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Haemoglobinopathies: Prevention, Diagnosis, Treatment and Management in Sub-Saharan Africa

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ABSTRACT

Hemoglobinopathies are a hereditary group of diseases that are characterized by qualitative changes in hemoglobin (sickle red blood cells) and quantitative changes in hemoglobin (thalassemia). Originally described in subtropical Africa, they are now found all over the world due to migration. Because of the limited available resources for the disease's care and prevention, their high frequency and clinical severity make them a serious public health burden, particularly in Africa. Sickle cell and thalassemia are categorized based on the specific defect in globin chains that are ineffectively produced. Developing nations like sub-Saharan Africa are faced with economic challenges, particularly, where problems are primarily caused by social and cultural backgrounds, along with the coexistence of infection and malnutrition, Therapeutic approaches and follow-up still pose challenges in these situations. Several European nations affected by hemoglobinopathies have successfully implemented an effective therapeutic regime, which is still lacking in sub-Saharan Africa. The aim of this review paper is to establish the current advanced techniques used for the treatment, prevention, diagnostic, and management of hemoglobinopathies and to ameliorate the burden of hemoglobinopathy in sub-Saharan Africa in the present and future.

KEYWORDS: Heamoglobinopathies, Sickle cell disease, Thalassemia, Treatment, Prevention, Managements, Sub-Saharan Africa.

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INTRODUCTION

The most prevalent single gene illnesses, with a carrier incidence of 7% in the global population, are inherited disorders of heamoglobin. They are particularly common in populations from the tropical and subtropical belts, and consist mainly of the α- and β- thalassaemias, and the haemoglobin variants S, C and E. (Ademola, 2015) Hemoglobinopathies, including sickle cell disease (SCD) and ß-thalassemia, are the most common genetic illnesses worldwide. Migration from high-prevalence areas has resulted in, and will likely continue to result in, an increase in patient numbers in Sub-Saharan Africa. In Nigeria, 20-25% of the population is genetically aberrant, and approximately 3% of kids are born with the condition. Individuals with SCD usually express HbS primarily due to the presence of the HbS allele, which codes for a valine residue instead of a glutamic acid residue at position 6 of ß-globin. Position 6 of valine, a hydrophobic amino acid, promotes the polymerization of deoxygenated HbS and the development of sickle-shaped Erythrocytes. After the hemoglobin switch, which occurs when HbF is switched to HbS during infancy, the resulted in chronic hemolysis, acute vasoocclusive crises, and a chronic vasculopathy that affects all organs (Odunvbun and Okolo, 2015)

The difficulties in treating haemoglobinopathies

The severity of SCD, like that of ß-thalassemia, is influenced by HbF expression and the presence of -thalassemia. This modulation is particularly visible in people who have, in addition to HbS, one allele of the ß-globin gene cluster that does not switch off HbF production during infancy ("hereditary persistence of fetal hemoglobin," HPFH)('Molecular Basis of Hereditary Persistence of Fetal Hemoglobin', no date). Since HbF effectively interferes with the polymerization of HbS, these individuals may exhibit little or no signs of SCD (Steinberg *et al.*, 2014). Moreover, pharmacologic induction of HbF has been shown to lessen the severity of SCD. Unfortunately, even large doses of HbF do not reliably ameliorate all SCD problems. Most SCD patients

die during their childhood if they do not receive sufficient medical treatment. More than 95% of patients reach maturity with the use of preventive antibiotics, immunizations, parent education, transcranial Doppler ultrasonography, red blood cell transfusions, and hydroxycarbamide (Telfer *et al.*, 2007). However, acute vasoocclusive episodes and persistent organ damage significantly affect quality of life. Even with best treatment, life expectancy is reduced by around two decades(Debaun *et al.*, 2018). Pharmacologic therapies that target many pathophysiologic processes of SCD have been developed in recent years and demonstrate clinically meaningful results(Gordeuk *et al.*, 2019). (Ademola, 2015) Combinations of these medications have the potential to significantly reduce SCD symptoms in many individuals, but they do not provide a cure and must be taken for a long time, if not forever. The only curative treatment now available is allogeneic stem cell transplantation, which is becoming regarded as "standard of care" when a sibling donor with an HLA-matched patient is available. However, stem cell transplantation from alternative donors—that is, unrelated or mismatched family donors—is linked with a substantial morbidity and even a mortality in the range of 10%. However, an imbalance of the hemoglobin is caused by thalassemia mutations, which can totally eliminate (ß0) or significantly diminish (ß+) the expression of the hemoglobin ß-chain. This imbalance results in extramedullary hematopoiesis and hyperplastic but inefficient erythropoiesis. The majority of patients with biallelic inactivation of the ß-globin gene have the clinical phenotype of thalassemia major, which is characterized by the requirement for regular lifelong red blood cell transfusions starting in early childhood (TDT). ß-Thalassemias are genetically diverse, with different mutations being common among different ethnic groups. (Antonis *et al*, 2022). Thalassemia that requires transfusions 10% to 15% of patients who have co-inherited -thalassemia mutations, genetic modifiers enhancing the production of -globin and fetal hemoglobin (HbF) beyond infancy, or mutations allowing for meaningful residual expression of ß-globin exhibit the phenotype of thalassemia intermedia. These individuals may infrequently need red blood cell transfusions, but because of their severe erythroid hyperplasia, they often experience skeletal abnormalities and iron overload later in life. Lately, the term "non-transfusion dependent thalassemia" has also been used to describe this clinical feature, possibly in an improper manner.

Red blood cell transfusions are the cornerstone of thalassemia therapy, along with rigorous iron chelation. While the majority of patients will mature into adults with proper care, the consequences of iron excess significantly reduce both quality of life and life expectancy(Nasa *et al.*, 2013). Allogeneic stem cell transplantation is recommended as soon as feasible, ideally before to the onset of iron overload, if an HLA-matched stem cell donor is available(Suarez *et al.*, 2018). Thalassemia has a significant financial cost, particularly in nations where the frequency is high. An adult patient's annual costs for iron chelation and red blood cell transfusions are predicted to surpass €30,000 (Mcquilten *et al.*, 2019).

The fundamentals of gene therapy for hemoglobinopathies

SCD and TDT are monogenic illnesses that are particularly receptive to gene therapy since the genetic abnormality must be addressed in hematopoietic stem cells that give birth to erythroid precursors exclusively. With a history of allogeneic stem cell transplantation spanning more than 50 years,(Bolan *et al.*, 2009) technologies for collecting and manipulating hematopoietic stem cells are widely available. Gene therapy (Payen *et al.*, 2017) has the potential to treat individuals with TDT and SCD even if no suitable stem cell donor is available and is not connected with the danger of Graft-versus-Host-Disease (GvHD). In compared to allogeneic stem cell transplantation, customized conditioning without the requirement for immunosuppression offers a lower risk of short- and long-term harm.

Gene treatments for hemoglobinopathies are all based on ex vivo modification of hematopoietic stem cells and mimic autologous stem cell transplantation (Fig. 1)(Payen *et al.*, 2017). A hemoglobinopathy can be cured if many requirements are satisfied. To begin, a significant quantity of hematopoietic stem cells must be collected and managed without compromising their ability to engraft, self-renew, proliferate, and differentiate. Second, the gene deficiency in the vast majority of stem cells must be rectified. Finally, the modified stem cells must be placed in an environment that supports survival and long-term growth, which necessitates myeloablative conditioning.

Thalassemias are distinguished by the reduced maturation capacity of erythroid precursors, which provides a selection advantage to those precursors whose genetic abnormality has been rectified by gene therapy. This effect was initially seen following allogeneic stem cell transplantation for thalassemia, when a percentage of 20% of all nucleated bone marrow cells sufficed to create only donor-derived erythrocytes (Andreani *et al.*, 2011). Nevertheless, this selective advantage is significantly less evident than that of corrected T-cells in severe combined immunodeficiency—one of the reasons why immunodeficiencies were historically the first targets of gene therapy

Newly developed self-inactivating (SIN) lentiviral vectors

Human immunodeficiency virus (HIV) auxiliary virulence factors and regulatory genes are removed from the viral genome to create the viral vectors employed in modern gene therapy techniques. The vesicular stomatitis virus, or VSV, normally replaces the envelope protein, and the gag, pol, and rev genes necessary for virus packing are also produced from a different plasmid in the packaging cell line. Once the viral DNA has been incorporated into the host genome, deletions in the viral LTR prevent virus replication.

A constitutively active promoter that is un-reliant on viral components like that takes over the LTR's promoter function. The endogenous promoter and regulatory sequences control how the therapeutic gene is expressed (Milone and Doherty, 2018).

These self-inactivating (SIN) lentiviral vectors randomly integrate without preference for regulatory areas in the host genome. Moreover, the absence of the LTRs reduces their ability to trigger nearby genes for integration. The likelihood of onogene activation is statistically low, but cannot be fully ruled out, with a vector copy number that normally approaches two to three per host genome. No cancer associated with lentiviral insertional mutagenesis has yet been identified in an increasing number (>300) of individuals treated for various genetic diseases in studies utilizing lentiviral. Nevertheless, the number of patients receiving lentiviral gene therapy for hemoglobinopathies is yet insufficient (about 10043) to be definite about the carcinogenic risk in this particular indication (Gaspar *et al.*, 2004).

Problems With ß-Hemoglobinopathies Gene Therapy

Despite the fact that self-inactivating lentiviral vectors provide a quick and potentially secure method of working with hematopoietic stem cells, clinically effective gene therapy still needs to optimize every step. In the past, bone marrow was the primary source of stem cells for gene therapy of hemoglobinopathies. Unfortunately, the number of hematopoietic stem cells collected from bone marrow is minimal and sometimes requires two or more harvesting procedures(Gaspar *et al.*, 2004). Moreover, in SCD patients, persistent inflammation may limit the ability of bone marrow stem cells to proliferate and undergo transduction. These factors have led to the replacement of bone marrow as the source of cells for gene therapy with hematopoietic stem cells isolated from peripheral blood (Bonifacino *et al.*, 2019).

The procedures for mobilizing hematopoietic stem cells for individuals with thalassemia are similar to those used for other purposes. Nevertheless, the manufacturing process needs significantly more cells than an autologous rescue after high dose chemotherapy does, therefore mobilization by chemotherapy is not recommended for benign disorders. For this reason, the most popular G-CSF and (off-label) plerixa for combination is employed. Compared to stem cells mobilized using other methods, those using this combination are easily transduced by lentiviral vectors and generate more ß-globin per gene copy(Lidonnici *et al.*, 2016). When G-CSF and plerixafor are combined to mobilize a gene, compared to other mobilization regimens, the therapeutic gene expresses at a higher level. This preference for integration into transcriptionally active chromatin regions could explain the difference in expression levels between the two mobilization methods.

G-CSF is not recommended for SCD patients, though, as it can produce deadly vaso-occlusive crises(Fitzhugh *et al.*, 2009). As a result, the sole mobilizing agent employed (off-label) is plerixafor (Negre *et al.*, 2015), which produces stem cells that are ideally suited for transduction and expression of the therapeutic ß-globin.

The specific sedimentation characteristics of blood cells in individuals with hemoglobinopathies must be taken into account throughout the apheresis technique. The stem cell fraction often has to be harvested with a significantly larger admixture of erythrocytes than other indications(Esrick *et al.*, 2018).

Transduced stem cells must be able to develop into an adequate number of erythroid precursors that generate as much ß-globin as a healthy ß-globin locus would in order to reverse the transfusion need in TDT via gene addition. After gene therapy, there is a correlation between the number of vector copies per genome in the peripheral blood and the number of vector copies in the altered stem cells (Marktel *et al.*, no date). Although if the fraction of transduced stem cells in the cell product and the consequent vector copy number in the peripheral circulation both enhance the amount of hemoglobin generated from the therapeutic gene,

The effectiveness of gene therapy is not entirely predicted by the properties of the cell product. The patient appears to be independent from red blood cell infusions with just one vector copy per periphal blood genome. As each cell would contain one vector copy, the optimal cellular product would have the lowest potential chance of insertional mutagenesis. The amount of therapeutic ß-globin needed to correct TDT is decreased if the patient has at least one ß+-globin allele that permits a residual expression of ß-globin, even if the outcome of gene therapy depends on several circumstances and cannot be easily anticipated. In this context, it is important to keep in mind that the amount of residual ß-globin chain production ranges from less than 5% to more than 20% depending on the kind of ß+-thalassemia mutation(Lidonnici *et al.*, 2016) In order to nearly completely transduce all of the graft's cells and achieve the ideal vector copy number, Optimizing the transduction process is necessary. The viral coat proteins,(Sarracino *et al.*, 2014) the use of growth factors and polycations such protamine,(Negre *et al.*, 2015) and the ratio of vector to target cells are all variables that can increase transduction efficiency. As a result, the amount of lentiviral vector needed can be significantly decreased by enriching hematopoietic stem cells using cell surface markers(Angeles, 2014). Strict quality checks must be performed on the graft to guarantee viability, sterility, and effective gene transfer into the host genome before it can be used again.

The myeloablative conditioning determines the immediate toxicity of gene therapy, which is similar to high dose chemotherapy with mucositis, bacterial infections, hemorrhage, and sinusoidal obstruction syndrome(Suarez *et al.*, 2018). Moreover, chemo conditioning has long-term effects, chief among them infertility and cancers brought on by the therapy(Santarone *et al.*, 2017) Other myeloablation techniques are now undergoing preclinical testing in order to avoid the toxicities of chemotherapy. They promise total

myeloablation with little to no systemic harm by using toxic immunological conjugates to deplete CD45+ or CD117+ precursors(Santarone *et al.*, 2017).

Most SCD patients have chronic vasculopathy, which increases the possibility of vasoocclusive crises during gene therapy. In order to lower the risk of acute problems, preventative procedures including exchange transfusion and anticonvulsive prophylaxis are required, much like with allogeneic stem cell transplantation(Kunz, Kulozik and Kulozik, no date)

In terms of allogeneic stem cell transplantation, gene-corrected stem cells are often administered intravenously.

(Suarez *et al.*, 2018)A direct intraosseous injection may lead to a more effective homing of stem cells, according to a mouse model. Although intraosseous injection of genetically altered stem cells was used to treat certain patients, neither of these application methods has been shown to be superior to the other in a controlled experiment.

When hematopoiesis is completely restored, many weeks of bone marrow aplasia following the injection of genetically altered stem cells pose the danger of infections and bleeding. Within three to six months, the therapeutic gene should fully express itself, and this can be associated with a decrease in the need for blood transfusions or, ideally, a total cessation of this need. 43,44 A selection for clones that most effectively express the transgene might be one explanation for the delay between the administration of the graft and complete production of therapeutic hemoglobin. Integration site analysis, which shows dynamic changes in the clonal composition even after years, can be used to track this clonal development of hematopoiesis produced from altered stem cells(Suarez *et al.*, 2018).

Hemoglobin separation enables the measurement of the therapeutic gene's expression in respect to endogenous ß-globin using vectors that code for ß-globin with an amino acid substitution(Suarez *et al.*, 2018). Furthermore, amino acid changes can be used to alter the therapeutic ß-physicochemical globin's characteristics in order to prevent HbS from polymerizing. The development of such therapeutic ß-globins addresses the pathophysiology of SCD at its core, just like enhanced HbF expression does.

Clinical outcomes of "gene insertion" for sickle cell disease and transfusion-dependent thalassemia

One of the first TDT patients treated with lentiviral gene addition in 2007 was able to stop needing blood transfusions, but they also had clonal hematopoiesis that was probably brought on by insertional mutagenesis and continued for years(Payen *et al.*, 2017).

Later, multiple experiments showed the effectiveness of thalassemia gene therapy without producing clonal hematopoiesis using modified vectors to lower the danger of insertional mutagenesis. Both employed lentiviral gene addition treatment, either with the GLOBE vector or the vectors BB305(Suarez *et al.*, 2018). Age of the patient and genotype were the two most significant indicators of gene therapy effectiveness.

According to Bluebird Bio's HGB-204 and HGB-205 studies, 12 of 13 evaluable patients with a non-ß0/ß0 genotype became independent from transfusions (median duration 38 months for HGB-204)(Suarez *et al.*, 2018). The following experiment, HGB-207, employed an enhanced transduction technique and achieved transfusion independence for at least six months in 10 of 11 patients. These findings led to the conditional licensing of gene therapy by lentiviral gene addition (ZyntegloTM) by the European Medicines Agency for TDT patients aged 12 years or older with a non-ß0/ß0 genotype and no HLA-matched sibling available as a stem cell donor(Payen *et al.*, 2017).

It was more challenging to treat TDT in patients with the ß0/ß0 genotype: All patients in the HGB204 experiment had their transfusion frequency decreased, however only four out of the eight evaluable patients attained full transfusion independence(First and Paper, 2021). Insufficient transduction efficiency with low vector copy numbers in the hematopoietic stem cells and an inadequate fraction of transduced stem cells were blamed for this less-than-ideal, although promising, outcome. Three of four TDT patients with a ß0/ß0 genotype remained transfusion-free for at least six months following gene therapy with an enhanced transduction method, according to preliminary results of the subsequent study HGB-212, however the follow-up is still too short to make firm conclusions. However, it should be highlighted that even those individuals who benefit from gene therapy and stop needing transfusions could not have fully recovered. In a study of eight of these individuals, there was still inefficient erythropoiesis present, despite improvement, and a marker profile showing disrupted iron homeostasis that probably led to incorrect iron resorption. These individuals could thus need to be watched for extramedullary erythropoiesis and might still need (expensive) iron chelation. Using the GLOBE vector, four of six children with TDT but not any of the three adults did not require transfusions. Just one of these patients, who had meaningful residual endogenous ß-globin production and a non-ß0/ß0 genotype, was effectively treated. The difference between children and adults was attributed by the authors to a deficiency in the stem cell niche brought on by repeated transfusions.

For SCD gene therapy to be effective, vaso-occlusive consequences such acute chest syndrome and pain crises must be reduced in frequency and severity. Ultimately, stop or even undo damage to end organs. If at least 30% of HbF is present in all erythrocytes, patients with compound heterozygosity for the HbS mutation plus a second allele that causes -globin to survive until maturity are symptom-free. 16 Similar to this, it is envisaged that pancellular production of a therapeutic ß-globin chain that interferes with HbS polymerization to the same degree may lessen SCD symptoms and stop its development.

This objective was only partially accomplished in the first patient who received lentiviral vector BB305-mediated gene addition therapy(First and Paper, 2021). Nevertheless, in additional individuals, the vector copy number and subsequently the expression of anti-sickling ß-globin were insufficient to significantly reduce SCD sequelae. In terms of the TDT trials, multiple degrees of optimization were required for the gene therapy strategy for SCD. The most recently treated group of patients expressed about 6 g/dl of therapeutic hemoglobin, dispersed in a pan cellular manner, using plerixafor for mobilization and enhanced technology for transduction. Laboratory parameters for hemolysis were expectedly normalized with this expression level of the anti-sickling ß-globin, and the incidence of Vaso occlusive consequences was decreased.

Beyond gene addition, gene therapy

In addition to "gene addition," a number of additional tactics either target the pathogenic mutation's direct repair or its neutralization via the insertion of genomic alterations in Trans. Although preclinical research has evolved significantly, there is very little clinical experience with these procedures. With the use of homology-directed repair, harmful mutations may be directly corrected by inserting a therapeutic DNA fragment (HDR). Nevertheless, the ineffectiveness of HDR un hematopoietic stem cells has so far prevented its application in hemoglobinopathies gene therapy(Suarez *et al.*, 2018).A different method of targeted base editing is to produce cytidine deaminases that are directed to a particular target sequence by short RNAs and cause C>T or G>A alterations. This method hasn't been used in a therapeutic setting yet due to limitations in the targeting of the "base editors" to a target sequence and because of off-target effects. Unlike TDT, where more than 300 distinct mutations in the ß-globin gene causing a thalassemia phenotype would need to be addressed, direct repair of the underlying ß-globin mutations in SCD is laborious and appealing until the technological challenges are resolved.

For this reason, none of the genome editing techniques now under development target the ß-globin gene and instead attempt to reactivate the -globin gene and promote the production of HbF. HbF is a completely functioning globin chain even in adult life and can replace the absent HbA in ß-thalassemia, despite having a lower oxygen affinity than HbA. The polymerization of deoxygenated HbS in SCD can be prevented or limited by HbF, providing a therapeutic target for both ß-hemoglobinopathies. When co-expressed with the HbS mutation, patients with hereditary persistence of fetal hemoglobin (HPFH) show that HbF may replace HbA without significant functional limitations15 and can prevent HbS from polymerizing. Both TDT and SCD may be cured using this indirect approach to altering globin synthesis, however healing ß-thalassemia will likely need a more significant reactivation of -globin chain synthesis than would probably be necessary for curing SCD(Payen *et al.*, 2017). Inactivating certain DNA sequences is the goal of the genome editing methods utilized in hematopoietic stem cells rather than correcting or inducing a particular mutation. Without the assistance of the CRISPR/Cas9 system's endogenous homology-directed repair activity, such an inactivation can be accomplished in a sequence-specific way. The CRISPR/Cas9 system uses prokaryotic enzymes that can identify and break down DNA sequences that bacteriophages have inserted into bacteria(Payen *et al.*, 2017). The endonuclease Cas9 is attracted by certain guide RNAs to particular regions of the genome, where it produces double strand breaks that nonhomologous end joining will repair. Little insertions or deletions caused by errors in the repair procedure inactivate the target gene. Targeted double strand breaks can also be introduced into DNA using nucleases that attach to the target sequence directly, without the use of a particular guide RNA. Similar to double strand breaks caused by Cas9.129, such Transcription Activator-Like Effector Nucleases (TALEN) or Zinc finger enzymes inactivate the corresponding gene. Genome editing is used in several methods to increase the production of HbF, the most direct of which is the insertion of activating mutations in the -globin promoter that prevent the binding of postnatal repressors (Fig. 4B)(First and Paper, 2021). BCL11A is the gene that gets edited the most commonly for ß-hemoglobinopathies. During the perinatal hemoglobin transition, the transcription factor encoded by BCL11A is necessary to suppress the production of -globin. BCL11A inactivation in erythroid precursors causes more HbF to be produced(Karponi *et al.*, 2016). Similar to LRF, disrupting its connection with the promoter of -globin causes HbF expression. LRF is another transcription factor that promotes the hemoglobin flip. By using a TALEN nuclease or a CRISPR/Cas9 nuclease to attack a lineage-specific enhancer of BCL11A, CL11A may be precisely inactivated in erythroid cells (Fig. 4C). This lineage specificity is significant since BCL11A is essential for the development of lymphoid139 and neuronal cells in addition to erythroid cells. There is a significant upregulation of HbF in erythroid progenitors produced from altered hematopoietic stem cells if BCL11A is disabled. The very first TDT and SCD patients received treatment with BCL11A inactivation using the CRISP/Cas9 system Preliminary results are favorable, despite the short follow-up and small sample sizes. Comparable to erythroid cells, HbF was induced in initial TDT patients who received these stem cells when BCL11A in hematopoetic stem cells was disrupted by Zink finger nucleases.Using lentiviral vectors to produce short hairpin (sh)RNAs in hematopoietic stem cells, there are further ways to induce HbF by suppressing BCL11A. (Fig. 4D). These shRNAs regulate their target gene's expression by specifically hybridizing to and degrading the target mRNA. As a potential therapy for SCD, downregulating BCL11A in hematopoietic stem cells via shRNA is currently being investigated(Bolan *et al.*, 2009). Initial findings of this "transcriptome editing" strategy in three SCD patients who were monitored for at least six months following gene therapy

revealed a lineage-specific activation of HbF and a reduction in hemolysis. Another option to "genome editing" is the production of a genetically modified Ldb1-Zink finger protein fusion protein from a lentiviral vector (Fig. 4E), which moves the ß-globin locus regulatory area close to the -globin gene. As a result, in vitro HbF expression is reactivated and surpasses HbS, which is consistent with the cure of SCD if realized in vivo(Bolan *et al.*, 2009).

The expression of a therapeutic gene product could be enhanced by combining many of the aforementioned methods. Such a combination, which may provide a success rate that much surpasses that of allogeneic stem cell transplantation, would, for example, involve the production of ß-globin by lentiviral gene addition and simultaneous repression of BCL11A via shRNAs expressed by the same vector.

Prevention and control Situation in the African region

Africa accounts for 60-70% of all births of infants with a serious hemoglobin issue, while having the fewest resources to deal with the problem. Though the numbers are increasing, advances in basic health care have enhanced the life of individuals with sickle-cell disease. (M. Angastiniotis *et al*.1995). An extensive survey of 16 000 randomly selected adults over the age of 15 from 30 states in Nigeria, revealed that 25.3% possessed the AS, AC, SS, or SC Hb genotypes. Under African conditions, providing counseling is the least expensive and most accessible helpful activity. Counseling for sickle cell disease comprises both psychological support for families and genetic counseling. (Brown *et al*. 2016). WHO has argued in favor of all-encompassing strategies (improving curative treatments, establishing prenatal diagnostics, developing carrier detection and counseling, improving education), as well as the requirement to create reference centers. To make sure that the strategy is relevant to various socio-cultural and political contexts, the value of volunteer groups and community organizations has also been underlined. (M. Angastiniotis *et al*.1995

Laboratory iagnosis of Heamoglobinopathies

Thalassemias and other haemoglobin disorders are among the most prevalent hereditary diseases, and the clinical laboratory is crucial for the accurate diagnosis of individuals who exhibit these abnormalities. Protein-based diagnostic methods, such as electrophoresis and chromatography, can be used to identify the majority of diseases. Genetic counseling is crucial to prevent negative outcomes since severe syndromes can arise from the inheritance of many globin genetic abnormalities. Protein-based methods cannot always detect potentially serious thalassemia disorders; in particular, α-thalassemia may be masked in the presence of β-thalassemia. Deletional forms of β-thalassemia are also sometimes difficult to diagnose definitively with standard methods. (Daniel, 2017). Haemoglobin electrophoresis, isoelectric focusing, and high-performance liquid chromatography are the three laboratory procedures most frequently used to diagnose sickle cell disease. (Kevin *et al*, 2021).Commonly available screening tests in Africa include sodium metabisulphite sickling test and sickle solubility tests. (Buser, 2017). Isoelectric focusing (IEF), High performance liquid chromatography (HPLC), and Haemoglobin electrophoresis (HBE) are the three often used assays. However, for clinical purposes, diagnosis typically entails screening (sickling or solubility test), followed by confirmation of the sickle phenotype using gel electrophoresis, IEF, or HPLC. (Makani *et al;*2013)

Screening Tests.

In the majority of hospitals in Africa, screening is conducted using the "sickling test," which entails making a thin blood film and placing it under hypoxic circumstances by adding sodium metabisulphite. This will result in RBCs containing HbS becoming deformed (i.e., forming sickle cells) as detected by light microscopy. A “positive” sickling test identifies the presence of sickled RBCs, which occurs in both homo- (SS) and heterozygous (AS) states. The sickle solubility test is another method used for screening which is based on the principle that HbS becomes insoluble when it is deoxygenated. SS-SCD or SCD involving other Hb types must be confirmed with further confirmatory tests when these screening assays are utilized. (Makani *et al*; 2013)

Confirmatory Tests.

These tests are based on the principle that various haemoglobin isoforms migrate in an electric field with varying velocities because they have varied total ionic charges. Heamoglobin electrophoresis can be done under alkaline or acidic conditions. HbA, HA2, HbF, and HbS migrate towards the anode under an electric field with different rate of mobility. During alkaline Hb electrophoresis the resolution between HbS and HbF can be poor, particularly in individuals with high HbF levels, for example, neonates. Under acidic conditions, HbF migrates relatively more rapidly and is therefore distinguishable from both HbA and HbS. Isoelectric focusing uses the same principles but is slightly more expensive than Hb electrophoresis. However, it is able to identify more Hb variants that would not be detected by electrophoresis. (WHO, 2012). HPLC uses cation exchange chromatography to identify the various hemoglobins in an individual. It has the advantage in that it can also accurately quantify the Hb levels. In resource rich countries, screening has largely been replaced by HPLC and confirmation is then done by IEF or HBE. This is mainly because HBE and IEF are labour intensive, time consuming and would not identify abnormal bands or quantify Hb. Furthermore, the quantification of Hb fractions by HPLC is used to monitor patients who are on Hydroxyurea therapy or exchange blood transfusion. (Makani *et al;* 2013)

Molecular Diagnosis.

The most popular molecular diagnosis of βS mutation, based on restriction enzyme digestion, is performed on HBB PCR products. The point mutation, which results in SCD, abolishes

the restriction site for the restriction enzyme *DdeI*. Digestion of DNA of individuals homozygous for HbAA would result in two fragments 188 bp and 192 bp. Analysis of heterozygous HbAS samples would result in three fragments one of 380 bp and the two digested fragments of 180 bp and 192 bp. Homozygous HbSS samples would result in 380 bp fragments being produced. This method is simple and cost effective and could be used for prenatal genetic diagnosis in African settings. (Makani *et al*; 2013)

Management.

The current management strategies for SCD could be considered under the public health principles of early diagnosis, primary prevention and prompt management of acute episodes and complications. The successes of early detection and prevention programmes in some North African countries should be imitated. Early diagnosis and primary prevention will constitute early detection of the disease coupled with counselling and use of Penicillin V and Folic acid. In areas where malaria is endemic, the use of treated mosquito bed-nets, indoor residual spraying and prompt treatment is highly recommended. (Daniel *et al.* 2013).There is convincing evidence to support the use of pneumococci vaccinations in lowering the morbidities and fatalities related to SCD patients. In the United States of American SCD patients benefit from this preventive intervention. GAVI since 2009 has begun a programme of introducing new vaccines like pneumococci vaccine into the EPI systems of some sub-Saharan counties like Gambia (2009) Mali (2011) and Ghana, Tanzania (2012). The introduction of vaccines like Pneumococcal and Haemophilus vaccines protect SCD children against these highly virulent encapsulated organisms would have a great impact on the quality of life and survival of SCD patients. (WHO, 2010). Hydroxyurea is safe, well-tolerated, and effective for children with SCA living in sub-Saharan Africa. Hydroxyurea optimization requires periodic dose escalation for weight gain and titration to mild myelosuppression. (Banu *et al*, 2020). Hydroxyurea, a cytotoxic has been found to improve the clinical course of the disease. The incidence and severity of most of the complications of the disease are remarkably reduced under hydroxyurea therapy. Lab toxicities from hydroxyurea are uncommon and typically asymptomatic, suggesting that routine CBC monitoring is needed only at 3-month intervals once a stable dose is achieved, more to optimize the dose than to identify incidental toxicities. This approach to optimizing hydroxyurea therapy will allow more widespread utilization in low-resource settings with limited laboratory monitoring. (Banu *et al*, 2020).Chronic blood transfusion is now indicated for SCD patients with higher than usual risk of stroke. However, the risk of iron overload should be considered in all patients on the regimen. As technology advances, the need to fund more research into interventions such as transplantation should be encouraged. (Daniel *et al.* 2013).

PERSPECTIVES

Red blood cell transfusions, iron chelation, and pharmacologic induction of HbF are all part of the current standard of care for TDT and SCD. These treatments must also be used to alleviate symptoms and side effects. More recent TDT therapies have been developed with the goal of enhancing erythroid precursor survival. 148 Allogeneic stem cell transplantation is the only proven curative treatment, albeit it can be linked with significant morbidity, such as graft versus host disease, infertility, and other long-term consequences, depending on the patient's age and the kind of donor. 104,105 While it is generally accepted that HLA-matched siblings should be used as the standard of care, for the majority of patients this is not an option, and transplantation from alternative donors still carries a risk of treatment-related mortality of 10% or more, which is deemed intolerable for non-malignant disorders. 34,37,109,149. Bypassing two of the most important drawbacks of allogeneic transplantation—limited donor availability and the danger of GvHD—gene therapy may be able to treat 80% of TDT patients with a non-ß0/ß0 genotype. After gene therapy for hemoglobinopathies, no known treatment-related deaths have been noted to yet. These findings have prompted the approval of the first gene treatment for transfusion-dependent thalassemia in Europe and provide the opportunity to evaluate the efficacy of gene therapy in a broader group of patients and under more realistic environmental factors. Gene therapy is presently not widely used due to two significant barriers. First, myeloablative conditioning is still needed in current regimens. Even though the cumulative dose of chemotherapy is lower than with allogeneic stem cell transplantation, long-term complications including infertility and the possibility of subsequent neoplasms will probably still be a problem. Second, gene therapy's complex logistics and high price tag150 restrict its application to individuals and healthcare systems with enough funding, while outright banning its application in nations with a high frequency of hemoglobinopathies that lack the infrastructure and resources needed. Naturally, the most crucial steps needed to expand the availability of this therapeutic option to more than a select few patients are the creation of conditioning regimens with lower toxicity and a reduction in the price of gene therapy.

CONCLUSION

The burden and prevalence of sickle cell disease in sub-Saharan Africa and around the world can be significantly reduced by sickle cell disease prevention strategies, such as primary prevention through neonatal screening and genetic counseling targeted at those who have the sickle cell trait.

Progress made in high-income and middle-income countries since 2000 has demonstrated that a suite of interventions anchored by newborn screening can raise the probability of survival for children with sickle cell disease to the same level as the general population.

The WHO Africa has recommended a set of public health interventions to reduce the burden of SCD in African region, namely, improving awareness, preventing the disease, early detection, improving the provision of health care for affected individuals by providing effective clinical, laboratory, diagnostic, and imaging facilities adapted to different levels of the health system, screening of newborns, training of health care workers, developing protocols for treatment, providing genetic counseling, patient support groups, advocacy, and research.

In this review paper, we have shown that progress in prevention, diagnosis and management of haemoglobinopathies in some sub-Saharan African countries has been encouraging

REFERENCES

I. Agarwal, A. (2021). CLINICAL PROFILE AND MID-TERM OUTCOME OF CHILDREN WITH SICKLE CELL DISEASE TREATED WITH HYDROXYUREA. PARIPEX INDIAN JOURNAL OF RESEARCH, pp.16–18. doi:https://doi.org/10.36106/paripex/8900707.

II. Andreani, M., Testi, M., Gaziev, J., Condello, R., Bontadini, A., Tazzari, P.L., Ricci, F., De Felice, L., Agostini, F., Fraboni, D., Ferrari, G., Battarra, M., Troiano, M., Sodani, P. and Lucarelli, G. (2010). Quantitatively different red cell/nucleated cell chimerism in patients with long-term, persistent hematopoietic mixed chimerism after bone marrow transplantation for thalassemia major or sickle cell disease. Haematologica, 96(1), pp.128–133. doi:https://doi.org/10.3324/haematol.2010.031013.

III. Ansong, D., Osei-Akoto, A., Ocloo, D. and Ohene-Frempong, K.O.-F. (2013). Sickle Cell Disease: Management options and challenges in developing countries. Mediterranean Journal of Hematology and Infectious Diseases, 5(1), p.e2013062. doi:https://doi.org/10.4084/mjhid.2013.062.

IV. Aygun, B., Tomlinson, G.A., McGann, P.T., Tshilolo, L., Williams, T.N., Olupot-Olupot, P., Santos, B., Stuber, S.E., Lane, A., Latham, T. and Ware, R.E. (2020). Optimizing Hydroxyurea Therapy with Reduced Laboratory Monitoring for Children with Sickle Cell Anemia in Sub-Saharan Africa: The Reach Experience. Blood, 136(Supplement 1), pp.17–17. doi:https://doi.org/10.1182/blood-2020-134180.

V. Baldwin, K., Urbinati, F., Romero, Z., Campo-Fernandez, B., Kaufman, M.L., Cooper, A.R., Masiuk, K., Hollis, R.P. and Kohn, D.B. (2015). Enrichment of Human Hematopoietic Stem/Progenitor Cells Facilitates Transduction for Stem Cell Gene Therapy. STEM CELLS, 33(5), pp.1532–1542. doi:https://doi.org/10.1002/stem.1957.

VI. Baumann, A.A., Belle, S.H., James, A. and King, A.A. (2018). Specifying sickle cell disease interventions: a study protocol of the Sickle Cell Disease Implementation Consortium (SCDIC). BMC Health Services Research, 18(1). doi:https://doi.org/10.1186/s12913-018-3297-1.

VII. Brown, T.M., Fee, E. and Stepanova, V. (2016). Halfdan Mahler: Architect and Defender of the World Health Organization 'Health for All by 2000' Declaration of 1978. American Journal of Public Health, 106(1), pp.38–39. doi:https://doi.org/10.2105/ajph.2015.302935.

VIII. Buser, J.M. (2017). The Need for Hematology Nurse Education in Low- and Middle-Income Countries: A Community Case Study in Tanzania. Frontiers in Public Health, 5. doi:https://doi.org/10.3389/fpubh.2017.00065.

IX. DeBaun, M.R., Ghafuri, D.L., Rodeghier, M., Maitra, P., Chaturvedi, S., Kassim, A. and Ataga, K.I. (2019). Decreased median survival of adults with sickle cell disease after adjusting for left truncation bias: a pooled analysis. Blood, [online] 133(6), pp.615–617. doi:https://doi.org/10.1182/blood-2018-10-880575.

X. Descotes, J. (2002). Preclinical safety evaluation of immunotherapeutics. Toxicology, 174(1), p.1. doi:https://doi.org/10.1016/s0300-483x(02)00050-1.

XI. Esoh, K., Wonkam-Tingang, E. and Wonkam, A. (2021). Sickle cell disease in sub-Saharan Africa: transferable strategies for prevention and care. The Lancet Haematology, 8(10), pp.e744–e755. doi:https://doi.org/10.1016/s2352-3026(21)00191-5.

XII. Esrick, E.B., Manis, J.P., Daley, H., Baricordi, C., Trébéden-Negre, H., Pierciey, F.J., Armant, M., Nikiforow, S., Heeney, M.M., London, W.B., Biasco, L., Asmal, M., Williams, D.A. and Biffi, A. (2018). Successful hematopoietic stem cell mobilization and apheresis collection using plerixafor alone in sickle cell patients. Blood Advances, 2(19), pp.2505–2512. doi:https://doi.org/10.1182/bloodadvances.2018016725.

XIII. Fitzhugh, C.D., Hsieh, M.M., Bolan, C.D., Saenz, C. and Tisdale, J.F. (2009). Granulocyte colony-stimulating factor (G-CSF) administration in individuals with sickle cell disease: time for a moratorium? Cytotherapy, [online] 11(4), pp.464–471. doi:https://doi.org/10.1080/14653240902849788.

XIV. Forget, B.G. (1998). Molecular basis of hereditary persistence of fetal hemoglobin. Annals of the New York Academy of Sciences, [online] 850, pp.38–44.

doi:https://doi.org/10.1111/j.1749-6632.1998.tb10460.x.

XV. Gene Therapy for X Linked Severe Combined Immunodeficiency. (2020). Case Medical Research. doi:https://doi.org/10.31525/ct1-nct04286815.

XVI. Graham, L.M. (2004). Sickle cell disease: Pulmonary management options. Pediatric Pulmonology, 37(S26), pp.191–193. doi:https://doi.org/10.1002/ppul.70102.

XVII. Hsieh, M.M., Kang, E.M., Fitzhugh, C.D., Link, M.B., Bolan, C.D., Kurlander, R., Childs, R.W., Rodgers, G.P., Powell, J.D. and Tisdale, J.F. (2009). Allogeneic Hematopoietic Stem-Cell Transplantation for Sickle Cell Disease. New England Journal of Medicine, 361(24), pp.2309–2317. doi:https://doi.org/10.1056/nejmoa0904971.

XVIII. Karponi, G., Psatha, N., Lederer, C.W., Adair, J.E., Zervou, F., Zogas, N., Kleanthous, M., Tsatalas, C., Anagnostopoulos, A., Sadelain, M., Rivière, I., Stamatoyannopoulos, G. and Yannaki, E. (2015). Plerixafor+G-CSF–mobilized CD34+ cells represent an optimal graft source for thalassemia gene therapy. Blood, 126(5), pp.616–619. doi:https://doi.org/10.1182/blood-2015-03-629618.

XIX. Kattamis, A., Kwiatkowski, J.L. and Aydinok, Y. (2022). Thalassaemia. The Lancet, 399(10343), pp.2310–2324. doi:https://doi.org/10.1016/s0140-6736(22)00536-0.

XX. Kunz, J.B. and Kulozik, A.E. (2020). Gene Therapy of the Hemoglobinopathies. HemaSphere, 4(5),p.e479. doi:https://doi.org/10.1097/hs9.000000000000047

XXI. La Nasa, G., Caocci, G., Efficace, F., Dessì, C., Vacca, A., Piras, E., Sanna, M., Marcias, M., Littera, R., Carcassi, C. and Lucarelli, G. (2013). Long-term health-related quality of life evaluated more than 20 years after hematopoietic stem cell transplantation for thalassemia. Blood, 122(13), pp.2262–2270. doi:https://doi.org/10.1182/blood-2013-05-502658.

XXII. Leonard, A., Bonifacino, A., Dominical, V.M., Luo, M., Haro-Mora, J.J., Demirci, S., Uchida, N., Pierciey, F.J. and Tisdale, J.F. (2019). Bone marrow characterization in sickle cell disease: inflammation and stress erythropoiesis lead to suboptimal CD34 recovery. British Journal of Haematology. doi:https://doi.org/10.1111/bjh.15902.

XXIII. Lidonnici, M.R., Aprile, A., Frittoli, M.C., Mandelli, G., Paleari, Y., Spinelli, A., Gentner, B., Zambelli, M., Parisi, C., Bellio, L., Cassinerio, E., Zanaboni, L., Cappellini, M.D., Ciceri, F., Marktel, S. and Ferrari, G. (2016). Plerixafor and G-CSF combination mobilizes hematopoietic stem and progenitors cells with a distinct transcriptional profile and a reduced in vivo homing capacity compared to plerixafor alone. Haematologica, 102(4), pp.e120–e124. doi:https://doi.org/10.3324/haematol.2016.154740.

XXIV. Magrin, E., Miccio, A. and Cavazzana, M. (2019). Lentiviral and genome-editing strategies for the treatment of β-hemoglobinopathies. Blood, 134(15), pp.1203–1213. doi:https://doi.org/10.1182/blood.2019000949.

XXV. Makani, J., Lyimo, M., Magesa, P. and Roberts, D.J. (2017a). Strengthening medical education in haematology and blood transfusion: postgraduate programmes in Tanzania. British Journal of Haematology, 177(6), pp.838–845. doi:https://doi.org/10.1111/bjh.14644.

XXVI. Makani, J., Lyimo, M., Magesa, P. and Roberts, D.J. (2017b). Strengthening medical education in haematology and blood transfusion: postgraduate programmes in Tanzania. British Journal of Haematology, 177(6), pp.838–845. doi:https://doi.org/10.1111/bjh.14644.

XXVII. Makani, J., Ofori-Acquah, S.F., Nnodu, O., Wonkam, A. and Ohene-Frempong, K. (2013). Sickle Cell Disease: New Opportunities and Challenges in Africa. The Scientific World Journal, [online] 2013, pp.1–16. doi:https://doi.org/10.1155/2013/193252.

XXVIII. Makani, J. and Roberts, D.J. (2016). Hematology in Africa. Hematology/Oncology Clinics of North America, 30(2), pp.457–475. doi:https://doi.org/10.1016/j.hoc.2015.12.002.

XXIX. Marktel, S., Scaramuzza, S., Cicalese, M.P., Giglio, F., Galimberti, S., Lidonnici, M.R., Calbi, V., Assanelli, A., Bernardo, M.E., Rossi, C., Calabria, A., Milani, R., Gattillo, S., Benedicenti, F., Spinozzi, G., Aprile, A., Bergami, A., Casiraghi, M., Consiglieri, G. and Masera, N. (2019). Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent ß-thalassemia. Nature Medicine, [online] 25(2), pp.234–241. doi:https://doi.org/10.1038/s41591-018-0301-6.

XXX. McQuilten, Z.K., Higgins, A.M., Burns, K., Chunilal, S., Dunstan, T., Haysom, H.E., Kaplan, Z., Rushford, K., Saxby, K., Tahiri, R., Waters, N. and Wood, E.M. (2019). The cost of blood: a study of the total cost of red blood cell transfusion in patients with β-thalassemia using time-driven activity-based costing. Transfusion, 59(11), pp.3386–3395. doi:https://doi.org/10.1111/trf.15558.

XXXI. Milone, M.C. and O'Doherty, U. (2018). Clinical use of lentiviral vectors. Leukemia, [online] 32(7), pp.1529–1541. doi:https://doi.org/10.1038/s41375-018-0106-0.

XXXII. Moher, D. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The

PRISMA Statement. Annals of Internal Medicine, [online] 151(4), p.264. doi:https://doi.org/10.7326/0003-4819-151-4-200908180-00135.

XXXIII. Mullan, F. and Frehywot, S. (2007). Non-physician clinicians in 47 sub-Saharan African countries. The Lancet, 370(9605), pp.2158–2163. doi:https://doi.org/10.1016/s0140-6736(07)60785-5.

XXXIV. Munkongdee, T., Chen, P., Winichagoon, P., Fucharoen, S. and Paiboonsukwong, K. (2020). Update in Laboratory Diagnosis of Thalassemia. Frontiers in Molecular Biosciences, [online] 7, p.74. doi:https://doi.org/10.3389/fmolb.2020.00074.

XXXV. Munn, Z., Moola, S., Lisy, K., Riitano, D. and Tufanaru, C. (2015). Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. International Journal of Evidence-Based Healthcare, 13(3), pp.147–153. doi:https://doi.org/10.1097/xeb.0000000000000054

XXXVI. Odunvbun, M. and Okolo, A. (2015). Implementing Comprehensive Health Care Management for Sickle Cell Disease in an African Setting. Nigerian Journal of Paediatrics, 42(4), p.298. doi:https://doi.org/10.4314/njp.v42i4.3.

XXXVII. Ribeil, J.-A., Hacein-Bey-Abina, S., Payen, E., Magnani, A., Semeraro, M., Magrin, E., Caccavelli, L., Neven, B., Bourget, P., El Nemer, W., Bartolucci, P., Weber, L., Puy, H., Meritet, J.-F., Grevent, D., Beuzard, Y., Chrétien, S., Lefebvre, T., Ross, R.W. and Negre, O. (2017). Gene Therapy in a Patient with Sickle Cell Disease. New England Journal of Medicine, [online] 376(9), pp.848–855. doi:https://doi.org/10.1056/nejmoa1609677.

XXXVIII. Sabath, D.E. (2017). Molecular Diagnosis of Thalassemias and Hemoglobinopathies. American Journal of Clinical Pathology, 148(1), pp.6–15. doi:https://doi.org/10.1093/ajcp/aqx047.

XXXIX. Santarone, S., Pepe, A., Meloni, A., Natale, A., Pistoia, L., Olioso, P., Papalinetti, G., Cuccia, L., Spasiano, A., Lisi, R., Di Ianni, M., Bonfini, T., Accorsi, P., Salvadori, S., Papola, F., Angelini, S. and Di Bartolomeo, P. (2017). Secondary solid cancer following hematopoietic cell transplantation in patients with thalassemia major. Bone Marrow Transplantation, 53(1), pp.39–43. doi:https://doi.org/10.1038/bmt.2017.214.

XL. Steinberg, M.H., Chui, D.H.K., Dover, G.J., Sebastiani, P. and Alsultan, A. (2014). Fetal hemoglobin in sickle cell anemia: a glass half full? Blood, 123(4), pp.481–485. doi:https://doi.org/10.1182/blood-2013-09-528067.

XLI. Thompson, A.A., Walters, M.C., Kwiatkowski, J., Rasko, J.E.J., Ribeil, J.-A., Hongeng, S., Magrin, E., Schiller, G.J., Payen, E., Semeraro, M., Moshous, D., Lefrere, F., Puy, H., Bourget, P., Magnani, A., Caccavelli, L., Diana, J.-S., Suarez, F., Monpoux, F. and Brousse, V. (2018). Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia. New England Journal of Medicine, 378(16),pp.1479–1493. doi:https://doi.org/10.1056/nejmoa1705342.

XLII. Tluway, F. and Makani, J. (2017). Sickle cell disease in Africa: an overview of the integrated approach to health, research, education and advocacy in Tanzania, 2004-2016. British Journal of Haematology, [online] 177(6), pp.919–929. doi:https://doi.org/10.1111/bjh.14594.

XLIII. Vichinsky, E., Hoppe, C.C., Ataga, K.I., Ware, R.E., Nduba, V., El-Beshlawy, A., Hassab, H., Achebe, M.M., Alkindi, S., Brown, R.C., Diuguid, D.L., Telfer, P., Tsitsikas, D.A., Elghandour, A., Gordeuk, V.R., Kanter, J., Abboud, M.R., Lehrer-Graiwer, J., Tonda, M. and Intondi, A. (2019). A Phase 3 Randomized Trial of Voxelotor in Sickle Cell Disease. New England Journal of Medicine. doi:https://doi.org/10.1056/nejmoa1903212.

XLIV. WHO (2017). Sickle Cell Disease. [online] WHO | Regional Office for Africa. Available at: https://www.afro.who.int/health-topics/sickle-cell-disease.