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Selecting patients with sickle cell disease for gene addition or gene editing-based therapeutic approaches: Report on behalf of a joint EHA Specialized Working Group and EBMT Hemoglobinopathies Working Party consensus conference

Lucia de Franceschi[1,2] | Franco Locatelli[3] | David Rees[4] | Christian Chabannon[5] | Jean-Hugues Dalle[6] | Stefano Rivella[7,8,9] | Achille Iolascon[10,11] | Stephan Lobitz[12] | Miguel R. Abboud[13] | Josu de la Fuente[14,15] | Pagona Flevari[16] | Emanuele Angelucci[17] | Mariane de Montalembert[18,19]

Correspondence: Emanuele Angelucci (emanuele.angelucci@hsanmartino.it); Mariane de Montalembert (mariane.demontal@aphp.fr)

ABSTRACT

Sickle cell disease (SCD) remains associated with reduced life expectancy and poor quality of life despite improvements observed in the last decades mostly related to comprehensive care, use of hydroxycarbamide, screening to identify patients at risk of strokes, and implementation of safe transfusion protocols. The course of the disease is highly variable, making it difficult to predict severity and response to therapy. Allogeneic hematopoietic stem cell transplantation potentially provides a cure with a relatively low rate of complications, but few patients have an HLA-identical sibling. The hopes of patients and healthcare providers have been raised after the initial excellent results of gene therapy studies. However, there is a strong contrast between the high expectations of families and patients and the limited availability of the product, which is technically complex and very expensive. In light of this consideration and of the limited data available on the long-term efficacy and toxicity of different gene therapy approaches, the European Hematology Association Red Cell & Iron Specialized Working Group (EHA SWG) and the hemoglobinopathy working part of the European Blood & Marrow Transplant (EBMT) Group have prioritized the development of recommendations for selection of patients with SCD who are good candidates for gene therapy. The decision-making algorithm was developed by a panel of experts in hemoglobinopathies and/or transplantation chosen by EHA SWG and EBMT, to discuss the selection of SCD patients for gene therapy and draw notes on the related clinical problems.

[1]Department of Engineering for Innovative Medicine, University of Verona, Verona, Italy
[2]Azienda Ospedaliera Universitaria integrata di Verona, Verona, Italy
[3]IRCCS Bambino Gesù Children's Hospital, Catholic University of the Sacred Heart, Rome, Italy
[4]School of Cancer and Pharmaceutical Sciences, Faculty of Life Sciences Medicine, King's College London, and Department of Haematological Medicine, King's College Hospital, London, UK
[5]Institut Paoli-Calmettes Comprehensive Cancer Center and Module Biotherapies du Centre d'Investigations Cliniques de Marseille, INSERM-Aix-Marseille Université AP-HM-IPC, CBT-1409, Marseille, France
[6]Pediatric Hematology and Immunoloy Department, Robert-Debré Academic Hospital, GHU AP-HP Nord Université Paris Cité, Paris, France
[7]Department of Pediatrics, Hematology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA
[8]Penn Institute for RNA Innovation, University of Pennsylvania, Philadelphia, Pennsylvania, USA
[9]Penn Institute for Regenerative Medicine (IRM), University of Pennsylvania, Philadelphia, Pennsylvania, USA
[10]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Naples, Italy
[11]CEINGE Biotecnologie Avanzate, Naples, Italy
[12]Pediatric Hematology & Oncology, Gemeinschaftsklinikum Mittelrhein, Koblenz, Germany
[13]Department of Pediatrics and Adolescent Medicine, American University of Beirut, Beirut, Lebanon
[14]Department of Immunology and Inflammation, Centre for Haematology, Imperial College London, London, UK
[15]Department of Paediatrics, Imperial College Healthcare NHS Trust, London, UK

INTRODUCTION

Sickle cell disease (SCD) is a hereditary red blood cell disorder that is diffused worldwide and is characterized by high mortality and morbidity in adult populations, even in high-income countries.[1] Up to now, treatment of SCD patients is mainly based on hydroxycarbamide (HC), either alone or in combination with new agents, or on potentially curative approaches represented by allogeneic hematopoietic stem cell transplantation (HSCT).[2] HSCT enables long-term correction of the hemopoiesis, resulting in overall survival (OS) rates exceeding 90%.[3,4] Donor availability is the main limiting factor in both the related and unrelated settings.[5,6] Transplantation across the human leukocyte antigen (HLA) barrier with cord units has led to disappointing results.[7,8] While matched unrelated donor (MUD) transplantation provides outcomes comparable to those obtained with HLA-identical sibling HSCT in thalassemia, there is limited benefit deriving from MUD transplantation in SCD.[9–11] Graft failure remains the most significant challenge when a donor other than an HLA-identical sibling is used.[12] Although recent advances in HLA-haploidentical transplantation have overcome most of these challenges, both OS and event-free survival (EFS) remain worse than those recorded when a compatible sibling is used as a donor.[13,14]

Gene therapy strategies for SCD represent immense hope for patients, but they are very expensive, with still incompletely elucidated risks, especially in the long term.[15] Although the cost is likely to decrease over time and greater clarity will emerge about safety, because of this uncertainty and also the bottlenecks related to the manufacturing capacity it is inevitable that, for at least the next decade, gene therapy will only be available to a minority of patients with SCD. It is therefore important to identify patients most likely to benefit from this approach. This process is particularly challenging in SCD, as this disorder is characterized by a variable and highly heterogeneous clinical course among patients. Thus, the European Hematology Association Red Cell & Iron Specialized Working Group (EHA SWG) and the European Blood and Marrow Transplant (EBMT) group have prioritized the development of recommendations for selecting good SCD candidates for gene therapy.

METHODS

A decision-making algorithm was developed by experts in hemoglobinopathies and/or transplantation chosen by EHA through the EHA Specialized Working Group on Red Cells and Iron, and by EBMT through the EBMT hemoglobinopathy working party (HWP), who discussed the selection of SCD patients for gene therapy/editing and analyzed the related clinical problems (Figure 1). Among the experts, some were involved in registrational studies for gene addition therapy/gene editing in SCD. The expert opinion has been prepared to be used by specialists in hemoglobinopathy centers of excellence.

The published literature (Medline, PubMed, Embase, and Cochrane Library) was searched for high-quality evidence to define the best candidates for gene therapy. The keywords used were SCD; bone marrow transplantation; gene therapy; gene editing; hematopoietic stem cell (HSC) transplantation; hemoglobinopathies; hepatitis; cardiomyopathy, pulmonary hypertension, kidney disease; sickle cell-related cerebrovascular disease; liver and thrombotic complications. The literature evaluation/scientific evidence was reported and discussed by the EHA-EBMT expert panel. The final version of the expert opinion will be uploaded onto the EHA and EBMT, websites. It was decided, at least in this first phase of access to gene therapy, to give priority to those patients in good clinical condition, without irreversible severe organ impairment and able to tolerate myeloablative conditioning, since these are the subjects who are likely to obtain the most clinical benefit with the least risk. As mentioned previously, this cautious approach is due to both the limited clinical experience obtained during clinical trials and the probably limited availability of gene therapy, at least initially. The patient priority criteria for access to gene therapy defined in this algorithm and supporting notes is likely to vary over time as new scientific evidence emerges, and therefore, the expert opinion will be reviewed regularly and updated when new clinical data are published and/or any changes are made to the European Medicines Agency (EMA) license for gene therapy. A part of the panel experts are also members of the EuroBloodNET (https://eurobloodnet.eu). At the kickoff meeting, the working group coordinators agreed with the panelists to allow the involvement of authors of registration studies, due to their extensive knowledge of the gene addition/editing approaches, the rarity of the disease in Europe, and the limited number of expert centers for gene therapy/gene editing for patients with hemoglobinopathies. A list created by the expert panel is included in the supplementary information, providing details on the requirements and recommendations for the identification of qualified gene therapy treatment centers (supplementary material).

RECENT AGENTS APPROVED BY THE EUROPEAN MEDICINES AGENCY (EMA)—FOOD AND DRUG ADMINISTRATION (FDA) TO TREAT SCD

The primary goal of treatment of SCD is to avoid acute complications and to protect all organ systems in the long term. Drugs used for this purpose should be as tolerable, effective, easy to use, and as inexpensive as possible. To date, the portfolio of therapeutic molecules for SCD is limited, and hydroxycarbamide (HC) remains the benchmark.[16]

Hydroxycarbamide (HC) is a ribonucleotide reductase inhibitor that improves SCD manifestations through several different mechanisms. In particular, HC: (i) increases hemoglobin F (HbF) expression; (ii) improves the rheological properties of blood by reducing chronic inflammatory processes at the endothelium, leading to downregulation of adhesion factors, and thus reducing cellular interactions; (iii) decreases reticulocyte and leukocyte/neutrophil counts and positively affects the biosynthesis of nitric oxide. HC is widely considered the standard of care for all patients irrespective of clinical severity. HC reduces the frequency of vaso-occlusive crises (VOC) and acute chest syndrome (ACS) episodes, and transfusion requirements, decreases long-term morbidity through protective effects on vital organs, and, most likely, increases life expectancy.[1,17–19] It is considered effective and safe for adults and children as young as 9 months of age. Of the few side effects, cytopenias are particularly relevant, according to individual tolerance. HC dosage is usually increased up to the maximum tolerated dose (MTD) as defined by a neutrophil count between 1 and 3×10^9/L, that is, mild neutropenia not associated with an increased risk of infection.[16] HC seems suitable for lifelong use. Some long-term effects of HC, particularly on male fertility, remain controversial. The

[16]Thalassemia Unit—Center of Expertise in Haemoglobinopathies, Laiko General Hospital, Athens, Greece
[17]UO Ematologia e Terapie Cellulari, IRCCS Ospedale Policlinico San Martino, Genova, Italy
[18]Department of General Pediatrics and Pediatric Infectious Diseases, Sickle Cell Center, Necker-Enfants Malades Hospital, Assistance Publique—Hôpitaux de Paris (AP-HP), Université Paris Cité, Paris, France
[19]Laboratory of Excellence GR-Ex, Paris, France

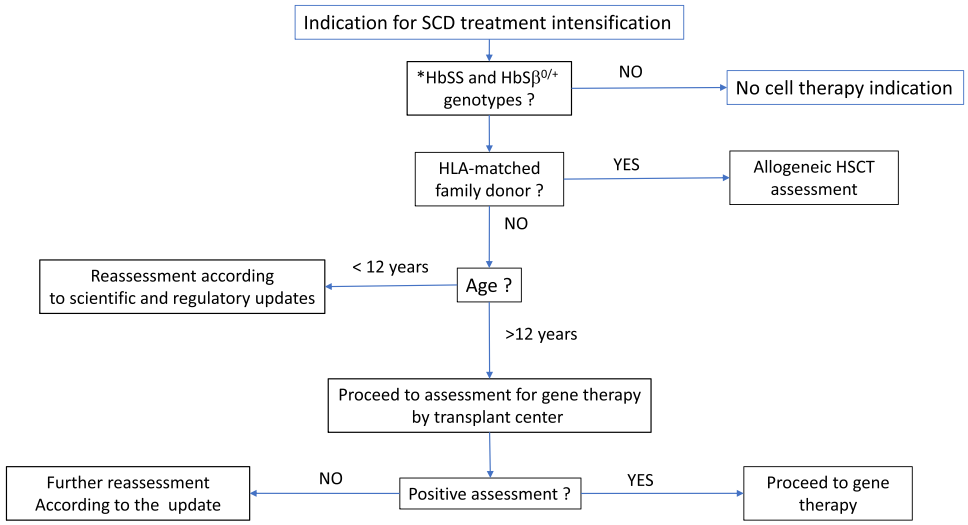

FIGURE 1 **Algorithm for the selection of patients with sickle cell disease (SCD), who might be candidates for gene therapy/gene editing approaches.** *HbSβ$^+$ with severe clinical presentation. Hb, hemoglobin; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation.

impact on ovarian reserve in women with SCD is under investigation.[20] It is also unclear whether treatment can be continued during pregnancy and lactation. Cancer risk does not appear to increase significantly with long-term HC treatment.[21] Overall, the benefits of HC clearly outweigh the risks in patients with SCD. However, in some patients, HC is either not effective or not effective enough. Overall, therapeutic adherence to life-long HC administration is limited. Alternative monotherapies or combinations of different agents with different modes of action are needed. New compounds are currently undergoing preclinical and clinical testing; details on these novel agents can be consulted in the relevant reviews.[22–24]

Voxelotor is a small, oral anti-sickling agent that binds to hemoglobin (Hb) and promotes a left shift in the P_{50} of HbS. Hence, HbS is stabilized and does not polymerize as quickly or at all. Voxelotor significantly increases hemoglobin levels in people with SCD, reduces the rate of hemolysis, and may have a positive effect on leg ulcers.[25,26] On September 25, 2024, the company producing voxelotor announced that it was voluntarily withdrawing all stocks of voxelotor for the treatment SCD, in all markets where it was approved and discontinuing all active voxelotor clinical trials and expanded access programs worldwide.

This decision was based on the totality of clinical data that now indicates that the overall benefit of voxelotor no longer outweighs the risk in the approved sickle cell patient population.

Crizanlizumab is a P-selectin inhibitor that binds to P-selectin on activated endothelial cells and platelets, preventing their interaction with P-selectin glycoprotein ligand 1 (PSGL-1) on most leukocytes and the PSGL-1-like receptor expressed on sickle cells. As a result of reduced cellular interactions, vascular occlusions become less likely. In the core publication, on which the approval in the U.S. and Europe was based, crizanlizumab was shown to significantly reduce the number of VOC.[27] However, in May 2023 the EMA's Committee for Medicinal Products for Human Use (CHMP) recommended revoking the marketing authorization for crizanlizumab, because the results of the STAND study, which randomized crizanlizumab vs. placebo, failed to show superiority of the drug.

L-Glutamine is a precursor molecule of nicotinamide adenine dinucleotide (NAD), involved in glutathione metabolism since it preserves NADPH levels required for glutathione recycling. Adequate L-glutamine levels can be maintained by external supply. The pivotal study demonstrated a significant reduction in pain crises and hospitalizations.[28] L-glutamine was approved by the FDA in 2017 but rejected by the EMA because of concern over the statistical significance of the results. The application for approval in Europe was withdrawn by the company in 2019. Notably, limited information is available on long-term use of L-glutamine supplementation. Thus, L-glutamine must be used with caution in both adult and pediatric SCD patients.

Encouraging data from early clinical trials evaluating pyruvate kinase activators (mitapivat, etavopivat) showed a decrease in the markers of hemolysis, improvement of anemia, and reduction of annualized rate of VOC in patients with SCD.[29]

ALLOGENEIC HSCT IN PATIENTS WITH SCD

Several studies analyzing a large number of patients documented that HLA-identical donor allogeneic HSCT is a potentially curative option endowed with great efficacy in preventing the tissue damage associated with vaso-occlusion occurring in SCD.[30–35]

In SCD, the main concern related to the use of allogeneic HSCT is the risk of graft-versus-host disease (GvHD). In the most comprehensive meta-analysis to date, the pooled acute GvHD (aGvH) rate was 20% (95% confidence interval [CI], 15%–25%), grade III–IV aGvHD rate was 4% (95% CI, 2% to 6%), chronic GvHD (cGvH) rate was 10% (95% CI, 7%–15%), and extensive cGvHD was 2% (95% CI, 1%–5%).[4] This latter complication mainly affects the patient's quality of life in the long term.[36]

Combined data from the Center for International Blood and Marrow Transplantation (CIBMTR), EBMT, and Eurocord has shown in the context of HLA-sibling HSCT unadjusted overall 5-year probabilities of OS and EFS of 92.9% (95% CI, 91.1%–94.6%) and 91.4% (95% CI, 89.6%–93.3%), respectively.[33] Age is the major determinant of mortality, with patients older than 14 years experiencing a worse outcome.[9,11,37] The risk of GvHD also increases with age at transplantation, whatever the donor source.[33,38] The EBMT dataset demonstrates an extremely low mortality rate, especially in children in the pre-scholar age.

HSCT enables abrogation of both VOC, ACS and stroke, allows improvement in growth velocity, stable or improved brain MRI appearances, and improved stenosis of cerebral vessels outcome in

stroke-free children as compared with those under chronic transfusion program.[34,35,38–40] Due to endothelial damage, SCD patients are at risk of developing posterior reversible encephalopathy syndrome (PRES) in the early post-transplant period because of the use of a calcineurin inhibitor as GvHD prophylaxis. Then, their blood pressure has to be closely monitored and any hypertension episode has to be treated without delay. The long-term follow-up appears similar for those patients transplanted for other underlying non-malignant diseases and requires multi-disciplinary care to identify long-term organ injury (https://www.ebmt.org/education/ebmt-handbook). Fertility impairment has to be considered, as myeloablation induces the risks of ovarian and testicular failure even when given during the pre-pubertal period.[41,42] Any available fertility preservation methods have to be discussed with the patient and family.

Experience with MUD donors, including unrelated cord blood (CB), is limited in SCD. In 2011, Ruggeri et al. reported the results of unrelated CB transplantation in 16 children with SCD. Overall and disease-free survivals were 94% and 50%, respectively, with lack of sustained engraftment of donor cells being the main cause of graft failure.[43] Regarding unrelated donor marrow transplantation from the registry, a Blood and Marrow Transplant Clinical Trial Network phase 2 trial, conducted from 2008 to 2014, enrolled 30 children aged 4 to 19 years transplanted from MUD after fludarabine-melphalan-alemtuzumab conditioning regimen. The graft rejection rate was 10% and the 1- and 2-year EFS rates were 76% and 69%, respectively. The 1-year cumulative incidence of cGvHD was 62%, with 38% of patients experiencing the extensive form of the complication.[10]

For these reasons, and because less than 20% of SCD patients have an HLA-matched sibling or unrelated donor, haplo-identical HSCT was widely developed in the last few years. Three different strategies have emerged (summarized in Table 1). The pooled aGvHD rate for haploidentical transplantation has been reported to be 14% (95% CI, 2% to 38%), while that for cGvHD is 11% (95% CI, 2% to 25%).[4] The risk of these complications seems to be higher in patients receiving a PTCy approach than in those given an *ex-vivo* T-cell depleted allograft.[4] TCRαβ haploidentical approach is associated with low rates of both grade III-IV aGvHD and cGvHD, while with PTCy, the current rates of aGvHD and cGvHD are about 30% and 10–20%, respectively. However, no comparative study with correct matched pairing is available. Additionally, TCRαβ depletion is both complex and expensive and could not be deployed widely. To note, due to its recent development, haplo-HSCT in SCD, using either PTCy or TCRαβ depletion, was mainly offered to older patients as compared to the majority of patients who received HSCT from an HLA-identical sibling donor. Then, at least in part, the worse results obtained with haploidentical transplantation may be explained by older age and higher comorbidity. The main concern with haplo-identical approaches still remains graft failure rate.[44,45]

Many protocols are applied to optimize the conditioning regimen. HSCT for SCD was established with busulfan-based conditioning, usually in combination with cyclophosphamide.[3,46] However, the finding that stable long-term mixed chimerism (with a majority of donor cells) is able to reverse the sickle phenotype enables consideration of reduction in the intensity of the conditioning regimen to minimize toxicity.[47] Cyclophosphamide replacement by fludarabine (+/− thiotepa) may contribute to reduced toxicity.[48]

Non-myeloablative (NMA) conditioning based on the use of alemtuzumab and low-dose Total Body Irradiation aims also to preserve fertility, although this benefit has yet to be confirmed in this population, and favors the application of HSCT to adults with end-organ damage.[49,50] Though preliminary results on the use of this approach in children are encouraging, it has not yet been possible to replicate the results in a multicenter study.[51] Furthermore, some cases of both myelodysplastic syndromes and acute leukemia were reported after NMA in patients affected with SCD. An increased incidence of hematologic malignancies has been reported in patients with SCD who underwent NMA allogeneic peripheral blood HSCT between 2004 and 2020.[52] As far as we know, myeloid neoplasms were never recorded after alkylating agents-based myeloablative-conditioning.[38] Different hypotheses to explain the occurrence of clonal disorders may be considered, related either to SCD itself or HSCT: non-eradication of heavily pre-treated hemopoiesis; chronic hypoxia; older patients at the time of HSCT. Notably, also non-transplanted SCD patients present slightly higher risk of such clonal proliferation as compared with the general population.[53,54]

TABLE 1 Different strategies in haplo-identical HSCT setting in SCD patients.

Strategy	Key points	Ref.
T-cell repleted haploidentical transplantation with the use of post-transplantation cyclophosphamide (PTCy)	Optimization of the original John Hopkins Group protocol while maintaining a reduced intensity approach, by the addition of thiotepa or an increase in the total body irradiation (TBI) dose from 300 to 400 cGy (has resulted in a reduction in graft failure to 6%–7%).	Luznik L, et al. *Seminars Oncol.* 2012[a]
T-cell depleted haploidentical transplantation with the use of either $CD3^+$ or TCRαβ and CD19+ depletion	Pilot studies of both approaches demonstrated the feasibility of SCD with the most recent data providing an OS of about 90%.	Handgretinger R, et al., *Expert Rev Clin Immunol.* 2022[b]; Foell J et al., *BMT* 2017[c]; Gaziev J et al., *Blood Adv.* 2018[d]; Foell J, et al., *BMT* 2019[e], Merli P, et al., *Blood Adv* 2022)[f]
CD34+ enrichment with T-cell add-back	Single-arm prospective phase 2 study, 19 patients; 2 year-EFS 84% (95% CI: 57–94.4)	Luznik L, et al. *Seminars Oncol.* 2012[a]

[a]Luznik L, O'Donnell PV, Fuchs EJ. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical bone marrow transplantation. *Semin Oncol.* 39:683-693. doi: 10.1053/j.seminoncol.2012.09.005.

[b]Handgretinger R, Arendt AM, Maier CP, Lang P. Ex vivo and in vivo T-cell depletion in allogenic transplantation: towards less or non-toxic conditioning regimen. *Expert Rev Clin Immunol.* 2022;18:1285-1296. doi. 10.1080/1744666X.2022.213 4857.

[c]Foell J, Pfirstinger B, Rehe K, Wolff D, Holler E, Corbacioglu S. Haploidentical stem cell transplantation with $CD3^+$-/$CD19^+$-depleted peripheral stem cells for patients with advanced stage sickle cell disease and no alternative donor: results of a pilot study. *Bone Marrow Transplant.* 2017;52:938-940. doi: 10.1038/bmt.2017.49.l.

[d]Gaziev J, Isgrò A, Sodani P, et al. Haploidentical HSCT for hemoglobinopathies: improved outcomes with TCRαβ$^+$/$CD19^+$-depleted grafts. *Blood Adv.* 13:263-270. doi: 10.1182/bloodadvances.2017012005.

[e]Foell J, Schulte JH, Pfirstinger B, et al. Haploidentical CD3 or α/β T-cell depleted HSCT in advanced stage sickle cell disease. *Bone Marrow Transplant.* 2019;54:1859-1867. doi: 10.1038/s41409-019-0550-0.

[f]Merli P, Pagliara D, Galaverna F, et al. TCR αβ/CD19 depleted HSCT from an HLA-haploidentical relative to treat children with different nonmalignant disorders. *Blood Adv.* 2022;6:281-292. doi: 10.1182/bloodadvances.2021005628.

Recommendations

Patient's age ≤12 years and use of an HLA-identical family donor are the variables associated with the best transplant outcomes in patients with SCD.[55] Given the very good results of HSCT using HLA-matched siblings, systematic assessment of intra-familial HLA compatibility must be recommended to discuss early the possibility of transplantation in symptomatic SCD children with an HLA-identical sibling.

GENE THERAPY/GENE EDITING IN SCD EXPERIMENTAL AND APPROVED GENETIC TREATMENTS

Current genetic treatments for the cure of SCD are based on two strategies: gene addition therapy and gene editing of autologous CD34+ cells, where CD34 expression represents a surrogate marker of HSCs (Figure 2). The first strategy uses lentiviral vectors to insert novel DNA sequences that can, directly or indirectly, increase the expression of a curative gene. These include the insertion of a therapeutic gene (i.e., the α- or β-globin genes) or a shRNA that increases expression of the γ-globin genes (by targeting the BCL11A mRNA) (Table IS in Supporting Information II).[15]

The second strategy uses, primarily but not exclusively, the CRISPR-Cas9 system that consists of a guide RNA (gRNA) complementary to the target genomic DNA sequence and a Cas9 nuclease.[56,57] After the recognition of the target sequence by the gRNA, the Cas9 nuclease is tied to the DNA-gRNA complex, generating double-strand breaks (DBS). This leads to the activation of various endogenous DNA repair mechanisms.[56,57] In human cells, the mechanism most active is the non-homologous end joining (NHEJ) one, which acts in an error-prone manner introducing small deletions and/or insertions (Indels) to the targeted genomic region, eventually disrupting the open reading frame.[58] In this setting, it has been utilized to disrupt the GATA1-binding site of the BCL11A erythroid enhancer to reactivate the synthesis of the γ-globin chains and, thus, of HbF.[59–62]

Alternatively, the CRISPR-Cas9 system has been modified to avoid the generation of DSBs. This includes the use of base editors, which are considered potentially safer than traditional gene editing approaches as they overcome the DSB-associated deleterious effects on genome integrity.[63–66] Using cytosine or adenosine base editors, base editing precisely generates targeted point mutations, without generating DSBs or requiring donor DNA templates.[63] Using this novel approach, additional sequences in the BCL11A erythroid-specific enhancer, β-globin promoter, or the SCD mutation can be targeted leading to the expression of HbF or normal HbA.[67–71]

More recently, a new approach based on the use of CRISP-Cas12 to target the promoter of the γ-chain gene has been reported with promising results.[72]

Patient selection and conditioning

Gene therapy protocols require a high number of CD34+ cells collected through apheresis. Due to the risk of hyper-viscosity and subsequent vaso-occlusive events induced by granulocyte-colony stimulating factor (G-CSF), this mobilizing agent is contra-indicated in SCD patients and mobilization is performed using only plerixafor. The total number of CD34+ cells collected with one round of mobilization is rarely sufficient, and many patients require more cycles of mobilization + apheresis.[62] This process requires weeks or months and may take longer than the recruitment of donors in an allogeneic setting. In addition, for a minority of patients (around 15%), cell collection fails, leading to gene therapy cancelation. It cannot be excluded that aging represents an additional challenge for obtaining enough stem cells for gene therapy drug production.[73]

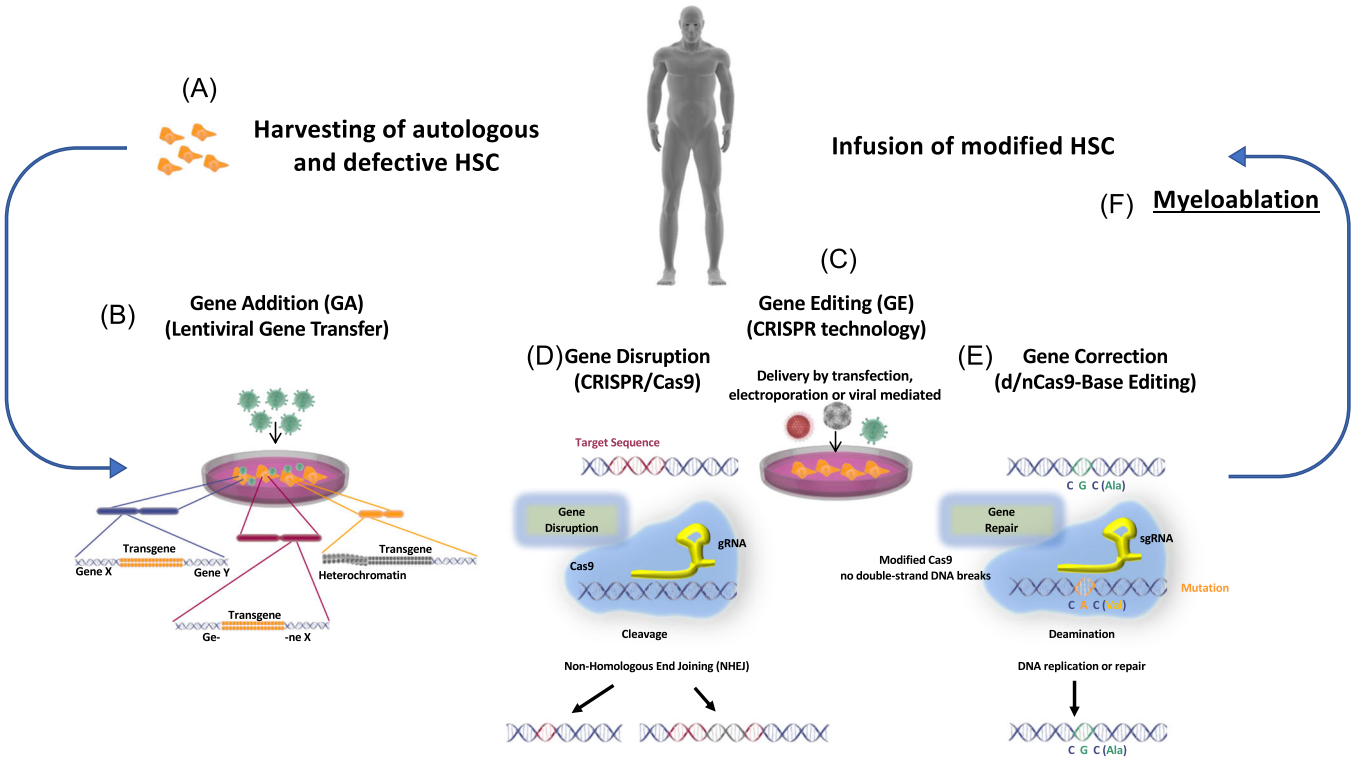

FIGURE 2 **Schematic diagram of the different strategies for gene therapy/gene editing approaches.** gRNA, guide RNA; HSC, hematopoietic stem cells.

Patients require myeloablation obtained through high-dose alkylating agent administration. Up to now, all SCD patients received a busulfan-based conditioning regimen (NCT02453477), which is associated with the same risk of ovarian and testicular failure as when given for allogeneic HSCT.

Follow-up data on gene therapy in patients with SCD

Since gene therapy has emerged as a new therapeutic possibility for SCD with lower treatment-associated risks and not limited by the availability of an HLA-identical donor, safety concerns are under continuous investigation. The observed adverse events and the toxicity profile are mostly associated and consistent with the myeloablative conditioning, but long-term data are needed as gene addition may lead to genome toxicity as the integration of the transgene may lead to clonal hematopoiesis and leukemia.[15,74–76] Moreover, the genome editing tools may trigger undesired effects on the target chromosome, such as large deletions and inversions, chromosomal truncations, chromothripsis, aneuploidy, loss of heterozygosity, and unintentional off-target effects, such as point mutations, deletions, insertions, inversions, and translocations, leading to clonal selection.[77–80] To fully understand and assess the risk of a delayed adverse event, participants in gene therapy trials will need to be monitored for an extended period of a total period of 15 years as requested by regulatory authorities.[53] It is important to mention that 2 SCD patients treated by gene addition have developed myelodysplastic syndrome (MDS) and leukemia.[15,81] which led to the transient suspension of the Bluebird bio gene therapy trial NCT02140554 (HGB-206 study) in February 2021. A participant in the HGB-206 trial had been diagnosed with MDS, which eventually transformed into acute myeloid leukemia (AML), 36 months post-LentiGlobin infusion.[82] It was determined that AML developed from busulfan conditioning due to the absence of vector integration in the leukemic cells. Another participant of the initial cohort of the HGB-206 study developed AML approximately 5.5 years after receiving LentiGlobin.[83] In the second case of AML, lentiviral integration was detected in leukemic cells, but further analysis revealed that the lentivirus insertion in close proximity to *VAMP-4*, a gene never found to be involved in leukemogenesis, was an unlikely cause of leukemia development. Notably, the two patients of group A of the HGB-206 study in which AML was developed had received an earlier version of the drug product, characterized by a lower transduction efficiency and transgene expression than current products.[82–84]

In addition to these secondary neoplasm case reports in SCD patients treated by gene therapy, the FDA recently alerted the medical community about the occurrence of T-cell malignancies in patients treated by CAR-T cells.[85] In these cases, one hypothesis is the clonal transformation of some transduced $CD3^+$ T-cells. Even though the gene therapy modalities differ from stem cell-based gene therapy to CAR-T cells, and from gene addition through lentivirus vector to gene editing by CRISPR-Caspase method, both physicians and patients have to be aware of the currently unknown risk level of using such new therapies.

Independently from receiving advanced therapies, patients with SCD are at increased risk of hematologic malignant conditions; several studies are underway to assess if SCD patients may be predisposed to clonal hematopoiesis due to previous hydroxycarbamide exposure or chronic inflammation.[53,75,76,81,86–90] If this is the case, patients treated with genome editing may also show clonal selection as the process to isolate and reinfuse the modified HSC is similar in gene addition and gene editing. Systematic screening for clones before gene therapy is a controversial issue limited by the lack of knowledge on which microarray to use, and what level of positivity should be considered as significant.

However, up to now, there is no evidence regarding such risks, but the number of treated patients and the follow-up is limited (Table 2).

Overall, although recent HSC-targeted gene-addition and gene-editing therapies for SCD have produced encouraging results, these therapies still face many challenges. Long-term follow-up is needed to assess efficacy and safety profile.[90]

MARKERS OF POOR PROGNOSIS THAT COULD BE USED TO SELECT PATIENTS FOR GENE THERAPY/EDITING

SCD is a highly variable condition, and it is difficult to reliably predict clinical severity.

A literature review was undertaken to identify prognostic markers that might be useful to identify patients at increased risk of severe complications who might benefit most from gene therapy/gene editing provided their organ function is preserved. We have identified the following factors of poor prognosis:

i) Genetic factors such as genotype, HbF expression, and alpha thalassemia.
ii) Non-genetic factors such as anemia and abnormal kidney function.

Genetic factors

HbSS and HbS/β^0 thalassemia genotypes

There are more than 15 different genotypes known to cause SCD. There is a clear difference between HbSS and HbSC genotypes, with those with HbSC suffering significantly fewer life- and organ-threatening complications, with the exception of retinopathy.[91] HbS/β^0 thalassemia causes severe SCD and is often grouped together with HbSS and referred to as sickle cell anemia (SCA). HbS/β^+ thalassemia varies widely depending on the severity of the β -thalassemia genetic variant.[92]

Lower fetal hemoglobin expression

Higher HbF levels reduce the rate of HbS polymerization and lead to many clinical benefits including improved survival.[93] Although HbF levels fall rapidly over the first two years of life, HbF levels in the first year of life are of prognostic significance.[94] Variants in three genes (*BCL11A*, *CMYB*, *HGB1/2*) can predict adult HbF levels fairly accurately, although so far this has not given much useful prognostic information and is less informative than measuring the HbF level.[95] β-globin haplotypes do not seem to contain prognostic information beyond their association with HbF levels.

Absence of α-thalassemia

In populations of African and Indian origin, 30%–40% of patients carry some form of α -thalassemia. The reduced erythrocyte hemoglobin concentration associated with α-thalassemia reduces rates of HbS polymerization, decreasing the severity of most complications, but not the incidence of hospital admissions, acute painful episodes, or increased life expectancy.[96–98]

The role of other genetic factors is less certain

Males with SCA have lower HbF levels and worse outcomes than females, with worse renal function, increased risk of cerebrovascular

TABLE 2 Main results of gene therapy/gene editing clinical trials, outcomes, and follow-up (see also Supporting Information II, Table IS).

Clinical Study/target gene/phase trial	Patient characteristics (age, gender, genotype)	Outcomes	Follow-up (time and long-term AE)	Ref.
BB305/β-globin gene ($\beta^{A\text{-}T87Q}$)/Phase 1-2 study (HGB-206, on-going)	Genotype: SS (100%) age: 24 years (12–42); 28 M/16 F 3 cohorts:			Ribeil JA, et al., *NEJM* 2017[a] Ref[84]
Betibeglogene autotemcel (beticel)/minigene encoding Beta-globin/lentiglobin (Zynteglo®; BlueBirdBio, EMA/FDA approved, however, no longer available outside the United States, after negotiations for reimbursement failed in major EU countries)	• A, *n* = 7, stem cell source: BM-$CD34^+$ cells	• Stable but insufficient expression of transgene in cohort A to significantly increase total Hb level and significantly decrease HbS level. Vaso-occlusive events decreased by 82.6% during the follow-up period	61.5 months (min–max: 55.5–66.1) 2 cases of fatal AML 1 not related to gene therapy (no lentiviral vector into the blasts) 1 unlikely related to gene therapy (LVV present but insertion site not considered as a trigger for AML)	
	• B, *n* = 2, stem cell sources: BM-$CD34^+$ cells	• Better results regarding both total Hb level increase, therapeutic Hb level expression, and HbS decrease. Vaso-occlusive events decreased by 79.1%% during the follow-up period	48.3 months in Group B1, and 44.4 months in Group B2	
	• C, *n* = 35, stem cell source: PBMC-$CD34^+$ cells	• All patients in group C engrafted • Median time to neutrophil engraftment 20 (12–35) days • Median time to platelet engraftment 36 (18–136) days • Hb level increased from 8.5 at the baseline to 11 g/dL • No vaso-occlusive event post gene therapy among 25 evaluable patients for the complete follow-up period	17.3 months (3.7–37.6 months) 1 patient deceased from SCD-cardiopulmonary disease 20 months post-infusion 2 patients presented with anemia and abnormal erythroid precursors without MDS nor insertional mutagenesis	
I8Hβ/γW/gamma globin lentiviral vector/Phase ½ pilot study MOMENTUM study with ARU-1801[a]	*n* = 4 pts (reported during meetings, 7 in total?) Median age: 26 years (19–35) Stem cell source: either BM or PBSC-$CD34^+$ cells	Transient neutropenia and thrombocytopenia (median duration 7 days) 80%–93% reduction in annualized VOEs in the first two patients and complete absence of VOEs (100% reduction) in the next two patients	≥12 months No adverse event so far	Grimley M, et al., *Blood* 2021[c]
Program was discontinued[a]		42% HbF^{G16D} mean expression level.		
BCH-BB694 BCL11A shmiR/shRNA reactivating the gamma-globin through BCL11 repression/ pilot study	*n* = 6 (with more than 6 months follow-up) + 2 (below 6 months) genotype: SS (*n* = 5); $S\beta^0$ (*n* = 1) median age: 18 years (7–25); M/F: 4/2 Stem cell source: PBMC-$CD34^+$ cells	No data was published about detailed hematological reconstitution but all patients were engrafted. 5/6 patients are free of transfusion with a median total Hb level increase from 9.3 to 11.4 g/dL For all 6 patients, median HbF/(HbF+HbS) of 30.5% (range, 20.4 to 41.3) F-cells among untransfused red cells was 70.8% (range, 58.9 to 93.6)	Median follow-up: 18 months (12–29) One patient experienced recurrent priapism episodes from 4 to 8 months post-gene therapy infusion	Esrick EB, et al. *N Engl J Med.* 2021[b]
CLIMB-SCD 121/BCL11A reactivating the gamma-globin/phase 3	*n* = 44 genotype: SS, $S\beta^0$ age 21 (12–35): 12: ≥12 ≤ 18 years; 32: ≥18 ≤ 35 years M/F 24/20	Time to neutrophil engraftment: 27 (15–40) days Time to platelet engraftment: 35 (23–126) days 97% free from VOCs (12 consecutive months) 100% free from hospitalization for severe VOCs in 12 consecutive months Hb>11 g/dL Pan-cellular distribution of HbF (>93% cells)	8.8–48.1 months	Frangoul H et al., *N Engl J Med* ref[62]

[a]Ribeil JA, Hacein-Bey-Abina S, Payen E, et al. Gene therapy in a patient with sickle cell disease. *N Engl J Med.* 2017;376:848-855. doi: 10.1056/NEJMoa1609677.

[b]Esrick EB, Lehmann LE, Biffi A, et al. Post-transcriptional genetic silencing of BCL11A to treat sickle cell disease. *N Engl J Med.* 2021;384:205–215, doi:10.1056/NEJMoa2029392.

[c]Grimley M, Asnani M, Shresta A, et al. Safety and efficacy of Aru-1801 in patients with sickle cell disease: early results from the phase 1/2 momentum study of a modified gamma globin gene therapy and reduced intensity conditioning. *Blood.* 2021;138(suppl 1):3970. doi.org/10.1182/blood–2021-147 469.

disease, and increased hospital admissions.[99] Glucose-6-phosphate dehydrogenase (G6PD) deficiency, which is an X-linked inherited disorder, is possibly associated with an increased risk of cerebrovascular disease and anemia.[100] This may explain some of the gender-related differences in SCD, but equally could have no causative role, and just be a surrogate marker of being male. Variants in the *UDGT1A(UGT1A)* gene are associated with increased jaundice and gallstones, and the G1 and G2 variants of the *APOL1* gene are associated with nephropathy.[101,102] Although many other candidate genes for adverse outcomes have been reported, none of these have been validated or are in routine clinical use.

Non-genetic factors

Anemia and abnormal kidney function

Many routinely performed blood and urine tests are correlated with outcomes in SCD, and also with each other (Table IIS, Supporting Information). Anemia has been strongly associated with cerebral and renal impairment.[103] Although these measurements may give some useful information, none are routinely used or have an established role in predicting outcomes.

RECOMMENDATIONS FOR GENE THERAPY/EDITING TO BE CONSIDERED FOR PATIENTS WHO HAVE

Global inclusion criteria

- Genotype HbSS, HbS/β^0 thalassemia, and severe HbS/β^+ thalassemia (patients with HbS/β^+ thalassemia and frequent or severe complications of SCD, low HbA levels (typically less than 30%) and low HbF levels) according to the inclusion criteria of the registrative trial.
- Baseline HbF <30% (it is less likely that further increasing HbF in patients with baseline HbF >30% will induce a significant benefit).
- No HLA-matched family donor.
- Agrees in principle to annual controls/monitoring for as long as requested by health Authorities (15 years).
- Appropriate documentation of the patient's medical history.
- Age: So far, gene editing-gene therapy products have only been trialed in those over the age of 12 years. The panel:
  - o considers offering gene therapy/editing above the age of 2 years, when additional data and longer follow-up will become available.
  - o does not currently recommend gene therapy approaches in patients older than 45 years because of the lack of data on this patient population in the clinical trials conducted so far.[104] The panel agrees that any gene therapy approach beyond the 35-year threshold should be conducted progressively (e.g., by gradually expanding the age of patients) and in well-defined centers with experience in the disease and possibly in a controlled experimental environment.

Vaso-occlusive crises (VOC) and acute chest syndrome (ACS)

Pain is the hallmark of SCD and is responsible for multiple hospitalizations and impaired quality of life. A large proportion of patients still suffer from recurrent VOCs in spite of the existing therapies.[105] ACS is a major cause of death in adult patients with SCD and is not fully prevented by currently available therapies, as well.[106] Previous trials have considered as indications for gene therapy/gene editing having at least 2 VOC per year during the 2 years before screening and failure of HC, and/or having at least 2 ACS in 2 previous years.

Recommendations

- at least two VOCs requiring hospitalization per year in the two previous years with no response to HC at the MTD, either alone or in combination with other treatments
- Recurrence of ACS in spite of HC at the MTD.

The panel proposes these recommendations irrespective of the presumed quality of adherence to the prescribed hydroxycarbamide regime. While VOC and ACS are the major criteria having led so far to enroll patients in gene therapy/gene editing trials, the panel also considers that other patients and disease characteristics should be taken into consideration to decide on access to advanced therapies.

ORGAN DAMAGE

Organ damage is responsible for acute and chronic co-morbidities and is associated with decreased life expectancy.[107] Evidence of chronic organ damage is an indication for supportive treatment intensification to prevent further worsening. At the same time, potentially curative therapy should be offered, before organ impairment has progressed, precluding the administration of myeloablative conditioning.

Cardiovascular disease

Background: In SCD, the main cardiovascular manifestations are early diastolic dysfunction, hypertrophic cardiomyopathy, and pulmonary hypertension (PH).[108]

Diastolic dysfunction is early described in both children and adults with SCD.[108] A large amount of evidence suggests that high tricuspid regurgitant jet (TRJ) velocities, as measured by echocardiography, are associated in adults with adverse outcomes in SCD, and in particular increased risk of PH and death.[109] Whenever TRJ > 29 mmHg is documented, patients should undergo a right cardiac catheterization study to confirm the presence of a true PH.[110,111]

Recommendations

Gene therapy/gene editing can be considered for patients who have:

- Diastolic dysfunction in the absence of restrictive myocardiopathy
- PH is defined as a mean pulmonary arterial pressure between 25–29 mmHg defined by heart cardiac catheterization.

The following conditions should be considered as exclusion criteria because they are potentially associated with an increased risk of treatment-associated fatal events:

- Patients with New York Heart Association severity classification of III or above.
- PH with pulmonary arterial pressure >30 mmHg defined by heart cardiac catheterization with or without specific treatment.
- Significant arrhythmia requiring therapy as defined by the European Heart Rhythm Association guideline (https://academic.oup.com/europace/article/23/10/1612/6247 378).

- Myocardial ischemia in the previous 12 months as defined by the European Society of Cardiology (ESC) guidelines (https://www.escardio.org/Guidelines).
- Restrictive cardiomyopathy as defined by ESC guidelines.

Liver disease

Background: The liver is a target organ for SCD with complications mostly related to the sickling of red blood cells, resulting in sinusoidal obstruction and ischemia of hepatocytes, as well as to ischemia of bile ducts leading to cholangiopathy, and hemolysis-related hyperbilirubinemia promoting cholelithiasis. Liver damage in SCD may also be due to iron overload, viruses, notably hepatitis B and C virus (HBC and HCV), or autoimmune disorders.[112] The main acute manifestations are sickle cell hepatic vaso-occlusion, sickle cell intrahepatic cholestasis, and hepatic sequestration, while chronic manifestations include cholelithiasis, sickle cell cholangiopathy, auto-immune hepatitis, viral hepatitis, and iron overload.[113] In a cohort of 3500 adult patients, liver failure was considered a cause of death in 7% of cases.[114]

Since SCD patients are frequently exposed to different regimens of red cell transfusion, liver iron concentration (LIC) should be assessed before gene therapy is undertaken and appropriate iron-chelation treatment should be started whenever iron overload is documented.[115]

Concerning HCV and HBV active infection, the panel recommends using the guidelines for use for myeloablative HSCTs. The degree of liver fibrosis should also be determined as part of the work-up for gene therapy.[116,117]

Recommendations

- Patients with a history of sickle cell-related chronic cholangiopathy/chronic cholestatic hepatopathy without hepatic failure **are eligible** for gene therapy approaches.

The following conditions should be considered as exclusion criteria:

- Chronic HBV and HCV infection as defined by the European Association for the Study of Liver (EASL) guidelines (https://easl.eu/wp-content/uploads/2018/10/HepB-English-report.pdf, https://easl.eu/wp-content/uploads/2020/10/EASL-recommendations-on-treatment-of-hepatitis-C). Patients with positive viral DNA/RNA must be excluded. Patients with occult HBV infection as defined by EASL guidelines, will not be excluded, provided that they accept appropriate prophylaxis and have no other contraindication to the treatment. Patients with a history of HCV infection after spontaneous seroconversion or after anti-viral eradication will not be excluded as well, provided that they have no hepatic or extra-hepatic contraindication.[117]
- Liver fibrosis grade ≥3 (Child-Pugh scoring system).
- Liver cirrhosis.
- If LIC >7 mg/Fe/g liver, iron chelation therapy should be started until LIC <7 mg/Fe/gr liver.[104] More than LIC per se, it is the condition of the liver parenchyma that has relevance; thus, a high LIC should lead to a biopsy, and the degree of fibrosis to contraindication.

Kidney disease

Background: The kidney is involved early in the natural history of the disease. Indeed, non-selective proteinuria has been reported in 50% of patients with SCD between 36 and 45 years of age.[118] Small studies have reported beneficial effects of angiotensin-converting enzyme inhibitors (ACE-) or angiotensin receptor blockers (ARB) on proteinuria or macroalbuminuria without significant changes in SCD kidney disease.[119]

SCD-related kidney disease results in advanced renal failure in almost 4.2% of adult patients with the SS genotype with a median age of 23.1 years. Chronic kidney disease (CKD) is more common in SS and $S\beta^0$ patients than in SC subjects.[118]

Recommendations

Gene therapy/gene editing approaches can be considered for patients who have:

- Chronic kidney disease ≤ stage 2 according to the guidelines on kidney disease (https://kdigo.org/guidelines/ckd-evaluation-and-management), with or without ACE or ARB treatment.
- Urine albumin/creatinine ratio >30 mg/mmol without renal failure

The following conditions should be considered as exclusion criteria:

- CKD stage 3-4 or higher
- End-stage renal disease (ESRD) under hemodialysis

Cerebrovascular disease

Background: Sickle cell cerebrovascular disease causes a severe and disabling group of complications in patients with SCD. The main clinical cerebrovascular manifestations are: (i) overt ischemic stroke; (ii) hemorrhagic strokes; (iii) overt stroke and silent cerebral infarct (SCI); (iv) SCI; (iv) *Moyamoya* disease.[120] Cerebrovascular disease in patients with SCD has a negative impact on neurocognitive function, leading to defects in attention, memory, motor-vison, and cognitive processes.[120]

The imaging techniques to screen for or follow up on SCD cerebrovascular disease might be used to evaluate the increased risk of a particular complication.

- Transcranial Doppler (TCD) measurements of blood velocities in the circle of Willis: increased velocities identify children at increased risk of overt ischemic stroke, which can be largely prevented by regular blood transfusions,[121]
- MRI of the brain identifies SCIs, which cause significant problems themselves, but also are associated with an increased risk of other neurological complications,[122]
- MRA of the brain identifies more advanced cerebrovascular disease than TCD scanning. The appearance of *Moyamoya* and aneurysms are particularly associated with advanced cerebrovascular disease, hemorrhagic strokes, and progressive deterioration despite optimal treatment.[123]

Recommendations

Patients with SCD and persistently abnormal TCD velocities despite HU at MTD or with cerebrovascular disease treated with regular blood transfusions are **candidates** for gene therapy approaches. These patients were initially excluded from studies of gene addition and gene editing; thus, it should be clearly explained to potential candidates that the impact of these treatments on TCD velocity and cerebral vasculopathy is unknown.

The following condition should be considered as exclusion criteria:

- Severe cerebrovascular disease *with Moyamoya*

Erythrocyte alloimmunization and hemolytic transfusion reactions (HTRs)

Background: Erythrocyte alloimmunization can lead to patients becoming untransfusable and the occurrence of life-threatening hemolytic transfusion reactions.[124] HTRs were responsible for 6% of deaths in a retrospective review of 3500 SCD patients in which 198 deaths were recorded.[114]

Recommendations

SCD Patients with a history of HTRs may be **eligible** for gene therapy approaches, although for the time being we have no data on the safety/efficacy of the approach. These patients have in fact been excluded from trials, due to concerns about further alloimmunization during the procedure. There are no large-scale publications on the benefit-risk ratio of HSCT in patients with a history of HTR, with only a few experiences of both good and bad results. We recommend that each case is discussed on an individualized basis by a multi-disciplinary team, that uncertainties are explained to patients, and that risk-mitigating measures are taken (e.g. administration of rituximab and/or intravenous immunoglobulins).

Thrombophilia status in SCD

Background: Increased thrombotic risk is recognized in patients with SCD compared to a healthy population.[125] Since a peripheral blood stem mobilization step is part of the procedure for gene therapy and SCD patients may provoke a recurrence of venous thromboembolism (VTE), such as deep venous thrombosis (DVT) or pulmonary embolisms (PE), patients with a history of thrombotic events should be identified and their thrombophilia status assessed before considered eligible patients for gene therapy.[104]

Recommendations

- Patients with "low risk" thrombophilia screen, with moderate reductions in protein C, S, and anti-thrombin levels or/and increased markers of thrombin generation such as D-dimer, are not excluded from gene therapy, since these are likely to be related to the synergic effects of sickle cell liver dysfunction and sickle cell-related inflammatory vasculopathy.
- Patients with VTE can be offered gene therapy if the clinician feels that anticoagulation can be safely managed during the expected period of thrombocytopenia post-preparative regimen.

The following condition should be considered as exclusion criteria:

- Lupus anticoagulant (LAC) or anti-phospholipids.[104]

Autoimmune diseases and SCD

Background: Patients with SCD may have an increased prevalence of auto-immune diseases.[126] These conditions may enhance inflammation-related tissue damage and SCD-related organ dysfunctions, and SCD limits the use of steroids, which may provoke VOCs.

Recommendations

Patients with co-existent auto-immune disease (e.g. Rheumatoid arthritis, Systemic Lupus Erythematosus, auto-immune hepatitis) may be eligible for gene therapy approaches unless they are at an advanced stage with severe organ damage, which is judged to make the procedure unsafe.

SUMMARY OF RECOMMENDATIONS

An algorithm for selecting patients with SCD candidates to receive gene therapy/gene editing approaches is proposed in Figure 1. Reassessments, notably of genotypes, age, and indications will be performed regularly by the EHA SWG/EBMT expert panel, according to scientific and regulatory updates.

Table 3 shows both the current inclusion and exclusion criteria and the panel's proposals for new indications. The panel also

TABLE 3 Summary.

Regulatory agencies	Potential future candidates	Not eligible patients
• Patients followed by a comprehensive center for hemoglobinopathies • Genotype HbSS, HbS/β^0 thalassemia, and severe HbS/β^+ thalassemia • No HLA-matched family donor • Patients over 12 years of age • 2 VOC requiring hospitalization/year in the 2 previous years with no response to HC at MTD, either alone or in combination with other treatments • Having at least 2 ACS in the prior 2 years	• Patients followed by a comprehensive center for hemoglobinopathies • Genotype HbSS, HbS/β^0 thalassemia, and severe HbS/β^+ thalassemia • No HLA-matched family donor • Patients aged more than 2 years and <45 years • at least 2 hospitalized VOCs per year in the 2 previous years with no response to HC at the MTD, either alone or in combination with other treatments • Recurrence of ACS in spite of HC at MTD • Diastolic dysfunction in the absence of restrictive myocardiopathy • PH defined as a mean pulmonary arterial pressure between 25–29 mmHg defined by heart cardiac catheterization • Chronic cholangiopathy/hepatopathy without hepatic failure • Chronic kidney disease ≤2 stage with or without ACE or ARB treatment. • Urine albumin/creatinine ratio >30 mg/mmol without renal failure • Persistently abnormal TCD velocities despite HU at MTD • Significant cerebrovascular disease treated with regular blood transfusions • History of HTR/severe hemolytic reaction? • Co-existent auto-immune disease	• Baseline HbF > 30% • Organ dysfunction not compatible with myeloablative conditioning regimen • Active infection (HBV, HCV, HIV) • Patients with NYHA III or above • PH with pulmonary arterial pressure >30 mmHg defined by heart cardiac catheterization • Significant arrhythmia requiring therapy • Myocardial ischemia in the previous 12 months • Restrictive myocardiopathy • Chronic HBV and HCV infection • Liver fibrosis grade ≥3 • Liver cirrhosis • If LIC >7 mg/Fe/gr liver, iron chelation therapy should be started until LIC <7 mg/Fe/gr liver • CKD stage 3–4 or higher • End-stage renal disease (ESRD) under hemodialysis • Severe cerebrovascular disease with moyamoya • Lupus anticoagulant (LAC) or anti-phospholipids

Abbreviations: HC, hydroxycarbamide; HTR, hemolytic transfusion reaction; MTD, maximum tolerated dose; TCD, transcranial Doppler; VOC, vaso-occlusive crises.

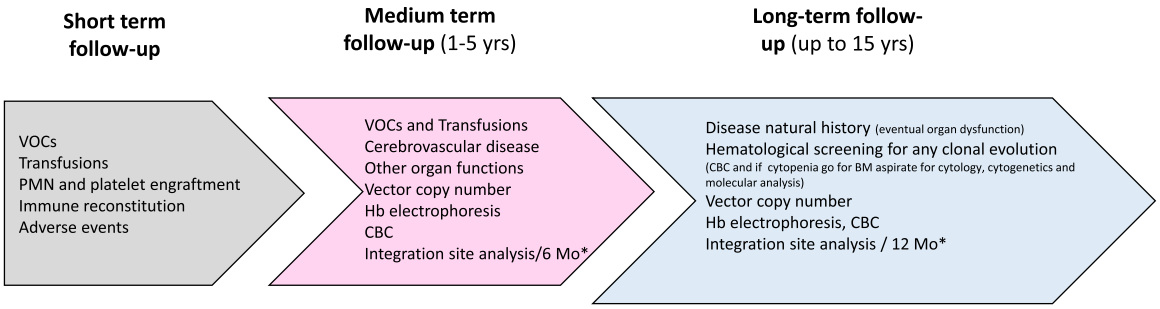

FIGURE 3 **Assessments for short-, medium-, and long-term follow-up**. *According to FDA recommendation for betibeclogen autotemcel. BM, bone marrow; CBC, complete blood count; Hb, hemoglobin; Mo, month; PMN, polymorphonuclear cells; VOC, vaso-occlusive crisis.

proposed the recommended assessments for short-, medium-, and long-term follow-up in Figure 3.

In conclusion, the careful selection of patients with SCD for gene therapy and gene editing approaches is mandatory, not only because of the technical nature and high costs involved but also because of the limited number and capacity of units capable of producing and infusing the treatment. Concerning the costs, the wholesale acquisition cost for a single dose of gene therapy (insertion and gene editing) ranges from $2.2 million to $2.8 million. Source: AnalySource® Monthly. January 5, 2024. Reprinted with permission by First Databank, Inc. All rights reserved. ©2024. www.fdbhealth.com/drug-pricing-policy). The cost of the Zynteglo (beti-cel) product led to delays in the commercialization in Europe of the first gene therapy medicinal product indicated for patients with transfusion-dependent thalassemia, despite conditional approval by the European Medicines Agency.[127] Worryingly, today allogeneic stem cell transplantation is not available in almost all African countries, and gene therapy is likely to be even less accessible.

On the other hand, the very nature of SCD makes it difficult to select patients. Unlike thalassemia, where the course of the disease is fairly uniform, SCD is characterized by variability in severity and unpredictability of prognosis. In the future, artificial intelligence might make it easier to identify the patients most at risk of complications, and who will be the best candidates for gene therapy approaches.[128]

AUTHOR CONTRIBUTIONS

All the authors have contributed to the writing of the manuscript and have accepted this last version.

CONFLICT OF INTEREST STATEMENT

Lucia de Franceschi: Agios and Bristol research grants, Roche consultant. Franco Locatelli: Speaker's bureau for Amgen, Miltenyi, Novartis, BMS, Gilead, SOBI and served on an advisory board for Amgen, Novartis, Sanofi and Vertex Pharmaceuticals Incorporated. Stefano Rivella: Scientific advisory board member of Ionis Pharmaceuticals, Meira GTx, Vifor, and Disc Medicine. Present-last 5 years: Stefano Rivella has been or is a consultant for GSK, BMS, Incyte, Cambridge Healthcare Res, Celgene Corporation, Catenion, First Manhattan Co., FORMA Therapeutics, Ghost Tree Capital, Keros Therapeutics, Noble insight, Protagonist Therapeutics, Sanofi Aventis U.S., Slingshot Insight, Spexis AG, Techspert.io, BVF Partners L.P., Rallybio, LLC, ven-Bio Select LLC, ExpertConnect LLC, LifeSci Capital. Stephan Lobitz: Consultant—Novartis, Vertex, Agios, Global blood therapeutics; Grant/contract: Novartis; Data and safety monitoring: Vertex. Miguel R. Abboud: Vertex: Data monitoring committee; Pfizer: Research funding; Emmaus: Speaker honoraria; Novo Nordisk: Research funding and preceptorship speaker; Novartis: Research funding; Roche: Travel support; Agios: research funding and Advisory board Josu de la Fuente: Steering Committee: Sanofi, VERTEX, Sangamo; Advisory Board: bluebird bio, VERTEX, Novartis, BEAM Therapeutics, Jazz, Roche, MAAT Pharma; Speaker Fees: Jazz, VERTEX; Research Grant: bluebird bio. Emanuele Angelucci: Data Monitoring Committee chair for Vertex and BMS, DMC member for Vifor; Consultant for Menarini-Stemline and Sanofi; Participation in advisory board for Regeneron. Received travel support from AbbVie, Sanofi, and GILEAD. Mariane de Montalembert: Vertex, Theravia, Novartis, Vifor Boards.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ORCID

Mariane de Montalembert http://orcid.org/0000-0002-4900-4753

SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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