LETTERS
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Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with Fanconi anemia

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Fanconi anemia (FA) is a DNA repair syndrome generated by mutations in any of the 22 FA genes discovered to date[1,2]. Mutations in *FANCA* account for more than 60% of FA cases worldwide[3,4]. Clinically, FA is associated with congenital abnormalities and cancer predisposition. However, bone marrow failure is the primary pathological feature of FA that becomes evident in 70–80% of patients with FA during the first decade of life[3,5]. In this clinical study (ClinicalTrials.gov, NCT03157804; European Clinical Trials Database, 2011-006100-12), we demonstrate that lentiviral-mediated hematopoietic gene therapy reproducibly confers engraftment and proliferation advantages of gene-corrected hematopoietic stem cells (HSCs) in non-conditioned patients with FA subtype A. Insertion-site analyses revealed the multipotent nature of corrected HSCs and showed that the repopulation advantage of these cells was not due to genotoxic integrations of the therapeutic provirus. Phenotypic correction of blood and bone marrow cells was shown by the acquired resistance of hematopoietic progenitors and T lymphocytes to DNA cross-linking agents. Additionally, an arrest of bone marrow failure progression was observed in patients with the highest levels of gene marking. The progressive engraftment of corrected HSCs in non-conditioned patients with FA supports that gene therapy should constitute an innovative low-toxicity therapeutic option for this life-threatening disorder.

Although the very low number of hematopoietic stem cells (HSCs) in patients with Fanconi anemia (FA)[7] has limited the collection of HSCs in previous gene therapy trials[8–10], the proliferative advantage observed in naturally reverted hematopoietic stem and progenitor cells (HSPCs) from mosaic patients with FA[11–14] suggested that the infusion of low numbers of gene-corrected HSCs might be sufficient for their engraftment after autologous transplantation. Nevertheless, none of the gene therapy trials conducted to date have shown engraftment of corrected HSCs in patients with FA[9,10]. Several factors may account for these negative results, including the collection of low numbers of HSCs from bone marrow (BM) or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (PB), the use of prolonged transduction protocols with gamma-retroviral vectors, or the absence of patients' conditioning before the infusion of transduced cells (see review in ref. [15]).

The use of CD34$^+$ cells mobilized with G-CSF and plerixafor, together with the short transduction of these cells with *FANCA* lentiviral vectors, enabled us to demonstrate the repopulating potential of gene-corrected FA subtype A (FA-A) HSCs in a xenogenic transplantation model[16]. On the basis of our preclinical observations, we developed the current clinical trial in which gene-corrected HSCs were reinfused in patients with FA without any cytotoxic conditioning regimen.

CD34$^+$ cells were obtained from pediatric patients (aged 3–6 years) before the development of severe BM failure (BMF) (see Extended Data Fig. 1 and the main inclusion criteria of the FANCOSTEM-1 HSC mobilization trial in the Methods). In contrast, because the primary objective of the FANCOLEN-1 gene therapy trial was to evaluate the safety of the therapeutic strategy, only

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