CELL THERAPY

CRISPR/Cas9-edited HSPC therapy for hemoglobinopathies

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β-hemoglobinopathies are inherited disorders caused by mutations in the *β-globin* gene. Sickle cell disease, for example, is the result of deficient hemoglobin (Hb); patients with β-thalassemia produce insufficient amounts of the oxygen-carrying protein. To date, allogeneic transplantation of hematopoietic stem and progenitor cells (HSPC) is the only curative treatment available for hemoglobinopathies, but alternative therapeutic approaches are under investigation. One promising option is to use gene-editing technologies to reactivate fetal Hb (HbF); HbF can then act as a substitute for aberrant adult Hb. The findings of a new study describing persistent HbF reactivation after engraftment of CRISPR/Cas9-edited $CD34^+$ HSPC in a nonhuman primate (NHP) autologous transplantation model might bring us a step closer to the clinical translation of gene-edited HSPC therapy to patients.

The gene-editing strategy was designed to generate mutations in the HbF-associated locus (HBG) containing the binding site for B-cell lymphoma/leukemia 11A (BCL11A), an HbF repressor controlling the fetal to adult Hb switch. Three rhesus macaques were infused with CRISPR/Cas9-treated $CD34^+$ HSPCs after total body irradiation. The number of gene-edited nucleated cells stabilized after 1 month (8—27% of cells in peripheral blood (PB) during the entire follow-up period of > 1year), resulting in persistent HbF reactivation in transplanted animals. Compared with control transplant recipients showing <1.5% of cells expressing HbF (F cells) in PB at 250 days after treatment, all CRISPR/Cas9-edited animals expressed 6—18% of F cells during follow-up. HbF reactivation was further confirmed by longitudinal measurement of ϒ-globin with high-performance liquid chromatography (HPLC).

Similar results were observed in a second cohort of three rhesus macaques infused with a CRISPR/Cas9-edited stem cell-enriched $CD34^+$ subpopulation of $CD34^+CD90^+CD45RA^-$ cells, although the number of gene-edited cells transplanted in the second cohort was reduced by 10 fold. These results confirm that the $CD34^+CD90^+CD45RA^-$ subset is the main cell type contributing to multilineage long-term engraftment. Targeting that subset could be a new strategy to reduce the need for editing reagents without affecting engraftment, hematopoietic reconstitution, or HbF reactivation.

So far, the transplanted rhesus animals have been monitored for up to 1.5 years with no adverse effects detected. In the discussion of the study, the investigators conclude: "The conservation of the $CD34^+CD90^+CD45RA^-$phenotype and the *HBG* CRISPR-Cas9 gRNA target site between NHP and human, combined with the use of a highly clinically relevant large-animal model for stem cell gene therapy and transplantation, should facilitate the direct translation of this approach to patients."

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