

MEDICINE

A Comeback for Gene Therapy

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Gene therapy has recently had some important successes in treating severe inherited diseases (1–3) after years of skepticism from the scientific community and neglect by the pharmaceutical industry. On page 818 in this issue, Cartier *et al.* (4) report another major advance—the successful first clinical testing of an HIV-derived vector in hematopoietic stem cell (HSC)-based gene therapy. The procedure was used to treat a severe neurodegenerative disease, X-linked adrenoleukodystrophy (ALD), and the results indicate stable expression of a therapeutic gene in a substantial fraction of patients' hematopoietic cells, as well as clinical benefits.

Lentiviral vectors (including HIV-derived) were developed to overcome the inability of gamma-retroviral vectors to infect nondividing cells (5), and have become a widely used gene transfer tool with the potential to extend the reach of gene therapy applications. Several studies have shown that lentiviral vectors transduce HSCs more efficiently than gamma-retroviral vectors (6–11). Cartier *et al.* tested this strategy for treating childhood ALD, a fatal disorder of the central nervous system caused by mutations in *ABCD1*, a peroxisomal transporter gene. The transporter functions in the turnover of myelin (lipid-rich material that insulates neurons) in oligodendrocytes and microglia, and its deficiency leads to demyelination and consequent nervous system dysfunction. Disease progression can be arrested by allogeneic HSC transplantation (cells are from a normal donor), which enables functional myelo-monocytic cells derived from a donor's HSCs to migrate into the recipient's central nervous system and replace diseased microglia cells, thus relieving lipid storage (12).

Gene therapy may provide an alternative treatment if a sufficient number of autologous HSCs

(the stem cell donor is also the patient/recipient) are corrected by gene transfer and successfully engrafted into the patient. But an important question is whether the therapeutic efficacy is comparable to that of transplantation. Also, although the design of a lentiviral vector and its integration preference into the stem cell genome alleviate the risks (11, 13–16) of mutagenesis (and leukemia) that have been observed with gamma-retroviral vectors (2, 17, 18), whether this holds up when lentiviral vectors are used in clinical trials has not been known.

In the study by Cartier *et al.*, two ALD-affected boys were infused with autologous HSCs that were corrected *ex vivo* with an HIV-derived vector expressing *ABCD1* (see the figure). *ABCD1* protein was sta-

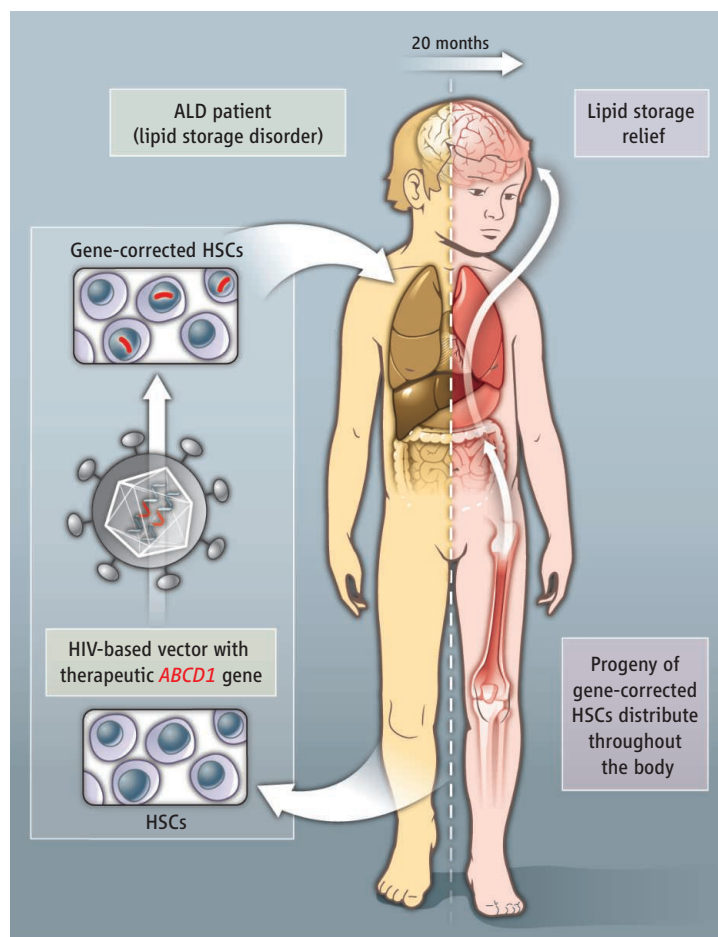
A lentivirus was used as a vector in hemato-poietic stem cells to treat a neurodegenerative disease in a clinical gene therapy trial.

bly expressed in 9 to 14% of granulocytes, monocytes, T and B cells, and bone marrow progenitors in both patients throughout the 24 to 30 months of follow-up, respectively. Cerebral demyelination was arrested 14 to 16 months after engraftment, and neurological and cognitive functions remained stable, an outcome comparable to that of successful HSC transplantation.

Were bona fide HSCs transduced by the lentiviral vector? Mapping integration sites helps to address this question as each site represents a unique genetic marker for tracking the clonal activity of a transduced cell. Up to 4% of all identified vector integration sites were found in both lymphoid and myeloid cells, often at several time points after engraftment. This is close to long-sought evidence for transduction and engraftment of self-renewing, multipotent HSCs.

Was gene transfer into HSCs efficient? As only up to 14% of the cells in each lineage stably expressed *ABCD1*, there is room for improvement. Higher vector titers, which will become available as lentiviral vector manufacturing improves, will likely boost this figure. However, it is difficult to compare earlier gene therapy trials with that of Cartier *et al.*, in which the patient's HSCs were ablated to favor engraftment of the gene-corrected HSCs. Earlier trials based on gamma-retroviral vectors used no or less intense bone marrow conditioning and exploited the growth advantage of gene-corrected cells to favor their engraftment and expansion *in vivo* (1, 2, 17).

Long-term engraftment of hematopoietic cells transduced by gamma-retroviral vectors is often characterized by the appearance of clonal cells bearing vector integration in genes involved in growth control and/or oncogenesis. This



Promising treatment. Progeny of HSCs that were engineered to carry the correct version of a gene (through the integration of a lentiviral vector) distribute throughout the body. Cartier *et al.* show that some cells replaced diseased microglia in the brain and relieved lipid storage in patients suffering from ALD.

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may indicate positive selection of clones with increased growth potential caused by vector insertion (2, 17, 18). Is lentiviral vector integration more neutral? Cartier *et al.* report a reassuring picture of highly polyclonal hematopoietic cells transduced with the *ABCD1* gene, which is maintained throughout the follow-up time without evidence for sustained expansion of individual clones or enrichment of common integration sites. But when the authors compared the distribution of integration sites in cells before infusion and after engraftment, they observed an enrichment of integration sites at some gene classes after engraftment. This may suggest that integration was not completely neutral. It may also reflect differences in integration preference between the short-lived progenitors, which constitute most of the cells infused into the patients, and the rare HSCs whose progeny engraft the patients long-term. Longer follow-up and additional testing in this and other diseases will better establish the safety features of lentiviral vectors and how they can be influenced by conditions specific to each study design.

If most lentiviral vector integration is neutral to cell behavior, the tracking of integra-

tion site distribution in the different cell lineages reported by Cartier *et al.* may be a first glimpse of live hematopoiesis in humans at the clonal level. The authors used a combination of approaches to maximize the coverage of integration sites in each sample and alleviate the retrieval biases imposed by the DNA restriction and amplification steps of the procedure. This technological rigor will likely become a gold standard for future HSC-based gene therapy trials.

Gene therapy of ALD in the study of Cartier *et al.* provided a benefit similar to that of allogeneic HSC transplantation, despite a relatively low level of gene correction. This unexpected finding indicates that enhanced efficacy in relieving lipid storage may be attained with cells that overexpress the therapeutic gene as compared to normal donor cells. It also suggests that microglia cells might be replaced by infused short-lived progenitors that contain a higher proportion of gene-corrected cells than HSCs. These scenarios might eventually position HSC-based gene therapy as a preferable treatment option for ALD, as it abrogates the morbidity associated with the allogeneic source of HSCs in conventional transplantation. Furthermore,

improved HSC transduction protocols may overcome the need for bone marrow conditioning. Although many questions remain to be fully settled, this study clearly supports further testing of HSC-based gene therapy in ALD and other diseases and represents a long-sought rewarding achievement in the field of gene therapy.

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ECOLOGY

Biodiversity and Climate Change

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Over the past decade, several models have been developed to predict the impact of climate change on biodiversity. Results from these models have suggested some alarming consequences of climate change for biodiversity, predicting, for example, that in the next century many plants and animals will go extinct (1) and there could be a large-scale dieback of tropical rainforests (2). However, caution may be required in interpreting results from these models, not least because their coarse spatial scales fail to capture topography or “microclimatic buffering” and they often do not consider the full acclimation capacity of plants and animals (3). Several recent studies indicate that taking these factors into consideration can seriously alter the model predictions (4–7).

In one study, Randin *et al.* assessed the influence of spatial scale on the accuracy of

bioclimatic model predictions of habitat losses for alpine plant species in the Swiss Alps (4). A coarse European-scale model (with 16 km by 16 km grid cells) predicted a loss of all suitable habitats during the 21st century, whereas a model run using local-scale data (25 m by 25 m grid cells) predicted persistence of suitable habitats for up to 100% of plant species. The authors attributed these differences to the failure of the coarser spatial-scale model to capture local topographic diversity, as well as the complexity of spatial patterns in climate driven by topography.

Luoto and Heikkinen reached a similar conclusion in their study of the predictive accuracy of bioclimatic envelope models (which model the relation between current climate variables and present-day species distributions) on the future distribution of 100 European butterfly species (5). A model that included climate and topographical heterogeneity (such as elevational range) predicted only half of the species losses in mountainous areas for the period from 2051 to 2080 in comparison to a climate-

only model. In contrast, the number of species predicted to disappear from flatlands doubled in the climate-topography model relative to the climate-only model. The two studies suggest that habitat heterogeneity resulting from topographic diversity may be essential for persistence of biota in a future changing climate.

Highly contrasting predictions have also been obtained when bioclimatic models of tropical biomes included the physiological effects of elevated atmospheric CO₂ concentrations and temperature on trees (6). Many studies have indicated that increased atmospheric CO₂ affects photosynthesis rates and enhances net primary productivity—more so in tropical than in temperate regions—yet previous climate-vegetation simulations did not take this into account.

To address these issues, Lapola *et al.* (6) developed a new potential-vegetation model for tropical South America that includes CO₂ fertilization effects. They then drove this model with different climate scenarios for the end of the 21st century from 14 coupled

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