

Current status of gene therapy in hemoglobinopathies

Diana Jean-Sébastien

Clinical Investigation Center of Biotherapy, Hôpital Necker, Assistance Publique Hôpitaux de Paris, Paris, France

Introduction

The first successful gene therapy clinical trials have revealed the potential of genetic engineering of hematopoietic stem and progenitor cells (HSPCs) as a viable substitute for allogeneic transplantation in monogenic hematological diseases. The evolving scope of gene therapy applications and the potential of other molecular biology strategies instills hope for a revolution in gene transfer and vector utilization in sickle cell diseases (SCD). The improvements in our knowledge of the genetic mechanisms involved in the physiopathology of SCD have driven the development of new, potentially curative gene therapies, painting a promising future for the field.

Gene addition

The first gene therapy strategy for hemoglobinopathies featured gene addition through the lentiviral (LV)-based transduction of HSPCs. This strategy was first tested in 2006 in a phase 1/2 clinical trial in β -thalassemia patients (LG001, NCT01745120)¹. The HPV569 vector contained a transgene encoding the β A-T87Q-globin, which has the same amino acid in position 87 as the fetal γ -globin with antisickling properties. This vector was further optimized to increase transduction efficiency, and the first successful gene therapy procedures using an improved vector (BB305) were reported in 2017 and 2018 for SCD and β -thalassemia, respectively^{2,3}. Results from the phase 3 study with 49 patients treated with the LentiGlobin investigational treatment in the United States and Europe (HGB-210, NCT04293185, sponsored by Bluebird Bio) were presented during ASH 2023. Similarly, a lentivirus coding for a modified fetal

γ -globin is also being tested in a phase 1/2 clinical trial in people with SCD (NCT02186418). Both trial's preliminary results show a reduction in volatile organic compounds following treatment⁴.

Two other transgene vectors (GLOBE-AS3 and β AS3-FB) encoding the modified AS3 β -globin are being tested in phase 1/2 clinical trials (NCT02247843 and NCT03964792). The β -globin contains three beneficial antisickling amino acids that inhibit the contact required for hemoglobin S (HbS) polymerization (T87Q and E22A) or increase the affinity for α -globin (G16D). Finally, an LV coding for a short hairpin RNA that reduces BCL11A expression has shown its effectiveness in reactivating HbF expression and reducing the signs and symptoms of SCD in six patients (NCT03282656)⁵.

Despite encouraging results, gene addition by a viral vector has some limitations. The amount of Hb resulting from a single vector copy is far from that required for a favorable clinical outcome. However, increasing the number of LV integrations per HSPC might also increase the risk of insertional mutagenesis. Recently, three SCD patients in the HGB-206 study developed myelodysplastic syndrome or acute myeloid leukemia; however, this was probably linked to the accumulation of mutations in hematopoietic stem cells (HSCs) before gene therapy rather than vector insertion.

Nuclease-based genome editing

The advancement of molecular biology strategies is poised to revolutionize gene transfer and vector utilization. Cutting-edge gene-editing techniques harness novel enzymatic and repair mechanisms to modulate gene expression and effect precisely targeted DNA

Correspondence:

Diana Jean-Sébastien

E-mail: jean-sebastien.diana@aphp.fr

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modifications. These innovative technologies promise to correct, replace, or suppress gene expression while mitigating the toxicity associated with genetic material integration. Due to its high prevalence and single causal mutation, SCD is a good target for developing gene correction strategies.

CRISPR-Cas9 is an adaptive defense system prokaryotes use to cleave non-self nucleic acid sequences. In particular, it has been exploited to develop genome-editing tools capable of generating double-strand breaks (DSBs) at a target locus within the genome. In the CRISPR-Cas9 system, a guide RNA (gRNA) drives the Cas9 nuclease to a specific region in the genome. Other nucleases (such as zinc finger nucleases) have also been developed and used as genome-editing tools with DSB.

DSB repair through non-homologous end joining (NHEJ) results in small insertions or deletions (indels) around the target site and can be exploited to inactivate genes or regulatory sequences. By contrast, adding an exogenous DNA template with homology to the target sequence triggers homology-directed repair (HDR) and thus makes it possible to replace one sequence with another precisely⁶.

Gene correction

Efficient HbB gene correction by CRISPR-Cas9 and HDR has been demonstrated *in vitro*. However, HDR competes with other repair mechanisms, such as NHEJ. Correction of the mutation can, therefore, be accompanied by a high frequency of alleles harboring indels. The indel-induced frameshift mutations can generate a β -thalassemic phenotype. Furthermore, the HDR pathway is weakly active in quiescent cells, such as long-term repopulating HSCs targeted in gene therapy approaches.

In 2021, two-phase 1/2 clinical trials of the safety of a nuclease-based editing strategy for correcting the SCD mutation in HSCs were announced. First, a clinical trial conducted by the University of California will include nine patients (NCT04774536) using single-stranded oligodeoxynucleotides as DNA donor templates. Second, a trial sponsored by Graphite Bio will consist of approximately 15 patients (NCT04819841) and use the adeno-associated virus (AAV6) vector to deliver the donor template.

Gene disruption

Producing high HbF levels relieves the signs and symptoms of SCD. Consequently, several research

groups have sought to reactivate HbF expression by either decreasing the expression of transcription factors that repress HBG genes (e.g., BCL11A) or disrupting the BCL11A and linear reduction factor factors' binding sites at HBG promoters. One therapeutic strategy has been designed to downregulate BCL11A by targeting its erythroid-specific enhancer. This strategy explicitly represses BCL11A expression in the erythroid lineage and thus decreases inhibition of HBG expression. With promising results, Sangamo Therapeutics launched a clinical treatment trial based on existing zinc finger nucleases in patients with SCD (NCT03653247). Another clinical approach, the vertex pharmaceuticals clinical trials (NCT03745287), used the Cas9 nuclease to disrupt the BCL11A enhancer by introducing indels. This strategy showed preliminary encouraging results. After treatment, the patient presented a high level of editing cells in bone marrow cells and peripheral blood nucleated cells, with a pan-cellular expression of HbF. The results were confirmed in 31 SCD patients and prompted a phase 3 clinical trial initiation in a dozen pediatric patients (NCT05329649).

Limitations of nuclease-based genome editing

Despite the remarkable advances in nuclease-mediated strategies for SCD, several limitations and concerns must be addressed to make genome editing safer. Off-target effects are still a significant concern in therapeutic applications. In fact, gRNA hybridization can tolerate a few mismatches and might modify the expression of non-targeted genes. Off-target DSBs also might trigger complex chromosomal rearrangements, such as translocations between on-target and off-target sites. Genome-editing strategies are designed to correct a gene sequence through HDR and can suffer from a low editing rate when the NHEJ pathway is involved. Finally, CRISPR-Cas9 nuclease treatment can activate the p53-mediated DNA damage response pathway. This arrest affects HSC function and reduces the cells' repopulating after transplant.

Conclusion

SCD is a significant candidate monogenic disease for developing gene therapies. Addition strategies successfully treated SCD, but they had some limitations in efficacy and safety. The discovery of the protective role of HbF allowed the development of disruption genome-editing strategies to reactivate the HbG genes.

New research and clinical development of genome correction by CRISPR Cas9 will evaluate the efficacy, on-target, and off-target safety of DSB in HSPC and HDR efficacy. However, such approaches can potentially damage the ability of HSCs to engraft long-term following *ex vivo* editing due to the DNA damage response by the stem cells to both CRISPR/Cas9 and AAV6 vectors. Moreover, there is a pressing need to enhance the editing rate for human applications, as the on-target editing efficiency on HSPCs with HDR. These issues highlight the need for improved methods of delivering gene therapy or gene repair vectors without inducing mutagenesis or affecting hematopoietic engraftment. For the next technological steps, new DSB-free base and prime editing strategies are in the pipeline to overcome the potential genotoxicity caused by nucleases.

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