



The Present Condition of Sickle Cell Disease: An Overview of Stem Cell Transplantation as a Cure

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Abstract

Treatment of sickle cell disease (SCD) remains largely palliative. While it can enhance living standards, persons having SCD still suffer from extreme sickling crises, end-organ destruction, and reduced life expectancy. Increasing research has resulted in the recognition and advancement of stem cell transplantation and gene therapy as possible solutions for SCDs. However, there have been various factors that have hindered their clinical application. The more advantageous of the two, stem cell transplantation, is constrained by a small donor pool, transplant difficulties, and eligibility requirements. The current article reviewed the literature on SCDs, current treatment options, and more particularly the progress of stem cell transplants. It outlined various challenges of stem cell transplant and proposed ways to increase the donor pool using alternative strategies and modifications of regimen conditioning with minimal transplant-related toxicities and associated complications.

Keywords

- ▶ cord blood
- ▶ haploidentical
- ▶ gene therapy
- ▶ stem cell transplantation

Introduction

A series of inherited red blood cell (RBC) disorders known as sickle cell disease (SCD) is a structural abnormality of hemoglobin leading to the characteristic shape—"the sickle." Herrick JB first described the medical condition in 1910 as peculiar elongated and sickle-shaped.¹ SCD occurs most frequently among people in Sub-Saharan Africa, and less regularly in regions of the Middle East, the Indian subcontinent, Mediterranean regions, and people of African origin. Globally, over 300,000 children are annually born with this disease with approximately 150,000 deaths per year; as a result, it has been identified as a worldwide health issue.² The average lifespan eagerness of individuals with SCD is reported to be 40 to 50 years, which is often shorter for persons with homozygous HbS or HbS/β0 than for persons with compound heterozygous.³

The sickle hemoglobin, under normal conditions, combines with oxygen or carbon dioxide forming a biconcave shape termed premeniscocytes, which cannot be differenti-

ated from the healthy RBCs.⁴ Premeniscocytes have randomly distributed hemoglobin and are as flexible as healthy cells. However, when the oxygen or carbon dioxide is removed, hemoglobin is transformed into an uncombined state, and the premeniscocyte undergoes sickling. Ingram established the genetic basis of the condition in 1958 and showed that the change from glutamic acid (GAG) to valine (GTG) at position six of the hemoglobin-globin chain is what causes the disease.⁵ The amino acid substitution results from the point mutation of the hemoglobin molecule. SCD can occur when one inherits homozygous HbS (HbSS) or compound heterozygous with β-thalassemia mutations (HbS/β0-thalassemia and HbS/β+ -thalassemia, and other structural β-globin variants such as HbC-African Americans, HbD-Indian/Pakistan, HbE-Asian, HbO-Arab).

Persons with SCD may suffer from acute or chronic complications. Severe complications of SCD include but are not limited to acute chest syndrome (ACS), hepatic and splenic sequestration, painful episodes, and stroke. The disease may also lead to life-threatening chronic

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complications such as osteomyelitis, and damage or injury to the liver, lungs, kidney, brain, eyes, and heart. The current disorder-altering strategies of SCD that cure and manage these problems include crizanlizumab, hydroxyurea (HU), *L*-glutamine, RBC transfusion, and voxelotor. However, the only promising cure, to date, remains an allogeneic hematopoietic stem cell transplant which has shown excellent potential recently.^{6–8} Gene therapy is also a potential therapeutic strategy currently under investigation. In this article, we outlined the advances and constraints of hematopoietic stem cell immigration as a remedy for SCD. The article also highlighted strategies to improve the procedure through regimen modification to reduce transplant-related toxicities and associated complications based on meticulous considerations of current research outcomes.

Pathophysiology of Sickle Cell Disease in Brief

SCD has a complicated pathway of contributory pathologies, and although polymerization of sickle cells is central in vaso-occlusive crises, the hypothesis that it is solely responsible for vascular obstruction and sickle cell-related painful crises is no longer valid. In a low condition of oxygen concentration, intracellular hemoglobin becomes insoluble and polymerizes into tubulin fibers producing the sickling shape.⁹ The inflexible and rigid cells aggregate and block tissue-depriving cells of blood flow and oxygen, leading to ischemic damage or tissue necrosis.

Sickle cells' reduced antioxidant capability, mediated by faulty intracellular signaling pathways, insufficient nitric oxide (NO), and adenosine triphosphate, may result in oxidative damage. The highly reactive oxygen species may damage cellular membrane proteins and cause hemolysis. The abnormal membrane proteins at the cell surface contribute to contact among sickle cells and normal RBCs, and other blood cells. The release of hemoglobin into plasma traps NO and adversely lowers NO content since arginase-1 activity, responsible for NO production, is lower in sickle cells. The lower NO concentration causes vasoconstriction of the vessels contributing to ACS, cardiac ischemia, and stroke.

Additionally, sickle cells have dysregulated microRNAs, which suppress crucial RNA molecules, contributing to defective erythropoiesis. Adhesive receptors such as RBC intercellular adhesion molecule-4 and basal cell adhesion molecule may become abnormally activated as a result of the process. These surface molecules operate as a bridge connecting sickle cell and endothelium, leukocytes, and platelets. Other adhesive proteins such as mitogen-activated protein kinase ERK 1/2, E, and P selectin are upregulated in SCD and contribute to disease pathology and severity.¹⁰

Aside from the interaction between sickle cells and leukocytes and platelets caused by adhesive proteins on sickle cells, interleukin (IL)-4, IL-10, macrophage inflammatory protein (MIP-1 α), and tumor necrosis factor- α are all examples of inflammatory cytokines, increased in SCD resulting in the extreme inflammation and painful episodes associated with the disease.^{11,12}

As a result of the multiple contributory pathways of the disease pathology, it is challenging to strategize therapy that can address all the mechanisms and pathways. There have been short-term and long-term treatment therapies that address specific path(s) of the disease pathology. Curative strategies have also been postulated. However, there are adverse limitations to such procedures, and detailed studies ought to be performed.

Current Treatment Options

The majority of SCD therapies are still mainly available for palliative and while they may improve quality of life, persons with SCD may still suffer from extreme sickling crises, end-organ damage/injury, and reduced life expectancy. Current therapies for SCD include fetal hemoglobin-inducing agent-HU, RBC transfusion, *L*-glutamine, antiadhesive agent crizanlizumab, and hemoglobin oxygen-affinity modulator voxelotor. Crizanlizumab and voxelotor were granted accelerated approval based on their effect on a surrogate endpoint; therefore, further research and trials are required to verify and establish their clinical benefits.

Hydroxyurea

HU, also certain hydroxycarbamide, is an antineoplastic oral drug used to treat chronic myelogenous leukemia, melanoma, and inoperable ovarian cancer. Dresler and Stein¹³ first created the chemical compound in 1869, and the United States Food and Drug Authority (USFDA) officially recognized it as an anticancer agent in 1967. The drug received approval from the FDA for use in treating adults with SCD in 1998 based on the evidence of HU-inducing HbF in the mid-1980s.^{14,15} Various studies have supported the drug's effect on reducing acute painful episodes and ACS and inducing HbF.¹⁶

Although HU is widely believed to induce HbF synthesis, the mechanism of action for HbF induction remains not completely established. However, the well-established mechanism of action of HU as an HbF inducer is the blockage of synthesis of deoxyribonucleotides, DNA synthesis, and repair through reversible inhibition of ribonucleotide reductase.¹⁷ The inhibition of ribonucleotide reductase in the S-phase, where DNA synthesis is highly expressed, leads to the transitory arrest of erythropoiesis. The recovery from the arrested state leads to stress erythropoiesis consisting of erythropoietin induction and enlistment of early erythroid progenitors that maintain their HbF-producing ability, resulting in mature erythrocytes with actively expressing *g*-gene.¹⁸ The erythroid stress-related HbF expression may happen because of the alteration in the erythroid environment such as a change in erythropoietic kinetics, signal transduction, or others.

L-Glutamine

The USFDA 2017 announced the commercial availability of *L*-glutamine (under the brand "Endari") as the second drug for the treatment of SCD.¹⁹ The syntheses of glutathione, nicotinamide adenine dinucleotide, and arginine all begin with

glutamine, compounds central to the protection of RBCs against oxidative stress or damage and maintenance of normal vascular tone.²⁰ *L*-Glutamine is absorbed and metabolized by sickle RBCs at a higher rate that exceeds the amount the body produces. The mechanistic use of the drug is to supplement the low *L*-glutamine levels caused by the decreased redox potential of sickle RBCs which may lead to oxidative stress or damage to the cell. By increasing naturally occurring redox agents like nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide hydrogenase, supplementing the low levels of *L*-glutamine prevents oxidative stress. By neutralizing the oxidative stress in sickle RBCs and making the cells flexible to deliver oxygen throughout the body, *L*-glutamine has been demonstrated to drastically reduce the frequency of vaso-occlusive episodes and incidence of ACS with negligible adverse effects. In addition, *L*-glutamine acts as an antioxidant to protect RBC protein and lipids against oxidation, RBCs fragility, and phosphatidylserine exposure.²¹

Crizanlizumab

On November 15, 2019, crizanlizumab was also approved for treating SCD after several reports of a medication that reduces the incidence of vaso-occlusive crises in adults and adolescents 16 years old with SCD.²² A humanized monoclonal antibody called crizanlizumab binds to P-selectin, which is expressed on the surface of platelets and endothelial cells.^{23,24} These cells express P-selectin during activation processes like inflammation.^{25,26} This starts the leukocytes' adhesion to the endothelium through the leukocytes' P-selectin glycoprotein ligand 1 (PSGL-1). Activated platelets may also bind to PSGL-1 on the surface of leukocytes. Normal RBCs do not express PSGL-1 on their surfaces. Sickle RBCs are, on the other hand, known to possess surface glycoproteins like PSGL-1 which adheres to activated endothelium, forming an aggregate that obstructs the vasculature, tissue ischemia, and vaso-occlusion.²⁷ Crizanlizumab, an anti-P-selectin monoclonal antibody, inhibits the binding of PSGL-1, thereby preventing interactions with leukocytes and sickle cells. Inhibition of these interactions also eliminates subsequent local hypoxia that may cause a rise in P-selectin expression in the endothelium, HbS polymerization, and severe vaso-occlusive crises.

Voxelotor

Based on the evidence of boosting Hb, the USFDA also granted accelerated approval to voxelotor, a Hb oxygen-affinity modulator, on November 19, 2019. The compound inhibits HbS polymerization and the sickling of HbS-containing RBCs. The mechanism of voxelotor in reducing the sickling of RBC is through increasing the proportions of oxygenated HbS in SCD patients. Oxygenated HbS has a biconcave shape like normal Hb, and is flexible and randomly distributed, thereby preventing vaso-occlusion. The drug safety reported by Hutchaleelaha et al was high with over 30% of cases of vomiting and nausea, 50% with diarrhea, and others with gastroenteritis and headache.²⁸

RBC Transfusion

About 90% of adults with SCD must have received at least one RBC transfusion making blood transfusion a mainstay of treatment for SCD along with HU.²⁹ RBC transfusion can be given to treat complications of sickle cell or as an intermittent preventive treatment to protect against complications.³⁰ Blood transfusion is, in some cases, given to treat stroke in children with abnormal transcranial Doppler.³¹ Transfusion increases the oxygen capacity of the blood, especially in people with anemic sexually transmitted diseases (STDs), and reduces the complications of vaso-occlusion. People with severe anemia usually require a simple transfusion in which normal blood cells are given without the removal of one's blood. However, with mild anemia, exchange transfusion may be necessary to lower the concentration of HbS through dilution. RBC transfusion may follow either a restriction policy where a simple transfusion is given to reach a prespecified Hb target or a liberal policy in which transfusion reduces HbS percentage below a preselected threshold.³² Although there are risks associated with RBC transfusion, including the transmission of transfusion-associated infections, alloimmunization, iron overload, acute or delayed hemolytic transfusion, and more difficult compatibility testing, it has generally improved the quality of life for people with SCD. Therefore, the benefits and risks must be assessed before transfusion.

Hematopoietic Stem Cell Transplantation

Although advances have been achieved in treatment to control complications and crises of SCD, the safety and accessibility of these drugs have always been a universal issue. To date, hematopoietic stem cell transplantation (HSCT) or bone marrow transplantation remains the only cure for SCD with gene therapy also considered a promising strategy. Considering how skeptical most health care professionals and SCD patients are about current treatments, curative therapy should have been an accomplished discovery. However, HSCT with its several concerns including the selection of patients (end-organ injury, disease severity, and age), risk of short-term toxicity, long-term adverse effects (graft-vs-host disease [GVHD], sterility), graft failure, selection of conditioning regimen, and availability of suitable donor has limited its prospects. This article focuses on current advances made in HSCT, especially to overcome such limitations, recent challenges, and the way forward.

Several factors may be considered in the selection of patients for HSCT. The eligibility for HSCT, in contrast, is based on individual benefits-and-risks analysis. The procedure is only recommended for patients for whom a compatible donor is available, and the advantages of recovery exceed the dangers of transplant-associated complications. Age and suitable donors are the most significant factors in the mortality and success of HSCT. Studies show that pediatric persons with human leukocyte antigen (HLA)-matched sibling donors using a myeloablative conditioning regimen is, by far, the most successful transplant.³³ A global study of HLA-identical sibling HSCT revealed 5-year overall survival (OS) rates of 95 and 81% for patients under the age of 16 and those

Table 1 Listing some major ongoing and recently completed hematopoietic stem cell transplantation clinical trials in sickle cell disease

Recruitment status	Study type	Allocation	Clinical phase	Clinical trial identifier
Not yet recruiting	Interventional	Randomized	Phase 3	NCT05392894
Not yet recruiting	Interventional	Nonrandomized	Phase 3	NCT04046705
Completed	Interventional	N/A	Phase 3	NCT01877837
Completed	Interventional	Nonrandomized	Phase 3	NCT00176852
Recruiting	Interventional	N/A	Phase 3	NCT04293185

who were 16 years old, respectively. Event-free survival (EFS) rates were correspondingly 93 and 81%. The hazard ratio for treatment failure (graft rejection or death) increased by 9% with each additional year of age, and the hazard ratio for acute GVHD increased by 4% with each additional year.³⁴ ▶**Table 1** enlists the major ongoing and recently completed clinical trials for SCDs.

Selection of Conditioning Regimen

Myeloablative regimen, the standard conditioning for HSCT, has been associated with transplant-related toxicities, veno-occlusive disease of the liver, hemorrhage, secondary malignancy, graft failure, and sterility.^{35–37} Attempts in preventing these adverse effects mostly seen in adults have resulted in newer regimens such as nonmyeloablative and reduced-intensity conditioning regimens. HSCT using these approaches is aimed at producing mixed chimerism for stable disease control. However, an engraftment threshold that allows the improvement of the disease remains controversial. Previous studies have also heavily associated these approaches with a greater engraftment dropout rate, a higher frequency of GVHD, and prolonged immunosuppression which put patients at risk of infections.³⁸ A study that has been replicated in other studies prompted the use of an alemtuzumab nonmyeloablative regimen using HLA-matched sibling HSCT with no GVHD or transplant-related mortality and an EFS rate of 90%.^{39,40}

Matched Unrelated Donor

With the chances of an individual finding a matched sibling donor within the 16 to 20% range, efforts have been made to increase the number of transplant donors available to most SCD individuals. Advances in HSCT have led to the use of matched unrelated, cord blood, and haploidentical donors. A study using a reduced intensity conditioning regimen including alemtuzumab, fludarabine, and melphalan reported a 10% graft rejection, 76 and 69% incidence of 1- and 2-year EFS, respectively, and corresponding 86 and 79% rates of OS, using a matched unrelated donor. The rate of acute GvHD on day 100 was 28% and that of chronic GVHD was 62% in the first year. Severe GvHD-related death was also reported in the second year.⁴¹

In 2019, a prospective study on adolescents and young adults, 17 to 36 years, was conducted using a reduced-intensity regimen with both HLA-identical sibling donors

and HLA-allele-matched unrelated donors. The progress and success of unrelated transplants were still very low. One-year OS rates for sibling and unrelated transplants were 94 and 80%, respectively, and the corresponding EFS values were of 94 and 60%.⁴²

Cord Blood Donor

The use of unrelated cord blood for the treatment of SCD is currently discouraged due to the high rate of graft rejection, GVHD, and posttransplant infections. A study conducted by Kamani and colleagues in 2012 using reduced-intensity conditioning with alemtuzumab, fludarabine, and melphalan resulted in a 1-year EFS of 37.5%, acute GVHD of 25%, and chronic extensive GVHD of 12.5%.⁴³ Despite EFS not meeting the prespecified target, the reduced-intensity conditioning used in the study was reported to have a positive safety profile and therefore new approaches with improved engraftment can be adopted for unrelated cord transplants in persons with severe SCD.

A more recent study tested the previous reduced-intensity conditioning regimen with thiotepa in nine children to see the effect on engraftment. The findings showed increasing engraftment with 1-year EFS and OS rates of 78 and 100%, respectively, but the incidence of acute GVHD and viral infections of 44 and 78% were still very high.^{44,45}

Contrary to unrelated cord blood, transplanting related cord blood has had excellent results in SCD. According to reports, the 6-year EFS and OS for SCD patients who received HLA-identical sibling cord blood were 90 and 97%, respectively. Graft failure was noted in 10.4% of patients, with acute GVHD occurring in 11% of the 96 patients and no incidence of chronic severe GVHD.⁴⁶ Aside from the difficulty in obtaining HLA-identical related cord blood for the majority of SCD patients, there have been reports of significant delay in the time of engraftment for neutrophils and platelets, which place SCD patients at increased risk of viral infection.⁴⁷

Haploidentical Donor

With continuous efforts to expand the HSCT donor pool, haploidentical transplants, which use half-matched donors, have seen major improvements in the conditioning regimen with promising outcomes. Haploidentical transplants have previously been associated with high transplant morbidity, graft failure, and extensive GVHD. Also, the transplant procedure of haploidentical cells has itself been reported to cause a decline in cardiac, pulmonary, and renal functions.

Haploidentical transplant uses half-matched donor cells or one HLA-matched haplotype, making donors easily accessible.⁴⁸

For patients with significant organ damage, an alemtuzumab nonmyeloablative regimen with and without post-transplant cyclophosphamide was utilized to create a secure HLA-haploidentical peripheral blood stem cell transplant strategy.^{49,50} The conditioning regimen without cyclophosphamide had all patients rejecting graft after 7 months of transplantation. In patients that received cyclophosphamide, graft failure decreased significantly to 50%, and acute and chronic GVHD was diagnosed in 16%. Two of the 21 patients died from congestive heart failure, infections, and pulmonary hypertension. In a similar study with a thiotepa-augmented nonmyeloablative regimen, 93% stable engraftment and 100% OS were achieved after 6 months. Five of the 15 patients that received cyclophosphamide developed acute GVHD and 6 patients had chronic GVHD. Other complications reported in the study were mainly asymptomatic viral infection, occurring in nine patients, and one case each of typhlitis, gastritis, and gastrointestinal bleeding.⁵¹⁻⁵³

A recent study by Foell et al, using CD3/CD19 and ab/CD19 T cell-depleted haploidentical cells achieved 100% primary engraftment.⁵⁴ The recorded rates of EFS and OS were 100 and 88%, respectively, where all individuals who survive are devoid of SCD-related morbidity and comorbidities. The incidence of GVHD was considerably high with 28 and 16% developing acute GVHD and chronic GVHD, respectively. Viral reactivation was reported in 52%, and 4% each with macrophage activation syndrome and veno-occlusive disease of the liver.⁵⁵

Challenges of the Transplantation Therapy at Present

The medical industry is expanding rapidly, with fresh concepts for ensuring safety and simplifying difficult procedures such as HSCT treatment, which has a crucial function in developing drugs and bioscience exploration and is anticipated to enhance significantly.⁵⁶ Even though clinical and preclinical investigations have affirmed the efficacy of HSCT in the treatment of SCDs, there remain a few drawbacks and possible adverse reactions, such as a vast stem cell manufacturing, prospective allograft denial of cells, threat of cell tumorigenesis, grafting or survivorship effectiveness of cell transplantation, obstacles with the route of administration, and targeting issues. Many of these factors hinder HSCTs' quick clinical translation and use.⁵⁷ GVHD is the major immunological obstacle to allogeneic HSCT effectiveness. With a mortality rate of over 20%, GVHD is the second largest fatality cause in allogeneic HSCT patients, after only primary illness mortality. Acute GVHD affects 20 to 70% of patients, while persistent GVHD, the leading cause of morbidity following allogeneic HSCT, can impact more than 50% of patients. The transfer of alloreactive donor T cells inside the stem cell transplant causes both acute and chronic GVHD, although their etiology and clinical characteristics are unique.⁵⁸

Further obstacles in obtaining HSCT include the slow aging of culture cells, repetitive cell extraction and infusion, expense, safety, and ethical concerns. Next, the acquired exogenous stem cells should be allogeneic; immunological rejection following cell transplantation is a possible barrier in the use of HSCTs.^{59,60} Allogeneic stem cells, like any other tissue or organ transplant, might be denied by the host immune system. The primary cause of this is that the transplanted tissue does not match the host's HLA. Normally, host T lymphocytes identify MHC class 1-protein antigens on other cells; if they do not match, the immune system is triggered, and the transplanted cells are attacked.^{61,62} Additionally, the possible tumorigenicity of transplanted foreign stem cells is the most obvious issue with cell therapy. When put into a live host, stem cells, particularly embryonic stem (ES) cells or induced pluripotent stem (iPS) cells, can create teratomas or malignant tumors.^{63,64} According to research, iPS cells produced from B6 mouse embryonic fibroblasts endure immunological rejection and develop teratomas after being transplanted into B6 mice. Even though no major immune rejection occurs, ES cells generated from B6 mice cause teratogenesis in mice.⁶⁵ Concurrently, the tumorigenicity of iPS cells is linked to c-Myc gene insertion site mutagenesis and the permanent expression of reprogrammed foreign genes.^{66,67} In allogeneic transplantation, a small number of contaminated foreign cells may also promote carcinogenesis, and even very tiny levels of contaminating undifferentiated ES cells have been observed to create tumors in mice.⁶⁸ Luckily, extensive steps are already being made across the world to establish regulatory standards and regulations to assure the safety of patients. HSCTs will have a substantial impact on human wellness in the coming years.

Conclusion

Despite recent breakthroughs in the care of people with SCD, the mechanism of action, safety, and clinical benefits of these drugs remain elusive to most scientists. Universal clinical trials are needed to be initiated and performed under strict conditions and analysis to firmly establish the safety and benefits of these drugs. Gene therapy as a cure for SCD is an interesting and potential research area but its safety and efficacy are yet to be established. For those with SCD, allogeneic HSCT is the only treatment option. However, concerns about donor availability and transplant-related complications had limited the use of HSCT. Recent studies have discovered new approaches to expand the donor pool and prevent some transplant-associated complications. Most of these approaches met the prespecified target of an EFS rate; however, the incidence of transplant-related diseases was considerably high.

Aside from using HLA-matched sibling donors, all efforts to expand the donor pool have resulted in a high incidence of transplant-related conditions that are most deadly to recipients. However, reports from studies using haploidentical peripheral stem cells are encouraging and demonstrate a safer and more feasible procedure that could be adopted as an alternative cure for SCD patients. Also, different

conditioning regimens have been shown to result in transplant-related toxicities. Therefore, to determine the optimum regimen for diverse donor cells with various therapeutic alternatives to SCD, substantial research is needed.

Conflict of Interest

The author declares no competing interests.

References

- Herrick JB. Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. *JAMA* 2014;312(10):1063
- Chakravorty S, Williams TN. Sickle cell disease: a neglected chronic disease of increasing global health importance. *Arch Dis Child* 2015;100(01):48–53
- Pauling L, Itano HA, Singer SJ, Wells IC. Sickle cell anemia a molecular disease. *Science* 1949;110(2865):543–548
- Ingram VM. Abnormal human haemoglobins. I. The comparison of normal human and sickle-cell haemoglobins by fingerprinting. *Biochim Biophys Acta* 1958;28(03):539–545
- Embury SH. The interaction of coexistent α -thalassemia and sickle cell anemia: a model for the clinical and cellular results of diminished polymerization? *Ann N Y Acad Sci* 1985;445(01):37–44
- Tanabe P, Spratling R, Smith D, Grissom P, Hulihan M. CE: Understanding the complications of sickle cell disease. *Am J Nurs* 2019;119(06):26–35
- Trivedi K, Hussain MS, Mohapatra C. Role of glutamine as an ergogenic amino acid during fatigue. *J Clinic Med Rev Rep* 2022;4(02):1–6
- Hussain MS, Tyagi S, Khatri H, Singh S. Stem cell therapy for myocardial infarction: a mini-review. *Asian J Pharmaceut Res Dev* 2022;10(02):122–124
- Kaul DK, Finnegan E, Barabino GA. Sickle red cell-endothelium interactions. *Microcirculation* 2009;16(01):97–111
- Pathare A, Kindi SA, Daar S, Dennison D. Cytokines in sickle cell disease. *Hematology* 2003;8(05):329–337
- Knight-Perry J, DeBaun MR, Strunk RC, Field JJ. Leukotriene pathway in sickle cell disease: a potential target for directed therapy. *Expert Rev Hematol* 2009;2(01):57–68
- McGann PT, Ware RE. Hydroxyurea therapy for sickle cell anemia. *Expert Opin Drug Saf* 2015;14(11):1749–1758
- Dresler WF, Stein R. Ueber den hydroxylharnstoff. *ustus Liebigs Annalen der Chemie* 1869;150(2):242–252
- Platt OS, Orkin SH, Dover G, Beardsley GP, Miller B, Nathan DG. Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. *J Clin Invest* 1984;74(02):652–656
- Habara A, Steinberg MH. Minireview: Genetic basis of heterogeneity and severity in sickle cell disease. *Exp Biol Med (Maywood)* 2016;241(07):689–696
- Charache S, Terrin ML, Moore RD, et al; Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. *N Engl J Med* 1995;332(20):1317–1322
- Ware RE, Eggleston B, Redding-Lallinger R, et al. Predictors of fetal hemoglobin response in children with sickle cell anemia receiving hydroxyurea therapy. *Blood* 2002;99(01):10–14
- Strouse JJ, Lanzkron S, Beach MC, et al. Hydroxyurea for sickle cell disease: a systematic review for efficacy and toxicity in children. *Pediatrics* 2008;122(06):1332–1342
- Chapman TR, Kinsella TJ. Ribonucleotide reductase inhibitors: a new look at an old target for radiosensitization. *Front Oncol* 2012;1:56
- Foong WC, Loh CK, Ho JJ, Lau DS. Foetal haemoglobin inducers for reducing blood transfusion in non-transfusion-dependent beta-thalassaemias. *Cochrane Database Syst Rev* 2023;1(01):CD013767
- Ho JA, Pickens CV, Gamcsik MP, Colvin OM, Ware RE. In vitro induction of fetal hemoglobin in human erythroid progenitor cells. *Exp Hematol* 2003;31(07):586–591
- Ali MA, Ahmad A, Chaudry H, et al. Efficacy and safety of recently approved drugs for sickle cell disease: a review of clinical trials. *Exp Hematol* 2020;92:11–18.e1
- Pavan AR, Dos Santos JL. Advances in sickle cell disease treatments. *Curr Med Chem* 2021;28(10):2008–2032
- Quinn CT. l-Glutamine for sickle cell anemia: more questions than answers. *Blood* 2018;132(07):689–693
- Ballas SK. The evolving pharmacotherapeutic landscape for the treatment of sickle cell disease. *Mediterr J Hematol Infect Dis* 2020;12(01):e2020010
- Torres L, Conran N. Emerging pharmacotherapeutic approaches for the management of sickle cell disease. *Expert Opin Pharmacother* 2019;20(02):173–186
- Ataga KI, Kutlar A, Kanter J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. *N Engl J Med* 2017;376(05):429–439
- Gutsaeva DR, Parkerson JB, Yerigenahally SD, et al. Inhibition of cell adhesion by anti-P-selectin aptamer: a new potential therapeutic agent for sickle cell disease. *Blood* 2011;117(02):727–735
- Matsui NM, Borsig L, Rosen SD, Yaghamai M, Varki A, Embury SH. P-selectin mediates the adhesion of sickle erythrocytes to the endothelium. *Blood* 2001;98(06):1955–1962
- Hutchaleelaha A, Patel M, Washington C, et al. Pharmacokinetics and pharmacodynamics of voxelotor (GBT440) in healthy adults and patients with sickle cell disease. *Br J Clin Pharmacol* 2019;85(06):1290–1302
- Chou ST. Transfusion therapy for sickle cell disease: a balancing act. *Hematology Am Soc Hematol Educ Program* 2013;2013:439–446
- Yawn BP, Buchanan GR, Afenyi-Annan AN, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. *JAMA* 2014;312(10):1033–1048
- Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. *N Engl J Med* 1998;339(01):5–11
- Bernaudin F, Socie G, Kuentz M, et al; SFGM-TC. Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. *Blood* 2007;110(07):2749–2756
- Vermynen C, Cornu G, Ferster A, et al. Haematopoietic stem cell transplantation for sickle cell anaemia: the first 50 patients transplanted in Belgium. *Bone Marrow Transplant* 1998;22(01):1–6
- Gluckman E, Cappelli B, Bernaudin F, et al; Eurocord, the Pediatric Working Party of the European Society for Blood and Marrow Transplantation, and the Center for International Blood and Marrow Transplant Research. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. *Blood* 2017;129(11):1548–1556
- Bhatia M, Sheth S. Hematopoietic stem cell transplantation in sickle cell disease: patient selection and special considerations. *J Blood Med* 2015;6:229–238
- Gluckman E. Allogeneic transplantation strategies including haploidentical transplantation in sickle cell disease. *Hematology Am Soc Hematol Educ Program* 2013;2013:370–376
- Shenoy S. Hematopoietic stem-cell transplantation for sickle cell disease: current evidence and opinions. *Ther Adv Hematol* 2013;4(05):335–344
- Nickel RS, Flegel WA, Adams SD, et al. The impact of pre-existing HLA and red blood cell antibodies on transfusion support and engraftment in sickle cell disease after nonmyeloablative hematopoietic stem cell transplantation from HLA-matched sibling donors: a prospective, single-center, observational study. *EClinicalMedicine* 2020;24:100432

- 41 Saraf SL, Oh AL, Patel PR, et al. Nonmyeloablative stem cell transplantation with alemtuzumab/low-dose irradiation to cure and improve the quality of life of adults with sickle cell disease. *Biol Blood Marrow Transplant* 2016;22(03):441–448
- 42 Guilcher GMT, Monagel DA, Nettel-Aguirre A, et al. Nonmyeloablative matched sibling donor hematopoietic cell transplantation in children and adolescents with sickle cell disease. *Biol Blood Marrow Transplant* 2019;25(06):1179–1186
- 43 McPherson ME, Anderson AR, Haight AE, et al. Transfusion management of sickle cell patients during bone marrow transplantation with matched sibling donor. *Transfusion* 2009;49(09):1977–1986
- 44 Kodish E, Lantos J, Stocking C, Singer PA, Siegler M, Johnson FL. Bone marrow transplantation for sickle cell disease. A study of parents' decisions. *N Engl J Med* 1991;325(19):1349–1353
- 45 Shenoy S, Eapen M, Panepinto JA, et al. A trial of unrelated donor marrow transplantation for children with severe sickle cell disease. *Blood* 2016;128(21):2561–2567
- 46 Krishnamurti L, Neuberger DS, Sullivan KM, et al. Bone marrow transplantation for adolescents and young adults with sickle cell disease: results of a prospective multicenter pilot study. *Am J Hematol* 2019;94(04):446–454
- 47 Kamani NR, Walters MC, Carter 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(08):1265–1272
- 48 Abraham A, Cluster A, Jacobsohn D, et al. Unrelated umbilical cord blood transplantation for sickle cell disease following reduced-intensity conditioning: results of a phase I trial. *Biol Blood Marrow Transplant* 2017;23(09):1587–1592
- 49 Locatelli F, Kabbara N, Ruggeri A, et al; Eurocord and European Blood and Marrow Transplantation (EBMT) group. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. *Blood* 2013;122(06):1072–1078
- 50 Fitzhugh CD, Hsieh MM, Taylor T, et al. Cyclophosphamide improves engraftment in patients with SCD and severe organ damage who undergo haploidentical PBSCT. *Blood Adv* 2017;1(11):652–661
- 51 Kurtzberg J. Update on umbilical cord blood transplantation. *Curr Opin Pediatr* 2009;21(01):22–29
- 52 Mehta RS, Dave H, Bollard CM, Shpall EJ. Engineering cord blood to improve engraftment after cord blood transplant. *Stem Cell Investig* 2017;4(06):41
- 53 Munoz J, Shah N, Rezvani K, et al. Concise review: umbilical cord blood transplantation: past, present, and future. *Stem Cells Transl Med* 2014;3(12):1435–1443
- 54 Foell J, Kleinschmidt K, Jakob M, Troeger A, Corbacioglu S. Alternative donor: $\alpha\beta$ /CD19 T-cell-depleted haploidentical hematopoietic stem cell transplantation for sickle cell disease. *Hematol Oncol Stem Cell Ther* 2020;13(02):98–105
- 55 de la Fuente J, Dhedin N, Koyama T, 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(06):1197–1209
- 56 Gögel S, Gubernator M, Minger SL. Progress and prospects: stem cells and neurological diseases. *Gene Ther* 2011;18(01):1–6
- 57 Zhang GL, Zhu ZH, Wang YZ. Neural stem cell transplantation therapy for brain ischemic stroke: review and perspectives. *World J Stem Cells* 2019;11(10):817–830
- 58 McDonald-Hyman C, Turka LA, Blazar BR. Advances and challenges in immunotherapy for solid organ and bone marrow transplantation. *Sci Transl Med* 2015;7(280):280rv2
- 59 Strecker JK, Olk J, Hoppen M, et al. Combining growth factor and bone marrow cell therapy induces bleeding and alters immune response after stroke in mice. *Stroke* 2016;47(03):852–862
- 60 Broughton BR, Lim R, Arumugam TV, Drummond GR, Wallace EM, Sobey CG. Post-stroke inflammation and the potential efficacy of novel stem cell therapies: focus on amnion epithelial cells. *Front Cell Neurosci* 2013;6:66
- 61 Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC. CNS immune privilege: hiding in plain sight. *Immunol Rev* 2006;213(01):48–65
- 62 Galea I, Bechmann I, Perry VH. What is immune privilege (not)? *Trends Immunol* 2007;28(01):12–18
- 63 Newman MB, Misiuta I, Willing AE, et al. Tumorigenicity issues of embryonic carcinoma-derived stem cells: relevance to surgical trials using NT2 and hNT neural cells. *Stem Cells Dev* 2005;14(01):29–43
- 64 Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. *Nature* 2011;474(7350):212–215
- 65 Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer* 2011;11(04):268–277
- 66 Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. *Adv Cancer Res* 2008;100:133–158
- 67 Menendez S, Camus S, Herreria A, et al. Increased dosage of tumor suppressors limits the tumorigenicity of iPS cells without affecting their pluripotency. *Aging Cell* 2012;11(01):41–50
- 68 Lawrenz B, Schiller H, Willbold E, Ruediger M, Muhs A, Esser S. Highly sensitive biosafety model for stem-cell-derived grafts. *Cytotherapy* 2004;6(03):212–222