Therapy for Sickle Cell Disease

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Sickle cell disease (SCD) is a severe hereditary form of anemia that results from a single mutation in the sixth codon of the gene encoding the β-globin chain (from glutamic acid to valine) of the adult Hb tetramer (α2β2), which is prone to polymerization at low oxygen levels.

Keywords: cell therapy ; gene therapy ; anemia

1. SCD

SCD is a severe hereditary form of anemia that results from a single mutation in the sixth codon of the gene encoding the β-globin chain (from glutamic acid to valine) of the adult Hb tetramer (α2β2), which is prone to polymerization at low oxygen levels [1]. It is an autosomal recessive disorder caused by mutations in the Hb subunit β (*HBB*) gene, which encodes the β-globin subunit of HbA. It is characterized by the presence of RBCs that contain hemoglobin S (HbS) without additional normal HbA [2]. SCD may occur as sickle cell anemia (SCA), HbSC disease, and HbS-thalassemia. Genotypically, SCA has two sickle β (β^S) alleles; HbSC disease has one sickle β allele and one β^C allele, which has another type of mutation; while HbS-thalassemia has one sickle β and one β null allele. Polymerized sickle Hb (HbS, α2βS2) leads to abnormalities in the RBC biconcave architecture and flexibility, resulting in crescent-shaped cells with an enhanced adherence to the vascular endothelium; these abnormal shaped RBCs obstruct blood flow, and are prone to hemolysis [3]. The terminology of SCD is used for all anemias caused by mutations in the *β-globin* gene. SCA is the most common form of SCD, representing 70% of SCD cases in African patients [4].

2. Epidemiology

An epidemiological study showed that 300,000 children with SCD are born every year worldwide [5]. Another study revealed that approximately 100,000 US nationals have SCD [6]. The areas with the highest prevalence of SCD are India, the Democratic Republic of Congo, and Nigeria [7]. In most areas of Africa, SCD affects approximately 1% of all births, and approximately 6–15% of affected children die before their fifth birthday. Approximately 50–90% of children develop SCD in Africa [8]. In Sudan, the prevalence of SCA ranges from 0.8% (Central Sudan) to 30.4% (Western Sudan). A study showed that the prevalence of SCA in Sudan is approximately 2–30.4% [9][10].

Mortality and morbidity caused by SCA in childhood are high. In the Unites States, the median survival is 48 years for women and 42 years for men [11], though advances in treatment options may increase survival, and a report from a London Hospital showed a median survival of 67 years [4]. A prospective cohort study in Kenya showed that children with SCD (58 per 1000 children/years) have a higher mortality than those without SCD (2.4 per 1000 children/years) [12].

3. Pathogenesis

The main pathogenic processes of SCD include HbS polymerization, dehydration, vaso-occlusion (VOC), hemolysis-mediated endothelial dysfunction, and sterile inflammation.

The polymerization of sickle Hb due to deoxygenation or hypoxic events is the most crucial and basic molecular pathogenic mechanism of SCD. This polymerization initiates the arrangement of contiguous strands that cause the formation of misshaped or crescent-shaped RBCs. This polymerization also initiates downstream events, such as alterations of the function and structure of the RBC membrane, deteriorations in vasoactivity, the unbalanced distribution of RBC volume, and increased RBC attachment to the endothelium [13][14].

The main etiology of the dehydration of sickled erythrocytes in SCD involves the activation of cytokines, endothelin 1 (ET-1), and prostaglandin E2 (PGE2) in the Gardos pathway [13]. The reticulocyte-rich fractions of sickled blood exhibit a high expression of K-Cl cotransporter isoform 2 (KCC2), which maintains optimal K^+-Cl^- cotransport [15] and cation fluxes (Ca^{2+}

ion entry), which are induced in a deoxygenation state [16]. In tissues with a high oxygen demand, the intraerythrocyte deoxygenation process causes hydrophobic motifs of deoxygenated HbS tetramers to be exposed [17].

In SCD, increased cell adhesion to the endothelium mediates VOC and biorheological disorders. Blood rheology is dependent on the viscosity of the plasma and hematocrit, as well as the deformability of erythrocytes. Because of decreased sickle erythrocyte deformability and persistent hemolysis owing to dehydration and Hb polymerization, increased plasma viscosity impedes the flow of blood through the venules of tissues with a high oxygen demand. Sickle erythrocytes have inadequate deformability and are sequestered in the microcirculation, causing transient VOC [18][19].

VOC can be triggered by multiple factors, such as stress, inflammation, ischemia, hemolysis, activated endothelial cells, increased plasma viscosity, and diminished blood flow [20]. E- and P-selectin, which are endothelial selectins, also play an important role in VOC. Random precapillary obstruction by a small number of dense sickle RBCs (SS-RBCs) also contributes to VOC. Epinephrine can activate intercellular adhesion molecule 1 (ICAM-4; an LW blood group glycoprotein), which is an RBC adhesion receptor that mediates SS-RBC adhesion to endothelial α-v-β-3 integrin. VOC enhances ischemia-reperfusion injury [21][22][23]. In addition, patrolling monocytes are reduced in recent VOC episodes. Patrolling monocytes, which scavenge debris and damaged cells from the vasculature, have higher levels of the anti-inflammatory heme oxygenase 1 (HO-1) enzyme, which degrades heme. Patrolling monocytes expressing HO-1 protect the SCD vasculature from ongoing hemolytic insult and vaso-occlusion [24].

Endothelial dysfunction is a pathognomonic factor related to SCD, and is caused by the upregulation of P- and E-selectin, ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and major leukocyte chemoattractants (i.e., keratinocyte-derived chemokine in mice or interleukin-8 in humans) on endothelial cells [17]. Repeated vascular damage resulting from VOC may cause endothelial cell dysfunction through a mechanism resembling ischemia/reperfusion injury [21].

Together with heme and iron, endothelial cells are key players and have an important role in SCD oxidative damage through several actions. First, they are involved in the formation of robust oxidizing species, such as ferryl-Hb and •OH, through the H_2O_2-dependent Fenton reaction. Second, increased platelet adhesion may activate endothelial cells. Third, increased inflammatory cytokine production (interleukin (IL)-1-β, IL-6, and IL-18, and tumor necrosis factor (TNF)-α), inflammasome activation, and the stimulation of Toll-like receptor-4 (TLR4) in endothelial cells occur through NF-κB-linked pathways. In addition, there is reduced anti-inflammatory cytokine IL-10 that is correlated with the frequency and severity of VOC [25]. Fourth, activated neutrophils may influence endothelial cells and serve as neutrophil extracellular traps (NETs) for RBCs and platelets. Fifth, the expression of adhesion molecules such as P-selectin, E-selectin, ICAM-1, and VCAM-1 is increased; these are all markers of endothelial dysfunction, and can serve as receptors for leukocytes (monocytes, neutrophils, and lymphocytes). Sixth, blood coagulation is triggered by the release of intraluminal tissue factor from endothelial cells—the tissue factor then binds to factor VIIa, promoting the coagulation cascade [26].

The association of sterile inflammation with SCD-related morbidity suggests that anti-inflammatory paradigms are treatments for SCD [27]. Sterile inflammation may be promoted by damage-associated molecular patterns, such as heme, Hsp70, ATP, cyclophilin A, mtDNA, HMGB1, extracellular DNA, and S100A8, which may play important roles in SCD inflammatory mechanisms [28].

CXCL1 is a fundamental inflammatory biomarker of VOC [29]. The administration of CXCL1 exogenously is adequate to activate VOC, and the suppression of the CXCL1 receptor (namely CXCR2) may prevent VOC development because of the hemolytic transfusion reaction. The direct suppression of this pathway may be a novel therapy for VOC [30].

SCD features a vicious circle between inflammation and abnormal RBC rheology [31], which modulates clinical severity in patients. Chronic inflammation leads to organ dysfunction in SCD patients. High activation of coagulation factors, endothelium, monocytes, neutrophils, and platelets are key factors in this vicious cycle. Different strategies have been used to determine the effect of these factors in SCD patients [32].

4. Clinical Features of SCD

SCD or SCA has various clinical manifestations, such as asplenia, severe infection, episodes of VOC, neurological changes, priapism, stroke, acute chest syndrome, renal failure, pain crisis, and pulmonary hypertension [33]. On the basis of a retrospective study in children with SCA in Rio de Janeiro, their clinical features are infection (27–35%), hemolytic crisis (25%), splenic sequestration (17–21%), painful events (12–16%), and hand–foot syndrome (12–16%) [34].

5. Current Management of SCD

The current management approaches for SCD are hematopoietic stem cell transplantation (HSCT), various drugs, and other strategies [35].

5.1. HSCT

Allogeneic human leukocyte antigen (HLA)-matched HSCT is the only curative treatment for SCD. It is curative in 90–95% of SCD patients [36]. Matched sibling donor (MSD) HSCT is a curative strategy for all patients aged ≤ 5 years old as well as older pediatric patients older between 5 to 18 years old presenting with SCD-derived complications. When performed in time, HSCT can establish donor-derived erythropoiesis and stabilize or even restore the function of affected organs in patients with SCD. Haploidentical-bone marrow transplantation (BMT) with posttransplant cyclophosphamide (PTCy) has emerged as a viable and safe option for HSCT in patients with severe SCD, and it results in a lower rate of graft versus host disease (GVHD), but improvements are required to increase the low engraftment rate. When ex vivo-expanded, partially mismatched unrelated cord blood (UCB) grafts are used, rapid donor cell engraftment may lead to a reduction in SCD severity. The risks of infections and severe GVHD must be minimized [17][26][27][28].

Following HSCT, SCD patients can develop mixed chimerism (MC), that is, the co-existence of host- and donor-derived cells; however, the clinical control of SCD can still be ensured. In an SCD patient with MC, the expression of the apoptotic regulator Fas was significantly higher in the recipient erythroblasts and RBCs than in the donor erythroblasts and RBCs, suggesting that SCD “ineffective” erythroid cells undergo apoptosis, whereas donor cells have a survival advantage. Stable donor chimerism greater than 25% is associated with the resolution of SCD-related symptoms [29][30][31].

Pilot studies of BMT for the management of young symptomatic SCD patients showed a low transplantation-related mortality and resolution of the underlying disease. Two-thirds of BMT recipients remained VOC-free over 2 years of follow-up, but transplant-related complications, including GvHD, occurred with a high frequency. Currently, 35 clinical trials registered at ClinicalTrials.gov (accessed on 1 April 2021) are studying allogeneic BMT in patients with SCD [32][33][34].

To reduce the graft failure rate of T-cell-replete haploidentical transplantation, pretransplant immune and myelosuppression (PTIS), which involves the use of cyclophosphamide, dexamethasone, and fludarabine, followed by the augmented John Hopkins protocol with thiotepa-based conditioning and plerixafor-based mobilization in healthy donors, is effective and safe for treating patients with SCD, with minimal risk of graft failure and low GVHD rates [37].

5.2. SCD Management Approaches Other Than HSCT

The current drug management approaches related to SCD are classified into four categories based on their target. The first category is of drug compounds that target erythrocyte rheology, reduce polymerization, and improve cellular hydration. Novel approaches for inhibiting HbS polymerization may be achieved through several mechanisms, such as activating HbF synthesis, increasing oxygen (O_2) affinity, inhibiting intermolecular connections in sickle strands in RBCs by decreasing the concentration of 2,3-diphosphoglycerate, and decreasing the intracellular Hb concentration. A decline in the intracellular Hb concentration generally occurs in patients who have developed iron deficiency [16]. Examples of these drugs are hydroxyurea, senicapoc, metformin, Aes-103, and voxelotor. The second category contains drug compounds that reduce VOC and cellular adhesion, such as hydroxyurea, crizanlizumab, rivipansel, Ig (intravenous), tinzaparin, dalteparin, sevuparin, eptifibatide, NKTT120, ticagrelor, and prasugrel. The third category includes drug compounds that improve endothelial dysfunction, including hydroxyurea, l-glutamine, haptoglobin, arginine, oral or intravenous nitrite, inhaled nitric oxide, and antioxidants. The fourth category contains drug compounds that improve sterile inflammation, including hemopexin/haptoglobin, MP4CO, various antioxidants, DNAse-1, canakinumab, montelukast, simvastatin, and anakinra, as well as TLR4 inhibition. Drugs that are approved by US Food and Drug Administration (FDA) for SCD are hydroxyurea, l-glutamine, and crizanlizumab [38]. Despite the availability of standard therapies and novel management approaches (i.e., hydroxyurea, blood transfusion, hydration, and pain-relieving medicines), patients continue to experience long-term complications from the disease [39]. In addition, the safety and efficacy of related medicines are still under investigation in clinical trials (Table 1).

Table 1. Current clinical trials for sickle cell disease (SCD) treatments [38][40][41][42][43][44][45].

Trial Phase(s)	Drug Compounds (and Explanation)
Phase I RCT	Metformin, Aes-103, SCD-101 (NCT02380079), and NKTT120 (NCT01783691)

Trial Phase(s)	Drug Compounds (and Explanation)
Phase I	Ambrisentan (NCT02712346) Decitabine + tetrahydrouridine or THU (NCT01685515) Plerixafor mobilization and apheresis (NCT03226691–multicenter study) Citrulline * (NCT02314689, NCT02697240) Zileuton (NCT01136941–SAOL) Panobinostat or LBH589 (NCT01245179) INCB059872 (NCT03132324)
Phase I/II RCT	Voxelotor or GBT440 (NCT02850406)
Phase I/II	Arginine (NCT02447874–open-label randomized crossover design), rivipansel or GMI-1070 (NCT00911495–SAOL, NCT01119833–RDBPC, and NCT02187003–RDBPC), omega-3 fatty acids (NCT02947100–SAOL), *N*-acetylcysteine (NCT01800526–SAOL), and Simvastatin (NCT0050802–SAOL, NCT01702246–SAOL)
Phase II RCT	Crizanlizumab, rivipansel, intravenous Ig, or IVIG (NCT01757418–RDBPC), dalteparin, sevuparin, eptifibatide, prasugrel, haptoglobin, oral or intravenous nitrite, inhaled nitric oxide, hemopexin/haptoglobin, MP4CO, various antioxidants, canakinumab, montelukast, and simvastatin.
Phase II	Atorvastatin (NCT01732718), Arginine (NCT01796678–RDBPC, NCT02536170–RDBPC, and NCT00004412–open-label randomized design), Mometasone (NCT02061202), Montelukast (NCT01960413), Omega-3 fatty acids (NCT02973360–RDBPC), AMD 3100 or Mozobil (plerixafor) (NCT00075335), Riociguat (NCT02633397–RDBPC), and IW-1701 (NCT03285178–RDBPC).
Phase III RCT	Arginine, senicapoc, tinzaparin, ticagrelor, rivipansel (GMI-1070), crizanlizumab (NCT03814746), and antioxidants.
Phase III	Glutamine (NCT01179217–RDBPC), Omega-3 fatty acids (NCT02525107, NCT02604368), and *N*-acetylcysteine (NCT01849016–RDBPC).

Trial Phase(s)	Drug Compounds (and Explanation)
FDA-approved	l-glutamine, hydroxyurea, crizanlizumab, or SEG101 (NCT1895361–RDBPC, NCT03264989–SAOL, and NCT03474965–SAOL).
Under investigation	TLR4 inhibition, DNAse-1, anakinra, and vitamin D.

* Study drug administered during acute VOC, SAOL: single-arm open-label, RDBPC: randomized double-blind placebo-controlled.

Hydroxyurea is a ribonucleotide reductase inhibitor [4], and is regarded as a drug of choice for improving endothelial dysfunction (as a nitric oxide donor) and red cell rheology. Beneficial effects include the induction of fetal hemoglobin (HbF) for inhibiting erythrocyte adhesion, conceivably ensuring nitric oxide donor bioavailability; myelosuppression (resulting in the reduced availability of leukocytes, reticulocytes, and platelets) and reduced frequency of acute pain; and alleviation of acute chest syndrome, reduced hospital admissions, and reduced blood transfusions. It may also protect against progressive organ damage, including nephropathy [40][46]. Moreover, clinical trials in infants and toddlers have shown very promising results [4]. However, as the most prominent side effect of hydroxyurea is in adult men with SCD, fertility problems are still an important concern worldwide [47].

5.3. GT Approaches for SCD

A GT approach toward SCD was developed in which HbF production is permanently enhanced using various vectors. One vector is the lentiviral vector (LV), which induces the overproduction of γ-globin. LVs have been proven to efficiently transmit complex globin expression cassettes containing transcriptional regulatory sequences from the β-globin locus control region, which are required for a high expression. A similar approach has been used in studies involving mouse models of SCD. The overexpression of a β-globin polypeptide containing specific point mutations was designed to optimize the antisickling activity and led to the improvement of SCD in two models. In BERK SCD mice, efficient γ-globin LV gene transfer resulted in steady-state HSCs. Moreover, a clinically relevant forward-oriented β-globin–expressing vector, with six-fold higher vector titers and a four- to ten-fold higher transduction efficiency, was tested for the long-term repopulation of hematopoietic cells in humanized mice and rhesus macaques. The insertion of the Rev response element enabled the retention of intron 2, and β-globin production was observed in macaques transplanted with human SCD CD34+ cells. Another vector is the adenoviral vector. In vivo HSC GT using the bimodular HDAd5/35++ vector cured SCD in a mouse model. Compared with HDAd vectors with either γ-globin addition or CRISPR-Cas9 reactivation units, in vivo HSC transduction of CD46/Townes mice with the HDAd combo resulted in significantly higher γ-globin production in RBCs, which reached 30% of the production of adult human α and βS chains, and resulted in complete phenotypic correction for SCD. Some clinical trials (NCT02247843, NCT02140554, and NCT02186418) are ongoing and are using lentiviral strategies to induce HbF [35][37][38][39].

Another approach is the knock down of BCL11A, which is a repressor of γ-globin expression and HbF production, in adult erythrocytes. The inhibition of BCL11A was effective for inducing HbF. This is initial proof that shmiR-based gene knockdown results in an advantageous risk–benefit profile in SCD. Moreover, inactivation of the transcription factor BCL11A in a humanized and transgenic SCD mouse model resulted in the adjustment of the pathologic and hematologic defect that was related to SCD [48].

5.4. Engineered Stem Cell Approach for SCD

The engineered stem cell approach for SCD consists of three processes. The first is harvesting autologous HSPCs from bone marrow or peripheral blood. The second is genetically modifying sickle HSPCs by gene editing. The third approach involves transplanting the gene corrected HSPCs back into the patient after chemotherapy conditioning. Gene-modified HSPC graft repopulates the hematopoietic stem compartment, producing genetically corrected RBC progeny. Sickle HSPCs can be genetically modified through zinc finger nucleosomes (ZFNs), transcription activator-like effector nucleases (TALENs), or clustered regularly interspaced short palindromic repeats/CRISPR-associated nuclease 9 (CRISPR/Cas9) techniques (NCT03745287) [40][41][42][43].

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