largest low-oxygen zones are located in the eastern tropical Pacific Ocean (ETP). Yang *et al.* use the ocean component of an Earth-system model to investigate interannual (year to year) variations in the rate of water-column denitrification in response to the El Niño–Southern Oscillation (ENSO) in the ETP.

The ENSO phenomenon is a periodic reorganization of density, oxygen and nutrient distributions in the upper regions of the ETP that occurs in response to wind-driven changes in ocean circulation[7,8]. During the El Niño phase of the ENSO cycle, the upper ocean's mean oxygen concentration increases, and the nutrient supply to the surface ocean decreases. The opposite occurs during the La Niña phase of the ENSO (Fig. 1).

Yang *et al.* report large interannual variations in denitrification in the ETP in response to the ENSO. In their model, denitrification peaks at 70% above average rates during La Niña, and drops as low as 70% below average during El Niño. The variations do not scale linearly with the associated changes in the volume of low-oxygen zones (±2%), or in export production — the amount of organic matter formed by primary production that sinks from the surface ocean, which varies by ±6%. In their detailed analysis, Yang *et al.* show that the amplification of modest changes in low-oxygen volume and export production to much larger variations in denitrification occurs because denitrification hotspots form at the upper boundary of low-oxygen waters during La Niña, but are eliminated during El Niño, when the upper boundary recedes to greater depths (Fig. 1). This mechanism has been hinted at previously[9].

The current findings have important implications for our ability to quantify the present oceanic nitrogen budget, and to make projections of future budgets using Earth-system models. First, the large changes in denitrification rate observed over interannual timescales make it difficult to estimate denitrification rates in the ETP from the sparse observations presently available. Estimates of the long-term mean rates will be higher when based on observations made during La Niña, lower when based on observations during El Niño, and will simply be inaccurate if based on observations made during different ENSO phases.

Second, simple scaling arguments suggesting that global denitrification flux in the water column increases with the volume of low-oxygen water (see refs 10 and 11, for example) are questionable. In fact, an upward movement of the upper boundary of low-oxygen waters could lead to more denitrification even if the low-oxygen volume shrinks. The amplification documented by Yang *et al.* suggests that the match or mismatch of an increased organic-matter supply with low-oxygen conditions, rather than low-oxygen volume, determines water-column denitrification rates.

The challenge for oceanographers is now twofold. Better spatial and temporal coverage of ocean observations is needed to confirm the relationship between physical driving forces and the biological responses described by Yang and colleagues. The rapid maturation of autonomous sensor technology provides realistic prospects of accomplishing this. For example, the proposed Biogeochemical-Argo programme[12] could obtain observations of subsurface ocean properties at high spatial and temporal resolution, using a global array of floats. In addition, the ability of Earth-system models to accurately simulate the observed denitrification amplification must be verified and, if necessary, improved. ■

Katja Fennel *is in the Department of Oceanography, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada.*
e-mail: katja.fennel@dal.ca

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GENE THERAPY

Transgenic stem cells replace skin

The treatment of a patient affected by an incurable genetic skin disease demonstrates the efficacy, feasibility and safety of replacing almost the whole skin using genetically corrected stem cells. SEE ARTICLE P.327

MARIACELESTE ARAGONA & CÉDRIC BLANPAIN

Stem-cell and gene therapies are often considered to be the future of medicine, but there have been many barriers to putting these approaches into practice. Indeed, there are few examples of truly useful human stem-cell therapies[1]. On page 327, Hirsch *et al.*[2] describe a success in this area — the use of gene therapy to correct the cells of a child who had a devastating genetic disease associated with skin blistering.

The skin is composed of the epidermis, which acts as a barrier against the external environment, and the underlying dermis, in which the epidermis is firmly anchored, conferring elasticity and mechanical resistance[3]. In the disease epidermolysis bullosa, genetic mutations prevent normal epidermal resistance or anchoring[4], making the skin fragile. Mechanical stress and minor trauma provoke epidermal fragmentation or detachment from the dermis, causing skin blistering and ulcers. This produces chronic, painful and untreatable wounds, and ultimately leads to skin cancers, infection and sometimes death[4]. There is currently no cure.

The group that performed the current study previously used gene therapy to treat a mild form of epidermolysis bullosa caused by mutations in the gene laminin β3 (*LAMB3*), which encodes an epidermal anchoring protein[5]. In that study, the authors isolated a small piece of epidermis from a patient and added a normal version of *LAMB3* to the isolated epidermal cells, using a retroviral vector to carry the gene into the cells' nuclei. The vector integrated into each cell's genome, enabling normal *LAMB3* expression. The group grew the genetically corrected cells *in vitro* to form a larger piece of epidermis that they transplanted onto the patient's leg, where it engrafted.

Hirsch *et al.* have taken this strategy much further. A seven-year-old child who had an extremely severe form of epidermolysis bullosa caused by *LAMB3* mutations was admitted to hospital in a life-threatening condition, having lost almost his entire skin. The authors took a 4-square-centimetre biopsy from an unaffected part of his skin, genetically corrected cells using a retroviral vector carrying *LAMB3*, and grew the corrected cell population to obtain 0.85 m^2 of transgenic epidermal grafts. They replaced 80% of the patient's skin with the grafts in three separate operations (Fig. 1). After 21 months' follow-up, the child seemed to have made a

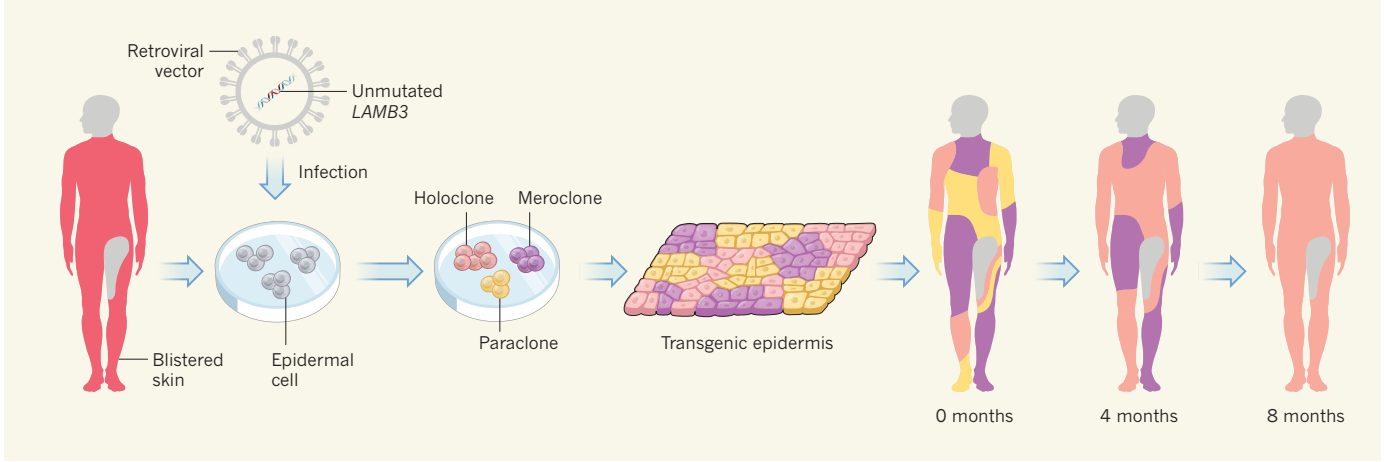

Figure 1 | Gene therapy to treat a skin disease. Hirsch *et al.*[2] used gene therapy to treat a child who had lost 80% of his skin owing to epidermolysis bullosa, a skin-blistering disease caused by mutation of the gene *LAMB3*. The authors isolated epidermal cells from a non-blistering skin region and corrected the cells by infecting them with a retrovirus that carried unmutated *LAMB3*. *In vitro* growth of epidermal cells produces three types of colony: holoclones, which are proliferative and contain stem cells; differentiated paraclone colonies; and meroclones, which are in an intermediate state of differentiation. Further growth produces sheets of transgenic epidermis derived from these colonies that can be transplanted back to the patient. The skin completely regenerates about once a month, with differentiated cells being replaced — after four months, the authors found that many paraclone and meroclone colonies from the initial transplant had been lost, and by eight months, almost the entire skin was derived from the initial holoclones. Thus, skin is maintained by a few stem cells.

full recovery, with no blistering. His skin was resistant to stress and healed normally.

One possible complication of gene therapy is that, because the vector integrates into the host genome at random sites, it might disrupt essential genes or trigger overexpression of genes that control tumour development. To investigate this possibility, Hirsch *et al.* sequenced DNA from the patient's genetically corrected skin. Sequencing revealed that most integrations occurred in non-protein-coding sequences. The genes that contained integrated retroviral vectors are not known to be directly involved in cancer, demonstrating the safety of the approach.

Next, the authors compared integration patterns in the corrected *in vitro* cultures with patterns in the regenerated epidermis *in vivo*, to determine whether particular patterns (for example, integration into promoter sequences that drive gene expression) conferred a survival advantage that might predispose to cancer in the future. They found similar patterns in both conditions, indicating that neither the culture protocol nor natural skin-cell turnover led to preferential survival and expansion of particular cell subsets. Finally, the researchers found no sign of autoantibody production against the transgene, which would lead to the rejection of the graft, further demonstrating the safety of the approach.

The epidermis is completely renewed about once a month[3]. Whether renewal is ensured by stem cells at the top of a cellular hierarchy, or whether all proliferative cells behave as equipotent progenitors, choosing randomly between proliferation and differentiation, is a matter of debate[6]. Culturing epidermal cells *in vitro* produces three types of cell colony — holoclones, paraclones and meroclones. Holoclones are proliferative colonies composed of undifferentiated cells that have self-renewal capacity; paraclones are differentiated cells that have little renewal capacity; and meroclones are intermediate between the two[7]. Although it has been hypothesized that holoclone colonies contain epidermal stem cells, this relationship has not been formally demonstrated.

Hirsch *et al.* mapped the positions of viral integration sites in the genomes of holoclones *in vitro*, and compared them with the integration sites retained in cells from the child's skin four and eight months after grafting. There were many fewer different integration sites in the biopsied cells taken at four months than in the initial cultures. By contrast, many insertion sites were retained between the four- and eight-month samples. These data suggest that most cells present in the initial culture are lost over the first four months, with only a few stem cells contributing to long-term epidermal maintenance.

Moreover, there was a massive increase in the frequency at which the *in vitro* holoclone integration sites appeared in the rejuvenated skin over time (this skin contained not only holoclones, but also newly formed paraclones and meroclones). Thus, holoclone colonies contain stem cells that repopulate the regenerated skin. After eight months, almost the entire epidermis was derived from holoclones. Clearly, a few long-lived stem cells sustain the human epidermis.

Hirsch and colleagues' study demonstrates the feasibility and safety of replacing the entire epidermis using combined stem-cell and gene therapy. In addition, the work provides insights into the cellular hierarchy that governs epidermal maintenance in humans. But there are several considerations to be addressed before rolling the treatment out to other patients.

Epidermolysis bullosa can be caused by mutations in different genes, not all of which will be easy to correct. Strategies such as the use of CRISPR–Cas9 gene-editing technology will be needed to correct some mutations. It will also be necessary to adapt the procedure to different sites in the body, in particular in people who have less-severe skin conditions. The treatment might be more effective in children, whose stem cells have higher renewal potential and who have less total skin to replace, than in adults.

Finally, longer-term follow-up of the child in the current study and other patients will be needed, to ensure that there are no adverse consequences — for example, the development of skin cancers or changes that lead to the loss of transgene expression in some cells, which could result in blistered zones. Nonetheless, the authors' work marks a major step forward in the quest to use stem-cell therapies to treat disease. ■

Mariaceleste Aragona *and* **Cédric Blanpain**
are in the Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles, 1070 Brussels, Belgium.
e-mail: cedric.blanpain@ulb.ac.be

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