**ABSTRACT**

## *Life-saving regeneration of the entire human epidermis by transgenic stem cells*

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Laminin beta3-deficient generalized Junctional Epidermolyis Bullosa is the first genetic disease targeted by transplantation of epidermal cultures originated from transgenic epidermal stem cells. Three patients were treated with autologous epidermal cultures transduced with a MLV-derived retroviral vector carrying the *LAMB3* cDNA under the control of the viral LTR. Several skin biopsies were taken from each patients to perform histological analysis, immunofluorescence, *in situ* hybridization and genome-wide analysis of the retroviral integration sites. The regenerated epidermis was normal-looking, remained mechanically stable throughout the entire follow-up period and did not form blisters, even upon shear force. We observed a proper expression and location of laminin 332 in the basal lamina. *In situ* hybridization performed using vector-specific *LAMB3* probes showed homogenous expression of *LAMB3* mRNA in all epidermal layers, confirming that the regenerated epidermis consists only of transgenic keratinocytes. Histological analysis showed a normal and fully differentiated epidermis with a normal dermal-epidermal junction. Electron Microscopy confirmed the presence of well-defined, organized hemidesmosomes comparable to those of healthy controls. In particular, we report life-saving regeneration of the entire epidermis on a seven-year-old JEB child. The proviral integration pattern was maintained in vivo and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained not by equipotent progenitors, but by a limited number of long-lived stem cells, detected as holoclones, that can extensively self-renew in vitro and in vivo and produce progenitors that replenish terminally differentiated keratinocytes.