Open Access

OBM Transplantation

Review

Present and Future Strategies with Curative Intent for Hereditary Hemoglobinopathies

Vanessa G. Oliveira [1,*], Filipa Saraiva [2], Fátima Costa [2], Aida Botelho de Sousa [2]

1. Transfusion Medicine Unit, Hospital Prof. Doutor Fernando Fonseca, Amadora, Portugal; E-Mail: vanessa.oliveira@hff.min-saude.pt
2. Hematology and Hematopoietic Stem Cell Transplant Unit, Hospital Santo António dos Capuchos, Centro Hospitalar Universitário de Lisboa Central (CHULC), Lisbon, Portugal; E-Mails: filipa.msaraiva@gmail.com; fatimacosta70@gmail.com; aida.botelho@chlc.min-saude.pt

* **Correspondence:** Vanessa Oliveira; E-Mail: vanessa.oliveira@hff.min-saude.pt

Academic Editor: Yasuhiko Sugawara

OBM Transplantation
2019, volume 3, issue 3
doi:10.21926/obm.transplant.1903086

Received: February 27, 2019
Accepted: August 30, 2019
Published: September 23, 2019

Abstract

Nowadays, hematopoietic stem cell transplantation (HSCT) is a common procedure in hematology units within reference centers, mainly for the treatment of hematological malignancies such as multiple myeloma, lymphoma, and acute leukemia. Nevertheless, HSCT has much wider applications, namely in autoimmune diseases, congenital metabolic defects, and hemoglobinopathies. Thalassemia major and sickle cell disease make up the most frequent hereditary hemoglobinopathies worldwide. Despite advances on the prevention and treatment of complications related to these diseases, the only curative approach available resides in allogeneic HSCT. The main challenges of this treatment remain focused on the toxicity of pre-transplant conditioning regimens and short-term transplant related complications like graft-versus-host disease, infections, and disease recurrence. Thus, it is crucial to establish a balance between the risk vs benefit of HSCT for each patient and follow the available guidelines for both diseases. Recently, gene therapy has become a real alternative to allogeneic HSCT. Recent advances in molecular biology methods have provided more accurate and reliable gene editing techniques such as the CRISP/CAS9 system. The long-term outcome of gene manipulation procedures remains uncertain, especially in

the immune system of the host. This review will focus on HSCT and gene therapy in hereditary hemoglobinopathies.

Keywords
Hemoglobinopathies; transplantation; bone marrow; sickle cell disease

1. Introduction

In recent decades, hematopoietic stem cell transplantation (HSCT) has greatly evolved to become a routinely established treatment for hematopoietic malignancies and some congenital or acquired diseases. In 2015 the Worldwide Network for Blood and Marrow Transplantation (WBMT) published the results of a retrospective observational study reporting about one million hematopoietic stem cell transplants, both autologous and allogeneic [1], performed from 2006 to 2014 worldwide amongst World Health Organization (WHO) member states. Around 1.4% of the allogeneic hematopoietic stem cell transplants performed in Europe in 2017 were in the clinical setting of hemoglobinopathies [2]. From 2006 to 2013, the East Mediterranean/African region registered an increase of 200% in HSCT for hemoglobinopathies [3].

Sickle cell disease (SCD) and β-thalassemia are the most common hereditary hemoglobinopathies. It is estimated that around 311,000 neonates /year worldwide are born with SCD [4, 5] and around 56,000 with thalassemia major (TM) [6].

These patients exhibit inadequate erythropoiesis and their peripheral red blood cells (RBC) display shortened life spans, which leads to anaemia. They often rely on chronic blood transfusions ultimately suffering from iron overload and its subsequent consequences on heart and liver function. About 75% of children affected by these diseases are born in low-income countries and most will probably die before the age of 5 years [6]. With increasing migration, predictions indicate that these patients will be of important health and economical concern for high–income countries in the near future.

Children suffering from congenital hemoglobinopathies who are born in high-income countries can live decades; nevertheless, they often have a poor quality of life, attributed not only to the chronic treatments they must endure, but also to the cumulative sequelae these diseases inflict on their physical and psychological development. HSCT for patients with congenital hemoglobinopathies, namely SCD and β-thalassemia, is the only curative treatment available at the moment.

This review will focus on HSCT in the context of genetic haemoglobin disorders SCD and β-thalassemia major. We will briefly describe these pathologies and summarize the recent advances in HSCT, namely donor types, conditioning regimens, graft-versus-host-disease (GVHD), and late effects after HSCT. We will finish with an overview of the current status of gene therapy applied in these settings.

2. Pathology and Clinics

2.1 Sickle Cell Disease

Haemoglobin S (Hb S) is a variant of adult haemoglobin (Hb A) that results from a point mutation which leads to the substitution of a glutamic acid for a valine at the sixth position of the β-globin chain. This mutation causes changes in stability and solubility leading to the formation of polymers in situations of low oxygenation, reducing the life span of the red blood cell (haemolysis) and contributing to a vaso-occlusion phenomena with ongoing ischemia-perfusion injury, chronic organ damage, and decreased life-expectancy. Sickle cell disease is a generic term used to denote a group of diseases characterized by the presence of haemoglobin S in the absence of HbA1 production. This classification excludes HbS carrier HbAS, which is the heterozygous form where the concentration of HbS is always lower than the concentration of HbA and it is mainly asymptomatic. However, although HbAS stands as a protective condition for malaria infection, carriers can suffer from exertional rhabdomyolysis and other non-fatal exercise complications, progressing to end-stage renal disease in chronic kidney disease and elevated risk of venous thromboembolism and arterial events [7]. The homozygous state (HbSS) represents the most serious phenotype of sickle cell disease [8-10].

Children may have their growth and development impaired by chronic anemia, malnutrition, increased metabolic rate, and renal loss of nutrients. Other complications of the disease can be divided in two main groups: those related to large-vessel vasculopathy (cerebrovascular disease, pulmonary hypertension, priapism, and retinopathy) and those caused by progressive ischemic organ damage (hyposplenism, renal failure, bone disease, heart failure,–and liver damage). Infections are another important concern in this population and a major cause for the increased morbidity and mortality rates, especially in children where they are the main cause of premature deaths. This is due to functional asplenia and progressive ischemic involution of the spleen that provokes an increased susceptibility to bacterial infections (namely *Pneumococcus* and *Haemophilus influenzae*). These situations can be overcome with vaccination and antibiotic prophylaxis, which in high-income countries led to a childhood mortality rate now close to the one of the general population. Another potentially life-threatening situation in children is acute splenic sequestration, which consists of sequestration of red blood cells resulting in acute anemia, hypovolemia, splenomegaly, thrombocytopenia, and reticulocytosis. There is also an increased risk of stroke, but early screening with transcranial doppler (TCD) ultrasonography is an important feature to reduce the incidence of this complication [11, 12].

2.2 Thalassemia Major

Thalassemia represents a heterogeneous group of hypochromic and microcytic anaemias whose diagnosis requires the exclusion of iron deficiency anaemia, which is the most common type of anaemia worldwide affecting all ages. Thalassemia is characterized by diminished or absent production of alpha or beta globin chains, which alters the normal balance of chains, leading to intracellular accumulation of the non-affected chain and resulting in pathological effects such as the early destruction of the erythroid precursors before reaching the state of complete maturation. The erythrocytes that overcome this maturation disorder have abundant precipitates of excess globin chains that invariably decrease their circulating life span (haemolysis). In severe

forms, ineffective erythropoiesis results in the expansion of bone marrow, leading to the distortion of bone structure and to extramedullary activity with enlargement of the hematopoietic sites. Additionally, chronic anaemia, severe ineffective erythropoiesis, and hypoxia also cause iron overload due to increased intestinal absorption of iron, ultimately leading to the formation of reactive oxygen species that can cause tissue damage, organ dysfunction, and death.

Beta-thalassemia usually results from point mutations in globin genes located on the short arm of chromosome 11 [8, 10, 13]. The clinical severity is also a function of the number and type of genetic defects, ranging from less severe forms such as beta thalassemia trait which is asymptomatic and characterized by mild microcytic, hypochromic anaemia, to more severe forms like beta thalassemia major in which patients suffer from severe anaemia from infancy. Between these two extremes lies beta thalassemia intermedia with a variable phenotype between the two aforementioned [10, 13].

Children with beta thalassemia major and some with beta thalassemia intermedia typically manifest failure to thrive, progressive anaemia that evolves to a transfusion-dependent condition, and hepatosplenomegaly as a manifestation of extramedullary haematopoiesis. During their life time, they are confronted with many complications, some derived from the disease itself and others from treatment. Reduced bone mineral density occurs in over 30% of children with beta thalassemia major. They experience growth abnormalities from chronic anaemia, hypermetabolic states, endocrine abnormalities, and iron overload. The latter, particularly, is a major concern because it can lead to a wide range of complications from gonadal dysfunction, cirrhosis, and endocrine disorders. Furthermore, deposits of excess iron occur in cardiac muscle leading to cardiac dysfunction which is a common cause of death for these patients. Beta thalassemia major patients also have a multifactor increased risk of infection [10].

2.3 Treatment Strategies

Supportive treatment is the only option for patients with beta thalassemia intermedia or major. Treatment strategies involve frequent blood transfusions combined with chelation therapy. For sickle cell disease the mainstay treatment is hydroxyurea, which induces HbF synthesis preventing polymerization of HbS and consequently improving blood rheology, reducing adhesive interactions, increasing total haemoglobin concentration, and decreasing platelet, reticulocyte, and neutrophil counts. Altogether these events will diminish the frequency of vaso-occlusive crisis and subsequently lead to fewer blood transfusions and a reduction of acute chest syndrome events, preserving organ function. Ultimately this will diminish the need for hospitalizations and decrease mortality. Ongoing studies hypothesize that hydroxyurea may be used in nontransfusion-dependent beta thalassemia patients [14].

Splenomegaly with hypersplenism is a common problem in thalassemia due to severe anaemia and extramedullary haematopoiesis leading to exacerbation of haemolysis and thereby worsening anaemia and increasing transfusion requirements. In these situations splenectomy is recommended, not only to decrease blood consumption and blood transfusions but also to improve iron overload and to minimize extramedullary haematopoiesis and growth failure. However, splenectomised patients carry an increased risk of infection, pulmonary hypertension, heart failure, and thrombosis. Splenectomy should be avoided in children less than 5 years of age because pneumococcal bacteraemia is more common in young children. Prior to surgery,

immunizations against Pneumococcus, Haemophilus influenzae, and Meningococcus are necessary, and post-splenectomy antibiotic prophylaxis can be considered although this approach is not consensual.

Transfusion therapy should be started early in life to alleviate symptomatic anaemia, suppress ineffective erythropoiesis, improve growth, reduce iron overload from increased gastrointestinal absorption, and prolong life. Specifically, in the case of sickle cell disease, it reduces HbS and can prevent several complications, namely stroke, vaso-occlusive crisis, and acute chest syndrome. Associated complications include the risk of transfusion-transmitted infections (primarily hepatitis B and C) and transfusional hemosiderosis which still remains the leading cause of mortality due to cardiac complications in thalassemia. Iron chelation therapy in childhood improves survival and quality of life, and recommendations suggest beginning with an oral chelator given its high oral bioavailability, longer half-life (enabling once daily dosing), and ability to better chelate cardiac iron. However, side effects include skin rash, nausea, and diarrhea, which decreases compliance to the treatment. Furthermore, it is an expensive treatment which is unaffordable in most developing countries [10, 12, 15].

3. Bone Marrow Transplantation

HSCT is the only available treatment with a curative intent in both diseases regardless of whether or not gene therapy and gene editing approaches become a valid option in the near future. Recommendations suggest that if a suitable donor is available, HSCT should be considered during childhood in SCD. On the contrary, in TM, HSCT is the only curative treatment available and its efficacy has been established [16].

Despite this, HSCT is a therapeutic option taken cautiously and considered mainly for patients who have failed all other treatment strategies and in whom disease complications have importantly diminished their quality of life. Despite major advances performed to lessen HSCT toxicity and to increase its success rates, the fact that patients proposed for HSCT suffer from severe complications translates into poor post-transplant outcomes, mainly due to infections, graft rejection, acute and chronic GVHD (aGCVH, cGVHD), and other post-transplant side-effects leading to significant morbidity and mortality rates.

3.1 Sickle Cell Disease

In 1984 the first patient with SCD was transplanted [17]. According to the European Society for Blood and Bone Marrow Transplantation (EBMT) activity survey, in 2016 in Europe there were 138 transplants in patients with SCD, of which 101 were from HLA-identical donors [18].

Awareness of the importance of neonatal screening tests and advances in the treatment of SCD, especially with the administration of hydroxyurea, have greatly decreased child mortality [19, 20]. Nowadays, 95% of children with SCD reach adulthood, albeit in most cases at the expense of an accumulation of serious complications which render them incapable of complying with a normal daily routine, leading inevitably to a decreased survival in adulthood. In fact, although child mortality has decreased, in adulthood, mortality has increased 1% [21, 22] due in part to the accumulation of several episodes of acute illness throughout life which invariably lead to end-organ damage [23].

Despite its curative potential, allogeneic HSCT was regarded in the early 90s as an intervention of limited usage not only due to the lack of a suitable donor but also due to the inherent risk of transplant-related complications which could ultimately lead to death. Despite the immense distress that this disease imposes on patients with its high morbidity, for a long time, HSCT was restricted to patients with severe disease related complications [24]. In the last decades we have witnessed an increase in safety of transplant related procedures and a reduction of transplant related mortality which inevitably shifted the balance of risk versus benefit towards more favorable HSCT outcomes when compared to quality of life without transplantation. These changes took time and were slowly reached through the development of several clinical trials which consequently changed clinical indications of HSCT in SCD [25-27].

Approximately 22 years ago, criteria for bone marrow transplantation included previous manifestations of severe disease such as stroke, recurrent acute chest syndrome, and vaso-occlusive crisis. Furthermore, this procedure was restricted to children less than 16 years of age with an HLA-identical related donor [24]. Independently of the disease status, in this group of patients the estimated overall survival (OS) and event-free survival (EFS) at 4 years was 91% and 73%, respectively (Table 1) [24].

In 1998, Vermylen et. al. published results of HLA-identical HSCT performed in 50 children divided into two groups according to the severity of complications [28]. The myeloablative conditioning regimen consisted of busulfan (Bu)+cyclophosphamide (Cy) (BuCy) and either total lymphoid irradiation (TLI) or anti-thymocyte globulin (ATG). This study demonstrated that OS and EFS at 11 years were higher in the group with no history of previous disease related complications (100% and 93%, respectively), compared to the group with serious disease complications (88% and 76%, respectively) [28]. These results were corroborated by a study involving 50 children transplanted with matched sibling marrow allografts between 1991 and 1999 [29]. The conditioning regimen used was BuCy and either ATG or alemtuzumab and the estimated OS and EFS at 8 years were 94% and 84%, respectively. In both studies, a major concern after HSCT was gonadal dysfunction [28] and amenorrhoea in females, especially in HSCT performed during peri-puberty years [28]. Other complications like pulmonary function and cerebral vasculopathy remained stable or were improved after HSCT (Table 1) [29].

Patients were treated prophylactically with cyclosporine (CSP) with or without methotrexate (MTX) for a minimum of 6 months after transplantation [28, 29] to minimize the occurrence of GVHD [28]. In the trial of Walters et al. patients also received prednisone which might explain the better outcomes obtained concerning the occurrence of GVHD (12%) [29]. In this study, levels of haemoglobin S (HbS) were maintained at 30% prior to HSCT to minimize the occurrence of cerebrovascular disease and improve transplantation outcomes (Table 1) [29].

The selection of the conditioning regimen used in HSCT in SCD patients should take into consideration that contrary to malignant hematologic patients, the effect of graft versus disease is not required.

A study involving 30 transplant centers and a total of 67 patients using mostly the BuCy myeloablative regimen (94% of patients) reported an OS and disease free survival (DFS) at 5 years of 97% and 85%, respectively, with an overall probability of graft rejection at day 100 of 10% and 22% (aGVHD and cGVHD, respectively) (Table 1) [30].

Similarly, studies from Bernaudin et. al. involving 87 patients transplanted from 1988 to 2004 under the classical BuCy conditioning regimen showed an OS and an EFS of 93% and 86%,

respectively [31]. Interestingly, this group reported an overall cumulative incidence of rejection of 7% at 5 years partly due to the high cumulative incidence of rejection found in the group of patients transplanted before 1992 (22.6%). These patients also exhibited low mixed chimerism (5%-49% donor cells) which prompted clinicians to suggest the inclusion of ATG in the standard conditioning regimen for patients transplanted from 1992 onwards [31]. Adaptations of these conditioning regimens using BuCy+ATG were implemented by other groups obtaining similar results in incidence of rejection and stable mixed chimerism (Table 1) [32].

A direct comparison between studies from different centers is always difficult to perform because several variables are modified at the same time. Nevertheless, in 2014, an attempt was performed to directly compare the BuCy and the BuCy+ATG regimens but the number of patients in each group was very small and no differences were found in DFS between the two arms of the study (50% BuCY and 66.7% BuCy+ATG). Interestingly, the authors found that in patients previously treated with hydroxyurea (HU), DFS was greatly increased (97.4%) and no rejection was observed [33]. Another group in Rome published very encouraging results in 40 SCD transplanted patients, using BuCy+ATG and obtaining a DFS of 91% concomitant with sustained engraftment and 100% donor chimerism (Table 1) [34].

Altogether these observations suggest that not only does the inclusion of ATG in conditioning regimens seem to reduce the incidence of rejection by increasing chimerism, but also that treatment of SCD patients with HU prior to transplantation reduces graft rejection.

Busulfan is considered one of the main myeloablation drugs in HSCT for hemoglobinopathies; however, it carries a degree of interindividual variability and higher clearance in children [35]. Inclusion of busulfan in conditioning regimens is decisive for a successful engraftment [36] but the dose administered is limited by its side effects, namely, sinusoidal obstructive syndrome, seizures, and increased overall transplant related mortality (TRM) [37-39].

A high incidence of neurological complications was observed after HSCT in SCD patients [40, 41], which prompted clinicians to review the guidelines and include the need for anticonvulsant prophylaxis initiated before and after administration of busulfan and for a period of at least 6 months post-HSCT. Targeted Bu therapy performed in 25 SCD patients undergoing HSCT has demonstrated that increasing the dose of Bu, administered either orally or intravenously (iv), allowed for OS and DFS rates of 96% each, at a median follow-up of 4.9 years reporting no graft rejection [42]. In this study, 16% of patients presented with seizures and although 32% of patients developed sinusoidal obstructive syndrome, the authors found no statistically significant correlation with the cumulative Bu exposure despite a trend towards aggravation of this condition in patients receiving orally administered Bu (Table 1) [42]. Despite the improved transplantation outcomes using a higher dosage of Bu, long-term effects like gonadal dysfunction remain an important concern with this conditioning regimen.

With this in mind, the fine tuning of Bu dosing seems crucial to minimize side-effects while at the same time avoiding graft rejection. A published study using 16 SCD patients who underwent HLA-matched sibling donor HSCT assessed a different dose of Bu and showed excellent results with 100% OS and EFS at 3 years, 13% grade I-II aGVHD, no grade III-IV aGVHD, or cGVHD and no sickle cell related complications post-transplant (Table 1) [43]. Unfortunately, this regimen was not able to avoid gonadal dysfunction in some of these patients.

In an attempt to improve HSCT outcomes while minimizing toxicity of myeloablative therapy, other conditioning regimens were tested. A combination of Bu+fludarabine+alemtuzumab was

administered to 18 patients with SCD before HLA-matched sibling donor HSCT with an OS and EFS of 100% evaluated at 2 years [44]. Furthermore, no sinusoidal obstructive syndrome was found and pulmonary, cardiac, and neurological functions were stable or improved. Incidence of aGVHD was 17% and cGVHD was 11%, comparable to previous studies under the classical condition regimen of Bu-Cy-ATG, which represents a step forward for the minimization or even ablation of HSCT side-effects [44]. Similarly, a study involving 14 patients assessed the feasibility of decreasing the doses of Bu and Cy while maintaining the dose of ATG constant and introducing a fourth agent, fludarabine. These authors reported that successful engraftment could be achieved with lower doses of Cy if concomitant administration of fludarabine was used (Table 1) [45]. Unfortunately, it was not possible to further reduce the dose of Bu, which was the main objective of this study, but it is worth mentioning that the number of subjects in each group was too small to establish definitive conclusions from this multicenter trial.

Other strategies have been tried in an attempt to avoid the usage of Bu. Strocchio et. al. used a treosulfan + thiotepa + fludarabine conditioning regimen in 15 children with SCD transplanted with hematopoietic stem cells from an HLA-matched sibling or unrelated donor showing an OS and DFS of 100% and 93%, respectively and no grade II-IV GVHD. In this cohort study no grade III-IV regimen-related toxicity was observed for either group (treated with the Treo regimen or the classical Bu regimen), a finding that the authors attributed to their vast experience in Bu-level monitoring and strict dose adjustment (Table 1) [46]. Thus, this study showed non-inferiority results when compared to a classical regimen using Bu.

Side-effects of classical myeloablative conditioning regimens, especially gonadal dysfunction [47], limit the application of HSCT as a routine procedure for SCD patients. A published report using a non-myeloablative conditioning regimen consisting of total-body irradiation (TBI) of 300 rad, alemtuzumab and sirolimus in 30 SCD patients (children and adults) enlisted from 2004-2013 showed no aGVHD or cGVHD up to two years post-transplant with an OS of 97% and DFS of 87%, although with 13% graft failure (4 out of 30 patients) (Table 1) [48]. In 2015 another group reported the usage of alemtuzumab, fludarabine, and melphalan in 43 SCD pediatric patients undergoing HSCT from an HLA-matched sibling donor with OS of 93% and EFS of nearly 91% at a median follow-up of 3.42 years, although with a 13% incidence of cGVHD (Table 1) [49]. The effect of these non-myeloablative regimens in gonadal function remains to be assessed. Recommendations from 2014 from an international expert panel suggest the usage of Bu+Cy+ATG as the standard conditioning regimen for HSCT in SCD and thalassemia major [50].

It is now considered beneficial that children with SCD and a matched sibling donor should receive an HSCT as early as possible, preferably at pre-school age [50, 51]. Older patients tend to have more disease related complications which might contribute to worse HSCT outcomes [28].

An international retrospective survey including Eurocord, EBMT, and the Center for International Blood & Marrow Research (CIBMTR) reviewed 1000 children and adults transplanted from an HLA-identical sibling donor from 1986 up to 2013, and found an inverse correlation between the age of the patient and the 5-year EFS (93% for <16 years and 81% for ≥16 years, $p<0.001$) and OS (95% for <16 years and 81% for ≥16 years, $p<0.001$). In addition, patients ≥16 years exhibited a higher risk of aGVHD and higher hazard ratios for death and treatment failure with increasing age [52].

Table 1 Comparison of outcomes of HSCT in several clinical trials in sickle cell disease patients.

Reference	Patients	Stem cells	Donor	Conditioning	OS	EFS/DFS	aGVHD	cGVHD	Graft rejection (or failure)	TRM or OM
Walters *et al*., [24]	22	BM	Identical	Bu+Cy+ATG	91	73/84	9	4.5	18	9
Vermylen *et al*., [28]	50	BM,CB	Identical	Bu+Cy, Bu+TLI, Bu+ATG, Bu	93	82/85	40	20	10	4
Walters *et al*. [29]	50	BM	Identical	Bu+Cy+ ATG or AL	94	84/-	8	4	10	6.2
Panepinto *et al*. [30]	67	BM,CB,PB	Identical	Bu+Cy other regimens	97	-/85	10	22	13	4.4
Bernaudin *et al*. [31]	87	BM,CB,PB	Identical	Bu+Cy±ATG	93	86/-	20	12.6	7	6.9
Majumdar *et al*. [32]	10	BM,CB,PB	Identical	Bu+Cy+ATG	90	77/-	36.3	45.4	9	10
Dedeken *et al*. [33]	50	BM,CB,PB	Identical	Bu+Cy±ATG	94	85.6/-	22	20	8	4
Lucarelli *et al*. [34]	40	BM	Identical	Bu+Cy+ATG Bu+Cy+Flu	91	-/91	50	5	2.5	9
McPherson *et al*. [42]	27	BM	Identical	Bu+Cy+ATG	96	-/96	12	ND	0	3.7
Maheshwari *et al*. [43]	16	BM	Identical	Bu+Cy+ATG	100	100/-	13	ND	0	0
Bhatia *et al*. [44]	18	BM,CB	Identical	Bu+Flu+AL	100	100/-	17	11	0	0

Horan *et al.* [45]	14	BM,CB	Identical	Bu+Cy+Flu-ATG	ND	-/100	14	14	7	0
Strocchio *et al.* [46]	30	BM,CB,PB	MRD, MUD	Treo+Thio+Flu	100	-/93	17	ND	7	0
Hsieh *et al.* [48]	30	PB	MRD	AL+TBI+Sir	97	-/87	0	0	4	1
King *et al.* [49]	52	BM,CB	MRD	AL+Flu+Mel	93	91/-	23	13	1.9	5.7
Gluckman *et al.* [52]	1000	BM,CB,PB	Identical	MAC and non-MAC	92.9	91.4/-	14.8	14.3	2.3	7
Saraf *et al.*[53]	10	PBSC	Haploidentical	ATG+Flu+Cy+TBI	87.5	-/87.5	25	12.5	12.5	12.5

BM- bone marrow; CB- cord blood; PBSC- peripheral blood stem cells; MUD - matched unrelated donor; MRD – matched related donor (including matched sibling); Identical – HLA identical sibling; aGVHD – acute graft-versus-host-disease grade II-IV; cGVHD – chronic graft-versus-host-disease ; TRM –transplant related mortality; OM – overall mortality; Bu- busulphan; Cy- cyclophosphamide; Flu- fludarabine; TBI- total body irradiation; ATG- anti-thymocyte globulin; Thio- thiotepa; Treo- treosulfan; Dexa- dexamethasone; AZA- azathioprine; HU- hydroxyurea; TT-; MAC- myeloablative conditioning; RIC- reduced intensity conditioning. Numbers represent percentages.

3.1.1 Source of HSCs

Donor stem cells can be obtained from bone marrow (BM), peripheral blood (PBSC), or umbilical cord blood (CB). Early studies on HSCT in SCD used BM or CB as the source of hematopoietic stem cells. CB has some advantages, namely, cells are readily available from CB banks, latent viral infection is rare, collection poses no risk for the donor, and, more importantly, it allows for HLA-mismatches and carries a lower risk of GVHD and relapse which is partially attributed to the more immature nature of these cells. Nevertheless, CB cells have some disadvantages that limit their usage, especially the delay in engraftment and consequently in immune reconstitution, increasing the risk of viral infection which subsequently may influence graft failure. Furthermore, additional donations from the same donor are not available and cell dose is an important issue because the volume of cells collected is usually small and may not have sufficient stem cell numbers for HSCT in adults [54].

Following on this subject, Bernaudin *et al*. reported no GVHD amongst patients receiving CB as the source of HSC [31]. If a matched sibling donor is available, recommendations from an international expert panel suggest that HSCT for SCD should be considered using either BM or CB stem cells [50].

PBSC carry a greater risk of GVHD [55] because they contain mature T cells. For SCD patients the graft versus disease effect is not required, thus PBSC can be T cell depleted and/or $CD34^+$ selected after collection and prior to transplantation, if necessary. Engraftment and immune reconstitution using PBSC are faster than with CB cells allowing the patient a faster recovery of hematopoiesis which may decrease the risk of infections during aplasia and graft failure [54]. Recent studies from 2018 reported that PBSC from a haploidentical donor can be transplanted in adults with SCD resulting in stable engraftment and very low incidence of GVHD; however, this study included only 10 patients and a larger, multicenter, ideally prospective, trial including more patients should confirm these observations (Table 1) [53]. Thus, deciding which type of donor stem cells to use depends not only on the availability of an HLA-matched sibling donor but also on the clinical situation of the patient and on the urgency of the HSCT. Most clinical trials performed in children using HLA-identical sibling donors have focused on the usage of BM or CB stem cells.

3.1.2 Unrelated and Haploidentical Donors

Finding an HLA-identical sibling donor is the major limitation of HSCT in SCD patients. It is estimated that less than 30% of these patients will have an HLA-identical sibling donor [56]. In these situations, stem cells have to be obtained from HLA-matched unrelated donors or HLA-haploidentical related donors. The probability of finding a matched unrelated donor varies with the racial and ethnic group of the patient and it has been shown to be around 18% and 19% for the African and African-American population, respectively [57].

These alternative donor sources carry an increased risk of GVHD and graft rejection, especially when reduced-intensity conditioning regimens are used (Table 2) [58].

If taken from BM, stem cells of HLA-haploidentical or HLA-matched related donors transplanted into children and adults under non-myeloablative regimens induced 43% of graft failure [59]. On the other hand, transplantation of stem cells from HLA-matched related donors into SCD patients treated with a myeloablative regimen showed no graft rejection or failure; however, these

patients suffered a progressive decline in renal, pulmonary, and cardiac function, which, by its characteristics, the authors attributed to toxicity of the conditioning regimen used (Table 2) [60].

A regimen of GVHD prophylaxis consisting of calcineurin inhibitor, methotrexate, and methylprednisolone was used in SCD patients after transplantation of BM from HLA-matched unrelated donors. These patients had been treated with a conditioning regimen containing alemtuzumab, fludarabine, and melphalan and obtained an OS and EFS at 2 years of 79% and 69%, respectively, but registered an incidence of cGVHD at 1 year of 62%, of which 38% reported extensive GVHD and 7 GVHD related deaths (Table 2) [61].

A retrospective study of 19 patients receiving BM stem cells or PBSC either from HLA-identical sibling donors (47.3%), HLA-matched unrelated donors (31.5%), or α/β T cell depleted related haploidentical donors (21%) reported an OS at 2-years of 89%, 83%, and 75%, respectively. The authors found an overall incidence of aGVHD of 26% with no significant difference in the group of haploidentical transplants irrespective of the source of stem cells used [62]. Other studies with haploidentical donors reported good engraftment times and low incidence of GVHD using BM or PBSC [53, 63] as sources of hematopoietic stem cells.

A fine balance between stable engraftment and GVHD prophylaxis is one of the main concerns in HSCT using haploidentical donor stem cells. Schemes of post-transplant GVHD prophylaxis also contribute to graft failure and strategies to overcome this conundrum are necessary. A regimen based on ATG+Bu+fludarabine preceded by an immunosuppressive conditioning of fludarabine+dexamethasone yielded promising results if used with a scheme of GVHD prophylaxis based on Cy+tacrolimus+mycophenolate mofetil (Table 2) [64]. The side effects of such an aggressive regimen in long-term organ function remain to be assessed. Furthermore, eligibility for such a conditioning requires good organic reserves to allow such level of immunosuppression. The complexity of HSCT from haploidentical donors is demonstrated by the modest, but very relevant findings, using a conditioning of ATG+ Cy+fludarabine+TBI (3Gy) and Cy+sirolimus+mycophenolate mofetil for GVHD prophylaxis, with around 87% of stable engraftment and only 1 patient (out of a total of 8 adult patients) developing cGVHD [53].

These studies indicate that HSCT using HLA-matched unrelated donors or haploidentical donors should still be considered as a future alternative for SCD patients without an HLA-identical sibling donor. Nevertheless, at the moment, this procedure requires additional clinical studies before its widespread use.

Determining the best conditioning regimen and the best GVHD prophylaxis is crucial for the routine clinical application of HSCT in this setting. At the moment at least 4 clinical trials are recruiting patients in the USA. These trials target different aspects of the HSCT procedure. Some intend to use BM stem cells, others PBSC. Conditioning regimens will also be studied as well as GVHD prophylaxis. The likely major limitation of these studies is always the same: the small number of patients recruited in most studies. Despite these issues, HSCT should be pursued in an attempt to improve the technique so it can be safely applied routinely to SCD patients in the future.

Table 2 Comparison of outcomes of HSCT in several clinical trials in sickle cell disease and thalassemia major patients using hematopoietic stem cells from different sources.

Reference	Patients	Stem cells	Donor	Conditioning	OS	EFS/DFS	aGVHD	cGVHD	Graft rejection (or failure)	TRM or OM
Kamani *et al.* [58]	8	CB	MUD	AL+Flu+Mel	87.5	37.5/-	25	12.5	42.8	12.5
Bolanos-Meade *et al.* [59]	17	BM	Haploidentical, MRD	Flu+Cy+ATG+TBI	14	-/64.7	5.9	0	0	0
Dallas *et al.* [60]	14	BM,CB	MRD	Bu+Cy+ATG	93	-/93	28	21	38	0
Dallas *et al.* [60]	8	PBSC	Haploidentical	Flu+Thio+Bu+ATG+OKT3,HU+AZA+Thio+Cy+OKT3	75	-/38	50	37.5	10	25
Shenoy *et al.* [61]	30	BM	MUD	AL+Flu+Mel	79	69/-	28	62	10	23.3
Khazal *et al.* [62]	19	PBSC, BM	Identical, MUD, haploidentical	Several	84.2	26/-	10.5	10.5	-	-
de la Fuente *et al.* [65]	18	BM	Haploidentical	Cy+Flu+ATG+TBI± thio	100	>90	27.7	5	5	11

Fitzhugh *et al.* [63]	23	PBSC	Haploidentical	AL+TBI+Sir+Cy	87	0 (cohort 1)[#], 25 (cohort 2), 50 (cohort 3)	8.7	4.3	100 (cohort 1)[#], 12.5 (cohort 2), 17 (cohort 3)	0
Pawlowska *et al.* [64]	4	PBSC	Haploidentical	ATG+Bu+Flu preceded by Flu+Dexa	*	*	25*	75*	0*	0*

BM- bone marrow; CB- cord blood; PBSC- peripheral blood stem cells; MUD - matched unrelated donor; MRD – matched related donor (including matched sibling); Identical – HLA identical sibling; aGVHD – acute graft-versus-host-disease grade II-IV; cGVHD – chronic graft-versus-host-disease ; TRM –transplant related mortality; OM – overall mortality; Bu- busulphan; Cy- cyclophosphamide; Flu- fludarabine; TBI- total body irradiation; ATG- anti-thymocyte globulin; AL- alemtuzumab; Sir- sirolimus; TIB- total body irradiation; OKT3- muromonab CD3 OKT3: Mel- melphalan; Thio- thiotepa; Treo- treosulfan; Dexa- dexamethasone; AZA- azathioprine; HU- hydroxyurea; TT-; MAC- myeloablative conditioning; RIC- reduced intensity conditioning; [#] each cohort refers to a different concentration of cyclophosphamide; * study with only 4 patients. Numbers represent percentages.

3.2 Thalassemia Major

Contrary to the controversy still surrounding HSCT in SCD, in TM this procedure is widely accepted and even recommended as early as possible in children who depend on chronic blood transfusions and for whom an HLA identical sibling donor is available [50]. In 2016 in Europe, 335 bone marrow transplants were performed in patients with thalassemia, from which 203 were from HLA identical donors [18].

The first publications on HSCT performed in TM patients dates from the 80s and 90s [66-68]. In these early studies is included the "Pesaro Experience" which identified 3 classes of risk for patients with TM which could be used to correlate the medical status at the time of transplantation with the predicted outcome post-procedure [67, 69]. These 3 classes are based on the presence of three criteria: degree of hepatomegaly, degree of portal fibrosis, and quality of chelation therapy before the transplant. Patients for whom all three criteria are adverse are included in Class 3; if none of these criteria are adverse they belong to Class 1; with one adverse criteria or any combination of two, patients are included in Class 2 [69]. This group reported EFS of 95%, 84%, and 64% for patients of Class 1, 2, and 3, respectively, transplanted with BM from an HLA-identical donor [69] under a BuCy conditioning regimen. Similar results for Class 3 patients (EFS 62.2%) were obtained in a study in Turkey comprising of 245 children with TM receiving BM from an HLA-matched related donor (Table 3) [70].

Similarly, two pediatric centres in the UK reported their joint results with TM patients (most in Class 2 or 3) transplanted with BM from HLA-identical donors over a period of 10 years, showing an EFS of 82% despite 31% and around 14% of aGVHD grade II-IV and cGVHD, respectively [71]. A study with 197 patients showed that in fact Class 3 is a heterogeneous group that can be further subdivided, according to age and liver size, into two subgroups identifying a high-risk group with very poor HSCT outcomes (OS 39.01% and EFS 23.93%) [72]. These and other studies revealed similar results in what concerns the incidence of aGVHD and cGVHD with sinusoidal obstructive syndrome as one of the most threatening events but in general, these results revealed a low incidence of long-term post-transplantation side effects [73-75]. Most patients in these studies were submitted to myeloablative regimens with Bu (per os), Cy with or without ATG. Other conditioning regimens were developed using Bu (iv)+Cy with or without thiotepa or fludarabine in pediatric patients with values of OS as high as 97% and low rejection rates (around 2% and 5%) (Table 3) [76, 77].

To minimize rejection in Class 3 pediatric patients, other immune suppressive conditioning regimens were tested. One of the strategies adopted included a scheme of hyper-transfusion combined with hydroxyurea, azathioprine, and fludarabine a few weeks prior to the classic BuCy conditioning regimen. This group reported 93% OS and 85% EFS with only 8% graft rejection in 33 patients and minimal aGVHD (Table 3) [78].

Busulfan is considered a major agent for myeloablation, but its concentration must be balanced to achieve minimal mortality and morbidity due to its side-effects while maintaining high enough concentrations to minimize graft rejection [36, 39]. Due to the difficulty experienced to achieve the correct concentration of busulfan, especially in children, some groups have tried equally effective and less toxic regimens using thiotepa+treosulfan+fludarabine obtaining OS from 93% to 78.5%, EFS from 84% to 71.4%, and graft rejection from 9% [79] to 7% [80]. It is extremely difficult to compare these and other studies because they differ on the source of stem cells used and some

even used several sources of stem cells within the same study [77, 80]; furthermore, in some trials both HLA-identical related and unrelated donors [77, 79] are used and Class 3 patients vary in number for each study (Table 3) [75, 79, 80].

A national French multicenter study published results on HSCT performed from 1985 to 2007 in a total of 108 patients transplanted from HLA-identical sibling donors and conditioned with BuCy with or without ATG. The authors found a decrease in graft rejection from 35% to 10% when ATG was included in the conditioning regimen and around 82% EFS in patients with ATG versus 55% EFS without ATG [81]. An Italian group proposed the addition of ATG in conditioning regimens for high-risk TM patients, i.e., Class 3 pediatric patients, adults, patients receiving hematopoietic stem cells from unrelated donors, or patients receiving a second HSCT (Table 3) [67, 78, 82, 83].

An alternative strategy would be to perform a risk assessment for each patient and tailor conditioning regimens for each individual score, which was the strategy adopted by Hussein AA et al [84]. This group used a myeloablative conditioning regimen consisting of BuCy+ATG in Class 1 and 2 patients whereas Class 3 patients were treated with a reduced intensity conditioning consisting of Bu+fludarabine+TLI (total lymphoid irradiation)+ATG. OS and EFS were around 97% and 90%, respectively, for myeloablative conditioning regimen and 100% and 77%, respectively, for reduced intensity conditioning regimen (Table 3) [84].

A retrospective non-interventional study analysed data from the hemoglobinopathy registry of the European Society for Blood and Bone Marrow Transplantation from 2000 to 2010 in a total of 1493 patients undergoing HSCT for TM [16]. Their findings showed that if patients were transplanted below age 14, from a matched sibling donor or a matched family donor, an OS >90% and an EFS >83% could be expected.

3.2.1 Source of HSCs

In most studies mentioned so far for HSCT in TM, authors analysed heterogeneous groups of patients regardless of the source of stem cells used, PBSC or BM. As for SCD, a comparison of hematopoietic transplantation outcomes amongst different stem cell sources is of crucial importance.

Publications have shown that Class 1 and 2 TM children treated with BuCy and transplanted with HLA-matched PBSC or BM had no differences in 2-year OS (83% and 89%, respectively) and EFS (76% for both groups) despite a higher incidence of aGVHD grades II-IV (72% with PBSC vs 55% BM) and cGVHD (48% with PBSC vs 19% BM) in patients transplanted with PBSC [85]. GVHD prophylaxis with cyclosporine and methotrexate showed a higher incidence of GVHD (especially cGVHD) in patients transplanted with PBSC, highlighting the need for alternative strategies for the prevention of GVHD (Table 3) [85, 86].

Implementation of a triple immunosuppression protocol (cyclosporine, methotrexate, and prednisolone) for prophylaxis and treatment of GVHD in Class 3 BM and PBSC transplanted patients proved efficient with no statistically significant differences found between aGVHD (grade II-IV 26% PBSC vs 30% BM) and cGVHD (30% PBSC vs 24% BM) in the two groups of patients, i.e., with BM or PBSC (Table 3) [87].

As for SCD, in TM patients HSCT can be performed using CB cells with very good outcomes. Despite slower neutrophil and platelet recovery times, overall, patients transplanted with CB stem

cells exhibit less acute and chronic GVHD with similar OS and DFS as compared to patients transplanted with BM (Table 3) [88].

3.2.2 Unrelated and Haploidentical Donors

The availability of a matched sibling donor is the major limitation to HSCT in TM patients. The advances and successes of conditioning regimens have allowed the extension of the application of HSCT to TM patients without an HLA matched sibling donor. Bone marrow from matched unrelated donors has been used successfully in HSCT of TM patents treated with BuCy with an OS of 79%, EFS of 66%, and 13% of graft rejection, despite 41% of aGVHD and 25% of cGVHD (Table 3) [89]. The study also reported that 83% of the patients who died (5 out of 6) were Class 3 patients.

In 2014 a group published its results with 98 TM patients divided into two groups receiving matched-related or unrelated donor BM and showed an EFS of 88% and 82%, respectively, with an OS of 94% similar to both groups [90]. The major pitfall of this study is that it included Class 3 patients that were treated with a reduced intensity conditioning regimen and a group treated with a myeloablative conditioning regimen but each of these groups included recipients of related and unrelated donor stem cells. Furthermore, within the myeloablative conditioning regimen group, conditioning was different for recipients of related or unrelated stem cells. In spite of this, the authors showed very good results not only for TM patients transplanted with stem cells from unrelated donors as described above, but also for class 3 patients treated with a reduced intensity regimen with OS and EFS of 90% each [90]. In 2018 a study reported results on 51 class 1 and 2 TM patients treated with BuCy+fludarabine+tiothepa+ATG receiving HLA-matched HSCT from unrelated donors with an OS and EFS of 98% and 92%, respectively and only 2 patients experiencing graft rejection (Table 3) [91].

The use of HSC from unrelated donors broadens the window of application of HSCT to TM patients without an available HLA-identical sibling or HLA matched family member. However, due to the high rates of acute GVHD, these procedures should be performed in experienced centers and, preferably, in class 1 and 2 TM patients. Furthermore, a 10/10 or 9/10 match between donor and recipient should be the minimum requirement.

Unfortunately some patients do not find an HLA-matched unrelated donor and in this case alternative donors need to be used. In these situations the only solution is to rely on haploidentical donors. Advances in conditioning regimens and in treatments for acute and chronic GVHD will probably make it possible to look at this class of donors as a real option in the future. At the moment, early studies using heavy conditioning regimens including BuCy + fludarabine + tiothepa + ATG and T cell depleted PBSC have shown promising results despite high rates of graft rejection (Table 3) [92-94].

Table 3 Comparison of outcomes of HSCT in several clinical trials in thalassemia major patients.

Reference	Patients	Stem cells	Donor	Conditioning	OS	EFS/DFS	aGVHD	cGVHD	Graft rejection (or failure)	TRM or OM
Yesilipek *et al.* [70]	245	BM, PBSC, CB	MRD	Several	85	-/68	13.5	12.5	31.8	7.75
Lawson *et al.* [71]	55	BM	Identical, MRD	Bu+Campath, ATG or Flu	94.5	-/81.8	31	14.5	13.2	5.4
Mathews *et al.* [72]	197	BM	MRD	Cy+Bu or RIC	39 (high risk class 3) 78.3 (low risk class 3)	23.9 (high risk class 3) 70.3 (low risk class 3)	-	-	17 (high risk class 3) 14 (low risk class 3)	53.6 (high risk class 3) 20 (low risk class 3)
Di Bartolomeo *et al.*[73]	115	BM	MRD	Cy+Bu	89.2	-/85.7	37	17	6.7	8.7
Ullah *et al.*[74]	48	BM	MRD	Bu+Cy, HU+AZA+ Flu+Bu	79	-/75	35.4	8.3	10.4	20.8
Sabloff *et al.* [75]	179	BM	MRD	Bu+Cy± ATG	91 (class 2) 64 (class 3)	88 (class 2) 62 (class 3)	38	13	9.4	6.1

Gaziev *et al.* [76]	71	BM	Phenotipically identical parent	Bu iv different doses, preceded by HU+AZA	91	87	30	12	5	8.5
Li *et al.* [77]	82	BM, PBSC BM+CB	MRD, MUD	Bu+Cy+Flu+ Thio	92.3 (PBSC), 90 (BM)	90.4 (PBSC), 83.3 (BM)	9.6 (PBSC), 3.6 (BM)	0	1.9 (PBSC), 6.9 (BM)	7.7 (PBSC), 10 (BM)
Sodani *et al.* [78]	33	BM	Identical	By+Cy+ preceded by HU+AZA+Flu	93/85	85/-	9	ND	8	6
Bernardo *et al.* [79]	60	BM	Identical, MUD	Flu+Thio+ Treo	93/84	-/84	14	1.7	9	7
Choudhary *et al.* [80]	28	BM, PBSC, CB+BM	MRD, MUD	Flu+Thio+ Treo	71.4	-/78.5	14	7.1	7.1	21.4
Galambrun *et al.* [81]	108	BM, PBSC, CB+BM	Identical	Bu+Cy±ATG	86.8	-/69.4 (83 post 1994)	20.4	11.1	10	12
Lucarelli *et al.* [82]	107	BM	Identical	Bu+Cy	66	-/62	-	-	4	28
Gaziev *et al.* [83]	122	BM	Identical	Bu+Cy (Group A)Hu+AZA+ Flu (Group B)	66 (Group A), 67 (Group B)	62 (Group A), 67 (Group B)	20.5 (Group A),20 (Group B)	5.6 (Group A),13.3 (Group B)	4 (Group A), 8 (Group B)	37 (Group A), 27 (Group B)

Hussein *et al.* [84]	44	PBSC	MRD	Bu+Cy+ATG, Bu+Flu+ATG+ TBI	97.8 (total), 96.8 (MAC), 100 (RIC)	86.4 (total), 90.3 (MAC), 77 (RIC)	20.5 (total), 16.1 (MAC), 30.8 (RIC)	16 (total), 19 (MAC), 7.6 (RIC)	11.3 (total), 6.4 (MAC), 23 (RIC),	2.3 (total) 3.2(MAC) 0 (RIC)
Ghavamzadeh *et al.* [85]	183	BM, PBSC	MRD	Bu+Cy	83 (PBSC), 89 (BM)	76 (PBSC), 76 (BM)	74 (PBSC), 57 (BM)	49 (PBSC), 17 (BM)	18 (PBSC), 9 (BM)	14 (PBSC), 9 (BM)
Iravani *et al.* [86]	52	BM, PBSC	Identical	Bu+Cy	80	65	47	28	5.8	13.5
Irfan *et al.* [87]	56	BM, PBSC	MRD	Bu+Cy+ALG	73 (BM), 67 (PBSC)	65 (BM), 55 (PBSC)	30 (BM), 26 PBSC	24 (BM), 30 PBSC	7 (BM), 15 (PBSC)	27 (BM), 33 (PBSC)
Locatelli *et al.* [88]	485	CB,BM	Identical	several	95 (BM), 97 (CB)	85 (BM), 83 (CB)/ 88 (BM), 83(CB)	21 (BM), 10 (CB)	11.8 (BM), 7.1 (CB)	7.4 (BM), 10.4 (CB)	4.6 (BM), 3.1 (CB)
La Nasa *et al.* [89]	32	BM	MUD	Bu+Cy, Bu+Cy+Thio	93	66	41	25	13	25
Anurathapan *et al.* [90]	98	BM	MRD, MUD	Flu+Bu, Cy+Flu+Bu	94	87	31.6	12.2	6	7.1
Karasu *et al.* [91]	51	BM, PBSC	MUD	BuCy+Flu+ Thio+ATG	98	92	12	0	3.9	1.9

Gaziev *et al.* [92]	14	PBSC	Haploidentical	ByCy+Thio	84	69	28	21	14	7
Sodani *et al.* [93]	31	BM, PBSC	Haploidentical	BuCy+Flu+Thio+ATG	93	70	0	0	23	7
Anurathapan *et al.* [94]	31	PBSC	Haploidentical	Flu+Dexa followed by ATG+Flu+Bu	95	94	29	16.1	6.4	3.2

BM- bone marrow; CB- cord blood; PBSC- peripheral blood stem cells; MUD - matched unrelated donor; MRD – matched related donor (including matched sibling); Identical – HLA identical sibling; Bu- busulphan; Cy- cyclophosphamide; Flu- fludarabine; TBI- total body irradiation; ATG- anti-thymocyte globulin; Thio- thiotepa; Treo- treosulfan; Dexa- dexamethasone; AZA- azathioprine; HU- hydroxyurea; TT-; MAC- myeloablative conditioning; RIC- reduced intensity conditionin; TRM – transplant related mortality; OM – overall mortality; aGVHD – acute graft-versus-host-disease grade II-IV; cGVHD – chronic graft-versus-host-disease. Numbers represent percentages.

4. Gene Therapy

TM and SCD are both hemoglobinopathies but the course of the disease differs greatly between the two. Moreover, these pathologies are extremely debilitating, greatly reducing the quality of life of patients and are a huge burden to healthcare systems. The only available cure is HSCT but this therapy comes with high risks and side effects either from immunosuppression, GVHD, or graft rejection. Furthermore, in most cases, no matched sibling donors are available which further increases the risks associated with this approach.

Recently, gene therapy arose as a new potential strategy to cure TM and SCD. Several groups have worked on gene transference into hematopoietic stem cells to cure hemoglobinopathies. The major goal is to achieve safe and efficient gene expression that would allow transplanted stem cells to safely and stably express the gene of interest.

Retroviral vectors were one of the available tools for gene transfer experiments. Although initially promising due to their stable integration into the genome, they proved inefficient because they are prone to variegation and silencing in stem cells [95]; furthermore, they mainly integrate dividing hematopoietic stem cells and their tendency to insert near transcriptional sites makes them more prone to alter the expression of endogenous genes and induce insertional oncogenesis [96].

Lentiviral vectors can insert larger amounts of DNA, integrate non-dividing cells, and have a bias towards integration into sites of gene bodies. Furthermore, they were designed with a self-inactivating strategy which minimizes activation of genes in the vicinity of the insertion point, increasing their safety profile [97].

Lentiviruses were amongst the first viral vectors to be tested successfully [98, 99]. In short, CD34+ autologous stem cells are obtained from peripheral blood by apheresis or from bone marrow, and ex vivo transduced with the lentiviral vector carrying the gene of interest. Afterwards, transduced autologous hematopoietic stem cells are transplanted (reinfused) into the recipient [98, 100].

In 2017, Ribeil J.A. and colleagues presented their results on the first SCD patient successfully transplanted with lentiviral transduced autologous CD34+ bone marrow cells achieving stable haemoglobin levels and transfusion independence for 6 months after transplantation [101]. No adverse events related to the lentiviral vector used were reported in this study.

In a very recent study, 22 TM patients were submitted to a myeloablative conditioning regimen with Bu and reinfused with autologous CD34+ cells transduced ex vivo with a lentiviral vector encoding a variant of haemoglobin A. At a median of 26 months after infusion most patients were red cell transfusion independent with haemoglobin levels ranging from 8.2 to 13.7 per decilitre. The adverse events observed were transplant related and not attributed to lentiviral gene therapy itself [102]. Numerous phase I-II clinical trials are ongoing and, in the future, patients lacking an HLA-identical donor will certainly be eligible and benefit from gene therapy [103, 104].

4.1 CRISP/CAS9 Technology

An important goal of gene therapy is to overcome the random insertion of viral vectors into the human genome. An ideal strategy would target the locus of insertion in the DNA and precisely place the gene carried by the viral vector at specific sites in the DNA. So, instead of using a gene addition procedure, we should rather adopt a gene editing approach.

One strategy to achieve this goal is by using the CRISP/Cas9 (clustered, regularly interspaced palindromic repeats-associated nuclease Cas9) technology. CRISP/Cas9 allows the introduction of site-specific double-strand DNA breaks into the human genome guided by a RNA molecule (gRNA) which recognises a specific sequence on the DNA and is bound to an endonuclease, Cas9, which cleaves the double stranded DNA.

Two different approaches were tested by researchers: 1) correction of the mutated β-globin gene, and 2) enhancing fetal haemoglobin production. In both, the major challenge is to choose the best approach to deliver CRISP/Cas9 gRNA inside the cells.

Hoban and colleagues have achieved 18% of gene modification in vitro in human CD34+ and progenitor cells using electroporation to insert the CRISP technology into the HSCs [105]. This strategy is less likely to succeed because as holes are introduced in the cell, it recognizes this as an external insult and reacts to correct it by destroying the RNA material inserted. As such, small percentages of modified cells should be expected.

Several approaches have been tested to correct the β-globin mutation using a viral vector to insert the CRISPR/Cas9 gRNA inside the cell, thus avoiding its destruction at cell entry. Results are modest, but promising. More frequently, an adenovirus is used to deliver the CRISP technology inside the hematopoietic cell. After enrichment for modified CD34+ cells, a percentage of 90% of gene corrected cells can be achieved [106].

Another group used an unmodified single gRNA together with a single stranded DNA oligonucleotide donor (ssODN) which allowed the correct placement and correction of the SCD mutation in HSCs without the need to resort to a viral vector for CRISP/Cas9 technology delivery [107].

More recently an improved CRISP/Cas9 system with higher on-target activity (HiFi Cas9) was used successfully to correct, ex vivo, the mutation causing SCD in human CD34+ hematopoietic and progenitor cells [108].

Rescuing fetal haemoglobin expression seems easier than correcting the β-globin gene. The reason is simple: expression of fetal haemoglobin is silenced through a set of genes, disrupting any of those genes will break its effect and automatically result in re-expression of fetal haemoglobin. This approach does not require homology-directed repair, because it does not involve correction of a mutation, but solely disruption of the expression of a repressor gene [109]. This approach is pursued by several pharmaceutical companies with promising results (CRISP Therapeutics, http://www.crisprtx.com; Editas Medicine, http://www.crisprtx.com).

Although encouraging, these are pre-clinical studies mainly with ex vivo transductions of hematopoietic stem cells and experiences in animal models. Hematopoietic stem cells are fragile and will require extensive in vitro manipulation in order to achieve β-globin gene correction followed by re-infusion into the host. It remains to be assessed if their potential to differentiate into the different subsets will be affected.

Other important issues concern the longevity of these cells after re-infusion, how they will repopulate the bone marrow, and how stable the edited gene expression will be.

5. Conclusion

Currently HSCT in SCD is only recommended for symptomatic pediatric patients with an HLA-identical sibling donor. The same happens with pediatric TM patients for whom HSCT is recommended before the development of iron overload and iron-related tissue damage [50]. At

the moment HSCT from unrelated donors for SCD and TM patients should only be considered in the context of controlled trials [50] due to the higher risk of graft rejection and cGVHD. Exceptions are pediatric TM patients with life-long controlled iron overload and a well-matched unrelated donor. This is a serious obstacle to the wide application of the procedure. Most patients do not have a matched sibling donor, so either they are enrolled in controlled HSCT trials or they must comply with the classic medical treatment with all its limitations.

Whenever possible BM should be used in HSCT for TM or SCD patients due to the higher rate of cGVHD observed with PBSC [50]. The prophylactic regimen recommended for prevention of cGVHD is a feature that also needs to be reviewed. The most widely used protocol includes MTX and cyclosporine but other associations using ATG or alemtuzumab should be tested in combination with other immunosuppressive drugs [50].

Consensus for HSCT in TM is easier to achieve because this pathology courses with more homogeneous clinical features and transfusion dependency is usually accepted as an indication for HSCT in these patients. On the contrary, in SCD there are substantial clinical differences between patients and disease development is often complicated and unexpected, so indications for HSCT in SCD are not so consensual.

In these hemoglobinopathies HSCT are allogeneic and considered the last treatment option. In haematological malignancies HSCT are mostly autologous and sometimes a first line treatment as soon as the patient is in remission. The fact that TM and SCD patients have more treatment options, although with a poor quality of life, sometimes deters them from risking HSCT.

In SCD it is also important to have in mind the late consequences of HSCT, namely gonadal dysfunction, which carries a high psychological burden for the remaining life of the patient. Would it be more beneficial to comply with a chronic transfusion program or to be transplanted with HSCT from HLA-identical donors? The DREPAGREFFE trial intends to answer this and other questions and it is surely a very important study whose results will be anxiously expected as soon as enough patients are recruited [110].

Furthermore, HSCT is a complex and very expensive technique only available in high income, developed countries, which requires expert teams and very well equipped centres, so it is not available for the majority of TM and especially SCD patients. The high cost of HSCT, when the majority of hemoglobinopathies occur in very low income countries, associated with the fact that it is not a first choice treatment probably explains why so few HSCT have been performed for these diseases. Nonetheless, these patients are a huge burden for healthcare systems. In the USA, it is estimated that a SCD patient at the age of 45 has cost $1 million in healthcare expenses so far [111].

The assumption that HSCT carries a considerable risk of mortality which may be superior to the one observed with supportive care in SCD patients imposes an enormous barrier to the acceptance of this procedure. The inherent risks of infertility and GVHD are additional obstacles to the wide acceptance of this procedure by patients and their parents. On the other hand, a successful transplant will enable normal erythropoiesis and a life free of SCD and its related life-long complications. Thus, the final questions are: do these patients show an improvement in quality of life? Will they remain disease free or is the risk still too high to take? More clinical trials need to be performed so we can answer these questions confidently, but surely HSCT is the only available option for some TM and SCD patients and it is an option that should seriously be considered whenever an HLA-identical donor is available.

New developments in gene therapy treatments bring hope to the application of new strategies for the cure of SCD or TM. So far, this approach has proved efficient and safe but long-term effects need to be studied and evaluated and at the present we can only be optimistic about this methodology as a promising future treatment.

Author Contributions

Vanessa G. Oliveira and Filipa Saraiva wrote the paper, Fátima Costa reviewed all sections, Aida Botelho de Sousa scientifically reviewed and corrected every section of the paper.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Gratwohl A, Pasquini MC, Aljurf M, Atsuta Y, Baldomero H, Foeken L, et al. One million haemopoietic stem-cell transplants: A retrospective observational study. Lancet Haematol. 2015; 2: e91-e100.
2. Passweg JR, Baldomero H, Basak GW, Chabannon C, Corbacioglu S, Duarte R, et al. The EBMT activity survey report 2017: A focus on allogeneic HCT for nonmalignant indications and on the use of non-HCT cell therapies. Bone Marrow Transplant. 2019. doi: 10.1038/s41409-019-0465-9.
3. Baldomero H, Aljurf M, Zaidi SZ, Hashmi SK, Ghavamzadeh A, Elhaddad A, et al. Narrowing the gap for hematopoietic stem cell transplantation in the East-Mediterranean/African region: Comparison with global HSCT indications and trends. Bone Marrow Transplant. 2019; 54: 402-417.
4. Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. Lancet. 2017; 390: 311-323.
5. Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Dewi M, et al. Global epidemiology of sickle haemoglobin in neonates: A contemporary geostatistical model-based map and population estimates. Lancet. 2013; 381: 142-151.
6. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008; 86: 480-487.
7. Naik RP, Haywood C. Sickle cell trait diagnosis: Clinical and social implications. Hematology Am Soc Hematol Educ Program. 2015; 2015: 160-167.
8. Lavouras LIdC. Hemoglobinopatias: Diagnóstico laboratorial e sua importância. Lisbon: University of Lisbon; 2015.
9. Costa SN, Madeira S, Sobral MA, Delgadinho G. Hemoglobinopatias em Portugal e a intervenção do médico de família. Rev Port Med Geral Fam. 2016; 32: 416-424.
10. Chonat S, Quinn CT. Current standards of care and long term outcomes for thalassemia and sickle cell disease. In: Gene and cell therapies for beta-globinopathies. Berlin: Springer; 2017. p. 59-87.
11. Coulet A, Shah N, Hunter L, Barral C, Altman RB. Extraction of genotype-phenotype-drug relationships from text: From entity recognition to bioinformatics application. Biocomputing 2010: World Scientific; 2010. p. 485-487.

12. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. N Engl J Med. 2017; 376: 1561-1573.
13. Ferreira F, Carvalho F, Costa V, Farinha N, Gil-da-Costa MJ, Norton L, et al. Anemias hereditárias. Acta Pediatr. 1998; 29: 435-439.
14. Algiraigri AH, Wright NA, Paolucci EO, Kassam A. Hydroxyurea for nontransfusion-dependent β-thalassemia: A systematic review and meta-analysis. Hematol Oncol Stem Cell Ther. 2017; 10: 116-125.
15. Piel FB, Weatherall DJ. The α-thalassemias. N Engl J Med. 2014; 371: 1908-1916.
16. Baronciani D, Angelucci E, Potschger U, Gaziev J, Yesilipek A, Zecca M, et al. Hemopoietic stem cell transplantation in thalassemia: A report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000–2010. Bone Marrow Transplant. 2016; 51: 536.
17. Johnson FL, Look AT, Gockerman J, Ruggiero MR, Dalla-Pozza L, Billings III FT. Bone-marrow transplantation in a patient with sickle-cell anemia. N Engl J Med. 1984; 311: 780-783.
18. Passweg JR, Baldomero H, Bader P, Basak GW, Bonini C, Duarte R, et al. Is the use of unrelated donor transplantation leveling off in Europe? The 2016 European Society for Blood and Marrow Transplant activity survey report. Bone Marrow Transplant. 2018; 53: 1139.
19. Wang WC, Ware RE, Miller ST, Iyer RV, Casella JF, Minniti CP, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: A multicentre, randomised, controlled trial (BABY HUG). Lancet. 2011; 377: 1663-1672.
20. Ware RE. How I use hydroxyurea to treat young patients with sickle cell anemia. Blood. 2010; 115: 5300-5311.
21. Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, et al. Management of sickle cell disease: Summary of the 2014 evidence-based report by expert panel members. Jama. 2014; 312: 1033-1048.
22. ASH. State of sickle cell disease, 2016 Report. Am Soc Hematol. 2016: 1-27.
23. Maitra P, Caughey M, Robinson L, Desai PC, Jones S, Nouraie M, et al. Risk factors for mortality in adult patients with sickle cell disease: A meta-analysis of studies in North America and Europe. Haematologica. 2017; 102: 626-636.
24. Walters MC, Patience M, Leisenring W, Eckman JR, Scott JP, Mentzer WC, et al. Bone marrow transplantation for sickle cell disease. N Engl J Med. 1996; 335: 369-376.
25. Henig I, Zuckerman T. Hematopoietic stem cell transplantation—50 years of evolution and future perspectives. Rambam Maimonides Med J. 2014; 5: e0028.
26. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010; 363: 2091-2101.
27. Norkin M, Wingard JR. Recent advances in hematopoietic stem cell transplantation. F1000Res. 2017; 6: 870.
28. Vermylen C, Cornu G, Ferster A, Brichard B, Ninane J, Ferrant A, et al. Haematopoietic stem cell transplantation for sickle cell anaemia: The first 50 patients transplanted in Belgium. Bone Marrow Transplant. 1998; 22: 1-6.
29. Walters MC, Storb R, Patience M, Leisenring W, Taylor T, Sanders JE, et al. Impact of bone marrow transplantation for symptomatic sickle cell disease: An interim report. Blood. 2000; 95: 1918-1924.

30. Panepinto JA, Walters MC, Carreras J, Marsh J, Bredeson CN, Gale RP, et al. Matched-related donor transplantation for sickle cell disease: Report from the Center for International Blood and Transplant Research. Br J Haematol. 2007; 137: 479-485.
31. Bernaudin F, Socie G, Kuentz M, Chevret S, Duval M, Bertrand Y, et al. Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. Blood. 2007; 110: 2749-2756.
32. Majumdar S, Robertson Z, Robinson A, Starnes S, Iyer R, Megason G. Outcome of hematopoietic cell transplantation in children with sickle cell disease, a single center's experience. Bone Marrow Transplant. 2010; 45: 895-900.
33. Dedeken L, Lê PQ, Azzi N, Brachet C, Heijmans C, Huybrechts S, et al. Haematopoietic stem cell transplantation for severe sickle cell disease in childhood: A single centre experience of 50 patients. Br J Haematol. 2014; 165: 402-408.
34. Lucarelli G, Isgrò A, Sodani P, Marziali M, Gaziev J, Paciaroni K, et al. Hematopoietic SCT for the Black African and non-Black African variants of sickle cell anemia. Bone Marrow Transplant. 2014; 49: 1376-1381.
35. Poonkuzhali B, Srivastava A, Quernin M, Dennison D, Aigrain E, Kanagasabapathy A, et al. Pharmacokinetics of oral busulphan in children with beta thalassaemia major undergoing allogeneic bone marrow transplantation. Bone Marrow Transplant. 1999; 24: 5-11
36. Chandy M, Balasubramanian P, Ramachandran S, Mathews V, George B, Dennison D, et al. Randomized trial of two different conditioning regimens for bone marrow transplantation in thalassemia–the role of busulfan pharmacokinetics in determining outcome. Bone Marrow Transplant. 2005; 36: 839-845.
37. Vassal G, Deroussent A, Hartmann O, Challine D, Benhamou E, Valteau-Couanet D, et al. Dose-dependent neurotoxicity of high-dose busulfan in children: A clinical and pharmacological study. Cancer Res. 1990; 50: 6203-6207.
38. Grochow LB, Jones RJ, Brundrett RB, Braine HG, Chen T-L, Saral R, et al. Pharmacokinetics of busulfan: Correlation with veno-occlusive disease in patients undergoing bone marrow transplantation. Cancer Chemother Pharmacol. 1989; 25: 55-61.
39. Ljungman P, Hassan M, Bekassy A, Ringden O, Öberg G. High busulfan concentrations are associated with increased transplant-related mortality in allogeneic bone marrow transplant patients. Bone Marrow Transplant. 1997; 20: 909-913.
40. Ferster A, Christophe C, Dan B, Devalck C, Sariban E. Neurologic complications after bone marrow transplantation for sickle cell anemia. Blood. 1995; 86: 408-409.
41. Walters MC, Sullivan KM, Bernaudin F, Souillet G, Vannier J-P, Johnson F, et al. Neurologic complications after allogeneic marrow transplantation for sickle cell anemia. Blood. 1995; 85: 879-884.
42. McPherson M, Hutcherson D, Olson E, Haight A, Horan J, Chiang K. Safety and efficacy of targeted busulfan therapy in children undergoing myeloablative matched sibling donor BMT for sickle cell disease. Bone Marrow Transplant. 2011; 46: 27-33.
43. Maheshwari S, Kassim A, Yeh R, Domm J, Calder C, Evans M, et al. Targeted Busulfan therapy with a steady-state concentration of 600–700 ng/mL in patients with sickle cell disease receiving HLA-identical sibling bone marrow transplant. Bone Marrow Transplant. 2014; 49: 366-369.

44. Bhatia M, Jin Z, Baker C, Geyer M, Radhakrishnan K, Morris E, et al. Reduced toxicity, myeloablative conditioning with BU, fludarabine, alemtuzumab and SCT from sibling donors in children with sickle cell disease. Bone Marrow Transplant. 2014; 49: 913-920.
45. Horan JT, Haight A, Dioguardi JL, Brown C, Grizzle A, Shelman C, et al. Using fludarabine to reduce exposure to alkylating agents in children with sickle cell disease receiving busulfan, cyclophosphamide, and antithymocyte globulin transplant conditioning: Results of a dose de-escalation trial. Biol Blood Marrow Transplant. 2015; 21: 900-905.
46. Strocchio L, Zecca M, Comoli P, Mina T, Giorgiani G, Giraldi E, et al. Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in children with sickle cell disease. Br J Haematol. 2015; 169: 726-736.
47. Walters MC, Hardy K, Edwards S, Adamkiewicz T, Barkovich J, Bernaudin F, et al. Pulmonary, gonadal, and central nervous system status after bone marrow transplantation for sickle cell disease. Biol Blood Marrow Transplant. 2010; 16: 263-272.
48. Hsieh MM, Fitzhugh CD, Weitzel RP, Link ME, Coles WA, Zhao X, et al. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. Jama. 2014; 312: 48-56.
49. King AA, Kamani N, Bunin N, Sahdev I, Brochstein J, Hayashi RJ, et al. Successful matched sibling donor marrow transplantation following reduced intensity conditioning in children with hemoglobinopathies. Am J Hematol. 2015; 90: 1093-1098.
50. Angelucci E, Matthes-Martin S, Baronciani D, Bernaudin F, Bonanomi S, Cappellini MD, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: Indications and management recommendations from an international expert panel. haematologica. 2014; 99: 811-820.
51. Walters MC, De Castro LM, Sullivan KM, Krishnamurti L, Kamani N, Bredeson C, et al. Indications and results of HLA-identical sibling hematopoietic cell transplantation for sickle cell disease. Biol Blood Marrow Transplant. 2016; 22: 207-211.
52. Gluckman E, Cappelli B, Bernaudin F, Labopin M, Volt F, Carreras J, et al. Sickle cell disease: An international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. Blood. 2017; 129: 1548-1556.
53. Saraf SL, Oh AL, Patel PR, Sweiss K, Koshy M, Campbell-Lee S, et al. Haploidentical peripheral blood stem cell transplantation demonstrates stable engraftment in adults with sickle cell disease. Biol Blood Marrow Transplant. 2018; 24: 1759-1765.
54. Seitz R, Hilger A, Heiden M. Bone marrow, peripheral blood, or umbilical cord blood: Does the source of allogeneic hematopoietic progenitor cells matter. J Blood Disord Transfus. 2012. doi: 10.4172/2155-9864.S1-007.
55. Bashey A, Zhang M-J, McCurdy SR, Martin AS, Argall T, Anasetti C, et al. Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell–replete haploidentical donor transplantation using post-transplant cyclophosphamide. J Clin Oncol. 2017; 35: 3002-3009.
56. Krishnamurti L, Abel S, Maiers M, Flesch S. Availability of unrelated donors for hematopoietic stem cell transplantation for hemoglobinopathies. Bone Marrow Transplant. 2003; 31: 547-550.
57. Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the US registry. N Engl J Med. 2014; 371: 339-348.

58. Kamani NR, Walters MC, Carter S, Aquino V, Brochstein JA, Chaudhury S, et al. Unrelated donor cord blood transplantation for children with severe sickle cell disease: Results of one cohort from the phase II study from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). Biol Blood Marrow Transplant. 2012; 18: 1265-1272.
59. Bolaños-Meade J, Fuchs EJ, Luznik L, Lanzkron SM, Gamper CJ, Jones RJ, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. Blood. 2012; 120: 4285-4291.
60. Dallas MH, Triplett B, Shook DR, Hartford C, Srinivasan A, Laver J, et al. Long-term outcome and evaluation of organ function in pediatric patients undergoing haploidentical and matched related hematopoietic cell transplantation for sickle cell disease. Biol Blood Marrow Transplant. 2013; 19: 820-830.
61. Shenoy S, Eapen M, Panepinto JA, Logan BR, Wu J, Abraham A, et al. A trial of unrelated donor marrow transplantation for children with severe sickle cell disease. Blood. 2016; 128: 2561-2567.
62. Khazal S, Fernandez P, Benard E, Shoberu B, Manwani D, Mahadeo KM. Allogeneic blood and marrow transplantation for pediatric patients with sickle cell anemia: An institutional experience. Blood. 2017; 130: 4581.
63. de la Fuente J, Dhedin N, Koyama T, Bernaudin F, Kuentz M, Karnik L, et al. Haploidentical bone marrow transplantation with post-transplantation cyclophosphamide plus thiotepa improves donor engraftment in patients with sickle cell anemia: Results of an International Learning Collaborative. Biol Blood Marrow Transplant. 2019; 25: 1197-1209.
64. Fitzhugh CD, Hsieh MM, Taylor T, Coles W, Roskom K, Wilson D, et al. Cyclophosphamide improves engraftment in patients with SCD and severe organ damage who undergo haploidentical PBSCT. Blood Adv. 2017; 1: 652-661.
65. Pawlowska AB, Cheng JC, Karras NA, Sun W, Wang LD, Bell AD, et al. HLA haploidentical stem cell transplant with pretransplant immunosuppression for patients with sickle cell disease. Biol Blood Marrow Transplant. 2018; 24: 185-189.
66. Thomas ED, Sanders JE, Buckner CD, Papayannopoulou T, Borgna-Pignatti C, De Stefano P, et al. Marrow transplantation for thalassaemia. Lancet. 1982; 320: 227-229.
67. Lucarelli G, Clift RA, Galimberti M, Polchi P, Angelucci E, Baronciani D, et al. Marrow transplantation for patients with thalassemia: Results in class 3 patients. Blood. 1996; 87: 2082-2088.
68. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, et al. Bone marrow transplantation in patients with thalassemia. N Engl J Med. 1990; 322: 417-421.
69. Lucarelli G, Galimberti M, Giardini C, Polchi P, Angelucci E, Baronciani D, et al. Bone marrow transplantation in thalassemia: The experience of Pesaro. Ann N Y Acad Sci. 1998; 850: 270-275.
70. Yesilipek MA, Ertem M, Cetin M, Öniz H, Kansoy S, Tanyeli A, et al. HLA-matched family hematopoetic stem cell transplantation in children with beta thalassemia major: The experience of the Turkish Pediatric Bone Marrow Transplantation Group. Pediatr Transplant. 2012; 16: 846-851.
71. Lawson SE, Roberts IA, Amrolia P, Dokal I, Szydlo R, Darbyshire PJ. Bone marrow transplantation for β-thalassaemia major: The UK experience in two paediatric centres. Br J Haematol. 2003; 120: 289-295.
72. Mathews V, George B, Deotare U, Lakshmi KM, Viswabandya A, Daniel D, et al. A new stratification strategy that identifies a subset of class III patients with an adverse prognosis

among children with β thalassemia major undergoing a matched related allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2007; 13: 889-894.
73. Di Bartolomeo P, Santarone S, Di Bartolomeo E, Olioso P, Bavaro P, Papalinetti G, et al. Long-term results of survival in patients with thalassemia major treated with bone marrow transplantation. Am J Hematol. 2008; 83: 528-530.
74. Ullah K, Khan B, Raza S, Ahmed P, Satti TM, Butt T, et al. Bone marrow transplant cure for β-thalassaemia major: Initial experience from a developing country. Ann Hematol. 2008; 87: 655-661.
75. Sabloff M, Chandy M, Wang Z, Logan BR, Ghavamzadeh A, Li C-K, et al. HLA-matched sibling bone marrow transplantation for β-thalassemia major. Blood. 2011; 117: 1745-1750.
76. Gaziev J, Nguyen L, Puozzo C, Mozzi AF, Casella M, Donnorso MP, et al. Novel pharmacokinetic behavior of intravenous busulfan in children with thalassemia undergoing hematopoietic stem cell transplantation: A prospective evaluation of pharmacokinetic and pharmacodynamic profile with therapeutic drug monitoring. Blood. 2010; 115: 4597-4604.
77. Li C, Wu X, Feng X, He Y, Liu H, Pei F, et al. A novel conditioning regimen improves outcomes in β-thalassemia major patients using unrelated donor peripheral blood stem cell transplantation. Blood. 2012; 120: 3875-3881.
78. Sodani P, Gaziev D, Polchi P, Erer B, Giardini C, Angelucci E, et al. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. Blood. 2004; 104: 1201-1203.
79. Bernardo ME, Piras E, Vacca A, Giorgiani G, Zecca M, Bertaina A, et al. Allogeneic hematopoietic stem cell transplantation in thalassemia major: Results of a reduced-toxicity conditioning regimen based on the use of treosulfan. Blood. 2012; 120: 473-476.
80. Choudhary D, Sharma SK, Gupta N, Kharya G, Pavecha P, Handoo A, et al. Treosulfan-thiotepa-fludarabine–based conditioning regimen for allogeneic transplantation in patients with thalassemia major: A single-center experience from north India. Biol Blood Marrow Transplant. 2013; 19: 492-495.
81. Galambrun C, Pondarré C, Bertrand Y, Loundou A, Bordigoni P, Frange P, et al. French multicenter 22-year experience in stem cell transplantation for beta-thalassemia major: lessons and future directions. Biol Blood Marrow Transplant. 2013; 19: 62-68.
82. Lucarelli G, Clift R, Galimberti M, Angelucci E, Giardini C, Baronciani D, et al. Bone marrow transplantation in adult thalassemic patients. Blood. 1999; 93: 1164-1167.
83. Gaziev J, Sodani P, Polchi P, Andreani M, Lucarelli G. Bone marrow transplantation in adults with thalassemia: Treatment and long-term follow-up. Ann N Y Acad Sci. 2005; 1054: 196-205.
84. Hussein AA, Al-Zaben A, Ghatasheh L, Natsheh A, Hammada T, Abdel-Rahman F, et al. Risk adopted allogeneic hematopoietic stem cell transplantation using a reduced intensity regimen for children with thalassemia major. Pediatr Blood Cancer. 2013; 60: 1345-1349.
85. Ghavamzadeh A, Iravani M, Ashouri A, Mousavi SA, Mahdavi N, Shamshiri A, et al. Peripheral blood versus bone marrow as a source of hematopoietic stem cells for allogeneic transplantation in children with class I and II beta thalassemia major. Biol Blood Marrow Transplant. 2008; 14: 301-308.
86. Iravani M, Tavakoli E, Babaie MH, Ashouri A, Khatami F, Ghavamzadeh A. Comparison of peripheral blood stem cell transplant with bone marrow transplant in class 3 thalassemic patients. Exp Clin Transplant. 2010; 8: 66-73.

87. Irfan M, Khalil Hashmi SA, Shamsi T, Farzana T, Ansari S, Panjwani V, et al. β-Thalassaemia major: Bone marrow versus peripheral blood stem cell transplantation. J Pak Med Assoc. 2008; 58: 107-110.
88. Locatelli F, Kabbara N, Ruggeri A, Ghavamzadeh A, Roberts I, Li CK, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood. 2013; 122: 1072-1078.
89. La Nasa G, Giardini C, Argiolu F, Locatelli F, Arras M, De Stefano P, et al. Unrelated donor bone marrow transplantation for thalassemia: The effect of extended haplotypes. Blood. 2002; 99: 4350-4356.
90. Anurathapan U, Pakakasama S, Mekjaruskul P, Sirachainan N, Songdej D, Chuansumrit A, et al. Outcomes of thalassemia patients undergoing hematopoietic stem cell transplantation by using a standard myeloablative versus a novel reduced-toxicity conditioning regimen according to a new risk stratification. Biol Blood Marrow Transplant. 2014; 20: 2066-2071.
91. Karasu G, Uygun V, Daloglu H, Kilic S, Ozturkmen S, Dincer Z, et al. Hematopoietic stem cell transplantation from unrelated donor in children with beta thalassemia major. Biol Blood Marrow Transplant. 2018; 24: S428-S429.
92. Gaziev J, Isgrò A, Sodani P, Paciaroni K, De Angelis G, Marziali M, et al. Haploidentical HSCT for hemoglobinopathies: Improved outcomes with TCRαβ+/CD19+-depleted grafts. Blood Adv. 2018; 2: 263-270.
93. Sodani P, Isgrò A, Gaziev J, Paciaroni K, Marziali M, Simone MD, et al. T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients. Pediatr Rep. 2011; 3: e13.
94. Anurathapan U, Hongeng S, Pakakasama S, Sirachainan N, Songdej D, Chuansumrit A, et al. Hematopoietic stem cell transplantation for homozygous β-thalassemia and β-thalassemia/hemoglobin E patients from haploidentical donors. Bone Marrow Transplant. 2016; 51: 813-818.
95. Rivella S, Sadelain M, editors. Genetic treatment of severe hemoglobinopathies: The combat against transgene variegation and transgene silencing. Semin Hematol. 1998; 35: 112-125.
96. Cattoglio C, Facchini G, Sartori D, Antonelli A, Miccio A, Cassani B, et al. Hot spots of retroviral integration in human CD34+ hematopoietic cells. Blood. 2007; 110: 1770-1778.
97. Miyoshi H, Blömer U, Takahashi M, Gage FH, Verma IM. Development of a self-inactivating lentivirus vector. J Virol. 1998; 72: 8150-8157.
98. Urbinati F, Wherley J, Geiger S, Fernandez BC, Kaufman ML, Cooper A, et al. Preclinical studies for a phase 1 clinical trial of autologous hematopoietic stem cell gene therapy for sickle cell disease. Cytotherapy. 2017; 19: 1096-1112.
99. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature. 2010; 467: 318.
100. Cavazzana M, Ribeil J-A, Payen E, Suarez F, Beuzard Y, Touzot F, et al. Outcomes of gene therapy for severe sickle disease and beta-thalassemia major via transplantation of autologous hematopoietic stem cells transduced ex vivo with a lentiviral beta AT87Q-globin vector. Blood. 2015; 126: 202.
101. Ribeil J-A, Hacein-Bey-Abina S, Payen E, Magnani A, Semeraro M, Magrin E, et al. Gene therapy in a patient with sickle cell disease. N Engl J Med. 2017; 376: 848-855.

102. Thompson AA, Walters MC, Kwiatkowski J, Rasko JE, Ribeil J-A, Hongeng S, et al. Gene therapy in patients with transfusion-dependent β-thalassemia. N Engl J Med. 2018; 378: 1479-1493.
103. Demirci S, Uchida N, Tisdale JF. Gene therapy for sickle cell disease: An update. Cytotherapy. 2018; 20: 899-910.
104. Sii-Felice K, Giorgi M, Leboulch P, Payen E. Hemoglobin disorders: Lentiviral gene therapy in the starting blocks to enter clinical practice. Exp Hematol. 2018; 64: 12-32.
105. Hoban MD, Lumaquin D, Kuo CY, Romero Z, Long J, Ho M, et al. CRISPR/Cas9-mediated correction of the sickle mutation in human CD34+ cells. Mol Ther. 2016; 24: 1561-1569.
106. Dever DP, Bak RO, Reinisch A, Camarena J, Washington G, Nicolas CE, et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature. 2016; 539: 384-389.
107. DeWitt MA, Magis W, Bray NL, Wang T, Berman JR, Urbinati F, et al. Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. Sci Transl Med. 2016; 8: 360ra134.
108. Vakulskas CA, Dever DP, Rettig GR, Turk R, Jacobi AM, Collingwood MA, et al. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. Nat Med. 2018; 24: 1216.
109. Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science. 2008; 322: 1839-1842.
110. Chevret S, Verlhac S, Ducros-Miralles E, Dalle J-H, De Latour RP, De Montalembert M, et al. Design of the DREPAGREFFE trial: A prospective controlled multicenter study evaluating the benefit of genoidentical hematopoietic stem cell transplantation over chronic transfusion in sickle cell anemia children detected to be at risk of stroke by transcranial Doppler (NCT 01340404). Contemp Clin Trials. 2017; 62: 91-104.
111. Kauf TL, Coates TD, Huazhi L, Mody-Patel N, Hartzema AG. The cost of health care for children and adults with sickle cell disease. Am J Hematol. 2009; 84: 323-327.