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Advances in managing sickle cell anemia: a systematic review

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ABSTRACT

Background: Despite the acceptance and approval of several medications and techniques to reduce vaso-occlusive episodes (VOEs), Hydroxyurea along with other analgesics have remained the primary treatment option for Sickle Cell Anaemia (SCA) in Nigeria. However, in terms of cost-effectiveness and fewer side effects, Niprisan® remains the preferred option. In this review, we discussed new drugs/technologies as well as previously approved medications that could ameliorate SCA aside from the options available in Nigeria; and we hope to inspire our readers by providing insights into new inventions to overcome current challenges in the field.

Methods: This review involved a comprehensive examination of existing literature on SCA treatments; specifically focusing on new pharmaceutical developments, innovative technologies, and previously approved medications on Google Scholar, PubMed, ResearchGate, EMBASE, and Cochrane database using SCA, novel therapies, Niprisan®, haematopoietic stem cell transplant, and gene therapy as search items. Additionally, the references of some retrieved articles were also searched. The literature retrieved included review articles, meta-analyses, clinical trials, and original research papers.

Conclusions: Advanced insights into the cellular and molecular basis of the sickle cell disease processes have unveiled several established/potential drug targets on which newer SCA therapies are based. These newer therapies have varied mechanisms ranging from Fetal haemoglobin (HbF) induction, RBC membrane stabilization, oxidative stress reduction, adhesion inhibition, reduction of inflammation, prevention of polymerization, and enhanced flow dynamics to gene-directed therapies with the potential for cure. This expounded review has highlighted real progress in SCA treatment. However, an improved survival rate will depend on the participation of clinical sites across the globe, as well as the availability of funds to support studies needed to confirm the safety and efficacy of these drugs.

1. Introduction

Sickle cell anaemia (SCA) is a hereditary, autosomal recessive monogenic disorder caused by a missense mutation in the HBB gene (that is the gene that encodes the beta subunit of the oxygen-carrying molecule, haemoglobin) with very few effective therapies[1]. It is linked to organ failure in kidneys, lungs, liver and the retina with a midpoint life expectancy of about 20 years lower than that of the general populace[2]. About 300,000 babies are born every year, and around 25 million humans are affected across the globe by this disorder[1]. This has made the United Nations General Assembly officially recognize SCA as a disease of global public health distress. It affects persons primarily of African ancestry[2]. Although Nigeria has the highest burden with SCA occurring in about 2 % of all births, it is widely spread across sub-Saharan Africa, the Middle East, India, the Caribbean, South and Central America, Europe, the United States, and some Mediterranean countries. Seasonal movement, trans-Atlantic slave trade, and carrier selection through their survival propel the global spread of SCA[3].

A lone nucleotide switch of thymine for adenine at the sixth codon of the β-globin gene situated at the short arm of the 11th chromosome is solely responsible for SCA[1]. This

mutation causes the swapping of valine for glutamic acid at the sixth amino acid location in the β globin chain resulting in atypical sickle haemoglobin (HbS) assembly which has the disposition to polymerize under a reduced oxygen saturation state such as in microcirculation[4]. The HbS polymers distort RBC cytoskeletal structures causing membrane protrusions that give rise to the typical poorly deformable sickle-shaped cells. A single polymerization episode results in a reversible decline in RBC deformability with an increase in mechanical fragility whereas, repeated polymerization cycles lead to irreparable damage to red blood cell (RBC) deformability. Along with polymerization, occlusion of blood vessels and haemolytic anaemia contribute to SCA pathophysiology[5]. Vascular endothelial dysfunction, functional nitric oxide insufficiency, inflammation, oxidative stress, ischaemia-reperfusion injury (IRI), hypercoagulability, enhanced neutrophil stickiness and platelet activation are additional pathophysiological processes that could lead to diverse complications[6]. HbSC and HbSβ-thalassemia ($HbSb^0$ and $HbSb^+$) are other types of sickling conditions of haemoglobin besides SCA which is the homozygous type (HbSS)[6]. Other rare sickling variants include haemoglobin SE, haemoglobin SO Arab, haemoglobin SD Los Angeles, and haemoglobin SG Philadelphia[3,6]. When the RBCs become sickle persistently, fragility, hemolysis, ischemia-reperfusion, post-capillary venules occlusion, and infarction are usual events that have injurious outcomes on the whole body. The disparity between a sickle cell and a normal RBC is that sickle cell has increased adhesion molecules that ease binding to endothelial walls; they haemolyze easily with compensatory reticulocytosis, resulting in local endothelial dysfunction.[1] The sickling and haemolytic properties of sickle RBC kindle an inflammatory cascade through reciprocations with the endothelium, the white blood cells, and platelets[5]. When RBC sickling and hemolysis occur repeatedly, acute and chronic organ damage at the cellular level ensues sometimes resulting in acute, unpredictable, and potentially life-threatening complications[7]. Acute pain (AP), Acute chest syndrome (ACS), and avascular necrosis are the result of vaso-occlusion (VOC) with ischemia, while haemolysis-linked endothelial impairment underlies stroke, pulmonary hypertension, priapism, and chronic leg ulcer[7]. Furthermore, events taking place in SCA lead to a boost in the generation of free radicals via the triggering of pro-oxidant enzymes, free release of haemoglobin, and heme from hemolysis which stimulates the Fenton's reaction, mitochondrial respiratory chain activity modification, and auto-oxidation of RBC. Abundant free radical generation increases oxidative stress in RBCs, endothelial cells, neutrophils, and platelets, presenting as multiorgan vasculopathy[6]. The clinical severity, and life expectancy in SCA vary widely: co-inheritance of genetic variants that determine the expression of HbF genes and α-thalassemia gene are associated with mild SCA phenotypes[2,4].

Indeed, the pathologic processes in SCA arise from complex molecular, cellular, tissue, and organ level interactions the understanding of which has unveiled real/potential drug targets on which newer more effective therapies are based.[1-7] Despite the high burden of SCA in Nigeria, many of the new modalities of managing this disease are not widely known or available. A lot of treatment regimens, each with its merits and demerits have been proposed, and it is forecasted that many more will be developed shortly to continue to boost the life expectancy of SCA patients. In this review, we explore both commercial and experimental therapies for SCA, and we aim to offer objective assessments of prevailing methods that have been reported. Because managing SCA is a growing field, it is possible that by the time this review is published, there will be more recent techniques available. Nevertheless, we hope that this review will not only provide a showcase of advances in managing SCA but will also inspire new inventions to overwhelm current challenges in the field.

2. Sickle cell anemia therapies

Niprisan®

It is a phytomedicine developed by the National Institute for Pharmaceutical Research and Development (NIPRD) for the management of SCA in the late 1990s in an effort to advance research of traditional herbal medicine. This herb is prepared using cold water extract of a mixture containing *Piper guineense* seeds, *Pterocarpus osum* stem, *Eugenia caryophyllata* fruit, *Sorghum bicolor* leaves, and trona. This recipe has been in use among the Yoruba people of Nigeria for ages to ameliorate the painful crisis associated with sickle cell anemia[8]. The result of the in vivo studies on the mechanisms of action of this herb revealed multiple effects that are beneficial for the treatment of SCA[9]. It was shown to contain compounds that increase the solubility of deoxy-Hb S, prolong the delay time before deoxy-Hb S polymerization, and shift the oxygen equilibrium curve of Hb S towards the left, thereby increasing the oxygen affinity of Hb S[9]. In a study conducted at The Children's Hospital of Philadelphia, in transgenic mice and in-patient subjects with sickle cell anemia, Niprisan® showed potential anti-sickling attributes; while findings from some Nigeria tertiary healthcare institutions showed that the drug can significantly reduce the number of vaso-occlusive crisis

without causing hemolysis or methemoglobin, accompanied by an increase in body weight in 60 % of the total number of patients enrolled in the study[8]. The biochemical pharmacology of Niprisan® reveals that the product impedes sickling and lessens the frequency and severity of SCA crisis in about 70 % of patients in phases II and III clinical trials with no sign of acute hepatic toxicity, as assessed by liver enzyme activity; nor renal function toxicity as evaluated for serum creatinine and blood urea nitrogen levels[9]. The demand for the product is high in Nigeria, India, and even in the US where it was granted orphan status by the US FDA. In 2005, the European Medicine Evaluation Agency also approved Orphan drug status for this product. The demand for Niprisan® has been on the rise to meet the increasing global demand by sufferers of SCA especially in developing countries due to its cost-effectiveness (~US$ 4.00/month compared to hydroxyurea at ~US$ 50.37/month)[10]. After the expiration of its patent in 2017, only a few generics such as Xickle (available in the US), and Sifcovan currently at the initial stage of clinical trials in Nigeria have been developed[2,3].

Hydroxyurea

It impedes the enzyme ribonucleotide reductase which aids the selection of high HbF-producing erythroid cells, a process that is traditionally inactivated shortly after birth[7]. For nearly 20 years, this drug was the only therapy approved by the US FDA for adults and babies with SCA[11]. In SCA, HbF restricts polymerization so that RBC rigidity and haemolysis are decreased, and eventually, anaemia is ameliorated. Hydroxyurea is known to increase nitric acid (a potent vasodilator) production, decrease RBC adhesion, and also decrease leucocyte counts (which contribute to vaso-occlusion)[7]. In a trial involving 152 adult subjects placed on hydroxyurea, 147 of them had their painful events reduced from 4.5 to 2.5 per year including acute chest syndrome (ACS). Usually, this drug is prescribed from 9 months of age for individuals with HbSS and HbSβ^0-thalassemia at an initial dose of 20 mg/kg daily, followed by a maintenance dose of 25-35 mg/kg, up to a maximum dose of 2500 mg daily[1]. Recent findings have shown that only a few percent of the eligible population could access this drug due to its cost, the lack of clinician expertise in SCA, and mistrust from SCA patients who have been underserved, discriminated against, or marginalized[12]. Common adverse effects of this medication include abdominal pain, nausea, diarrhea, and myelosuppression[7].

L-glutamine

An oral supplement of amino acid with a trait of enhancing NADPH production and decreasing reactive oxygen species in RBCs which in turn causes a reduction in sickling and RBC adhesivity[13]. This drug is proven to reduce acute pain (AP) crisis and hospitalization by 25 and 33 % respectively when compared to placebo in a study involving 152 subjects. It could also reduce ACS by 23 %, and it is recommended for patients with at least 2 painful crises per year irrespective of hydroxyurea therapy, and from age 5 and above. For patients whose weight is below 30 kg, the recommended dose is 5 g twice daily, while for those weighing 30 kg and above, a dose of 10-15 g is required daily[13]. Side effects of L-glutamine are flatulence, constipation, nausea, and abdominal pain.

Voxelotor

It is a haemoglobin oxygen-affinity modulator known to preserve the oxygenated state of haemoglobin in SCA by upgrading the binding of oxygen to HbS to reduce sickled hemoglobin polymerization thereby improving RBC deformability and blood viscosity and reducing haemolysis.[14]. In a randomized clinical trial of 198 patients between the ages of 12 and 65 with severe anemia, voxelotor increased haemoglobin production by about 1.0 g/dL[14]. Although its clinical effect in pain crisis or quality of life is currently unknown, Voxelotor is suitable for patients with low hemoglobin levels between 5.5 and 10.5 g/dL having at least one pain crisis per year irrespective of hydroxyurea therapy. A dose of between 600 to 1500 mg daily is appropriate depending on body weight. Adverse effects associated with this medication include skin rash, abdominal pain, nausea, diarrhea, and headache[14].

Small molecule pyruvate kinase activator therapy

Erythrocyte pyruvate kinase is the RBC-expressed isoform of the critical glycolytic enzyme pyruvate kinase. It is known to catalyze the last and rate-inhibiting step of glycolysis from phosphoenol-pyruvate to pyruvate while generating adenosine triphosphate (ATP) from adenosine diphosphate (ADP) which aids in the preservation of membrane integrity and RBC deformability. It is naturally activated by fructose biphosphate (FBP)[4].

Etavopivat is an experimental oral, small-molecule erythrocyte pyruvate kinase activator for SCA treatment. It was developed to improve the sickling of RBC in SCA patients via the reduction of 2,3-diphosphoglycerate, which inevitably enhances hemoglobin-oxygen affinity and further reduces hemoglobin polymerization sickling[4]. After administration of etavopivat daily for 5 days in nonhuman primates, ATP was seen to increase by 38 % from baseline; while in RBCs collected from SCA patients, hemoglobin-oxygen affinity increased, and sickling reduced[4].

Mitapivat also called **AG-348**, is another oral small molecule allosteric activator of the enzyme-pyruvate kinase that binds to distinct allosteric sites from FBP on the erythrocytic pyruvate kinase tetramer, allowing for the activation of both wild-type and mutant forms of the enzyme[15]. As a result of this mechanism, AG-348 offered promising features for use in hemolytic anaemias. It has been granted orphan drug designation by the US FDA for SCA, pyruvate kinase deficiency, and thalassemia. Several clinical trials for this drug have been completed, and others are still ongoing[15].

Proinflammatory cytokine inhibitors

Inflammation is a vital part of the pathophysiology of SCA. It has a far-reaching effect on SCA affecting virtually all organs or systems. Drugs targeting discrete pathways of inflammation therefore offer an appealing therapeutic plan to ameliorate many of the clinical events in SCA. Briefly, we will be discussing the prospect of targeting multiple inflammatory pathways enmeshed in the pathogenesis of SCA with a focus on new therapeutics:

Hemopexin (Hx) inhibits cytokine release from heme-stimulated macrophages, reduces expression of endothelial P-selectin/von Willebrand factor, and possibly induces hepatocyte Heme oxygenase-1 (HO-1) expression, all of which contribute to reducing inflammation and vaso-occlusion. In preclinical analysis Hx prophylaxis limited ACS and prevented stasis in intravenous heme-challenged SCA mice[16]. In another study, Hx stopped SCA mice with prior features of ACS from advancing to respiratory failure[5]. Although there is no commercially available Hx preparation yet, the risk of either exaggerated adverse pharmacologic effects at the target of interest or adverse effects as a result of modulation of other targets biologically unrelated to the target of interest is validated[16].

Resatorvid (TAK-242), is a selective toll-like receptor 4 (TLR4) inhibitor, resulting in the blockade of the release of mediators of inflammation such as nuclear factor kappa B (NFkB) and endothelial adhesion molecules. In preclinical studies, it prevents ACS and mortality in SCA mice infused with heme.[16].

MCC950 is a small molecule selective Nucleotide oligomerization domain (NOD)-like receptor protein-3 (NLRP3) inflammasome inhibitor in a dose-related reaction. This drug was looked into and proven to be worthwhile in the preclinical prototype of multiple sclerosis[17]. Its usefulness in SCA is currently being looked into.

Canakinumab is a monoclonal antibody that carefully binds interleukin-1 beta (IL-Iβ) and is valuable in managing chronic inflammation linked to cryopyrin-associated periodic syndromes and may also be effective in preventing hemolysis-related end-organ damage in SCA[5].

Sulfasalazine blocks the activation of NFkB and causes a notable decline in the activation of circulating endothelial cells and expression of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and E-selectin; as well as improve significantly the blood flow in transgenic SCA mice. In the canonical pathway, NFkB plays a key role as a regulator of transcription that allows for the acquisition of pathogens and the production of proinflammatory cytokines and chemokines at the site of infection or injury.[18]. Persistent activation of NFkB typically is stimulated by ischemia-reperfusion injury as well as the activation of heme-induced TLR4, and the resultant effect is chronic inflammation seen in SCA. Sulfasalazine has displayed potential in patients with SCA when it was dispensed orally for about 4 weeks[19]. The treated patient demonstrated a striking reduction in endothelial cell expression of VCAM, ICAM, and E-selectin. Other inhibitors of NFkB signaling that have proven to be efficacious preclinically in transgenic sickle mice include polyhydroxyphenyl hydroxamic acid derivatives, **didox** (N-3,4-trihydroxybenzamide), and **trimidox** (N-3,4,5 tetrahydroxybenzenecarboximidamide HCI). These drugs act as antioxidants but also effectively inhibit NFkB activation by blocking selectively the phosphorylation and breakdown of Ikbα. These agents decrease leukocyte adhesion, and stasis, and enhance blood flow in transgenic SCA mice[19]. Also, **glucocorticoids** have been proven to impede NFkB via induction of IkBα, and also by the interaction of the glucocorticoid receptor with the p65 subunit of NFkB[20]. However, its use in ACS has remained controversial.

Benzimidazole derivative, **BRP-7** inhibits leukotriene biosynthesis in vitro and in vivo by targeting 5-Lipoxygenase (5-LPO)-activating protein (FLAP). BRP-7 blocks the formation of cellular leukotriene, prevents co-localization of FLAP with 5-LPO, and reduces inflammation. Generally, leukotrienes are associated with several events that occur in SCA such as constriction of the bronchi tree, excessive generation of mucus, edema, and inflammation, hyperactivity of the airways, and increased peripheral nociceptors sensitivity to trivial stimuli. Some trials showed this drug to reduce several inflammatory mediators such as Svcam-1, Sicam-1, and IL-6 in SCA[21]. **Zileuton** is a structural analogue of hydroxyurea which limits 5-LPO catalyzed synthesis of leukotrienes approved

by the US FDA for persons with asthma. This drug has also been shown to induce the expression of the γ-globin gene and enhance the levels of HbF in erythroid progenitors extracted from SCA patients[21].

Cysteinyl leukotriene (CysLT) level is elevated in steady state SCA, and this level correlates with the rate of pain event. **Montelukast** (a CysLT inhibitor) role in sickle vasculopathy and vaso-occlusion is currently been tested in patients already on stable doses of hydroxyurea, with the surrogate endpoint of a reduction in sVCAM-1 level. In this study, scientists look at whether montelukast has additive effects in patients already taking hydroxyurea, compared with study subjects placed on hydroxyurea alone[7].

Many proinflammatory perturbations in SCA encourage mast cell activation, and nitric oxide (NO) inhibits the activation of mast cells and inflammation. SCA is typified by a reduction of NO bioavailability, and a further reduction in the inhibitory effect of NO on the generation of IgE-dependent cytokines such as TNF-α, IL-4, and IL-6 by mast cells. Conversely, IRI, oxidative stress, and activation of TLR4, which are all increased in SCA enhanced mast cell activation and are linked with increased Fc epsilon receptor 1 (FcƐR1) expression[6]. In a preclinical model for SCA, **Imatinib** and **Cromolyn sodium** reduce neurogenic inflammation. Both agents are known inhibitors of substance-P (SP) and calcitonin gene-related peptide (CGRP) release from the skin and dorsal root ganglion of transgenic SCA mice. Previously it was proven that cromolyn sodium could exert an anti-sickling and possibly membrane-stabilizing effect on sickle red cells in vitro, while a combination of cromolyn and hydroxyurea produced a better improvement in pain scores and a great reduction in the number of sickle erythrocytes in an ex-vivo assay[6].

SCA patients have a large amount of circulating invariant natural killer T (iNKT) cells, which increase further by a large proportion during vaso-occlusive episodes (VOE). The activation of CD4+iNKT cells appears to be limited by cluster of differentiation 1d (CD1d) lipid presentation of antigen. Many techniques are currently being studied to restrict the activation of iNKT cells in SCA[22]. **Regadenoson** for example is an antibody against CD1d and causes iNKT cell depletion. A two-stage trial of Regadenoson in SCA patients using various doses infused continuously over 12 hours showed that the drug limits markers of iNKT cell activation with enhanced levels of adenosine A2a receptor (A2aR); and was well tolerated without severe side effects[22]. More clinical trials for this therapy are underway to ascertain its effectiveness in reducing the amount of iNKT cells and activation during painful VOE and ACS.

Prasugrel (a platelet activator) has shown promising data in sickle mice in which it partly attenuates both basal and agonist-stimulated platelet activation[6]. A study involving adults with SCA receiving this medication showed a significant reduction in biomarkers of platelet activation compared with placebo[6]. A trial to determine whether prasugrel has significant effect on the number of VOC events in a year is currently ongoing. **Ticagrelor** (another platelet activator) is also on trial to evaluate if it could reduce the number of days of pain and pain intensity[6].

Therapies targeting cell adhesion

These drugs target either the RBC or leucocytes or both. They are a very attractive therapeutic modality in SCA treatment because multiple adhesive interactions have been proven to contribute to vaso-occlusion[5]. They inhibit adhesion and/or activation of leucocytes that are otherwise recruited to inflamed vessels. Several studies have shown that inhibiting both P-selectin-mediated and E-selectin-mediated adhesion led to a reduction of vaso-occlusion in *in-vitro* and murine models[2].

Crizanlizumab for example, is a monoclonal antibody administered in opposition to P-selectin (an adhesion molecule found on activated platelets and endothelial cells that may relatively mediate vaso-oclusion). This drug was approved based on a phase II clinical trial of 198 patients between the ages of 16 and 65 with SCA who received the medication monthly as an infusion[7]. A maintenance dose of 5 mg/kg administered intravenously monthly was sufficient for patients who have had more than 2 episodes of pain crisis per year irrespective of hydroxyurea therapy. Side effects are infusion-related reactions such as abdominal pain, arthralgia, and nausea[7].

Intravenous γ globulin (IVIg) infusion caused a rapid decrease in adherent leucocytes, reduction in leucocyte-erythrocyte interactions, and increased microcirculatory blood flow as well as survival rate via IVIg's ability to inhibit the Fc gamma receptor III (FcγRIII), leading to the inhibition of neutrophil adhesion, reduction of RBC capture by leucocytes, and reduced macrophage-1 (Mac-1) activity as a result of recruitment of Src homology 2-containing tyrosine phosphatase-1 in a recently conducted study for vaso-occlusion[2].

Simvastatin has shown a dose-dependent downregulation of endothelial adhesion molecules, inflammatory mediators, tissue factor expression, and increased nitric oxide levels in a pilot study involving children with SCA[5]. This drug's ability to reduce the frequency of acute pain episodes is currently underway.

Rivipansel also known as **GMI-1070** inhibits E-selectin

and P-selectin which are highly expressed by the endothelial cells and platelets of SCA subjects. It is currently in the advanced stage of clinical trial for acute vaso-occlusive episodes. A study of this drug in sickle mice revealed that it could prevent VOC and reduce the severity of ongoing VOC[13]. At the early stage of clinical trial, SCA patients well tolerated this drug, with data suggesting the reduction of biomarkers of endothelial activation, leucocyte activation, and coagulation activation[13].

Se1G1 is a humanized monoclonal antibody against P-selectin. A phase I study of intravenous Se1G1 in healthy adult subjects showed the drug to be well tolerated and effective in limiting P-selectin activity for about 4 weeks, while the phase II trial prevented VOC events during 50 weeks of treatment every 28 days with or without Hydroxyurea in SCA patients with pain crisis[23].

Sevuparin is a derivative of low-molecular- weight heparin (LMWH) being developed to inhibit adhesive interactions via P-selectin. It retains the P-selectin binding domain of heparin but largely lacks anticoagulant activity. This drug is currently at an advanced stage of clinical trial for acute VOC treatment[24].

Tinzaparin is another derivative of LMWH that can reduce acute episodes of VOC. At a therapeutic dose, this drug was associated with significantly shorter periods of severe pain, overall duration of pain episodes, and overall duration of hospitalization. However, issues regarding potential study bias and standard of care make this study difficult to interpret[24].

The role of a β_2-adrenergic signaling pathway in activating BCAM/Lu and ICAM-4 RBC adhesion receptors has suggested that beta-blockers might reduce RBC adhesion and thus conferring a statutory effect on SCA. A phase II double-blind study involving 25 candidates showed **propranolol** to reduce the level of soluble E-selectin, P-selectin, ICAM-1, vascular cell adhesion molecule-1 (VCAM-1) compared with values for baseline and those of placebo[7].

Poloxamer 188 is a surfactant that alters the way cells and molecules behave in the presence of water. It enhances blood flow in microvasculature by inhibiting cell-cell and cell-endothelial interactions and reduces blood viscosity in SCA subjects. This substance efficacy was evaluated in a phase III trial, and a modest reduction in painful episodes was recorded. It is currently been tested for its ability to reduce the resolution time of VOC[7].

Linking of hypoxia and fetal hemoglobin

Hemoglobin (a sponge-like protein) takes up oxygen and allows RBC to transport it throughout the body. The adult haemoglobin comprises four subunits of protein (two α-globin and two β-globin). A mutation in β-globin usually causes SCA and β-thalassemia[25]. At the time of fetal development, γ-globin (which is another type of hemoglobin subunit) is expressed instead of β-globin. The γ-globin then links up with α-globin to form HbF. During birth, γ-globin expression is turned off while β-globin is turned on, resulting in a switch from HbF to adult hemoglobin[25]. A repeated expression of HbF after birth has been proven to relieve the symptoms of SCA, and a raised HbF level could cure this disease notwithstanding the presence of defective β-globin genes[1]. Many scientists across the globe are focusing their research interest on understanding how the perinatal switch from the γ-to-β-globin gene occurs and figuring out novel techniques to reverse it with drugs or genetic therapies. Some think that any therapy that could activate part of the cellular hypoxia response will limit sickling of RBC derived from adults with SCA, and some have been able to establish a direct link between hypoxia adaptation and HbF expression, and that the identification of γ-globin as a hypoxia-inducible factor (HIF) target gene supports the notion that HbF evolved as a protective mechanism against hypoxia[1]. A **Prolyl hydroxylase inhibitor** can cause the accumulation of HIF1 and then bind to a DNA regulatory region near the γ-globin gene to activate its transcription to produce HbF and prevent sickling of cells[25]. This drug also functions by stabilizing HIF proteins to stimulate the production of erythropoietin (a hormone that drives RBC production). It is currently in the late stage of clinical trial for SCA treatment[25].

Several drugs when administered together with hydroxyurea can elevate HbF levels. **Sodium butyrate** for example, when given as an infusion was shown to reduce pain episodes associated with SCA. Also, **pomalidomide** has demonstrated the ability to up-regulate HbF production *in vitro* and in sickle mice[7].

Carbon monoxide is another potent anti-sickling agent that reverses HbS polymerization via attachment to Hb[6]. They do so by shifting the oxyhemoglobin dissociation curve to the left, or by blocking cell dehydration through Gardos channel induction. Although they failed to reduce the frequency of VOC in a phase III clinical study[6]. At very low concentrations, CO has also demonstrated anti-inflammatory effects[6]. **Sanguinate** is a bovine pegylated Hb product designed to reduce sickling by delivering CO to HbS and then transporting O_2[13]. This drug has been granted

orphan status and is currently undergoing a randomized, double-blind placebo-controlled trial in persons with SCA experiencing VOC. **Pegylated carboxyhemoglobin (MP4CO)** has been proven to induce hepatic heme oxygenase-1 activity and also limit the activation of NF-kB and microvascular stasis in sickled mice. **SCD-101** is a botanical drug at the clinical trial stage to ascertain its ability to inhibit sickling in an *in vivo* setting[13]. Direct thrombin and factor X inhibitors such as **rivaroxaban** and **apixaban** have been investigated for their effect on levels of sVCAM and IL-6, and daily pain scores respectively in outpatients with SCA[26].

Haematopoietic stem cell transplant (HSCT)

HSCT is potentially a curative approach in which sickle cell-producing stem cells in a patient's bone marrow are replaced by normal RBC-producing stem cells from a donor[27]. It can either be allogeneic with donor stem cells from another person usually HLA-matched sibling or autologous where the patient's stem cells (usually having been modified to correct the defect) are used[28]. The main indications of HSCT include a history of stroke, recurrent ACS, recurrent VOC, and poor response to hydroxyurea. There is a 90% chance of a cure when performed in children with HLA-identical siblings[27]. The challenges with this therapy have always been difficulty securing appropriate donors, cost, and having to grapple with transplant-related complications.

Gene directed therapy

Gene therapy to induce HbF: This involves infusing autologous stem cells that have been transduced with BCH-BB694, a lentiviral vector that codes for microRNA which silences the expression of BCL11A, a suppressor of the γ-globin gene that encodes HbF[29]. In a study involving 6 patients treated with this strategy, all had HbF expression of 20 % to 42 % at a median 18-month follow-up with no pain crisis[29]. This therapy could improve anemia to near normal thereby enhancing the quality of life. Side effects are transplanting-related morbidity.

Lentiglobin gene therapy involves transducing the patient's haematopoietic stem cells with BB305 lentiviral vector, a modified β globin gene that encodes β-globin chains with anti-sickling T87Q substitutions which are assembled with α-globins to form HbAT87Q, a fabricated haemoglobin with the anti-sickling property. These transduced stem cells are then infused back into the patient after a course of chemotherapy[26]. In a randomized clinical trial involving 35 patients with HbSS infused with Lentiglobin and followed up for 17.3 months, remodeled autologous stem cell engraftment happened in all 35 subjects culminating in a significant increase in Hb level from 8.5 g/dL to 11.0 g/dL, and a reduction in severe pain crisis rate from 3.5 to 0 episodes in a subset of 25 patients who had at least 4 vaso-occlusive events within 2 years of enrollment[26]. This therapy is ideal for patients without a matched sibling donor for HbSS or HbSβ⁰-thalassemia. Although, unexplained anemia with dysplastic features developed in 2 patients, broad interrogation disclosed that these changes were not consistent with myelodysplasia[26].

Prime editing for SCA treatment entails making precise nucleotide changes at the point of mutation on the β-globin gene without having to do double-strand DNA breaks and hence minimal potential genotoxicity[23]. A preclinical study showed this technology to be efficient as prime-edited haematopoietic stem and progenitor cells (HSPCs) from SCA patients transplanted into mice maintained the ability for engraftment and repopulated the bone marrow. Furthermore, normal adult hemoglobin production was present in about 45 % of circulating RBC 17 weeks post-transplant and did not sickle when exposed to hypoxia. Prime editing protocols will require time to adapt and optimize, such as designing the prime editing guide RNAs that will target the prime editing system to the specific DNA region and specify the desired edit[30]. The actual extent of the side effects of this technology remains largely unknown.

Base editing as a potential therapy for SCA: This is a novel gene editing technique that utilize a modified CRISPR/Cas9 platform to induce point mutation at a specified DNA sequence. This technology can either be used to correct SCA mutation or induce HbF synthesis. For example, adenosine base editing has been employed to induce HbF expression in SCA patient cells. This technique promotes the expression of fetal hemoglobin to a higher, more stable, and more uniform state than other genome editing technologies that use CRISPR/Cas9 nuclease in human hematopoietic stem cells. This was achieved by creating a new T-cell acute lymphocytic leukemia protein-1 (TAL1) transcription factor binding site that causes particularly strong induction of fetal hemoglobin[26]. The γ-globin (fetal hemoglobin) gene offers a good target for base editing because there are very specific mutations that can reactivate its expression to induce expression after birth thereby providing a one-size-fits-all treatment for all mutations caused by SCA[30]. The base edits were retained in engrafted blood stem cells from healthy donors and SCA patients by putting them into immunocompromised mice.

The first ever approval of a CRISPR-based gene-editing therapy was recently granted for **CASGEVY**

(exagamglogene autotemcel or exa-cel for short form) by the US FDA[29]. Previously it was granted conditional marketing authorization in Great Britain by the U.K. Medicines and Healthcare Products Regulatory Agency and by the National Health Regulatory Authority in Bahrain for patients 12 years of age and older with sickle cell disease characterized by recurrent vaso-occlusive crises or transfusion-dependent beta-thalassemia (TDT), for whom hematopoietic stem cell transplantation is appropriate and a human leukocyte antigen matched related hematopoietic stem cell donor is not available. It is currently under review by the European Medicines Agency and the Saudi Food and Drug Agency for both SCA and TDT. CASGEVY is a genome-edited cellular therapy consisting of autologous CD34+ hematopoietic stem cells (HSCs) edited by CRISPR/Cas9 technology at the erythroid-specific enhancer region of the BCL11A gene. It is intended for one-time administration via a hematopoietic stem cell transplant procedure made specifically for each patient where the patient's own CD34+ cells are modified to reduce BCL11A expression in erythroid lineage cells leading to increased HbF production, thereby eliminating VOCs in people with SCA[31].

Although base editing had fewer genotoxic events compared to other types of editing, more safety testing and optimization are needed to truly ascertain its safety in clinical therapy[32].

3. Conclusion

Newer approaches to SCA are actively being explored more than ever. With our expounded review, we have highlighted real progress in SCA treatment. However, an improved survival rate will depend on the participation of clinical sites across the globe, as well as the availability of funds to support studies needed to confirm the safety and efficacy of the drugs.

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