Gene Therapy for junctional epidermolysis bullosa (JEB)

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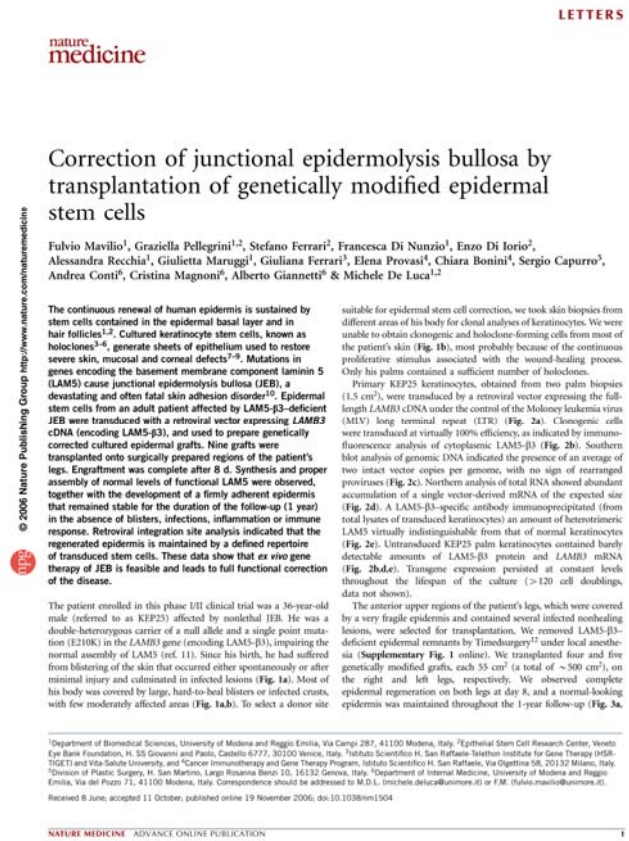
Regenerative medicine refers to innovative therapies aimed at the permanent restoration of diseased tissues and organs. Regeneration of self-renewing tissues requires specific adult stem cells, which are, in this case, epithelial stem cells genetically corrected for sane epidermal regeneration.
Centre for Regenerative Medicine, University of Modena and Reggio Emilia, has developed a gene therapy protocol for advanced therapy of junctional epidermolysis bullosa (JEB).



LETTERS

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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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1. Description of the product

Deficiency of the basement membrane component laminin-5 (LAM5) causes junctional epidermolysis bullosa (JEB), a severe and often fatal skin adhesion defect.
Autologous transplantation of epidermal stem cells isolated, genetically corrected and produced by the Centre for Regenerative Medicine, University of Modena and Reggio Emilia, may be the advanced and permanent therapy for JEB patients.
This protocol is propedeutic to the implementation of new clinical trials of gene therapies against other epithelial genetic diseases.

2. Innovative aspects of the product

Transcriptionally targeted lentiviral vectors efficiently transduced clonogenic stem/progenitor cells derived from a skin biopsy of a JEB patient, restored normal synthesis of LAM5 in cultured keratinocytes, and reconstituted normal adhesion properties in human skin equivalents transplanted onto immunodeficient mice. These vectors are therefore an effective, and potentially more safe, alternative to MLV-based retroviral vectors in gene therapy of JEB.

3. Main advantages of the offer

Up to now effective therapies for this type of pathology do not exist.

4. Technology keywords

human epithelial stem cells, cell and gene therapy, junctional epidermolysis bullosa (JEB), Laminin-5

5. Current stage of development

Laboratory tested, rapid advancement to clinical application

Technical and scientific publications

Mavilio F., Pellegrini G., Ferrari S., Di Nunzio F., Di Iorio E., Recchia A., Maruggi G., Ferrari G., Bonini C., Capurro S., Conti A., Magnoni C., Giannetti A. and De Luca M. (2006) Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. *Nat. Med.* 12:1397-1402

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