

Annals of Stem Cell Research

Review Article

Gene-Edited Haematopoietic Stem Cells for the Treatment of Inherited Haemoglobinopathies

Kovacs B^{1*}, Fontaine T² and Holmgren O³

¹Department of Cell and Tissue Engineering, Semmelweis University, Budapest, Hungary

²Department of Biomedical Engineering, Sorbonne University, Paris, France

³Department of Cell and Tissue Engineering, Karolinska Institute, Stockholm, Sweden

*Address for Correspondence: Balazs Kovacs, Department of Cell and Tissue Engineering, Semmelweis University, Budapest, Hungary; E-mail: balazs.kovacs@semmelweisuniversity.edu

Received: 18 May 2024; Accepted: 24 August 2024; Published: 15 November 2024; DOI: 10.5281/zenodo.22274335

Citation of this article: Kovacs B, Fontaine T, Holmgren O (2024) Gene-Edited Haematopoietic Stem Cells for the Treatment of Inherited Haemoglobinopathies. Ann Stem Cell Res, 7(1): 01-08.

Copyright: © 2024 Balazs Kovacs, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Sickle cell disease and beta-thalassaemia are the commonest monogenic disorders worldwide; the Global Burden of Disease 2021 analysis estimated 7.74 million people living with sickle cell disease.

Objective: To set out what the primary trial data show for lentiviral gene addition and nuclease editing of autologous haematopoietic stem cells, and what they leave open.

Methods: Narrative review of registrational trials, preclinical editing studies and safety reports.

Findings: In severe sickle cell disease, 29 of 30 evaluable patients (97%) were free of vaso-occlusive crises for at least 12 months, from 44 infused. In transfusion-dependent beta-thalassaemia, 32 of 35 (91%) achieved transfusion independence from 52 infused, with mean haemoglobin 13.1 g/dL.

Conclusions: The evidence supports durable correction by two distinct routes in small single-arm trials, but not long-term safety: the leukaemia signal is unexplained and off-target quantification incomplete.

Keywords: Haematopoietic stem cells; Gene editing; Sickle cell disease; Beta-thalassaemia; Fetal haemoglobin

Introduction

Sickle cell disease and beta-thalassaemia are the world's commonest monogenic disorders, and the affected gene's product is made almost exclusively in a lineage that can be removed, manipulated in culture and returned to the patient [1,2]. That has made them the proving ground for autologous haematopoietic stem and progenitor cell (HSPC) genetic therapy [3]. The Global Burden of Disease 2021 analysis estimated 7.74 million people (uncertainty interval 6.51-9.2 million) living with sickle cell disease in 2021, a 41.4% increase from 5.746 million in 2000, with 515,000 affected births, 34,400 cause-specific deaths against 376,000 deaths for which sickle cell disease was a contributing cause, and 81,100 deaths in children under five [4].

Two engineering strategies have produced durable clinical responses. The first adds a functional beta-globin transgene by lentiviral transduction; the second uses a nuclease to disable the developmental repression of fetal haemoglobin. Both were licensed only after allogeneic transplantation had established that replacing the marrow's erythroid output cures the phenotype, and both inherit that procedure's central liability: myeloablative conditioning. This review sets out what the trial data show, where the approaches converge, and which questions the published record does not yet answer. The emphasis is on denominators: patients infused, patients evaluable for the primary endpoint, and duration of follow-up. Those three numbers determine what can be inferred from single-arm trials in a disease with a decades-long natural history.

Fetal Haemoglobin as a Druggable Switch

The rationale for reactivating gamma-globin predates the tools that now do it: individuals carrying both a beta-globin mutation and a hereditary persistence of fetal haemoglobin allele are protected, marking the perinatal globin switch as a therapeutic target. BCL11A was then

shown to act as a developmental stage-specific repressor of the gamma-globin genes, with knockdown restoring HbF expression in adult erythroid cells [5]. BCL11A is not, however, a safe target for whole-gene disruption, being required in B lymphopoiesis and elsewhere. An intronic erythroid enhancer of BCL11A, carrying common variants associated with HbF level in genome-wide association studies, then proved dispensable outside erythroid cells [6]. Saturating Cas9 mutagenesis mapped its functional core to a narrow window around the +58 DNase I hypersensitive site and a GATA1 binding motif within it [7]. The clinical products that followed cut precisely there.

A parallel line of work attacks the gamma-globin promoters directly, recreating by nuclease editing the small deletions and point mutations that cause benign hereditary persistence of fetal haemoglobin [8]. Disrupting the binding site for the repressor LRF/ZBTB7A in the HBG promoters restores fetal haemoglobin synthesis and corrects the sickling phenotype in patient-derived cells [9]. Both routes converge on the same physiological endpoint but differ in what else they perturb: enhancer disruption removes a cis-regulatory element and leaves BCL11A intact elsewhere; promoter editing leaves BCL11A untouched altogether. No trial has compared them.

Nuclease Editing of the BCL11A Erythroid Enhancer in the Clinic

The first report of CRISPR-Cas9 editing in this indication described approximately 80% of BCL11A erythroid enhancer alleles modified in healthy-donor CD34+ cells after Cas9 ribonucleoprotein electroporation, no evidence of off-target editing, and outcomes in two patients, one with transfusion-dependent beta-thalassaemia (TDT) and one with sickle cell disease [10]. The pivotal datasets followed three years later. In severe sickle cell disease, 29 of 30 evaluable patients (97%; 95% CI 83-100) were free of vaso-occlusive crises for at least 12 consecutive months and 30 of 30 (100%; 95% CI 88-100) were free of

crisis-related hospitalisation, from 44 patients infused [11]. In TDT, 32 of 35 evaluable patients (91%; 95% CI 77-98; P<0.001 against a 50% null) achieved transfusion independence, from 52 infused, at a median follow-up of 20.4 months (range 2.1-48.1) [12].

Table 1: Ex vivo HSPC products for the beta-haemoglobinopathies, with the number of patients infused and the reported primary outcome on the evaluable subset [11,12,19,20,13,14].			
Product	Disease	Infused	Primary outcome
Exa-cel	SCD	44	29/30 (97%) VOC-free
Exa-cel	TDT	52	32/35 (91%) transfusion-free
Beti-cel	TDT	23	20/22 (91%) transfusion-free
Lovo-cel	SCD	35	25/25 severe VOs resolved
BCH-BB694	SCD	6	HbF/(F+S) 20.4-41.3%
OTQ923	SCD	3	HbF 21.8-25.0%

During transfusion independence, mean total haemoglobin was 13.1 g/dL and mean fetal haemoglobin 11.9 g/dL, with HbF distributed pancellularly across at least 94% of red cells (n=35) [12]. Pancellularity distinguishes this approach from pharmacological HbF induction: a mean HbF fraction confined to a minority of cells leaves an unprotected population; broadly distributed HbF raises the sickling threshold in essentially every erythrocyte.

Two smaller studies test the alternative HbF strategies. Post-transcriptional silencing of BCL11A by a lentivirally delivered erythroid-specific microRNA-adapted shRNA gave HbF/(F+S) ratios of 20.4-41.3% at latest follow-up, F-cells at 58.9-93.6% of untransfused red cells and 9.0-18.6 pg HbF per F-cell in six patients at a median 18 months (range 7-29) [13]. Editing the HBG1 and HBG2 promoters with a guide selected from a tiling screen of 72 gRNAs produced stable HbF of 21.8-25.0% and F-cells of 71-84.5% over 6-18 months in three patients [14]. An independent group reported BCL11A +58 enhancer editing in two children with beta0/beta0 TDT, with haemoglobin rising from 8.2 and 10.8 g/dL at screening to 15.0 and 14.0 g/dL at last visit, marrow editing persisting at 85.46% and 89.48%, and both children transfusion-independent

beyond 18 months [15]. Denominators of two, three and six are proof of mechanism, not efficacy estimates, but informative in one respect: the HbF fractions for shmiR knockdown and promoter editing, 20.4-41.3% and 21.8-25.0%, sit well below the 11.9 g/dL mean HbF for enhancer editing in thalassaemia, and the F-cell fractions, 58.9-93.6% and 71-84.5%, fall short of the at least 94% pancellularity of exa-cel [13,14,12]. The populations differ, sickle cell disease in the smaller studies and thalassaemia in the larger, so the comparison is suggestive rather than decisive, but such differences determine whether residual sickling persists.

Lentiviral Gene Addition: The Comparator That Set the Bar

Gene addition reached patients first. The index case in beta-thalassaemia achieved transfusion independence, but with a clonal dominance driven by vector insertion into HMGA2 that showed early that insertional effects here are real [16]. The approach was extended to sickle cell disease in a single patient using the anti-sickling betaA-T87Q transgene [17] and then to multicentre cohorts in TDT [18].

With betibeglogene autotemcel in non-beta0/beta0 TDT, 20 of 22 evaluable patients (91%) achieved transfusion independence, including 6 of 7 (86%) younger than 12 years, with average haemoglobin during transfusion independence of 11.7 g/dL; 23 patients were treated, at a median follow-up of 29.5 months [19]. In sickle cell disease, lovotibeglogene autotemcel raised median total haemoglobin from 8.5 g/dL at baseline to at least 11 g/dL from month 6 through month 36, with HbA-T87Q contributing at least 40% of total haemoglobin and present in a mean 85 +/- 8% of red cells across 35 patients infused at a median follow-up of 17.3 months (range 3.7-37.6) [20]. All 25 evaluable patients had complete resolution of severe vaso-occlusive events, against a median of 3.5 events per year (range 2.0-13.5) in the 24

months before enrolment, with no haematological cancers observed in up to 37.6 months [20]. Long-term follow-up of the HGB-205 cohort, seven patients aged 13-21 treated 4.6-7.9 years before analysis at a median 4.5 years of follow-up, found all four TDT patients transfusion-free but only two of three sickle cell patients in sustained clinical remission [21].

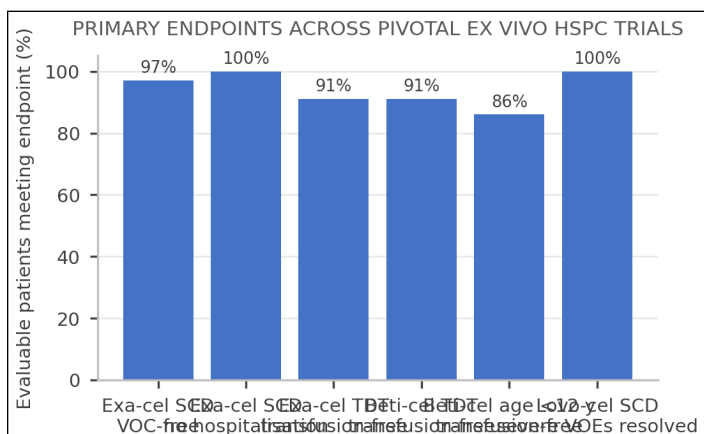


Figure 1: Proportion of evaluable patients meeting the primary endpoint in the pivotal ex vivo HSPC trials; denominators are evaluable subsets (30, 30, 35, 22, 7 and 25 patients) rather than all infused patients (44, 52, 23 and 35 infused) [11,12,19,20].

Sickle cell disease appears the harder indication for gene addition, consistent with the pathophysiology: thalassaemia requires only enough globin output to abolish the transfusion requirement, whereas sickle cell disease requires that the anti-sickling species outcompete HbS in individual cells throughout their lifespan. Gene addition and enhancer editing produce comparable headline response rates, and no randomised comparison exists. Nor are the response rates directly comparable: they are computed on evaluable subsets (30 of 44, 35 of 52, 22 of 23, 25 of 35) rather than on all infused patients, and evaluability criteria differ between protocols.

Base and Prime Editing: Repairing the Causal Allele

Homology-directed repair of the sickle allele in HSPCs, though demonstrated at the HBB locus with high efficiency in cultured cells, has been limited by the preference of long-term repopulating cells for

non-homologous end joining [22]. Optimised ribonucleoprotein delivery raised editing in true repopulating cells substantially [23], but the break-free editors are more consequential.

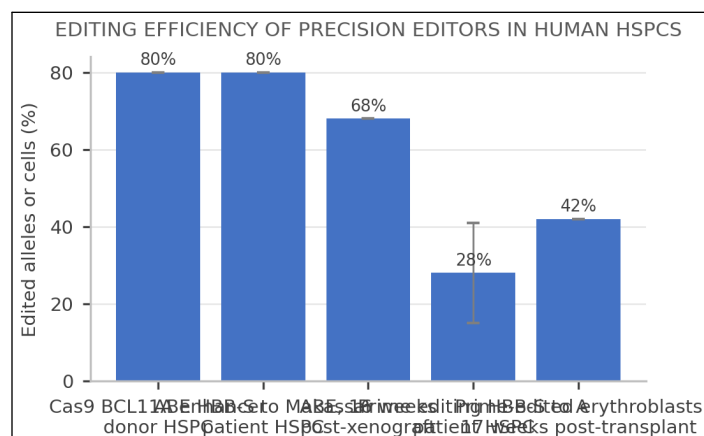


Figure 2: Editing efficiencies reported for nuclease, adenine base and prime editing in human HSPCs, the last two measured in patient cells from 4 sickle cell donors and after xenotransplantation in mice; the prime-editing bar shows the midpoint of a 15-41% range [10,24,27].

Adenine base editing converts the sickle codon to the benign Makassar variant without a double-strand break. In patient HSPCs, 80% of HBB-S alleles were converted ex vivo, 68% HBB-G persisted 16 weeks after xenotransplantation, hypoxia-induced sickling fell fivefold, and at least 20% editing sufficed for phenotypic rescue in mice [24]. Base editing has also targeted the gamma-globin promoter: homozygous -175A>G edited erythroid colonies expressed 81 +/- 7% HbF against 17 +/- 11% in unedited controls [25]. Therapeutic base editing of human HSPCs at the BCL11A enhancer motif has been demonstrated in parallel [26]. Prime editing, which writes the corrected sequence directly, restored HBB-S to HBB-A at 15-41% in patient HSPCs, with an average 42% of human erythroblasts and reticulocytes expressing HBB-A 17 weeks after transplantation across four donors and adult haemoglobin at 28-43% of normal levels [27].

These are mouse and xenograft data: no clinical outcome from a base- or prime-edited HSPC product in haemoglobinopathy is reported in the literature reviewed

here, and the efficiencies above should not be read as predictions of clinical benefit.

Genotoxicity, Conditioning and the Unmeasured Risks

Two malignancies in lentiviral sickle cell trials define the safety debate. A myelodysplastic syndrome was detected 36 months after infusion in a patient whose drug product carried 1.3 vector copies per diploid genome and whose peripheral-blood copy number was 0.08-0.15 over three years, with monosomy 7 and an abnormal 19p in 8 of 20 metaphases and approximately 10% malignant myeloblasts in marrow; the vector was not implicated [28]. A case of acute myeloid leukaemia in the same programme was similarly investigated [29]. The competing explanations, insertional mutagenesis, busulfan exposure, the intrinsic marrow stress of sickle cell disease, or some combination, have not been separated [30]. The question applies to nuclease editing as much as to gene addition, since both use the same conditioning.

For the editing products, off-target evidence is reassuring but not complete. Ultra-deep sequencing of Cas9-edited human HSPCs found no tumorigenic variants attributable to Cas9 except at an intentional positive-control guide, which produced a mean 19.3% variant allele frequency at EZH2 in two of three donors; a single SNP in the guide spacer abolished that off-target activity in the third donor, an incidental demonstration that off-target risk is genotype-dependent [31]. The clinical enhancer-editing report states no evidence of off-target editing [10], but no quantitative genome-wide off-target frequency for the clinical product has been published in the sources reviewed here. Table 2 sets these safety observations side by side with the number of patients or donors behind each.

Table 2: Reported genotoxicity and off-target findings across the ex vivo HSPC programmes, with the patient or donor denominator behind each observation [28,20,31,10].

Setting	Finding	Key value	N
Lentiviral SCD product	MDS at 36 months	Product VCN 1.3 c/dg	1
Lovo-cel, SCD	No haematological cancer	Up to 37.6 months	35
Cas9 HSPCs, deep sequencing	Off-target only at control guide	19.3% VAF at EZH2	2 of 3 donors
BCL11A enhancer, first report	No evidence of off-target editing	80% alleles edited	2

Conditioning remains the dominant near-term harm. Every pivotal trial cited used myeloablative busulfan, stated explicitly for exa-cel in TDT, beti-cel and OTQ923 [12,19,14]. Quantitative conditioning data, busulfan exposure targets, gonadal toxicity and infertility rates, and duration of neutropenia, are not established in the primary reports assembled here. Nor could median neutrophil or platelet engraftment days be verified; the TDT report states only that neutrophils and platelets engrafted in every patient [12]. A therapy whose principal toxicity is the preparative regimen will not reach the paediatric and low-resource populations carrying most of the burden until that regimen is replaced. Antibody-based conditioning and in vivo HSPC targeting are the obvious candidates, but neither has been reported in a haemoglobinopathy trial in the literature assembled here, and neither should be described as imminent.

Cost, Access and the Global Denominator

At an assumed price of \$2 million, an analysis of gene therapy versus common care for eligible United States patients with sickle cell disease produced incremental cost-effectiveness ratios of \$193,000 and \$427,000 per QALY under two alternative utility instruments from the health-sector perspective, and \$126,000 and \$281,000 per QALY from the societal perspective, with acceptable value-based prices of \$1 million to \$2.5 million [32]. The spread between the two instruments exceeds the distance from either to a conventional willingness-to-pay threshold, so the verdict currently depends on how quality of life in sickle cell disease is measured.

Set that against 7.74 million prevalent cases and 515,000 affected births per year, most of them outside high-income health systems [4]. A product requiring apheresis, GMP manufacturing, myeloablative conditioning and prolonged inpatient care addresses a numerator of hundreds. No verified data on realised list prices or low- and middle-income country access were available.

Conclusion

Ex vivo genetic modification of autologous HSPCs produces durable, clinically meaningful correction of both major beta-haemoglobinopathies, by two mechanistically distinct routes, at response rates of roughly 90-100% on evaluable subsets in single-arm trials of 23 to 52 infused patients [11,12,19,20]. Nothing in that record supports a claim of cure at population scale. Follow-up is measured in months to a few years, the leukaemia signal in the lentiviral programmes is unexplained [30], off-target quantification for clinical editing products is incomplete [31], and conditioning toxicity is unquantified in the primary reports. Base and prime editing are more precise and address the causal allele, but remain preclinical [24,27]. The two developments that would change the field are in vivo delivery to HSPCs and non-genotoxic conditioning; neither is yet established in patients.

References

1. Piel, F.B., Steinberg, M.H., Rees, D.C. (2017) Sickle Cell Disease. *New England Journal of Medicine*, 376(16): 1561-1573.
2. Taher, A.T., Weatherall, D.J., Cappellini, M.D. (2018) Thalassaemia. *The Lancet*, 391(10116): 155-167.
3. Ferrari, G., Thrasher, A.J., Aiuti, A. (2021) Gene therapy using haematopoietic stem and progenitor cells. *Nature Reviews Genetics*, 22(4): 216-234.
4. Thomson, A.M., McHugh, T.A., Oron, A.P., Teply, C., Lonberg, N., Vilchis Tella, V., et al. (2023) 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. *The Lancet Haematology*, 10(8): e585-e599.
5. Sankaran, V.G., Menne, T.F., Xu, J., Akie, T.E., Lettre, G., Van Handel, B., et al. (2008) Human Fetal Hemoglobin Expression Is Regulated by the Developmental Stage-Specific Repressor BCL11A. *Science*, 322(5909): 1839-1842.
6. Bauer, D.E., Kamran, S.C., Lessard, S., Xu, J., Fujiwara, Y., Lin, C., et al. (2013) An Erythroid Enhancer of BCL11A Subject to Genetic Variation Determines Fetal Hemoglobin Level. *Science*, 342(6155): 253-257.
7. Canver, M.C., Smith, E.C., Sher, F., Pinello, L., Sanjana, N.E., Shalem, O., et al. (2015) BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature*, 527(7577): 192-197.
8. Traxler, E.A., Yao, Y., Wang, Y.-D., Woodard, K.J., Kurita, R., Nakamura, Y., et al. (2016) A genome-editing strategy to treat beta-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition. *Nature Medicine*, 22(9): 987-990.
9. Weber, L., Frati, G., Felix, T., Hardouin, G., Casini, A., Wollenschlaeger, C., et al. (2020) Editing a gamma-globin repressor binding site restores fetal hemoglobin synthesis and corrects the sickle cell disease phenotype. *Science Advances*, 6(7): eaay9392.
10. Frangoul, H., Altshuler, D., Cappellini, M.D., Chen, Y.-S., Domm, J., Eustace, B.K., et al. (2021) CRISPR-Cas9 Gene Editing for Sickle Cell Disease and beta-Thalassemia. *New England Journal of Medicine*, 384(3): 252-260.
11. Frangoul, H., Locatelli, F., Sharma, A., Bhatia, M., Mapara, M.Y., Molinari, L., et al. (2024) Exagamglogene Autotemcel for Severe Sickle Cell Disease. *New England Journal of Medicine*, 390(18): 1649-1662.
12. Locatelli, F., Lang, P., Wall, D., Meisel, R., Corbacioglu, S., Li, A.M., et al. (2024) Exagamglogene Autotemcel for Transfusion-Dependent beta-Thalassemia. *New England*

- Journal of Medicine, 390(18): 1663-1676.
13. Esrick, E.B., Lehmann, L.E., Biffi, A., Achebe, M., Brendel, C., Ciuculescu, M.F., et al. (2021) Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease. *New England Journal of Medicine*, 384(3): 205-215.
14. Sharma, A., Boelens, J.J., Cancio, M., Hankins, J.S., Bhad, P., Azizy, M., et al. (2023) CRISPR-Cas9 Editing of the HBG1 and HBG2 Promoters to Treat Sickle Cell Disease. *New England Journal of Medicine*, 389(9): 820-832.
15. Fu, B., Liao, J., Chen, S., Li, W., Wang, Q., Hu, J., et al. (2022) CRISPR-Cas9-mediated gene editing of the BCL11A enhancer for pediatric beta0/beta0 transfusion-dependent beta-thalassemia. *Nature Medicine*, 28(8): 1573-1580.
16. Cavazzana-Calvo, M., Payen, E., Negre, O., Wang, G., Hehir, K., Fusil, F., et al. (2010) Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature*, 467(7313): 318-322.
17. Ribeil, J.-A., Hacein-Bey-Abina, S., Payen, E., Magnani, A., Semeraro, M., Magrin, E., et al. (2017) Gene Therapy in a Patient with Sickle Cell Disease. *New England Journal of Medicine*, 376(9): 848-855.
18. Thompson, A.A., Walters, M.C., Kwiatkowski, J.L., Rasko, J.E.J., Ribeil, J.-A., Hongeng, S., et al. (2018) Gene Therapy in Patients with Transfusion-Dependent beta-Thalassemia. *New England Journal of Medicine*, 378(16): 1479-1493.
19. Locatelli, F., Thompson, A.A., Kwiatkowski, J.L., Porter, J.B., Thrasher, A.J., Hongeng, S., et al. (2022) Betibeglogene Autotemcel Gene Therapy for Non-beta0/beta0 Genotype beta-Thalassemia. *New England Journal of Medicine*, 386(5): 415-427.
20. Kanter, J., Walters, M.C., Krishnamurti, L., Mapara, M.Y., Kwiatkowski, J.L., Rifkin-Zenenberg, S., et al. (2022) Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease. *New England Journal of Medicine*, 386(7): 617-628.
21. Magrin, E., Semeraro, M., Hebert, N., Joseph, L., Magnani, A., Chalumeau, A., et al. (2022) Long-term outcomes of lentiviral gene therapy for the beta-hemoglobinopathies: the HGB-205 trial. *Nature Medicine*, 28(1): 81-88.
22. Dever, D.P., Bak, R.O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C.E., et al. (2016) CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells. *Nature*, 539(7629): 384-389.
23. Wu, Y., Zeng, J., Roscoe, B.P., Liu, P., Yao, Q., Lazzarotto, C.R., et al. (2019) Highly efficient therapeutic gene editing of human hematopoietic stem cells. *Nature Medicine*, 25(5): 776-783.
24. Newby, G.A., Yen, J.S., Woodard, K.J., Mayuranathan, T., Lazzarotto, C.R., Li, Y., et al. (2021) Base editing of haematopoietic stem cells rescues sickle cell disease in mice. *Nature*, 595(7866): 295-302.
25. Mayuranathan, T., Newby, G.A., Feng, R., Yao, Y., Mayberry, K.D., Lazzarotto, C.R., et al. (2023) Potent and uniform fetal hemoglobin induction via base editing. *Nature Genetics*, 55(7): 1210-1220.
26. Zeng, J., Wu, Y., Ren, C., Bonanno, J., Shen, A.H., Shea, D., et al. (2020) Therapeutic base editing of human hematopoietic stem cells. *Nature Medicine*, 26(4): 535-541.
27. Everette, K.A., Newby, G.A., Levine, R.M., Mayberry, K., Jang, Y., Mayuranathan, T., et al. (2023) Ex vivo prime editing of patient haematopoietic stem cells rescues sickle-cell disease phenotypes after engraftment in mice. *Nature Biomedical Engineering*, 7(5): 616-628.
28. Hsieh, M.M., Bonner, M., Pierciey, F.J., Uchida, N., Rottman, J., Demopoulos, L., et al. (2020) Myelodysplastic syndrome unrelated to lentiviral vector in a patient treated with gene therapy for sickle cell disease. *Blood Advances*, 4(9): 2058-2063.
29. Goyal, S., Tisdale, J., Schmidt, M., Kanter, J., Jaroscak, J., Whitney, D., et al. (2022) Acute Myeloid Leukemia Case after Gene Therapy for Sickle Cell Disease. *New England Journal of Medicine*, 386(2): 138-147.
30. Jones, R.J., DeBaun, M.R. (2021) Leukemia after gene therapy for sickle cell disease: insertional mutagenesis, busulfan,

both, or neither. *Blood*, 138(11): 942-947.

31. Cromer, M.K., Barsan, V.V., Jaeger, E., Wang, M., Hampton, J.P., Chen, F., et al. (2022) Ultra-deep sequencing validates safety of CRISPR/Cas9 genome editing in human hematopoietic stem and progenitor cells. *Nature Communications*, 13(1): 4724.
32. Basu, A., Winn, A.N., Johnson, K.M., Jiao, B., Devine, B., Hankins, J.S., et al. (2024) Gene Therapy Versus Common Care for Eligible Individuals With Sickle Cell Disease in the United States. *Annals of Internal Medicine*, 177(2): 155-164.