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PII: S1473-0502(21)00009-4

DOI: https://doi.org/10.1016/j.transci.2021.103059

Reference: TRASCI 103059

To appear in: *Transfusion and Apheresis Science*

Please cite this article as: Justus DG, Manis JP, Parameters affecting successful stem cell collections for genetic therapies in Sickle Cell Disease, *Transfusion and Apheresis Science* (2021), doi: https://doi.org/10.1016/j.transci.2021.103059

Parameters affecting successful stem cell collections for genetic therapies in Sickle Cell Disease

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31 Pages, 2 Tables

Abstract

Emerging cellular therapies require the collection of peripheral blood hematopoietic stem cells (HSC) by apheresis for *in vitro* manipulation to accomplish gene addition or gene editing. These therapies require relatively large numbers of HSCs within a short time frame to generate an efficacious therapeutic product. This review focuses on the principal factors that affect collection outcomes, especially relevant to gene therapy for sickle cell disease.

Keywords: Gene therapy, gene editing, Sickle Cell Disease, stem cells

1. Introduction

Over the past two decades, great advances in vector technology and cell manipulation have brought gene therapy closer as a therapeutic option, especially for monogenetic hematopoietic diseases including primary immunodeficiencies, beta-thalassemia, and sickle cell disease (SCD). While allogeneic hematopoietic stem cell transplant (HSCT) remains the standard of care, in certain diseases such SCD, access to an HLA matched sibling translates to fewer than 20% in the population. A matched unrelated donor has been the only other option for approximately 1/3 of those patients with the corresponding additional risks of high grade GVHD [1] [2] from bone marrow and peripheral blood hematopoietic progenitor cells (HPCs), as well as graft failure in patients undergoing transplant using cord blood [3] [4]. Clearly, there is a need for additional therapeutic options and gene therapy has finally matured into a real possibility for some patients. Genetically manipulated autologous hematopoietic stem cells eliminates the need for an HLA matched donor, the risk of GVHD, and potentially graft rejection. Currently, gene therapy can be performed by ex-vivo or in-vivo strategies. In-vivo gene therapies involve the direct introduction of a vector-gene construct, usually in an adenovirus-associated vector (AAV) vector, directly to patient tissue. This approach directly delivers either a modified gene product or the machinery for gene editing to correct the monogenic blood disorders. Ex-vivo gene therapy which involves the collection and modification of autologous hematopoietic progenitor cells (HPCs) with therapeutic vectors, whereupon corrected cells are re-infused into the patient to achieve disease amelioration. Ex-vivo gene therapy relies on the optimization and coordination of mobilization,

collection, ex-vivo transduction of HPCs followed by tissue engraftment with appropriate conditioning and infusion techniques as well as mitigating post infusion adverse events in a disease and gene specific manner. Obtaining appropriate numbers of HPCs for manipulation is paramount for a successful drug product outcome. This review will focus on mobilization and collection of HPCs by apheresis, using SCD as an example to highlight the various process parameters that require optimization to provide a single curative treatment and reach a durable response.

2. Mobilization

The process of generating an ex-vivo modified gene therapy product begins with an effective method of mobilizing the HPCs from the bone marrow compartment to the intravascular space. This is particularly crucial in that a relatively large number of peripheral blood HPCs are needed in a relatively short period of time to allow for cell loss during ex vivo manipulation and to generate a therapeutic drug product. Two mobilizing agents that are currently being used include granulocyte colony-stimulating factor (G-CSF) and plerixafor.

2.1 Chemotherapy and GCSF

Since the 1970s, it has been known that the number of HPCs in the peripheral blood increases during the recovery phase following chemotherapy administration [5]. Cyclophosphamide has been an agent used for its chemo-mobilization effects and has been commonly used in combination with G-CSF to produce higher yields of stem cells resulting in fewer collection

procedures in patients with multiple myeloma receiving autologous stem cell transplant [6]. Cyclophosphamide's mobilizing capabilities stems from the release of proteases and the cleavage of vascular cell adhesion molecule-1 (VCAM-1) and C-X-C chemokine receptor type 4 (CXCR4), which are key adhesion molecules resulting in the release of hematopoietic stem cells into the peripheral blood [7, 8]. Despite cyclophosphamide's effective mobilization effects, its use in patients without malignant diseases is limited when its additional cytotoxic effects are not required given the potential toxicities related to its administration. G-CSF is a glycoprotein that stimulates the bone marrow to produce and release granulocytes and HPCs into the peripheral blood. G-CSF was first developed to reduce chemotherapy induced neutropenia and infections in cancer patients [9] which then became routine practice with the additional benefit of decreasing delays in chemotherapy regimens [10]. However, its ability to mobilize HPCs from the bone marrow to the peripheral blood revolutionized stem cell collection and HSCT after the mobilization of hematopoietic progenitor cells was discovered [11]. Subsequent clinical trials showed that adequate numbers of cells could be collected from normal donors and cancer patients [12] [13]. Since the development of gene therapy strategies, G-CSF has proven to be a safe and efficient way to mobilize HPCs for collection in monogenetic hematopoietic diseases such as beta-thalassemia [14]. However, in some cases, G-CSF is contraindicated in the mobilization of HPCs in patients with SCD [15] due to severe adverse effects including vaso-occlusive crises [16], severe acute chest syndrome, splenomegaly, and even death [17]. In certain conditions, G-CSF has a decreased efficiency likely secondary to decreased bone marrow reserve found in primary and idiopathic bone marrow failure syndromes such as Fanconi anemia (FA) [18] and aplastic anemia [19]. Given the need for additional or alternative mobilization strategies

to improve the safety and/or efficiency of HPC mobilization, multiple agents have been evaluated including the CXCR4 antagonist AMD3100 also known as plerixafor.

2.2 Plerixafor

Plerixafor is a molecule that reversibly inhibits binding of stromal cell-derived factor-1-alpha (SDF-1a), expressed on bone marrow stromal cells, to CXCR4, expressed on stem cells, resulting in mobilization of HPCs from the bone marrow into the peripheral blood. This effect was initially demonstrated as a single agent [20] [21] and then in addition to G-CSF which exhibited synergistic effects [22] [23] when being used for autologous HSCT collections. Plerixafor has also proven to be safe and effective as a single mobilization agent in patients requiring HPC collections for gene therapy for conditions such as beta-thalassemia [14] and SCD [24] as well as in synergy with G-CSF in patients with beta-thalassemia who do not have a sufficient response to either plerixafor or G-CSF alone [14] [25]. G-CSF is typically given subcutaneously at a dose of 10 mg/kg/day for 5 doses with the collection occurring closely following the fifth dose though a lower dose has been used in splenectomized patients with beta-thalassemia to prevent the adverse effect of hyperleukocytosis. Plerixafor has been found to be safe and effective at a dose of 240 ug/kg given via subcutaneous injection in patients with beta-thalassemia [14] and SCD [24, 26-28]. The effect of the timing of administration of plerixafor has also been evaluated in the healthy donor and SCD populations and it was found that peak concentrations of CD34+ cells in the peripheral blood occurred between 6 and 8 hours following administration in the healthy donors and by 4 hours in the SCD patients [27] [24]. Aside from diseases dictating which mobilization agents should not be used and when they should be

administered in relation to collections, some may necessitate the combination of G-CSF and plerixafor exploiting their synergistic effect due to a poor response to single mobilization agents, such as in patients with bone marrow failure syndromes like FA [18] [29] and patients with beta-thalassemia who are poor mobilizers in response to G-CSF alone [25]. All of these differences speak to the great importance of understanding the most efficient mobilization agent(s) and timing of peak CD34+ cells following their administration in a disease specific manner.

2.3 Novel Mobilization Agents

While current mobilization strategies are optimized, investigation into additional mobilization agents continues. GRO-beta and the very late antigen-4 (VLA4) small molecule antagonist are two agents in particular that have shown success in preclinical studies. GRO-beta is a CXCR2 agonist that induces MMP9 release from neutrophils which promotes freeing of HPCs from their bone marrow niche to the peripheral blood [30]. When combined with plerixafor and given to mice as a single administration, the number of mobilized HPCs peaking within 15 minutes was comparable to the multi-day G-CSF regimen and resulted in HPCs with a higher engraftment efficiency [31]. The VLA4 small molecule antagonist inhibits the alpha-4 beta-1 integrin which is involved in homing and retention of HPCs within the bone marrow microenvironment [32]. In mouse models, the VLA4 small molecule antagonist has been shown to rapidly mobilize HPCs to the peripheral blood when administered with plerixafor or a CXCR2 agonist [33]. These additional agents provide alternative options to the current mobilization strategies and could provide further customization of overall collection method needs based on disease and editing strategy.

3. Stem Cell Collection by Apheresis

Advances in gene editing and continued improvements in lentiviral gene therapy has provided multiple approaches to genetically treat monogenetic hematopoietic diseases including gene addition, gene editing and other genetic manipulations. To realize these promising genetic therapies, a sufficient number of HPCs must be obtained in a short period of time to enable cell manipulation and subsequent manufacture of the cellular therapy. While bone marrow harvest by needle aspiration has been the principal cellular method used to source HPC for gene therapy trials, it was found to be inefficient for certain patients, resulting in poor yields of low purity HSC. Additionally, this operative procedure was associated with significant morbidity including pain after each collection, and also required multiple rounds of harvesting to yield sufficient cells for manufacturing a cellular product. Attention has now turned to apheresis for collections of peripheral blood HPCs after a mobilization regimen.

Following HPC mobilization from the bone marrow to the peripheral blood, collection by leukapheresis is performed to collect cells for the desired therapy. While the ideal cell dose for gene therapy has not yet been identified, most trials now aim for about 10 $x10^6$ CD34+ cells/kg collected prior to cell purification, to generate a drug product that is between 2-5 $x10^6$ CD34+ cells/kg. Depending on the downstream modifications to the product, there is typically no upper limit to the number of cells to collect in a day. An aliquot of unmanipulated cells equivalent to that necessary for an autologous transplant (2-3 $x10^6$ CD34+ cells/kg) is typically obtained during apheresis. If the collection from one day of apheresis is not optimal for cell manipulation,

the product can be stored overnight and pooled with a second day of apheresis collection. Cell manipulation in the lab can also influence the number of cells targeted for collection. These factors include cell loss due to magnetic bead enrichment, cell sorting, electroporation, or expansion of cells in culture for transduction.

Additionally, achieving the high pre-manipulation target CD34+ cell number can be more difficult to accomplish due to a significant reduction in HPC collection efficiency secondary to the clinical manifestations of the underlying disease being treated. Many factors including the size and shape, and potentially age of red cells as well as the composition of nucleated peripheral blood cells may play a role during the collection procedure as suggested by recent studies [34, 35]. Most notably, peripheral blood HPC collections are particularly inefficient for patients with hemoglobinopathies, yet these same patients are also in great need of novel cellular therapy to treat their disease. Major clinical trials have been launched for gene therapy in SCD with a consequent challenge for Transfusion Medicine to direct HPC collections to allow therapies for these patients. As more pre-clinical studies and gene therapy clinical trials are performed, it has become more evident that selective pressures on the transplanted cell graft for expansion post-transplant can also play a significant role in determining the number of CD34+ cells collected for manipulation.

3.1 Safety

As G-CSF use as a mobilizing agent is contraindicated in patients with SCD, bone marrow (BM) harvest appeared as the only other method to procure stem cells for manufacture. While BM has been previously used to collect stem cells for gene therapy in SCD, multiple harvests were

required for an optimal product [36]. BM harvest was further evaluated for safety and efficacy [28] in a recent study of patients undergoing gene therapy for SCD, where 11 out of 11 patients developed adverse events (SAE) (mainly VOC and post-procedure pain). Additionally, a median of two bone marrow collection procedures were required to achieve the targeted stem cell number compared to one apheresis cycle likely owing to the higher CD34+ cells/kg per collection in the apheresis group (10.1 vs 5.1 $x10^6$ CD34+ cells/kg/cycle, $p<0.0001$)[28]. These data suggest that bone marrow collection is suboptimal in this patient population leaving apheresis with plerixafor mobilization currently as the leading viable collection procedure.

Trialists for SCD gene therapy thus turned to plerixafor to use as a sole mobilizing agent. Boulad et al[26] performed a dose escalation study of plerixafor in 15 patients who received either 80 ug/kg, 160 ug/kg, or the standard dose of 240 ug/kg. Hydroxyurea was continued in most of these patients to continue standard medical care and to prevent adverse events potentially related to plerixafor. Only 2 SAEs, both pain crises, were seen (one at a dose of 80 ug/kg and one at 240 ug/kg) in this cohort with an unclear link to plerixafor. Esrick and Manis et al[24] evaluated mobilization with plerixafor in 6 patients on chronic exchange transfusions and Lagresle-Peyrou et al[27] placed all 3 of the patients in their study on monthly transfusion therapy for at least 3 months prior to collection in an effort to ensure safety of the mobilization/apheresis procedure in SCD patients. No SAEs were reported in either study directly attributable to plerixafor. In a recent report, 7 out of 15 patients developed adverse events (AEs) (pain and/or electrolyte abnormalities) in the mobilization and apheresis collection group [28]. For all the reported studies, symptomatic hyperleukocytosis was not noted in any of the patients. Thus, these studies

suggest that the use of plerixafor as a mobilizing agent is safe in patients with SCD, especially in the setting of hydroxyurea or preparative exchange transfusions.

3.2 Mobilization

Plerixafor at standard doses alone has been used successfully as monotherapy in healthy donors, with a reported CD34+ cell peripheral blood count of 27.8 cells/ul at about 8 hours in one study[37], and a CD34+ cell count of 19 at 4 hours in a more recent report[38]. For studies in SCD, mobilization by plerixafor was also evaluated in these studies by measuring peak CD34+ cells/ul in the peripheral blood. Boulad et al[26] found that 8 of 15 patients with SCD achieved peripheral blood CD34+ cell count of at least 30 CD34+ cells/μL when looking at all dose ranges. Tisdale et al[28], Lagresle-Peyrou et al[27], and Esrick and Manis et al[24] demonstrated robust mobilization as measured by the peak peripheral CD34+ cells/ul following plerixafor administration (Table 1), though the interpretation of the actual peak value in these studies is confounded by the concomitant peripheral blood apheresis collection.

3.3 Timing of apheresis collection

Most studies focused on the safety of plerixafor administration and/or apheresis collection rather than closely examining the mobilization of CD34+ cells into the peripheral blood at multiple time points using plerixafor alone. Boulad et al[26] measured peripheral blood CD34+ cell counts at 6-12 hours and 20-24 hours post plerixafor administration which may underestimate the peak count, as well as the earliest time of mobilization. Esrick and Manis et al[24] compared two plerixafor doses (180 ug/kg and 240 ug/kg) with a rapid mobilization of CD34+ cells noted

at 4 hours after plerixafor administration. Similarly, Lagresle-Peyrou et al[27] also detected CD34+ cell mobilization with plerixafor at earlier than expected time points, with peak counts exceeding 80 CD34+ cells/ul noted at 2-3 hours after administration. In a larger series of patients, Tisdale et al[28] report high peripheral CD34+ cell counts immediately before apheresis which occurred between 4-6 hours. While it can be difficult to truly assess mobilization and peak peripheral CD34+ cell counts following plerixafor administration from these studies as collection occurred during the measurement of CD34+ cell mobilization, these data show a trend for earlier and potentially higher peripheral blood CD34+ cells in patients with SCD. Further studies are needed to understand both the magnitude and timing of stem cell mobilization in SCD, as well as the seemingly wide variation of responses.

3.4 Collection interface and efficiency

Patients with hemoglobinopathy or altered red cell size or shape have been reported to have lower efficiency HPC collections by apheresis. The mechanism accounting for this poor collection efficiency is unclear; however, collecting at a deeper interface appeared to improve recovery[14, 34, 39] [25]. In the study performed by Esrick and Manis et al[24], the first 2 patients collected at a standard HPC collection interface of about 3% hematocrit resulted in poor collection efficiencies despite robust mobilization with plerixafor. Subsequent subjects in this trial had collections performed at a deeper interface, 5-10%, with improved CD34+ cell yields. Tisdale et al[28] and Lagresle-Peyrou et al[27] also collected at a deeper interface reporting collection efficiencies (CE1) of 49% and <30% (ranging 24-30%). This evidence suggests HPC sedimentation in patients with SCD is altered and may be somewhat improved by collecting at a deeper buffy coat interface closer to where granulocytes and mature red cells sediment.

Nevertheless, further work is required to understand the altered stem cell sedimentation dynamics in SCD and to identify biomarkers that may identify patients at risk for low efficiency apheresis collection.

3.5 HSC characteristics

Phenotypic characterization of mobilized progenitor cells in patients with SCD is of significance to ensure that the manufactured cells generate a long-term functional graft. Tisdale et al[28] reported that the proportion of CD34+^{bright} (long-term repopulating) cells mobilized by plerixafor and collected by apheresis was significantly higher ($p<0.0001$) than the bone marrow collection product with medians of 96% and 58%, respectively, and no statistically significant difference was seen between healthy donors when mobilized by plerixafor and collected by apheresis ($p=0.18$). Lagresle-Peyrou et al[27] also showed that more primitive HSCs (CD34+CD38-CD45RA-CD133+) were present in the collected apheresis product following plerixafor mobilization in patients with SCD compared to bone marrow from healthy donors and SCD patients and healthy donors undergoing apheresis with GCSF or plerixafor mobilization. Esrick and Manis et al[24] employed a combination of surface markers to investigate the CD34+ cell subsets including the lineage uncommitted and committed sub-populations. They found that cells collected from SCD patients mobilized with plerixafor were enriched in HSCs when compared to healthy donors mobilized with GCSF, 9.7% and 3.7% ($p=0.0012$), respectively. Boulad et al[26] evaluated the CD34+ CD38-cells and found enrichment of these progenitors in the peripheral blood after mobilization.

These data speak to the increased mobilization and subsequent collection of the CD34+ cells with the highest potential of long-term repopulation capability following plerixafor

administration supporting its continued use. Additionally, the proportion of uncommitted HSCs in the product after collection could potentially be predictive of long-term engraftment of the manipulated product and may merit investigation.

4. Conditioning

While mobilization and collection of the appropriate number of HPCs together with efficient ex-vivo transduction/gene editing are crucial for a successful therapeutic outcome, engraftment and proliferation of the corrected cells must also be optimized. Therefore, conditioning regimens continue to be adjusted to facilitate engraftment and post infusion conditions augmented to enhance proliferation of corrected cells. In certain conditions, the transplanted "gene-corrected" cell may have a selective growth advantage over endogenous cells as is the case in primary immune deficiencies. In these conditions, fewer transferred HPC's or lymphocytes can expand to fill the immune compartment. Transplantation without conditioning has yielded excellent results in patients with severe combined immunodeficiency (SCID) [40]. In other conditions, selective pressure of genetically modified cells can also influence expansion of donor or corrected cells as demonstrated with the initial gene therapy trials for ADA-deficiency. The first transplants did not include pretransplant cytoreductive conditioning, and enzyme replacement therapy was continued, resulting in lower numbers of gene corrected HPCs than anticipated [41]. It was later determined that reduced intensity conditioning with low dose busulfan (4 mg/kg) improved long-term ADA production as it allowed for a higher level of engrafted gene corrected HPCs and the withholding of enzyme replacement therapy favored the expansion of those corrected cells [42] [43]. Still, for other diseases, there is growing interest in transferring more corrected cells to

overcome historical graft failures, as seen in transplant and gene therapy outcomes for chronic granulomatous disease [44]. These studies demonstrate the value of informing apheresis collection physicians of the need to tailor cell targets for specific diseases.

5. Correction of SCD as a model for a multimodal approach to gene therapy.

Many factors influence therapeutic outcome for novel cellular therapies including preparation for peripheral blood (PB) HPC collection, strategy for genetic modification, and transplantation of the target cell. Gene therapy for SCD exemplifies the need to tailor all aspects of cell collection and modification to improve outcome. PB HPC collection for patients with SCD requires coordinated attention starting with preparation for stem cell collection. The BM microenvironment appears disorganized in SCD, with a vasculopathy and a disruption of the HPC niche [45]. In these studies, red cell exchange in a mouse model of SCD normalized the BM HPC defect, suggesting that red cell exchange may do the same in some patients with the intent to undergo apheresis HPC collection. Such a preparative regimen may also potentially suppress stress erythropoiesis and improve engraftment. PB HPC collection in patients with SCD is impeded by limited mobilization approaches and by lower efficiency recovery of cells by apheresis. Nevertheless, this option is superior to that of a BM harvest which requires general anesthesia and is associated with fewer cells collected and may require repeated collections [28]. As outlined above, G-CSF is contraindicated in SCD and plerixafor remains the only viable option, with emerging candidate drugs in development. The cause for the lower efficiency of collection is not yet solved, though similar findings have been reported in other hemoglobinopathies such as beta-thalassemia [14]. For SCD, there are indications that collecting

at a deeper-color preferences during apheresis (closer to a granulocyte interface), may improve recovery efficiency [24] [27] though more studies are needed to understand this finding.

SCD has now emerged with diverse strategies for gene modification to overcome the defect derived from a single point mutation in the beta hemoglobin gene. Strategies include gene correction, gene addition, or induction of endogenous fetal hemoglobin. Some of the initial reports for gene addition for SCD used a lentiviral vector containing a modified anti-sickling beta globin. The initial report using this strategy revealed prolonged expression of the transgene with the anti-sickling globin detectable and an associated improvement in sickle crises [36]. There are several other open gene addition trials where either gamma globin or anti-sickling beta globin are introduced into HPC with encouraging outcomes as presented in meetings. The overall safety of lentiviral-based vectors, together with documented long-term expression of the transgene make this an appealing strategy (Table 2).

In addition to gene addition, lentiviral vectors are being used to induce fetal globin production, as persistence of fetal hemoglobin has been well associated with amelioration of SCD disease. Gamma globin is potently anti-sickling, and also partially circumvents the concerns associated with the stoichiometry of pairing of heterozygous species of beta hemoglobin, making it a good therapeutic candidate. BCL11A is a transcription factor that suppresses fetal globin production via a regulatory site adjacent to the gamma globin locus. Gene silencing has been accomplished by utilizing RNA interference (RNAi)-based gene therapy approaches through the generation of short hairpin RNAs (shRNA). These shRNAs were embedded into miRNA, termed shRNAmiR, and developed to target erythroid lineage-specific BCL11A knockdown resulting in HbF induction [46]. Currently, a clinical trial (NCT03282656) at our center is evaluating the single infusion of autologous bone marrow derived CD34+ HPCs transduced with the lentiviral vector

containing the shRNAmiR targeting BCL11A in patients with SCD with promising early results of long term HbF induction communicated. Erythroid specific promoters in the lentiviral vector exclusively target the interfering RNA only to erythroid cells, allowing for BCL11A depletion only during red cell development. Other trials targeting BCL11A through RNAi are currently in place (NCT04091737).

Other genetic approaches to correct SCD involve gene correction either through replacement of mutant alleles or through gene editing or higher resolution base editing. Homology mediated repair involves the introduction of a corrected allele into cells together with engineered nucleases (ZFNs or TALENs) that promote the homologous exchange of the mutant allele for wildtype sequence. Relatively large amounts of genetic material can be exchanged making this an attractive therapy for SCD but especially for diseases with many different mutations (beta-thalassemia) [47, 48]. Clinical trials are in progress with such a vector to replace mutant beta globin.

Globin gene repair remains the most tempting, with a direct correction of the single base mutation. This can be accomplished most attractively using Crispr based technology with a generation of tools that avoid double strand breaks while accomplishing the nucleotide correction [49] [50]. While many aspects of this strategy are still in pre-clinical stages, there is palpable excitement for the promise of this therapy. Traditional Crispr technology generates double strand breaks making precise edits only possible through homology mediated repair, or by deleting sequences via non-homologous end joining. The latter mechanism allows for imprecise repair, and is better suited for non-coding sequences, a feature that is being used to delete the BCL11A erythroid specific locus. By deleting the specific sequence which function only in erythroid cells

using Crispr/Cas9 tools, fetal hemoglobin production remains on and elevated in developing erythrocytes. Early reports of such a strategy in a single patient are encouraging for this approach (NCT03655678).

Several factors influence the choice of vectors for therapy including the efficiency of genetic modification, persistence of the genetic effect, and the ability of corrected cells to contribute to ameliorate SCD. Thus, chimerism between corrected and unmodified cells in SCD can influence clinical outcome. There has been a strong body of evidence showing that a relatively low percentage of healthy HPCs, ranging from 20-30% in allogeneic transplants, may provide enough healthy red cells to prevent symptoms of SCD [51] [52]. These results hold promise for genetically modified cells where not all of the manipulated cells have optimal modifications, but nevertheless being able to modify disease outcome. As stated above, improving conditioning regimens for transplantation as well as using preparative regimens to restore the bone marrow microenvironment in SCD may further improve outcomes.

Nevertheless, there are major challenges for widespread adoption of these technologies that are currently being addressed. Access to health care, cost of the technology, and patient selection require much attention. Adverse events related to transplantation, including the toxicity of conditioning regimens is not inconsequential. Gene therapy related insertional mutagenesis of the vector leading to oncologic transformation of cells in the earlier trials utilizing RV vectors treating SCID-X1, CGD, and WAS remains under constant watch, with an FDA mandate of a 15-year follow-up of observation after treatment. Gene editing based strategies also carry the potential concern for off-target effects inducing DNA damage caused by the engineered

nucleases. In the human genome, GUIDE-seq [53] and CIRCLE-seq [54] as well as other newer sequencing techniques are assessing these outcomes [55]. One last consideration may be the longevity of the genetically corrected cells, which remains to be determined. Nevertheless, the scientific enthusiasm to impact devastating diseases like SCD drives clinical trials to better understand the risks and benefits of these novel therapies. Support of these clinical trials to answer these questions have has come from many sources including the multimodal NHLBI based Cure Sickle Initiative as well as from industry, emphasizing the wide excitement in this emerging arena.

No disclosures.

Funding 1R21HL145636 and OT2HL152800 to JPM, and T32 HL066987 to DGJ.

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Table 1. Trials testing safety and efficacy of mobilization of peripheral blood hematopoietic stem cells by plerixafor

Study	Plerixafor Dose (ug/kg)	No. of Patients	Adverse Events	Peak* CD34+ Cells (per ul)
Boulad et al[26]	80	6	1	48, 7, 8, 18, 132, 37
	160	3	0	43, 27, 251
	240	6	1	95, 19, 31, 63, 30, 10
Tisdale et al[28]	240	15	7	99 (45-627)
Lagresle-Peyrou et al[27]	240	3	0	All 80+
Esrick, Manis et al[24]	180	3	0	36, 65, 31
	240	3	0	290, 27, 156

*Peak values are the highest values reported at predetermined timepoints either with or without concomitant apheresis.

Table 2. Active gene therapy clinical trials for sickle cell disease as of September 1, 2020.

Clinical Trial Number	Phase	Gene Manipulation Strategy	Vector	Conditioning
NCT02186418	Phase 1/2	Addition	Anti-sickling gamma-globinG16D lentiviral vector (ARU-1801)	Melphalan (RIC*)
NCT03282656	Phase 1	Editing	shRNAmiR targeting BCL11A lentiviral vector	Busulfan (myeloablative)
NCT02247843	Phase 1/2	Addition	Beta-AS3 anti-sickling globin lentiviral vector	Busulfan (myeloablative)
NCT03745287	Phase 1/2	Editing	CRISPR-Cas9 targeting BCL11A (CTX001)	Busulfan (myeloablative)
NCT04091737	Phase 1	Addition	Anti-sickling Gamma-	Melphalan (RIC*)

			globinG16D and short-hairpin RNA734 lentiviral vector (CSL200)	
NCT04293185	Phase 3	Addition	Beta-T87Q anti-sickling globin lentiviral vector (LentiGlobin BB305)	Busulfan (myeloablative)
NCT04443907	Phase 1/2	Editing	CRISPR-Cas9 targeting BCL11A (OTQ923 and HIX763)	

* Reduced intensity conditioning