Cure for thalassemia major – from allogeneic hematopoietic stem cell transplantation to gene therapy

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation has been well established for several decades as gene replacement therapy for patients with thalassemia major, and now offers very high rates of cure for patients who have access to this therapy. Outcomes have improved tremendously over the last decade, even in high-risk patients. The limited data available suggests that the long-term outcome is also excellent, with a >90% survival rate, but for the best results, hematopoietic stem cell transplantation should be offered early, before any end organ damage occurs. However, access to this therapy is limited in more than half the patients by the lack of suitable donors. Inadequate hematopoietic stem cell transplantation services and the high cost of therapy are other reasons for this limited access, particularly in those parts of the world which have a high prevalence of this condition. As a result, fewer than 10% of eligible patients are actually able to avail of this therapy. Other options for curative therapies are therefore needed. Recently, gene correction of autologous hematopoietic stem cells has been successfully established using lentiviral vectors, and several clinical trials have been initiated. A gene editing approach to correct the β-globin mutation or disrupt the *BCL11A* gene to increase fetal hemoglobin production has also been reported, and is expected to be introduced in clinical trials soon. Curative possibilities for the major hemoglobin disorders are expanding. Providing access to these therapies around the world will remain a challenge.

Introduction

Thalassemias are the most common human monogenic disorders related to the deficiency of the production of either the α- or β-globin chains.[1] β-thalassemia is a larger clinical problem because its homozygous form, thalassemia major, leads to severe morbidity and mortality due to very low endogenous hemoglobin levels which are incompatible with life.[2] Even though educating the society at large, mass screening, cohort counselling and prenatal diagnosis can be applied to very effectively reduce the incidence of β-thalassemia major, this has only been achieved in some countries.[3] More than 50,000 children with this disease are born worldwide each year, adding to the disease burden of this condition.[4] Hypertransfusion and iron chelation have been the mainstay of therapy for thalassemia major for nearly 50 years.[5] However, these therapies are often ineffective due to complications related to repeated transfusions and inadequate iron chelation. The main reasons for this are the lack of compliance with the planned therapy due to its logistic demands as well as the ongoing costs of chelation therapy. This approach, therefore, leads to significant morbidity and mortality with up to 50% of these patients developing significant organ dysfunction by the time they are adults, even in Western countries.[6] This figure is even higher for patients in developing countries.[7] The need for curative therapy for thalassemia major was addressed with the success of allogeneic hematopoietic stem cell transplantation (alloHSCT), which was initiated in the early 1980s as a way to replace the defective gene in such patients.[8] AlloHSCT

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remains the only widely available curative therapy for this condition at present. The best results are seen when alloHSCT is offered early, before complications related to iron overload or transfusion-transmitted infections set in, with survival rates of over 90% being reported in these patients.[9]

The outcomes of higher risk patients have also continuously improved over the last two decades, so that 80-90% long-term survival rates have been obtained even in this group of patients.[10,11] However, there are many challenges in offering alloHSCT as a therapy for these patients all over the world.[12] More recently, gene replacement in autologous hematopoietic stem cells (autoHSCs) using viral vectors has become a reality, witGST M1 null status'h several clinical trials showing its success and potential for wider use.[13] This article will address the status of alloHSCT for β-thalassemia major and also briefly review the newer options for gene correction in autoHSCs using different approaches.

Allogeneic Hematopoietic Stem Cell Transplantation

While the classical principles of alloHSCT remain the same in treating patients with thalassemia major, early results showed that there were special challenges related to pre-existing organ dysfunction, particularly involving the liver as well as the hyperactive immune system, probably related to repeated transfusions.[14] Given that most of these patients were children with a non-malignant disease, busulfan (14-16mg/kg total dose) and cyclophosphamide (160-200 mg/kg total dose) based conditioning was chosen, even though many of them had liver dysfunction related to iron overload or transfusion-related viral hepatitis.[8] Significant regimen-related toxicities (RRT) were observed if the doses of the drugs were intensified, while there was a high incidence of graft rejections if the doses were reduced. In high-risk patients, this led to mortality rates of up to 35% and rejection in nearly 30% of patients, resulting in long-term event-free survival of only about 50%.[15] High RRT was attributed to the sequential use of these two drugs, often with severe sinusoidal obstruction syndrome, particularly in those with significant liver dysfunction.[16] RRTs were also shown to be associated with certain genetic polymorphisms in the glutathione S transferase M1 (*GSTM1*) and the cytochrome P450 (*CYP450*) genes. Detailed pharmacogenetic studies showed that the *GST M1* null status and the *CYP2C9* gene polymorphisms affected the pharmacokinetics of busulfan and cyclophosphamide, with a possible impact on RRTs.[17,18] Therefore, modifications in the approach to alloHSCT for these patients were needed.

Over the last decade, the results of alloHSCT have improved significantly. This has been possible due to better risk stratification, more effective targeted dose adjustment of intravenous busulfan during conditioning, a modified conditioning regimen and continually improving supportive care.[19,20] Patients over 7 years of age with hepatomegaly of more than 5cm have been identified as an especially high-risk group who are candidates for/require novel approaches, particularly with regard to their conditioning regimen and preparation for transplant.[21,22]

Two approaches have been taken in this regard, one based on pre-transplant immunosuppression using fludarabine, and the other by introducing a longer gap between the use of busulfan and cyclophosphamide during conditioning[23,24] or using less toxic myeloablative agents such as treosulfan and avoiding cyclophosphamide completely.[11] All these modifications have led to significantly improved survival rates of nearly 80-90% in these high-risk patients (Table 1).[10,11,15,19,20,23-27]

While we have to appreciate the impact of successful alloHSCT on the lives of these patients, we need to continue to recognize the many challenges that persist with respect to the still significant morbidity and mortality associated with this procedure. All over the world a major constraint is the lack of access to this therapy related to the lack of a suitable donor. Matched related donors are generally available only for a third of patients with thalassemia major,[28] but in countries with larger families or communities with consanguineous marriages up to two-thirds of patients may have suitable related donors.[29] However, only a very small minority of the global patient population falls into this category. The need for alternative donors has therefore been explored in several ways - partially mismatched related donors,[30] related haploidentical transplants,[31,32] and matched unrelated donors, the latter of which could be adult humans or cryopreserved cord blood units in blood banks.[20,33] While good results have been achieved with alternative donors in some studies, experience is still limited and the outcomes variable. Therefore these approaches have not yet been translated into becoming the standard of care for thalassemia major.[28] Persisting concerns regarding high rejection rates and graft *versus* host disease (GvHD) need to be addressed through the evaluation of novel protocols in future studies.

If these challenges can be addressed, haploidentical alloHSCTs could become a major alternative to matched unrelated donors or cord blood transplants. This could be particularly advantageous in resource-constrained countries with large numbers of these patients, where access to unrelated donors is severely restricted due to lack of services and the associated costs.[32-34]

The aim of curative therapy for any disease is to eradicate it and also enable a normal life afterwards. With high survival rates in patients with thalassemia major undergoing alloHSCT, the next important issue relates to the management of pre-existing and late transplant-related complications and their impact on long-term survival.[6,15] Over 90% of patients who survive the first two years after alloHSCT are generally expected to become long-term survivors.[35] Late (more than 2 years) complications after HSCT for hematological malignancies and bone marrow failure syndromes are accounted for mostly by disease relapses, chronic GvHD, infections and second cancers.[36] In addition, particularly in children, growth retardation, multiple endocrine and other organ dysfunction and cognitive changes have also been noted, depending on their pre-existing co-morbidities at the time of alloHSCT, or if they had developed major post-transplant complications such as significant chronic GvHD. Since most of the alloHSCTs for β-thalassemia major involve children and adolescents, data on long-term outcomes becomes particularly important as they would be expected to have several decades of life post-HSCT. This is even more relevant in patients receiving repeated transfusions because many of them already have systemic complications related to chronic anemia, iron overload and resultant endocrine and metabolic dysfunction, in addition to transfusion-transmitted infections.[37] Organ dysfunction exists in almost

75% of patients undergoing alloHSCT for thalassemia major with endocrine, hepatic and cardiovascular disease accounting for nearly 30-40% each, along with a host of other organ dysfunctions.[34] Unfortunately, information on late complications in ex-thalassemic patients is very limited. The only very long-term (over 20 years) study of outcome after alloHSCT for thalassemia major has shown that patients who were well-managed before transplant can have a quality of life comparable to the normal population, and certainly better than those being managed conservatively with hypertransfusion and iron chelation alone.[38] Older age at alloHSCT and the development of significant chronic GvHD were found to be factors leading to a worse long-term outcome.

More data with a systematic evaluation of organ dysfunction as well as an analysis of possible pre-transplant contributing factors is therefore needed in ex-thalassemic patients. An important issue will be the extent of iron overload at HSCT and the effectiveness of iron chelation post-HSCT, as this is the major cause of organ dysfunction in these patients. This is another aspect of the long-term outcome in these patients which has not been adequately addressed.[39,40] Varied approaches to iron chelation have been reported in small numbers of patients after alloHSCT, ranging from phlebotomies if serum ferritin was >2000 ng/ml,[39] to using only iron chelators such as desferrioxamine at 40mg/kg or deferasirox at 20-30mg/kg.[41,42] The time at which chelation was initiated has also varied from shortly after engraftment to up to two years after HSCT. A more considered and coordinated approach is needed to initiate intensive iron depletion therapy as soon as possible after alloHSCT in order to reduce or stop continuing organ damage.

As the vast majority of these patients come from the Mediterranean, Middle-Eastern and Asia-Pacific regions, where there is poor access to optimal transfusion-chelation therapy pre-HSCT, particular attention should be paid to iron depletion therapy in the post-HSCT period,[7,40] as many patients develop significant organ dysfunction at an early age, often within the first decade. A clear strategy needs to be implemented for improving pre-HSCT management with hypertransfusion and iron chelation, selecting patients early for HSCT before any major organ dysfunction sets in, and for the post-HSCT management of these residual complications. This is particularly important for all those patients who were poorly chelated before HSCT, and are closer to puberty. Without such an approach, while better survival may be achieved even in older higher risk patients, it is unlikely that these patients will have a normal quality of life because of the pre-existing irreversible morbidities. In fact, they will end up requiring multidisciplinary management of those dysfunctions post-HSCT.

An additional feature after HSCT for major hemoglobin

Table 1. Major reported clinical studies that have attempted to improve the outcome of patients with class 3 thalassemia major[$].

	Year	N	Median Age (yrs) / (range)	Proportion in Class 3 (%)	Proportion in Class 3HR (%)	Major defining feature of change in protocol	Treatment related mortality (%)	Graft rejection (%)	EFS	OS (%)
Lucarelli *et al.*[15]*	1996	115	11 (3-16)	100	NA	Bu / Cy based regimen with reduction in Cy total dose from 200 mg/kg to 160 mg/kg	24	35	49	74
Sodani *et al.*[25]	2004	33	11 (5-16)	100	NA	Reduction in Cy dose to ≤160 mg/kg with addition of Flu. Additional therapy from day -45 with immunosuppression with Azt and suppression of erythropoiesis with HU	6	6	85	93
Gaziev *et al.*[19]	2010	71	9 (1.6–27)	57.3	NA	Intravenous Bu, dose adjustments with therapeutic drug monitoring	7	5	87	91
Chiesa *et al.*[26]	2010	53	8 (1-17)	47	NA	Intravenous Bu, dose adjustments with therapeutic drug monitoring	4	15	79	96
Chiesa *et al.*[26#]	2010	25	NA	100	NA	Intravenous Bu, dose adjustments with therapeutic drug monitoring	4	34	66	96
Bernardo *et al.*[10]	2012	60	7 (1-37)	27^	NA	Treo based conditioning regimen	7	9	84	93
Li *et al.*[20]	2012	82	6 (0.5-15)	NA	NA	Conditioning with age adjusted PK based IV Bu, Cy (110mg/kg), high-dose Flu (200mg/kg), Thio. Additional therapy from day -45 with immunosuppression with Azt and suppression of erythropoiesis with HU	8.5^	4^	88^	91^
Choudhary *et al.*[27]	2013	28	9.6 (2-18)	75	39	Treo based conditioning regimen	21	7	71	79
Anurathapan *et al.*[23]	2013	18	14 (10-18)	100	NA	Conditioning regimen of Flu &IV Bu Pre-conditioning immunosuppression therapy with Flu and Dexa for 1-2 months.	5	0	89	89
Mathews *et al.*[11]	2013	50	11 (2-21)	100	48	Treo based conditioning regimen with PBSC graft in 74%	12	8	79	87
Mathews *et al.*[11@]	2013[@]	24	12 (3-21)	100	100	Treo based conditioning regimen with PBSC graft in 74%	13	8	78	87
Gaziev *et al.*[24]	2016	37	10 (5-17)	100	NA	As in Sodani *et al.*[25] but with higher dose of Flu (150 mg/kg) and addition of Thio(10 mg/kg)	8	0	92	92

[$]Adapted from Mathews *et al.*[10]; *Only patients <17 years included in this table; [#]Subset of high-risk cases from same paper; ^Includes all adult cases as well (assumed to be Class 3); ^Includes low-risk patients also; [@]Subset of high-risk cases from same paper. Cy: Cyclophosphamide; Flu: Fludarabine; Dexa: Dexamethasone; Bu: Busulfan; Treo: Treosulfan; Azt: Azathioprine; HU: Hydroxyurea; Thio: Thiotepa. HR: high-risk; EFS: event-free survival; OS: overall survival; NA: not applicable; PK: pharmacokinetics; PBSC: peripheral blood stem cell.

disorders is the state of mixed chimerism. This refers to the persistence of significant levels of residual host cells (RHC) of the hematopoietic system post-HSCT classified as level 1 (<10%), level 2 (10-25%) and level 3 (>25%). Transient mixed chimerism (TMC) is not uncommon in the immediate post-HSCT period and can occur in 30-45% of patients in the first 100 days.[43] Depending on the level of RHC, patients with TMC may later become complete chimeras in 60-75% of cases for those at level 1, but in only 5-30% of cases of those with level 3. (Figure 1) However, these predictions are based on limited data. Therefore careful monitoring of TMC and correlation with the hemoglobin levels and other blood counts is necessary post-HSCT in patients with major hemoglobin disorders. It should also be noted that some patients develop persistent mixed chimerism (PMC), in which a significant component of RHC persist long-term without rejection of the graft.[44] In some patients, the RHC component could be much higher than 25% with an acceptable hemoglobin level. If stable, then it is obviously a case of PMC of different hematopoietic elements and immune tolerance in such a way that it does not affect the erythroid lineage adversely. T regulatory cells could play a role, but the exact mechanism of this phenomenon is not fully understood.[45] The assessment of subset chimerism, particularly of the erythroid and lymphoid lineage in more patients, as shown in Figure 2, could shed more light on the mechanisms involved.[46] While RHC levels below 50% in T cells has been reported to be associated with a very low risk of rejection, high levels of RHC in both T and NK cells are

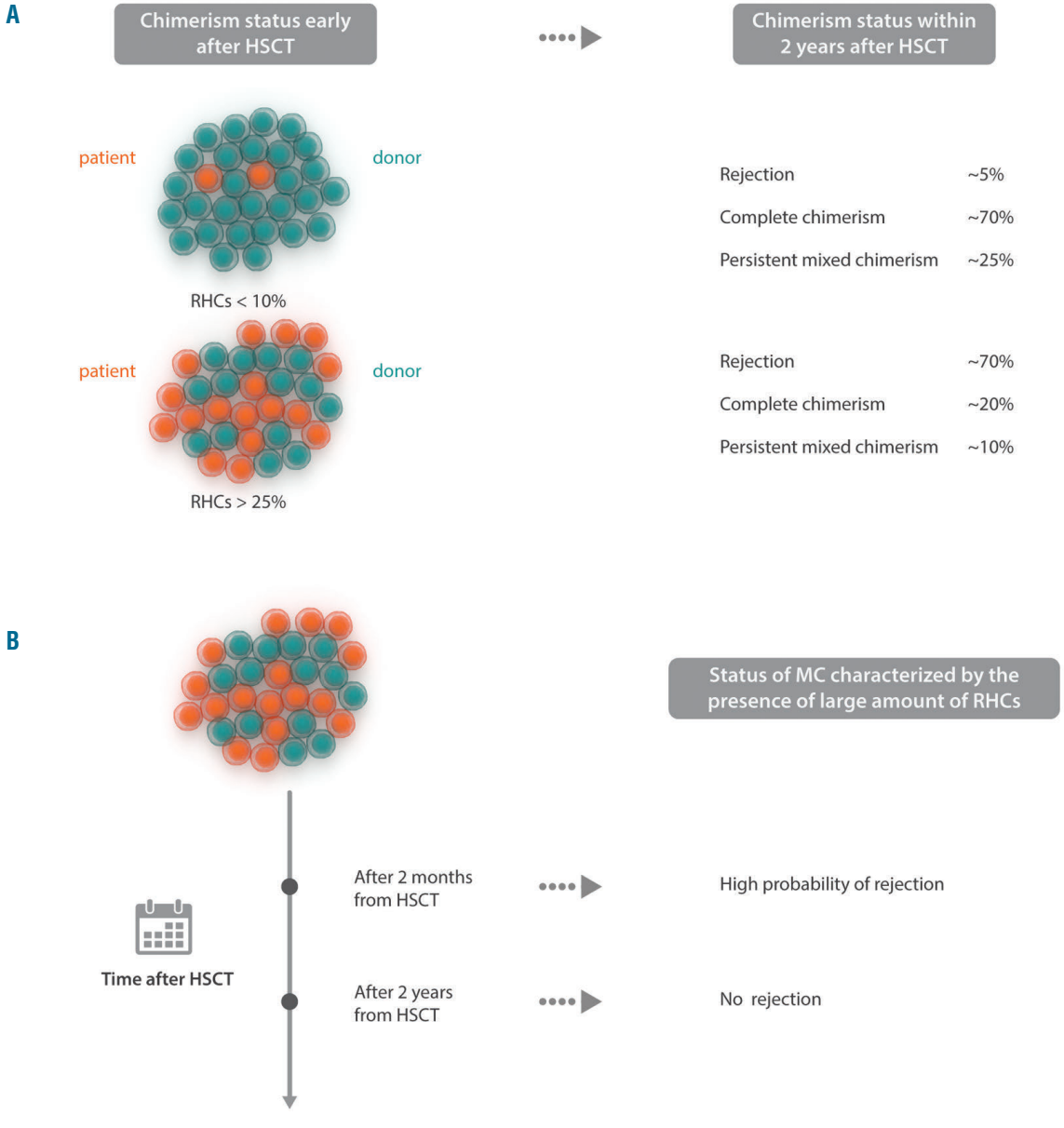

Figure 1. Evolution of chimerism after hematopoietic stem cell transplantation (HSCT). Early mixed chimerism is associated with higher risk of rejection while late chimerism often persists with a stable graft. RHCs: residual host cells. MC: mixed chimerism.

associated with rejections in up to 90% of patients.[47] These parameters also need further evaluation.

More HSCTs are now being done in the Asia-Pacific region for hemoglobin disorders than in any other part of the world,[48] given the high need and greater support from health care authorities as well as an increasing number of centers offering these services. It is therefore of the utmost importance and necessity to better assess the elements that will give the best long-term outcomes. This requires the complete evaluation and documentation of non-hematological complications pre-HSCT as well as the late post-transplant complications, in order that they can be appropriately managed to allow these ex-thalassemic patients to lead as normal a life as possible.[38] To this end, attention should be especially directed towards educating these families and the health care providers early about the potential complications and their prevention, as well as the importance of safe and effective transfusion-chelation programs. This will help keep patients in the best condition possible for alloHSCT to be undertaken. Indeed, our goal should be to offer alloHSCT to all patients with major hemoglobin disorders well before they become high-risk due to end-organ damage.

As we persist with our efforts to increase access to alloHSCT and its outcome, it is very important to note its limitations from a public health perspective. While more than 50,000 children with β-thalassemia major are added to the world population every year, less than 5,000 alloHSCTs have been reported so far for this condition worldwide over the last 30 years.[28] Even accounting for under-reporting, the actual number is likely to be well below 10,000 during this period. With the majority of these patients being in the Mediterranean, Middle-Eastern and Asia-Pacific regions of the world, access to this therapy remains highly restricted due to the lack of centers with trained personnel and facilities for HSCT services, and the inability of patients to access care due to the high costs, which very often have to be met by the patients or their families.[7,49] Recent data from the Asia Pacific Blood and Marrow Transplantation Registry shows that only about 450 alloHSCTs are being reported from the region every year, and perhaps less than 1,000 worldwide (personal communication, lida M, APBMT Registry) The enormity of this mismatch means that a different strategy is needed for effectively managing the large numbers of such patients in the world. Two approaches are necessary to address this problem. First, a preventative strategy through effective population screening, genetic counselling and prenatal diagnosis should be initiated in all countries where the prevalence of these conditions are high.[3] Second, the development of a more practical curative therapy, both in terms of technology, logistics and cost, if possible, such as gene correction in autoHSCs.[50] This will perhaps be the best way by which large numbers of patients who cannot avail of alloHSCT for various reasons will be able to access a curative therapy without the inherent risks and potential complications of alloHSCT.

Gene therapy for β-thalassemia major

Much effort has been put into developing gene therapy for several monogenic hematological disorders over the last several decades.[51] For those disorders where the defect is due to mutations in single genes that affect hemoglobin synthesis, the approach has been to introduce a normal

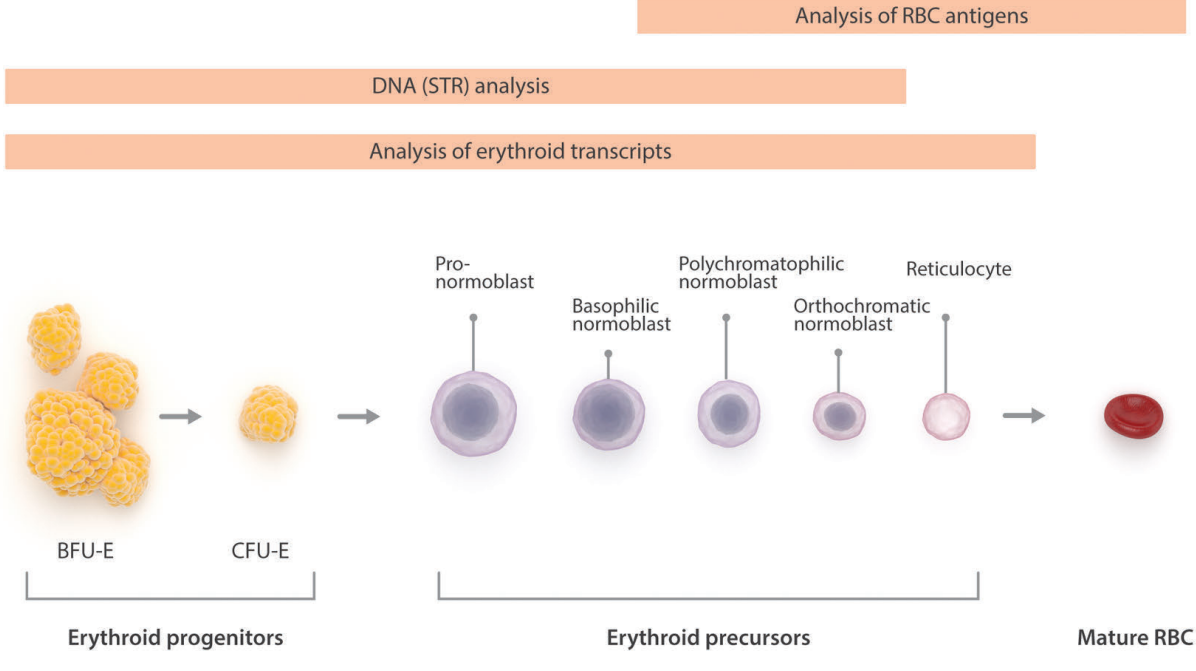

Figure 2. Methods to detect red cell chimerism. In patients with late persistent mixed chimerism, erythroid precursors may be predominantly donor while the non-erythroid cells may be more recipient in origin. Red cell chimerism can be measured by analyzing donor specific RBC antigens, STRs in erythroid DNA or nucleotide variations in erythroid transcripts. BFU-E: burst forming unit – erythroid; CFU-E: colony forming unit – erythroid; STR: short tandem repeats; RBC: red blood cell.

gene into autoHSCs and, more recently, to actually repair such genes with various genome editing tools.[52,53] The initial success achieved with the retroviral vectors used for efficient gene transfer into autoHSCs for different forms of severe combined immunodeficiency (SCID) disorders[53] was tempered by the development of clonal diseases and overt leukemia.[54] These vectors had intact long terminal repeats (LTRs) which included efficient and ubiquitous enhancers. While this aided the desirable high expression of the target transgene, it also led to undesirable genotoxicity.[53,54] The activation of oncogenes such as *LMO2* in the SCID trial led to the development of acute lymphoblastic leukemia in some of these patients. However, soon thereafter, the development of lentiviral vectors devoid of their pathogenic elements and with a self-inactivating (SIN) design provided a better alternative, not only in terms of its safety but also its ability to infect quiescent HSCs and carry larger transgene cassettes, which are needed for more effective expression in order to produce gram quantities of hemoglobin.[55] These advances in HSC-based gene therapy laid the foundation for its application in the major hemoglobin disorders.

The first successful gene therapy for a major β-globin disorder was in 2007 when a patient with β^{E}/β^{0}-thalassemia was treated.[56] The process involved the harvesting of autologous HSCs from the recipient, followed by *ex vivo* transduction of these cells with the lentiviral vector carrying the transgene. (Figure 3) If these transduced HSCs were found suitable following quality assessment to determine the number transduced as well as the vector copy numbers per HSC, they were used to perform an autologous HSCT after appropriate conditioning therapy to destroy existing HSCs. With a transduction efficiency of about 30% and only 10-20% of HSCs showing the transgene expression, and a busulfan-based myeloablative conditioning regimen in the initial patient, there was only limited expression of β-globin in the first year after gene therapy. This patient, therefore, required several transfusions during this time. In the second year, however, the transgene expression gradually improved and led to about 2-3 g/dL of transgene-associated HbA, resulting in the overall hemoglobin stabilizing at 8.5 to 9.0 g/dL and the patient becoming transfusion-independent.[57] However, this was also associated with a clonal expansion of erythroid cells (10-12%), with the insertion site being the high mobility group AT-hook 2 (*HMGA2*) locus. This clone peaked at 4% of hematopoietic cells at about 4 years, but has since declined to about 1% at 5 years without a reduction in total hemoglobin.[58] More recent data from this group has shown that with further improvements in the lentiviral vector leading to greater transduction efficiency and higher vector copy numbers, more than 15 patients have now

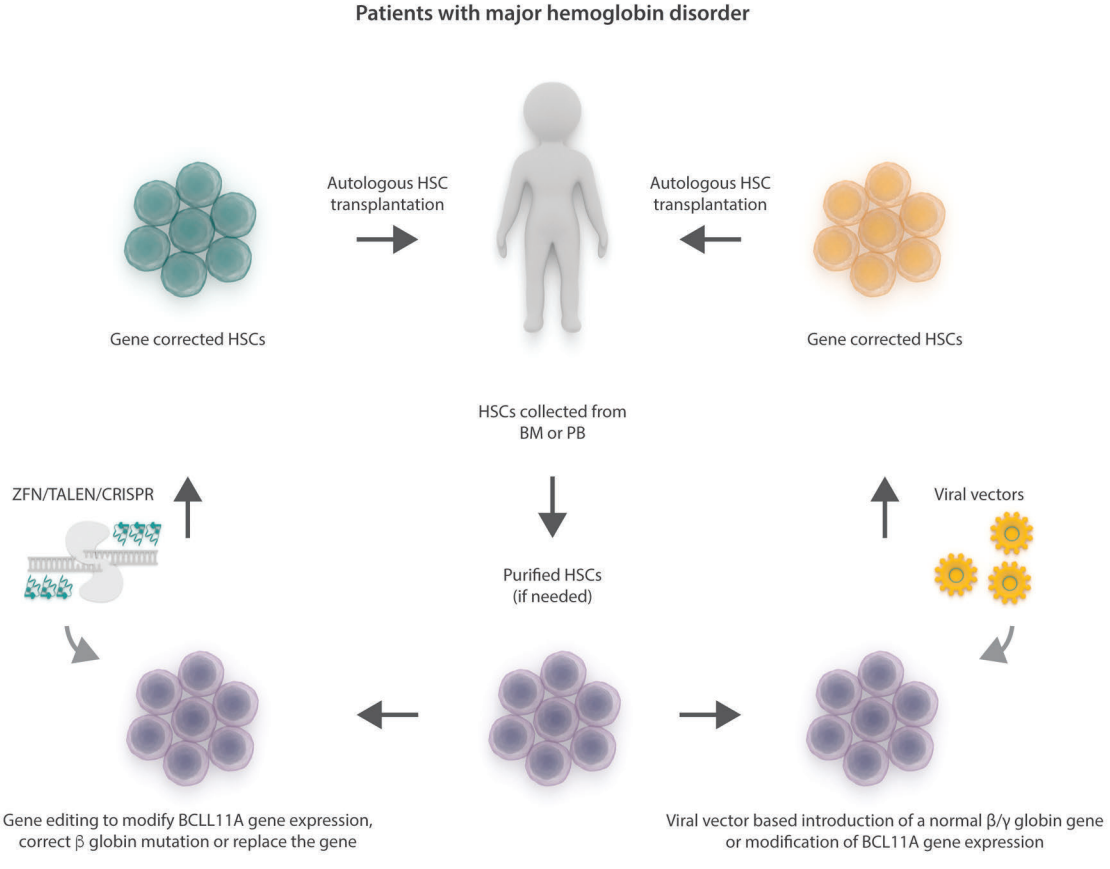

Figure 3. Overview of current approaches to gene therapy for the major hemoglobin disorders. Gene modifications may be through viral vectors or genome editing technologies to achieve the desired therapeutic effect. HSC: Hematopoietic stem cell; BM: Bone marrow; PB: Peripheral blood; ZFN: zinc finger nucleases; TALEN: transcription activator-like effectors with FokI nuclease; CRISPR: clustered regularly interspaced short palindromic repeats.

been treated.[59,60] Most of these patients achieved much higher transgene expression resulting in the hemoglobin increasing by 4-6 g/dl within the 2-5 months following gene therapy.[58,61] Significantly, no clonal events have been described thus far in these patients. The level of transgene-associated hemoglobin expression is related not only to the quality of transgene construct, but also to the efficiency of transduction itself with any vector, which in turn could be dependent on certain intrinsic properties of the particular HSC.[62] While much more needs to be learnt about effective gene therapy for major hemoglobin disorders, these results have certainly set the stage for further clinical trials to be undertaken. Several more studies are now open for patient recruitment.[63-66] Initial results continue to be encouraging and there have not been any significant safety concerns so far.

While this method of gene therapy for the major hemoglobin disorders relies on substituting a functional β-globin gene for a defective one, another approach could be to increase fetal hemoglobin (HbF) production by switching on the γ-globin gene expression which becomes gradually suppressed in the months following birth.[67] A major regulator of this phenomenon has been shown to be the BCL11A transcription factor which could itself be disrupted to reinitiate γ-globin gene expression through cell-intrinsic mechanisms.[68] This could then combine with the normal α–globin to produce enough HbF in these patients to effectively correct their anemia.[69] This approach has indeed been shown to be effective in animal models and cultured human HSC derived erythroid cells, where inactivation of the *BCL11A* gene increased HbF production to reverse sickle cell disease.[70,71] This has also now found clinical applications as described below.

There are many challenges ahead related to several aspects of gene therapy for the major hemoglobin disorders. Not least of these is the vector design, which is of utmost importance in order to ensure efficient transduction in HSCs to produce clinically meaningful high expression of the required globin chain that can then result in near-normalization of the hemoglobin in these patients. The success of these challenges needs to be achieved without dangerous genetic perturbations from the unavoidable random integration of the lentiviral vectors, which can lead to clonal hematopoietic disturbances with their own serious implications. Another aspect that can restrict success is the survival advantage of the transduced HSCs, given the fact that even with myeloablative conditioning, untransduced HSCs will also find their way back into the hematopoietic compartment in the patient, given the variable *ex vivo* transduction efficiency of the lentiviral vectors. Unlike alloHSCT, there is no donor immune system in this form of autologous stem cell therapy to give a survival advantage to the transduced transplanted HSCs with the functional β-globin gene. The dynamics that will determine the sustained presence of the transduced HSCs in the long-term are still unknown.

Genome editing for gene correction

Another approach to correct mutations in autoHSCs is through genome editing techniques using targeted nucleases, which can specifically target these sites and replace them with the normal sequence to bring back the wild-type functional configuration. These technologies include the zinc fingers nucleases (ZFN) and the transcription activator-like effector nucleases (TALENS) and more recently, the clustered regularly interspaced short palindromic repeats (CRISPR) with Cas9 nuclease system.[52] Conceptually, these powerful technologies allow for a

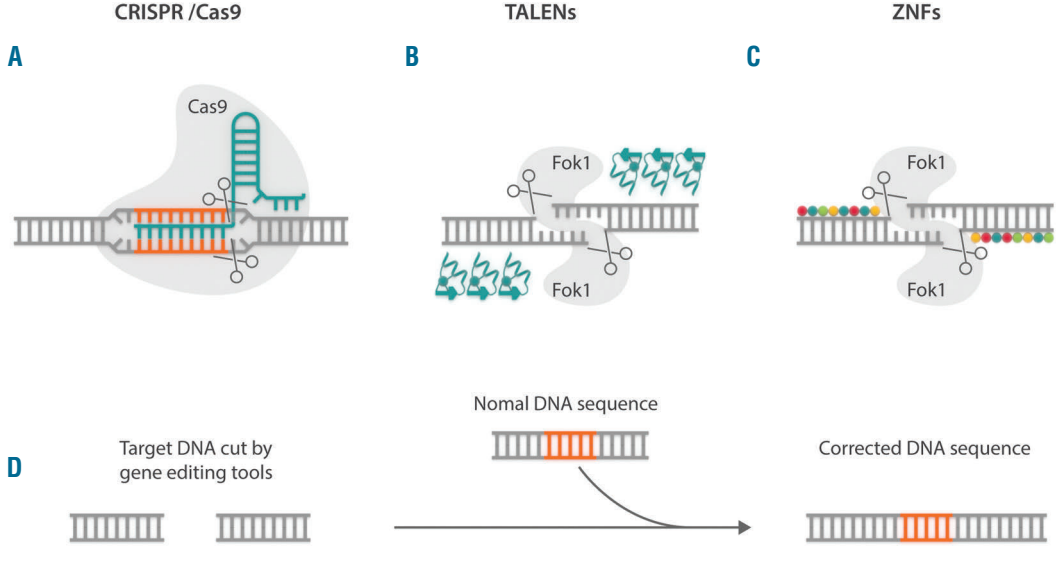

Figure 4. Genome editing techniques for correction of molecular defects. The three different gene editing techniques allow fixing of single mutations in the hemoglobin gene in patients with β-thalassemia major. ZFNs: zinc finger nucleases; TALENs: transcription activator-like effectors with FokI nuclease; CRISPR: clustered regularly interspaced short palindromic repeats.'

molecular 'cut and paste' approach to gene correction (Figure 4),[72] which could transform the entire approach to gene therapy for many diseases. In fact, the speed with which these applications are entering the clinic is remarkable.[73] The ability to efficiently and safely edit the genome to modify genes has indeed taken both therapeutics and diagnostics by storm. It has also given a new dimension to the potential of stem cell-based therapies to treat different diseases. A clinical trial has already been reported showing the safety and limited efficacy of the ZFN based system in altering the CCR5 receptor on lymphocytes from patients with HIV infection on highly active antiretroviral therapy (HAART) therapy.[74] Thus far however, the major challenge in using gene editing strategies in the correction of β–thalassemia is the low efficiency of these methods in targeted gene correction in HSCs. This is necessary for obtaining a large number of gene corrected HSCs to produce therapeutically significant levels of hemoglobin. However, a recent study that used improved methods to deliver ZFNs, showed that integrase-deficient lentiviral vectors to deliver ZFN messenger ribonucleic acids (mRNAs) and an oligonucleotide as a gene correction template had a very high efficiency of gene correction in HSCs obtained from patients with sickle cell disease.[75,76] A similar approach can be applied in β-thalassemia major.

The correction of β-globin gene mutations in induced pluripotent stem cells (iPSCs) derived from patients with thalassemia major is also possible.[77-81] One advantage of using iPSCs for gene correction is that it could then be possible to obtain a completely corrected clone of pluripotent stem cells, from which a large number of stem cells or other cells of interest could be derived for transplantation. However, so far it has not been possible to use iPSC-derived HSCs to engraft and provide sustained hematopoiesis.[82] On the other hand, an encouraging proof of concept comes from the report of using ZFN technology to correct the interleukin 2 receptor (*IL2R*) gene in diseased human HSCs and progenitors, and the demonstration in animal models of their ability to sustain adequate hematopoiesis after transplantation.[83] In fact, Sangamo BioSciences, Richmond, CA, USA, has recently obtained approval from the U.S. Food and Drug Administration (FDA) for the Investigational New Drug[11] application for SB-BCLmR-HSPC for the treatment of β-thalassemia, developed in collaboration with Biogen, Cambridge, MA, USA.[84] Data presented more recently by this group claimed about 70% efficiency of gene knockout using this approach in HSCs manipulated *ex vivo*.[85]

Clearly, these are very significant developments that have created more options for gene replacement or correction therapies for β-thalassemia major and other serious hemoglobin disorders (Figure 3). It may then be possible to move from alloHSCs as vehicles for the transfer of the normal gene to viral vectors or genome editing techniques being used to correct the gene defect in autoHSCs. One cannot underestimate the enormity of the work ahead in establishing the safety and efficacy of the latter approaches, but given the nature of the technology, it is not difficult to imagine that they hold great promise for providing platforms that could be much more amenable to developing cost-effective therapies for applications across the globe. At least they will not be limited by the much more difficult task of finding suitable donors for alloHSCT and the significant treatment-related immediate and long-term complications associated with it. However, these are very early days with regards to gene therapy, and many more clinical trials and follow-up studies will be needed to establish the long-term safety of the different approaches to gene therapy for the major hemoglobin disorders.

As with any technology-based options to therapeutics, intellectual property rights based restrictions on the use of effective technologies along with the cost and marketing policy related issues can lead to hugely restricted access to such therapies around the world.[86] Solutions will need to be found for those challenges if such situations do arise. Even with all these unresolved issues, there is no doubt that these are very exciting times for patients, physicians and scientists working in this field because after years of great effort, not only have the results of alloHSCT improved significantly for these patients, but several innovative gene correction therapies are also on the horizon.

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