative control. Effectors stimulated with allogeneic T cells (right panel) were challenged with the same T cells (white columns) and with autologous T cells (black columns). After 24 h, supernatants were harvested and IFN-γ quantified by ELISA.

is not far from the estimated stem cell content of a normal epidermis[3,20] and is compatible with the presence of an almost normal repertoire of genetically corrected epidermal stem cells in the regenerated skin.

Overall, 17 integrations (29%) were classified as intergenic, 21 (36%) were within the transcribed portion of 22 RefSeq genes, and 20 (34%) were at a distance of ≤30 kb upstream or downstream of 30 genes (**Supplementary Tables 1** and **2** online). Of the hit genes, 63% were expressed in keratinocytes cultured under the same conditions as used for transduction (**Supplementary Table 1**). Over 30% of the integrations occurred ≤10 kb upstream or downstream from transcription start sites, confirming the known integration preferences of gamma-retroviral vectors in human cells[21].

This study shows that genetically corrected epidermal stem cells regenerate a functional, self-renewing epidermis *in vivo*. Because Timedsurgery is moderately invasive and can be performed under local anesthesia[12], the replacement of large areas of JEB epidermis by *ex vivo* gene therapy appears feasible, and we are planning a systematic, stepwise replacement of the epidermis of patient KEP25 within the next 2–3 years.

Although epidermal cultures have been used for more than 20 years in the clinics[20,22], a formal proof of the engraftment of autologous cultured stem cells has been difficult to obtain. Using integrated proviruses as a marker of cell clonality, we have shown that a defined number of individual stem cells maintain their characteristics upon cultivation and transplantation, and sustain prolonged epidermal renewal *in vivo*, whereas most of the transit amplifying progenitors are lost within 1 month, owing to the normal epidermal turnover. This aspect is of considerable clinical relevance, as autologous graft failure in burn patients usually occurs within 1 month but almost never after 2 or more months. Our data indicate that early graft loss probably occurs when clonogenic stem cells (holoclones) are not maintained in the cultured epidermis and that only self-renewing stem cells sustain long-term skin regeneration[22].

A critical goal of this trial was to evaluate the safety of the clinical use of epidermal stem cells transduced by MLV-derived retroviral vectors, which have recently raised substantial safety concerns over the genotoxic risk associated with their uncontrolled insertion into the human genome. Insertional activation of a T-cell proto-oncogene has, in fact, been correlated with the occurrence of lymphoproliferative disorders in patients treated by gene therapy for X-linked severe combined immunodeficiency (X-SCID)[23]. However, no such serious adverse event has been reported in other clinical trials for X-SCID (ref. 24), adenosine deaminase–deficient SCID (ref. 25) or graft-versus-host disease[26], and retroviral insertion causes only a benign clonal amplification of hematopoietic progenitors in the context of chronic granulomatous disease[27]. Here we analyzed proviral integrations in skin biopsies obtained at two different time points during the patient's follow-up, and, although it is still too early to draw definitive conclusions, we obtained no evidence of clonal expansion or selection

of specific integration events *in vivo*. Likewise, we never observed clonal selection related to specific proviral integration *in vitro*, in many serially passaged cultures of normal or JEB-transduced keratinocytes (**Supplementary Fig. 2** online; and F.M., G.P. and M.D.L., unpublished observations). Finally, the recurrence of integration events in different areas of the transplanted skin and at different time points suggests that the number of independent genomic hits introduced into the patient with the graft is finite, potentially contributing to a lower risk of oncogene activation compared to a hematopoietic cell transplant.

The different forms of epidermolysis bullosa affect approximately 500,000 people worldwide (http://www.debra.org). The successful outcome of this first clinical trial would pave the road to gene therapy of other types of epidermolysis bullosa, as well as of other genodermatoses.

METHODS

Details are given in **Supplementary Methods** online.

Cell culture, retroviral vector and packaging cell line. 3T3-J2 cells and Am12-LAMB3 amphotropic packaging cells were grown as described[28,29]. Primary unaffected and JEB keratinocytes were cultivated on a feeder layer of lethally irradiated 3T3-J2 cells as described[28]. Clonogenic assays and clonal analysis were performed as described[3,5,29].

A retroviral vector expressing the 3.6-kb full-length laminin 5 *LAMB3* cDNA under the control of the MLV LTR was constructed in the MFG backbone[30] and integrated by transinfection in the amphotropic Gp+*env*Am12 packaging cell line[29]. A master cell bank of a high-titer packaging clone (Am12-LAMB3 2/8) was made under GMP/GLP standards by a qualified contractor (Molmed S.p.A).

Generation of genetically corrected epidermal sheets and surgical procedure. The phase I/II clinical trial was authorized by the Italian Ministry of Health and approved by the Ethical Review Board of the University of Modena in June 2005. A JEB patient, referred to as KEP25, was enrolled under informed consent. Keratinocytes from KEP25 were obtained from two full-thickness skin biopsies (1.5 cm^2) taken from the right and left palms, and cultivated as described[28]. Subconfluent primary cells were plated (6×10^3 cells/cm^2) onto a feeder layer (8×10^4 cells/cm^2) of lethally irradiated 3T3-J2 and Am12/LAMB3 2/8 cells in a 1:2 ratio, as described[28,29]. After 3 d, cells were transferred onto a 3T3-J2 feeder layer in nine 75-cm^2 flasks and grown to confluence. Grafts were detached with Dispase II (2.5 mg/ml) and prepared as described[12].

The recipient areas were cleaned with povidone-iodine solution and rinsed with a sterile saline solution. The patient's LAM5-deficient epidermis was removed under local anesthesia by programmed diathermosurgery (Timedsurgery, Korpo) as described[12]. Punch biopsies (0.4 cm) were taken 1, 4 and 6 months after transplantation.

Immunofluorescence, imunoprecipitation and western blot analysis. These assays were performed using the K140 monoclonal antibody to LAM5-β3 (anti–LAM5-β3), the sc-7651 antiserum to LAM5-β3 (SantaCruz Biotechnology), the D4B5 monoclonal antibody to LAM5-γ2 (anti–LAM5-γ2; Chemicon), the CD104 FITC-conjugated monoclonal antibody to β4 integrin (anti–β4 integrin; Serotec), and a rabbit antibody to p63α (ref. 17). Immunofluorescence analysis was performed on paraformaldehyde-fixed skin samples and keratinocyte colonies as described[17,28]. Confocal analyses were done with the LSM510 Confocal Analyzer (Zeiss). Immunoprecipitation was performed on subconfluent cells as described[28]. Western analysis was performed as described[16].

Cytotoxic T-cell assay. Cytotoxic T-cell assays were performed with peripheral blood mononuclear cells (PBMCs) obtained 1 and 4 months after transplantation, stimulated with autologous T cells transduced with the MFG-LAMB3 retroviral vector or with allogeneic T cells. Cytolytic activity was evaluated 10 d after the first and second stimulations by [^{51}Cr] release assay. IFN-γ secretion was evaluated 10 d after the third stimulation by a 24-h ELISA. In both assays, effectors stimulated with autologous T cells were challenged with MFG-LAMB3–transduced, autologous EBV cell lines at different effector/target (E/T) ratios.

In situ hybridization. Digoxigenin-labeled cRNAs were synthesized according to the manufacturer's instructions (DIG RNA Labeling Kit, Roche) and hybridization performed as previously described[17]. Primer pairs with Sp6/T7 promoter sequences (MWG Biotech) were used to obtain DNA templates for *in vitro* transcription. The following vector-specific primers were used: 5′-Sp6-AGTAACGCCATTTTGCAAGG-3′ (melting point (T_m) 60°C) and 5′-T7-AACAGAAGCGAGAAGCGAAC-3′ (T_m 58°C). Sense riboprobes were used as negative controls.

Southern, northern and PCR analyses. DNA was extracted from 1–5 × 10^6 cells, digested (10 μg) with different restriction enzymes, run on a 0.8% agarose gel, transferred to a nylon membrane (Duralon, Stratagene), and hybridized to a [^{32}P]-labeled LAM β3–specific probe. For PCR analysis, genomic DNA was amplified with the vector-specific primers 5′-TCGTACTCTATAGGCTTCAGC-3′ and 5′-AGACATGGAGTTGGAGCTGC-3′, and resolved on 1.3% agarose gels. Total RNA was extracted by the guanidine-HCl method, run on 0.8% agarose-formaldehyde gels, transferred by northern capillary blotting onto nylon membranes and hybridized to a LAM β3–specific probe.

Analysis of retroviral integration sites. Integration sites were cloned by LM-PCR, as described[26]. Briefly, genomic DNA was digested with *Mse*I and *Pst*I, and ligated to an *Mse*I double-strand linker. LM-PCR was performed with nested primers specific for the LTR and the linker. PCR products were shotgun-cloned by the TOPO TA cloning kit (Invitrogen) into libraries of integration junctions, which were sequenced to saturation. Sequences were mapped onto the human genome by the BLAT genome browser (UCSC Human Genome Project Working Draft, May 2004; http://www.genome.ucsc.edu). The gene expression profile of cultured keratinocytes was determined by Affymetrix microarray analysis of RNA isolated from 1–2 × 10^6 keratinocytes from KEP25 cultured under the same conditions as used for transduction, as previously described[26].

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

F.M., G.P. and M.D.L. designed and directed the study. G.P carried out the clonal analysis, transduced the patient's cells and prepared the skin implants. S.F, F.D.N., E.D.I. and G.M. carried out the hystological and molecular follow-up, G.F. and F.M. constructed the retroviral vectors and the packaging cell line, E.P. and C.B. performed the immunological analysis, S.C., A.C., C.M. and A.G. carried out the transplantation and were responsible for the clinical management of the patient.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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