

Cell-based Genetic Therapy for the Induction of Foetal Haemoglobin in Sickle Cell Disease and Transfusion-dependent β -thalassaemia

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Sickle cell disease and β -thalassaemia can be ameliorated or perhaps even 'cured' if most erythrocytes can be induced to contain high levels of foetal haemoglobin. While this is not yet possible using foetal haemoglobin-inducing drugs, it might be feasible with cell-based gene therapy. After mobilization of autologous haematopoietic stem and progenitor cells, these cells are engineered with vectors that lead to the derepression of foetal haemoglobin genes or add a globin gene that mimics the effects of foetal haemoglobin. Auto-transplantation after myeloablative conditioning is associated with levels of foetal haemoglobin or an antisickling haemoglobin of about 40% of total haemoglobin. In nearly every patient symptoms of sickle cell disease disappear; patients with β -thalassaemia rarely require blood transfusions. In both groups of patients anaemia is absent or minimal. Optimistically, treated patients appear functionally 'cured'. These are very early days of gene therapy. Less than 200 patients have been followed, usually for no more than 5 years. It is highly likely that improved methods that are being intensively studied will simplify gene therapy leading to its wider applicability for individuals with these very common monogenic diseases.

Keywords

BCL11A, F-cells, gene editing, sickle haemoglobin, sickle haemoglobin polymer, transfusion-dependent anaemia

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Mutations of the β -globin gene (*HBB*) cause sickle cell disease and β -thalassaemia, collectively named the β -haemoglobinopathies. As mankind's most common severe genetic diseases, where they are most prevalent, public health systems can be stressed.^{1–3} Asymptomatic heterozygous carriers are protected from severe *P. falciparum* infection. This has allowed the causative genes to reach polymorphic frequencies in some tropical and subtropical parts of the world.^{4,5} Treating the far less prevalent but seriously ill patients with homozygotic mutations is difficult and costly.

The pathophysiology of sickle cell disease is initiated by deoxy-sickle haemoglobin (HbS; $\alpha_2\beta^S_2$) polymerization. Insufficient β -globin synthesis in β -thalassaemia results in excessive toxic free α -globin leading to ineffective erythropoiesis, causing severe anaemia. Foetal haemoglobin (HbF; $\alpha_2\gamma_2$) prevents HbS polymerization. In β -thalassaemia, HbF compensates for the deficit of normal haemoglobin (HbA; $\alpha_2\beta_2$). With 30% HbF in the sickle erythrocyte, complications of sickle cell disease nearly disappear. Sufficient HbF in β -thalassaemia erythrocytes ameliorates ineffective erythropoiesis and haemolysis.^{1–3}

A single mutation in the first exon of *HBB* (β^7 GAG>GTG) causes sickle cell disease. This transversion replaces glutamic acid with valine. When paired with an α -globin chain, the sickle β -globin chain (β^{E7V}) forms HbS. The proximate cause of pathophysiology in sickle cell disease is the polymerization of HbS. The HbS polymer injures the sickle erythrocyte leading to vaso-occlusion and haemolysis. Homozygosity or compound heterozygosity for β -thalassaemia mutations, and sometimes compound heterozygosity for a thalassaemia mutation and a variant haemoglobin such as HbE, causes a β -thalassaemia phenotype. HbS is a haemoglobin variant; in β -thalassaemia the primary amino acid sequence of β -globin is almost always normal. β -thalassaemia mutations result in either insufficient (β^+) or a total absence (β^0) of β -globin gene expression. With this deficit in β -globin, excess α -globin becomes unstable, leading to erythroblast injury causing ineffective erythropoiesis, haemolytic anaemia and increased iron absorption.

Curing inherited diseases by somatic cell gene therapy was aspirational but now approaches reality.^{6,7} It had been hypothesized that the failure of a newborn's erythrocytes in sickle cell anaemia to sickle as readily as their mother's cells – even though the mother had sickle cell trait – as well as the paucity of symptoms in newborns with sickle cell anaemia, were attributable to the high HbF levels in infancy.⁸ Laboratory and clinical investigation suggested that if about one-third of the haemoglobin within a HbS-containing erythrocyte was HbF, this cell would be fully protected from polymer-induced damage. If nearly all erythrocytes contained sufficient HbF there would be few, if any, manifestations of sickle cell disease or β -thalassaemia. The phenotype of compound

heterozygotes for HbS and gene deletion types of hereditary persistence of HbF (HPFH) validates the ability of high levels of HbF in erythrocytes with high concentrations of HbS to prevent the phenotype of sickle cell disease. These individuals have about 30% HbF in most of their erythrocytes, with the remainder being HbS. Clinically they are usually well without presenting with anaemia.^{9,10} Understanding how HbF inhibited polymerization of HbS, knowing the residues in HbF responsible for this inhibition, finding how to best harvest, culture, genetically manipulate and transplant haematopoietic stem and progenitor cells (HSPCs), along with the development of programmable nucleases have led to the clinical approval of gene therapy for transfusion dependent β -thalassaemia and sickle cell disease.^{11,12}

In this manuscript, clinical gene therapy approaches to treat sickle cell disease and transfusion-dependent β -thalassaemia are described. HSPCs are obtained from patients with these diseases, corrected either by gene addition or genomic editing, and returned to the patient. Short-term results obtained showed disappearance of symptoms in patients with sickle cell disease and transfusion independence in patients with β -thalassaemia. Long-term efficacy and safety require additional follow up.

Inducing foetal haemoglobin

Haemoglobin gene expression switches during progression from embryo to foetus to adult. The switch starts midway through gestation as HbF production falls and HbA production increases. It is normally complete within months after birth. In normal adults, HbF constitutes <1% of total haemoglobin. Depending on the haplotype of the HbS gene, adults with sickle cell disease have between 5% and 16% HbF; homozygotes for β^0 -thalassaemia have 100% HbF, albeit at concentrations insufficient to sustain normal life.¹³ The controls of the switching process are partly understood. Switching is most dependent on the activation of HbF repressors like the genes *BCL11A* and *ZBTB7A*.¹³⁻¹⁵

Cell-based therapies inducing about 40% HbF, or a HbF-like haemoglobin in most erythrocytes, can – at least for the duration of the studies to date – 'cure', or 'nearly cure', β -haemoglobinopathies. In sickle cell anaemia, HbF exerts its powerful effect because it dilutes the concentration of HbS; more importantly, the chances of both the HbF tetramer and the hybrid tetramer $\alpha_2\gamma\beta^S$ entering the HbS polymer phase are nil.¹⁶ In β -thalassaemia, γ -globin pairs with α -globin, forming HbF.

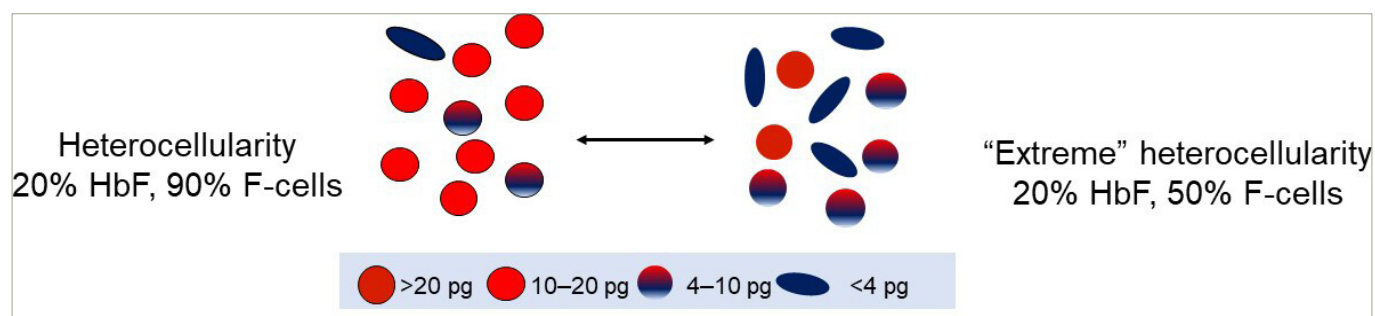
Erythrocytes with HbF detectable by fluorescent activated cell sorting (FACS) are called F-cells. They arise randomly from erythroblasts that give rise to both HbF-containing and non-HbF containing erythrocytes. F-cells are not foetal erythrocytes even though they have high levels of HbF.¹⁷

F-cells survive longer than non-HbF cells; in sickle cell anaemia 2–80% of red cells are F-cells. Hydroxyurea treatment increases the number of F-cells and the concentration of HbF/F-cell.

Normal erythrocytes contain ~30 pg of haemoglobin. The threshold for F-cell detection by FACS is 4–6 pg of HbF/F-cell. When HbF/F-cell is 9–12 pg, deoxyHbS polymerization should be prevented at physiologic venous and capillary O₂ saturations of 40–70%, affording the sickle erythrocyte nearly total protection from HbS polymer-induced damage. In HbS-HPFH where the total HbF level is ~30%, each cell has ~10 pg of HbF. These cells do not contain HbS polymer. Patients with sickle cell anaemia treated with hydroxyurea or patients with HbS- $\delta\beta$ -thalassaemia can have total HbF levels similar to those in HbS-HPFH, but are usually anaemic and symptomatic. The contrast between these conditions results from the pancellular distribution of HbF in HbS-HPFH versus its heterocellular distribution in the other disorders. Measuring HbF level or F-cells cannot predict disease complications in an individual because neither measure accounts for the variable distribution of HbF levels amongst F-cells. Modelling possible distributions of HbF/F-cell in patients with HbF levels of 5%, 10%, 20% and 30% showed that it is nearly impossible to have a clinically important number of fully protected F-cells when HbF is 5%; no more than 15% of cells can be protected when HbF is 10%. Most patients with these HbF levels have many complications. When HbF levels reach 20%, it is possible for nearly 25% of F-cells to have polymer-inhibiting HbF levels. HbF near 30% can be associated with relatively mild phenotype due to the possibility of up to 70% of cells being protected. The number of F-cells with polymer-inhibiting concentrations of HbF is likely to be a more important determinant of the features of sickle cell anaemia than the total number of F-cells or the concentration of HbF in the haemolysate (*Figure 1*).¹⁸

The HbF response in gene therapy differs from the HbF response to hydroxyurea. HbF levels above 20% are uncommon in adults treated with hydroxyurea. Macrocytosis with increased mean corpuscular haemoglobin (MCH) levels accompanies the HbF response. This permits greater heterogeneity of HbF among F-cells so that some F-cells can have very high HbF concentrations while more cells can have HbF concentrations below those needed for full protection. Macrocytosis, where mean corpuscular volume (MCV) increases from about 80 fL to about 90 fL is a feature of hydroxyurea treatment for children with sickle cell disease who have mean total haemoglobin levels of 9.5 g/dL and HbF of 31.5%.¹⁹ Macrocytosis is mild during HbF induction by gene therapy where pre- and post-treatment MCH levels have been similar. For example, following genomic editing of *BCL11A*, when HbF level was 44.4%, MCH and MCHC were 29.8 pg and 34.4 pg, respectively, compared with baseline levels of 29.1 pg and 34.1 pg.²⁰ Gene therapy increased

Figure 1: Heterocellularity of HbF expression: All HbF levels are not therapeutically equal



An example of HbF distribution in two individuals with HbF of 20%. On the left, 90% of cells are F-cells. Most have HbF between 10–20 pg. On the right is an example of 'extreme' heterocellularity, with 50% being F-cells and some containing HbF levels of >20 pg. HbF = foetal haemoglobin.

both F-cell number and HbF/F-cell; hydroxyurea increased F-cell numbers, but not γ -globin gene transcripts/F-cell.²¹ As HbF increased to 40% without a major increase in MCH, the near-pancellular expression of HbF at anti-sickling haemoglobin concentrations was inevitable given the observed haematologic constraints resulting in the correction of the sickle cell disease phenotype.²² Because HbF concentration/F-cell is not uniform, gene therapy-treated patients might have small populations of sickle erythrocytes with low HbF levels, which could account for the persistent low-grade haemolysis sometimes observed.

Clinical trials targeting HbF repressors by genomic editing

The genome editing repertoire is rapidly expanding. Gene editing using CRISPR-Cas9 dominates the field because of the ease of using guide RNAs directing the CRISPR complex to specific DNA sequences. Most sites in the genome can now be reached.^{23,24} Following double-strand DNA breaks, non-homologous end joining repair disrupts the normal sequence in the targeted area introducing small insertions or deletions. CRISPR-Cas9 can be targeted to disrupt specific erythroid enhancer motifs or binding sites for HbF repressor proteins, thus derepressing HbF production. In addition to the ability to create small deletions and insertions using 'traditional' CRISPR-Cas9 technology, it is also possible to achieve some single base edits by 'nicking' but not cleaving DNA.

In the first report of gene editing with CRISPR-Cas9 using a guide RNA specific for the erythroid enhancer region of *BCL11A*, a single infusion of autologous edited CD34⁺ cells (exa-cel) were given to patients with sickle cell anaemia and β -thalassaemia. Engraftment occurred after 30 days. In the patient with sickle cell disease with baseline HbF of 9.1%, HbF levels increased to 25.9% after 2 months and appeared to stabilize between 46–48% after 1 year. F-cells increased from 33.9% to 99.7%, total haemoglobin stabilized at 11–12 g/dL, and vaso-occlusive episodes stopped. The patient with transfusion-dependent β -thalassaemia, treated similarly, achieved HbF of >97% of the haemolysate with a haemoglobin concentration of 14 g/dL at 15 months.²⁰ These early results were extended into pivotal phase III trials of *ex vivo* editing of mobilized autologous CD34⁺ HSPCs from patients with sickle cell disease (CLIMB SCD-121; ClinicalTrials.gov identifier: NCT03745287) and transfusion dependent β -thalassaemia (CLIMB THAL-111; ClinicalTrials.gov identifier: NCT03655678).^{25,26} Patients were aged between 12 and 35 years. Patients with sickle cell disease had at least two episodes of acute pain in each of the 2 years prior to screening, while patients with thalassaemia with β^0/β^0 genotypes had a history of ≥ 100 mL/kg/year or ≥ 10 units/year of packed red blood cell transfusions in the previous 2 years. Prespecified interim

analyses evaluated efficacy and safety in two groups: a full analysis set of participants who received exa-cel infusion, and a primary efficacy set of participants followed for ≥ 16 months after exa-cel infusion (evaluable for primary and secondary endpoints). This interim analysis was carried out once the primary efficacy set included 27 participants in CLIMB THAL-111 trial and 17 participants in CLIMB SCD-121 trial. Study endpoints are shown in *Table 1*.

Both trials met the primary and key secondary endpoints. In thalassaemia, transfusion independence continued for up to 40.7 months; in sickle cell disease, episodes of acute pain disappeared, with no hospitalizations for sickle cell acute events recorded for up to 36.5 months. In previously transfusion-dependent β^0 -thalassaemia, 24 of 27 participants met the primary (TI12) and key secondary (TI6) endpoints ($p < 0.0001$), with normal mean haemoglobin and a mean transfusion-free duration of 20.5 months. Three participants who did not meet the primary and key secondary endpoints have either stopped transfusions or had 80% and 96% reduction in transfusions. In sickle cell disease, 16 of 17 participants (94.1%) met the primary endpoint (VF12) of being acute event-free for at least 12 months ($p = 0.0001$), with a mean acute event-free duration of 18.7 months. All 17 participants met the key secondary endpoint (HF12) of no in-patient hospitalizations for acute events for at least 12 months ($p < 0.0001$). In addition, all participants had early and sustained increases in total haemoglobin and HbF that was distributed nearly pancellularly. Markers of haemolysis normalized or decreased. Patient-reported quality-of-life measures improved by month 6 and were sustained through month 24. Stable high-level *BCL11A* editing was seen in bone marrow and peripheral blood: 84.4% and 74.4%, respectively, at 6 months, and 87.6% and 77.3%, respectively, at 24 months, suggesting that a long-lived HPSC was successfully modified and the effects of treatment should be durable. The safety profile of exa-cel was consistent with busulfan myeloablative conditioning and autologous haematopoietic stem cell transplantation based on the 83 participants who were dosed.²⁷ Available evidence, albeit limited, strongly suggests that induction of HbF to levels >40% in sickle cell disease will not adversely impact foetal development or normal physiology. In β^0 -thalassaemia homozygotes, the high oxygen affinity (low P_{50}) associated with 100% HbF may hypothetically cause babies to have a lower birth weight than normal, a far better outcome than the fraught pregnancies in transfusion-dependent β -thalassaemia.²⁸

In another gene therapeutic approach, engineered CD34⁺ cells were transduced with a lentiviral vector containing a 'foetal-like' HbA gene (*HbA^{T87Q}*) in 35 patients with sickle cell disease with at least four acute painful episodes in the 2 years before enrollment. In 25 patients evaluated

Table 1: Endpoints of CLIMB THAL-111 trial for transfusion-dependent β -thalassaemia and CLIMB SCD-121 trial for sickle cell disease²⁷

| | Transfusion-dependent β -thalassaemia | Sickle cell disease |
|---|--|---|
| Primary efficacy endpoint | Proportion of patients who are transfusion-independent for 12 consecutive months with average haemoglobin of ≥ 9 g/dL (TI12) | Proportion of participants free of severe VOCs for ≥ 12 consecutive months (VF12) |
| Key secondary endpoint | Proportion of patients who are transfusion-independent for 6 consecutive months with average haemoglobin of ≥ 9 g/dL (TI6) | Proportion of participants free from in-patient hospitalization for severe VOCs for ≥ 12 consecutive months (HF12) |
| Secondary & other efficacy endpoints | <ul style="list-style-type: none"> Total haemoglobin concentration HbF concentration F-cells Alleles with intended genetic modification in peripheral blood and CD34⁺ cells of the bone marrow Change over time in patient-reported outcome measures | |
| Safety endpoints | <ul style="list-style-type: none"> Neutrophil and platelet engraftment Safety and tolerability assessments, including adverse events, clinical laboratory values, and vital signs | |

CD34 = cluster of differentiation 34; HbF = foetal haemoglobin; VOC = vaso-occlusive crisis.

after 6 months, acute events stopped, haemoglobin increased to about 11 g/dL and 40% of total haemoglobin was HbA^{T87Q}, detectable in 85% of erythrocytes. Haemolysis was not eliminated, but was mild.²⁹ Similarly encouraging results with the same vector were found in 23 patients with non- β^0/β^0 , a genotype intrinsically milder than the homozygous β^0 genotype, where 91% of 20 evaluable patients had sustained average haemoglobin levels of 11.7 g/dL over 29.5 months (ClinicalTrials.gov identifier: NCT02906202).³⁰ The same treatment used in 9 patients with either β^0/β^0 or severe β^+ genotypes decreased transfusions by 73%, eliminating them in 3 patients.³¹

Knocking down *BCL11A* mRNA using an inhibitory microRNA delivered through lentiviral transduction of CD34⁺ cells, led to a 90% reduction of *BCL11A* mRNA with 60–70% γ -chain and HbF expression. In 6 patients with sickle cell disease followed for 7 to 29 months, HbF was 20.4–41.3%, levels of F-cells were 58.9–93.6%, haemoglobin levels were 9.3–11.4 g/dL and HbF/F-cell was calculated to be 9.0–18.6 pg; patients had few or no symptoms.³²

Disrupting the HbF gene promoters with CRISPR-Cas9 in 3 patients with sickle cell anaemia resulted in HbF levels of 22–29%, F-cell levels of 69.7–87.9% and continued mild haemolytic anaemia, with a reduction, but not cessation, of acute sickle vaso-occlusive events. These results, while short term in only 3 patients, seem inferior to those of the other three

reported gene therapy trials, illustrating the importance of achieving HbF levels high enough to force 'curative' levels of pancellularity.^{22,33}

Conclusion

Beyond these phase II/III clinical trials, the field is rapidly being transformed by *in vivo* delivery of base or prime editors via lipid nanoparticles or viral vectors directed to HPSCs, which target HbF repressor protein binding domains or the site of the HbS mutation.^{34–37} Ultimately, if cell-based or vectorized genetic modalities are safe, effective and similarly priced, how might one choose between them and allogeneic stem cell transplantation? Gene therapy does not require the immunosuppression associated with allogeneic stem cell transplantation. Off-target consequences of genome editing, adverse effects of semi-random integration of lentivirus and the sustainability of therapeutic effects need long-term investigation. Experience with allogeneic transplantation is far greater and matched-sibling transplants, especially in young children, provide excellent results.³⁸ Gene therapy will also have to compete with the improving prospects for haploidentical allogeneic transplantation. Myeloablative genotoxic conditioning is currently the standard for all cell-based therapeutics. For enhanced safety and greater acceptance, other forms of conditioning are being developed.^{33,39} Approved and soon to be approved gene therapies for β -haemoglobinopathies are likely to be the first steps in a long process leading to an effective 'curative' treatment for mankind's most common monogenic diseases. □

- Steinberg MH, Forget BG, Higgs DR, Weatherall DJ (eds). *Disorders of Hemoglobin: Genetics, Pathophysiology, Clinical Management*. 2nd ed. Cambridge: Cambridge University Press, 2009: 826. DOI: 10.1017/CBO9780511596582.
- Gladwin MT, Kato GJ, Novelli EM. Sickle Cell Disease. *McGraw Hill*. 2021;692.
- Weatherall DJ, Clegg JB (eds). *The Thalassemia Syndromes*. 4th ed. Wiley-Blackwell, 2008.
- Piel FB. The present and future global burden of the inherited disorders of hemoglobin. *Hematol Oncol Clin North Am*. 2016;30:327–41. DOI: 10.1016/j.hoc.2015.11.004.
- GBD 2021 Sickle Cell Collaborators. Sickle cell disease collaborators, global, regional, and national prevalence and mortality burden of sickle cell disease, 2000–2021: A systematic analysis from the global burden of disease study 2021. *Lancet Haematol*. 2023;10:e585–99. DOI: 10.1016/S2352-3026(23)00239-9.
- Mercola KE, Cline MJ. The potentials of inserting new genetic information. *N Engl J Med*. 1980;303:1297–300. DOI: 10.1056/NEJM198011273032211.
- Steinberg MH. Fetal-like hemoglobin in sickle cell anemia. *N Engl J Med*. 2022;386:689–91. DOI: 10.1056/NEJMe2119760.
- Watson J. The significance of the paucity of sickle cells in newborn Negro infants. *Am J Med Sci*. 1948;215:419–23. DOI: 10.1097/00000441-194804000-00008.
- Ngo DA, Aygun B, Akinsheye I, et al. Fetal haemoglobin levels and haematological characteristics of compound heterozygotes for haemoglobin S and deletional hereditary persistence of fetal haemoglobin. *Br J Haematol*. 2012;156:259–64. DOI: 10.1111/j.1365-2141.2011.08916.x.
- Steinberg MH. Fetal hemoglobin in sickle hemoglobinopathies: High Hbf genotypes and phenotypes. *J Clin Med*. 2020;9:3782. DOI: 10.3390/jcm9113782.
- Rubin R. New gene therapy for β -thalassaemia. *JAMA*. 2022;328:1030. DOI: 10.1001/jama.2022.14709.
- Larkin HD. Gene therapy for sickle cell disease, β -thalassaemia enters regulatory reviews. *JAMA*. 2022;328:1798. DOI: 10.1001/jama.2022.18448.
- Steinberg MH, Thein SL. Fetal hemoglobin (Hb F) in health and disease. UpToDate. 2022. Available at: www.uptodate.com/contents/fetal-hemoglobin-hb-f-in-health-and-disease?search=Fetal%20hemoglobin&source=search_result&selectedTitle=1-93&usage_type=default&displ_rank=1 (Date last accessed: 30 November 2023).
- Orkin SH. Molecular medicine: Found in translation. *Med*. 2021;2:122–36. DOI: 10.1016/j.medj.2020.12.011.
- Wienert B, Martyn GE, Funnell APW, et al. Wake-up sleepy gene: Reactivating fetal globin for beta-hemoglobinopathies. *Trends Genet*. 2018;34:927–40. DOI: 10.1016/j.tig.2018.09.004.
- Schechter AN, Noguchi CT. Sickle hemoglobin polymerization: structure-function correlates. In: Embury SH, Heibel RP, Mohandas N, Steinberg MH, (eds). *Sickle Cell Disease: Basic Principles and Clinical Practice*. Raven, NY, 1994:902.
- Khandros E, Blobel GA. Heterogeneity of fetal hemoglobin production in adult red blood cells. *Curr Opin Hematol*. 2021;28:164–70. DOI: 10.1097/MOH.0000000000000640.
- Steinberg MH, Chui DHK, Dover GJ, et al. Fetal hemoglobin in sickle cell anemia: A glass half full. *Blood*. 2014;123:481–5. DOI: 10.1182/blood-2013-09-528067.
- McGann PT, Ware RE. Hydroxyurea therapy for sickle cell anemia. *Expert Opin Drug Saf*. 2015;14:1749–58. DOI: 10.1517/14740338.2015.1088827.
- Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR-Cas9 gene editing for sickle cell disease and β -thalassaemia. *N Engl J Med*. 2021;384:252–60. DOI: 10.1056/NEJMoa2031054.
- Khandros E, Huang P, Peslak SA, et al. Understanding heterogeneity of fetal hemoglobin induction through comparative analysis of F and A erythroblasts. *Blood*. 2020;135:1957–68. DOI: 10.1182/blood.2020005058.
- Sebastiani P, Steinberg MH. Fetal hemoglobin per Erythrocyte (Hbf/F-cell) after gene therapy for sickle cell anemia. *Am J Hematol*. 2023;98:E32–4. DOI: 10.1002/ajh.26791.
- Doudna JA. The promise and challenge of therapeutic genome editing. *Nature*. 2020;578:229–36. DOI: 10.1038/s41586-020-1978-5.
- Walton RT, Christie KA, Whittaker MN, Kleinstiver BP. Unconstrained genome targeting with near-pamless engineered CRISPR-Cas9 variants. *Science*. 2020;368:290–6. DOI: 10.1126/science.aba8853.
- ClinicalTrials.gov. A safety and efficacy study evaluating CTX001 in subjects with severe sickle cell disease. ClinicalTrials.gov ID: NCT03745287. 2023. Available at: <https://clinicaltrials.gov/study/NCT03745287> (Date last accessed: 30 November 2023).
- ClinicalTrials.gov. A safety and efficacy study evaluating Ctx001 in subjects with transfusion-dependent β -thalassaemia. ClinicalTrials.gov ID: NCT03655678. 2023. Available at: <https://clinicaltrials.gov/study/NCT03655678> (Date last accessed: 30 November 2023).
- Locatelli F, Lang P, Corbacioglu S, et al. Transfusion independence and elimination of vaso-occlusive crises after exagamlogene autotemcel in transfusion-dependent β -thalassaemia and severe sickle cell disease. Presented at: 27th Congress of the European Hematology Association (EHA), June 9–17, 2022, Vienna, Austria.
- Steinberg MH. Fetal hemoglobin in β hemoglobinopathies: Is enough too much? *Am J Hematol*. 2022;97:676–8. DOI: 10.1002/ajh.26518.
- Kanter J, Walters MC, Krishnamurti L, et al. Biologic and clinical efficacy of lentiglobin for sickle cell disease. *N Engl J Med*. 2022;386:617–28. DOI: 10.1056/NEJMoa2117175.
- Locatelli F, Thompson AA, Kwiatkowski JL, et al. Betibeglogene autotemcel gene therapy for non- β^0/β^0 genotype β -thalassaemia. *N Engl J Med*. 2022;386:415–27. DOI: 10.1056/NEJMoa2113206.
- Thompson AA, Walters MC, Kwiatkowski J, et al. Gene therapy in patients with transfusion-dependent β -thalassaemia. *N Engl J Med*. 2018;378:1479–93. DOI: 10.1056/NEJMoa1705342.
- Esrick EB, Lehmann LE, Biffi A, et al. Post-transcriptional genetic silencing of *BCL11A* to treat sickle cell disease. *N Engl J Med*. 2021;384:205–15. DOI: 10.1056/NEJMoa2029392.
- Sharma A, Boelens J-J, Cancio M, et al. CRISPR-Cas9 editing of the *HBG1* and *HBG2* promoters to treat sickle cell disease. *N Engl J Med*. 2023;389:820–32. DOI: 10.1056/NEJMoa2215643.
- Breda L, Papp TE, Triebwasser MP, et al. *In vivo* hematopoietic stem cell modification by mRNA delivery. *Science*. 2023;381:436–43. DOI: 10.1126/science.ade6967.
- Li C, Georgakopoulou A, Newby GA, et al. *In vivo* HSC prime editing rescues sickle cell disease in a mouse model. *Blood*. 2023;141:2085–99. DOI: 10.1182/blood.2022018252.
- Shi D, Toyonaga S, Anderson DG. *In vivo* RNA delivery to hematopoietic stem and progenitor cells via targeted lipid nanoparticles. *Nano Lett*. 2023;23:2938–44. DOI: 10.1021/acs.nanolett.3c00304.
- Everette KA, Newby GA, Levine RM, et al. *Ex vivo* prime editing of patient hematopoietic stem cells rescues sickle-cell disease phenotypes after engraftment in mice. *Nat Biomed*. 2023;7:616–28. DOI: 10.1038/s41551-023-01026-0.
- Krishnamurti L. Should young children with sickle cell disease and an available human leukocyte antigen identical sibling donor be offered hematopoietic cell transplantation. *Hematol Oncol Stem Cell Ther*. 2020;13:53–7. DOI: 10.1016/j.hemonc.2019.12.008.
- Gao C, Schroeder JA, Xue F, et al. Nongenotoxic antibody-drug conjugate conditioning enables safe and effective platelet gene therapy of hemophilia A mice. *Blood Adv*. 2019;3:2700–11. DOI: 10.1182/bloodadvances.2019000516.