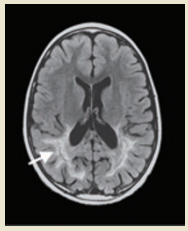
## Gene therapy learns its ABCDs



A gene therapy tested on two seven-year-old boys with X-linked adrenoleukodystrophy has halted the progression of this fatal demyelinating disease. The treatment used autologous CD34$^+$ peripheral blood mononuclear cells transduced *ex vivo* with a lentiviral vector carrying a normal copy of the defective gene, *ABCD1*. Earlier gene-therapy trials with γ-retroviral vectors had relied on an intrinsic selective advantage of the genetically corrected cells to ensure a sufficient level of engraftment. Because expression of *ABCD1* does not confer a growth advantage, Cartier *et al.* used full myeloablative conditioning to 'make room' for the corrected cells. From previous studies, they expected that corrected hematopoietic stem cells infused into the blood would reconstitute hematopoiesis in the bone marrow, differentiate into myeloid precursors that migrate to the brain, and differentiate further into microglial cells that express *ABCD1*. At 20–30 months after transplantation, up to 14% of several blood cell lineages and up to 18% of bone marrow CD34$^+$ cells expressed the transgene. Progressive brain demyelination arrested at 14–16 months. Importantly, corrected cells isolated at 24 and 30 months were polyclonal, showing little evidence that a small subset of transduced cells had acquired a selective advantage through growth-promoting viral integrations. (*Science* **326**, 818–823, 2009) *KA*

## Mapping drug promiscuity

Off-target drug interactions have long been the bugaboo of drug developers. But multiple interactions—polypharmacology—is not only a fact of life, but might be beneficial. Absent structures of all human targets, *in silico* techniques for predicting interactions, off and on target, have been developed. Now, Keiser and colleagues have applied their similarity ensemble approach (SEA) first described in these pages (*Nat. Biotechnol.* **25**, 197–206, 2007), to map out interactions between 3665 drugs (available or in development) against 65,000 ligands. SEA classifies targets by the similarity of compounds that bind to them, and reveals ligand-based similarities where they are not expected. Among 184 unprecedented interactions, thirty of which they verified experimentally, they found examples of new targets as the primary site of action, off-target binding that could explain side effects, and binding that was unrelated in sequence, structure or function. For example, the hallucinogen dimethyltryptamine was found to bind serotonergic receptors with a high probability, which suggests that what was believed to be the primary target (σ receptor) is probably incorrect. Some widely prescribed selective serum reuptake inhibitors like Paxil and Prozac were found to be β-blockers, which explains some of the side effects seen during rapid withdrawal. (*Nature* **462**, 175–181, 2009) *LD*

## New DNA-binding motifs

Transcription activator–like (TAL) effectors from plant pathogenic bacteria in the genus *Xanthomonas* contribute to many crop diseases by activating host genes. Moreover, certain crops have co-opted TAL effectors to trigger defense responses. Although the DNA-binding specificity of TAL effectors has long been known to be defined by a stretch of tandem imperfect amino acid repeats, the molecular basis of their interaction with DNA has remained enigmatic. Boch *et al.* and Moscou & Bogdanove independently report that the specificity of promoter activation by TAL effectors is determined by the ability of particular pairs of adjacent amino acids in each repeat to recognize a particular base pair in target DNA. Boch *et al.* demonstrate that the modular architecture of the specificity-determining domain enables the design of effectors with novel specificities. Moscou & Bogdanove show that the affinity of any repeat in a TAL effector, unlike zinc fingers, does not appear to be affected by its neighbor. The potential for identifying plant genes either involved in resisting or promoting diseases is extended by the demonstration by Römer *et al.* that functionally distinct DNA motifs recognized by separate TAL effectors retain their function when combined into a single promoter. Novel resistance genes activated by multiple TAL effectors could enable engineering of broad spectrum and durable disease resistance. (*Science*, published online October 29, 2009; doi:10.1126/science.11788111; doi:10.1126/science.1178817; *Proc. Natl. Acad. Sci. USA*, published online November 12; doi: 10.1074/pnas.0908812106) *PH*

## Reprogramming for all

When cultured fibroblasts are infected with viral vectors expressing the reprogramming genes *Oct4*, *Sox2*, *Klf4* and *c-Myc*, only a few rare cells convert to induced pluripotent stem (iPS) cells. This low efficiency has been attributed to the variability of viral delivery: every cell does not take up all four vectors, and those that do may not express the transgenes at the required doses. To control for this variability, Jaenisch and colleagues previously devised a "secondary" reprogramming system that generates large numbers of somatic cells with an identical viral integration pattern selected by its competence to produce iPS cells (*Nat. Biotechnol.* **26**, 916–924, 2008; *Nat. Biotechnol.* **27**, 169–171, 2009). But even with this system, in which reprogramming is induced simply by adding doxycycline, only a small minority of cells became iPS cells. This puzzling result is explored in a new study from the Jaenisch group. Using secondary pre-B-cells carrying a Nanog-GFP reporter, the authors cultured single cells in individual wells in the presence of doxycycline. After two weeks, cells in 3–5% of the wells expressed Nanog (a marker of iPS cells). But as the time in culture increased, the number of GFP$^+$ wells continued to rise, reaching 92% of the wells at 18 weeks. The variation in the kinetics of reprogramming was independent of transgene expression levels and cell growth rates. These findings suggest that all somatic cells are amenable to reprogramming. (*Nature* advance online publication, doi:10.1038/nature08592, November 8, 2009) *KA*

## Stackable paper cell culture

Cells in the human body grow in the presence of molecular gradients of nutrients and signals. To mimic this environment, Derda *et al.* culture cells in extracellular matrix hydrogel supported by the fibers within a sheet of paper. Stacking sheets of paper permeated with cells creates a three-dimensional growth environment in which the concentration of nutrients within the stack can be controlled. Unstacking the sheets allows the properties of cells at each layer to be assayed. Using this system, Derda *et al.* demonstrate that cell growth, histological staining markers and gene expression patterns accurately reflect the oxygen gradient available to cells at different layers in the stack. Paper-based cell culture stacks could be optimized with different types of paper for specialized applications, formatted to fit in microtiter plates to enable high-throughput assays and synthesized with chemical compounds on the paper to enable drug screening. (*Proc. Natl. Acad. Sci. USA* **106**, 18457–18462, 2009) *CM*

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