

## CLINICAL IMPLICATIONS OF BASIC RESEARCH

## A New Dawn for Stem-Cell Therapy

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Recently, the potential use of stem cells for regenerative medicine and for the treatment of genetic disease has rarely been out of the news. Discussion has focused mainly on the use of human embryonic stem cells, which in culture have the capacity to generate all cell types. However, initial hopes for stem-cell therapy have been somewhat dampened by both technical and ethical problems. Recent studies have therefore created a great deal of excitement. They show that fully differentiated somatic cells (such as skin fibroblasts) can be reprogrammed to make cells similar to embryonic stem cells, called induced pluripotent stem cells.<sup>1</sup> Now, Hanna et al.<sup>2</sup> have taken the next important step and have shown, by correcting a mouse model of sickle cell disease, how induced pluripotent stem cells might eventually be used to cure a human disease.

Sickle cell disease is one of the world's most common inherited anemias. About 1 in 500 persons of African descent has sickle cell disease, and it is also frequently seen in people from India, the Middle East, and some regions of the Mediterranean. Sickle cell disease results from the substitution of glutamic acid by valine at position 6 of the  $\beta$  chain of hemoglobin ( $\beta^{\text{Glu}6\text{Val}}$ ). The clinical manifestations of sickle cell disease arise from the tendency of sickle hemoglobin (also known as hemoglobin S [HbS] or  $\alpha_2\beta_2^{\text{S}}$ ) to polymerize at reduced oxygen tensions and deform red cells into the characteristic, rigid sickle cell shape. Such inflexible red cells cannot negotiate the microcirculation efficiently; this results in a chronic hemolytic anemia and intermittent vaso-occlusion, which affects a wide variety of organs. The clinical course is highly variable, but the complications are often life-threatening.

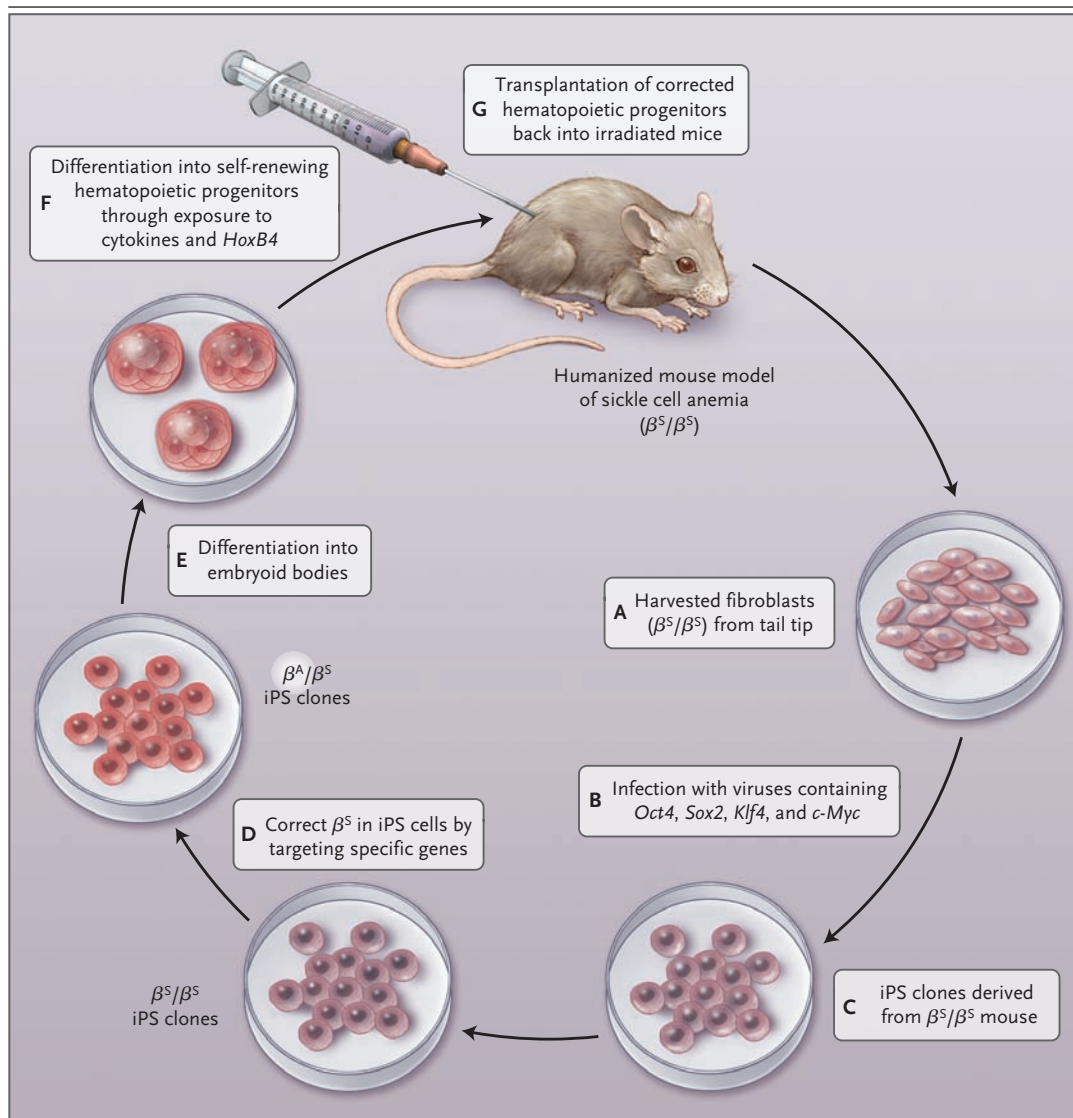
Fifty years of intensive investigation has had a disappointingly limited effect on the clinical management of the disease. The only cure for sickle cell disease is bone marrow (hematopoietic stem-cell) transplantation, which can legitimately be

called adult stem-cell therapy. To date, approximately 250 patients with sickle cell disease have been treated with hematopoietic stem-cell transplantation involving stem cells donated by an HLA-identical sibling. This approach is currently reserved for those with severe disease; the rate of disease-free survival is 85%. The main issues restricting the use of stem cells from an HLA-matched donor are immunologic and could be overcome if a patient's own somatic cells (such as skin fibroblasts) could be reprogrammed to make hematopoietic stem cells, the biologic equivalent of an alchemist's dream of turning lead into gold.

The first approach to attempt this reprogramming was ingenious but complicated. It involved removing the nucleus from a somatic cell and using it to replace the nucleus of a fertilized egg (so-called somatic-cell nuclear transfer). Factors remaining in the egg could then reprogram the somatic nucleus as the reconstituted egg developed into an early human embryo. Epiblast cells from this embryo could then be used to establish a "customized" embryonic stem cell that would be immunologically compatible with the donor of the somatic cell. Perhaps, not surprisingly, the technical difficulties and ethical complexities of this approach were always likely to render it impractical.<sup>3</sup>

Important breakthroughs initially came from the laboratories of Yamanaka<sup>1</sup> and, more recently, Thomson,<sup>4</sup> showing that mouse and human fibroblasts can be reprogrammed into cells similar to embryonic stem cells by introducing combinations of transcription factors (such as Oct4, Sox2, Klf4, Nanog, Lin28, and c-Myc) that are normally expressed in embryonic stem cells. They showed that such cells are pluripotent, but the question remained: could they differentiate into fully functional somatic cells and be used for cell therapy?

In a proof-of-principle experiment using a humanized mouse model of sickle cell anemia, Hanna et al. have now shown that such mice can be



**Figure 1. Curing Sickle Cell Anemia in a Humanized Mouse Model.**

Hanna et al.<sup>2</sup> recently described how induced pluripotent stem cells,<sup>1</sup> in this case derived from a mouse model of sickle cell disease, might be repaired and then used for cell therapy to cure the disease. The process involves four steps: the reprogramming of mutant donor fibroblasts with the sickle mutation ( $\beta^S$ ) into induced pluripotent stem cells (iPS cells) (A, B, and C), the ex vivo repair of the genetic defect through homologous recombination with the wild-type allele ( $\beta^A$ ) (D), the in vitro differentiation of the repaired induced pluripotent stem cells into hematopoietic progenitors (E and F), and the transplantation of these cells into affected donor mice after irradiation (G). All clinical and hematologic abnormalities in the mouse with sickle cell disease were corrected.

rescued after transplantation with the use of hematopoietic progenitors derived in vitro from autologous induced pluripotent stem cells (Fig. 1). First, induced pluripotent stem cells were made from a mouse with sickle cell disease, and the sickle mutation ( $\beta^S$ ) was corrected in the induced pluripotent stem cells by means of conventional homologous recombination. Next, the repaired induced

pluripotent stem cells were differentiated into cells similar to hematopoietic stem cells and transfected with *HoxB4*, which has previously been shown to confer engraftment potential on hematopoietic cells derived from embryonic stem cells.<sup>5</sup> Finally, these transfected cells were transplanted back into three isogenic mice with sickle cell disease. Stable engraftment resulted in the blood containing ap-

proximately 70% of cells derived from corrected induced pluripotent stem cells and 30% endogenous hematopoietic cells. Furthermore, the red-cell abnormalities, hemolytic anemia, and associated pathologic features were corrected.

This is a very exciting advance for the potential treatment of monogenic disorders such as sickle cell disease and for the development of cell therapy in general. However, many daunting problems remain to be solved before induced pluripotent stem cells could be used in human trials. For example, the reprogrammed hematopoietic stem cell-like cells used in these experiments are not the equivalent of the naturally occurring, long-term, repopulating hematopoietic stem cells currently used in clinical practice; initial evidence suggests they may not fully restore all mature blood-cell lineages.<sup>2,4</sup> Further work will be needed to develop robust and reliable protocols for differentiating human induced pluripotent stem cells into functional cells appropriate for cell therapy. In addition, it remains to be seen whether homologous recombination, the critical step necessary to correct any molecular defect, can be reliably achieved in human induced pluripotent stem cells.

Finally, there are many safety issues to overcome, mainly related to the potential of induced pluripotent stem cells to develop into malignant stem cells. Certainly, new tricks are already being developed to modify the methods used to generate

induced pluripotent stem cells, in particular those that avoid the prolonged use of oncogenes such as *c-Myc* and replace the use of retroviruses that are known to cause insertional mutagenesis, a problem that has bedeviled attempts at human gene therapy. Even if these goals could be achieved, we will still need to be sure that the transcriptional and epigenetic programs in induced pluripotent stem cells have not been incorrectly reprogrammed such that they are predisposed to malignant transformation. With these important caveats, we have good reason to be optimistic. This is a key step forward in the treatment of genetic disease and in regenerative medicine.

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