Elite Journal of Medicine. Volume 2 issue 6(2024), Pp. 56-71
https://epjournals.com/journals/EJM

Role of Nitric Oxide in Modulating Oxidative Stress in Sickle Cell Disease

Emmanuel Ifeanyi Obeagu

Department of Medical Laboratory Science, Kampala International University, Uganda

emmanuelobeagu@yahoo.com

Abstract

Nitric oxide (NO) is a critical regulatory molecule in Sickle Cell Disease (SCD), playing a dual role in modulating oxidative stress and influencing disease pathology. While NO is well-recognized for its vasodilatory effects and ability to reduce vaso-occlusive crises, it also engages in complex interactions with reactive oxygen species, which can both alleviate and exacerbate oxidative stress in SCD. This review explores the dual nature of NO in SCD, examining its mechanisms of action on red blood cells, vascular function, and the oxidative stress pathway. By highlighting both the beneficial and detrimental effects of NO, this review provides insights into current therapeutic strategies and future directions for NO-based treatments in SCD. Nitric oxide, produced mainly by endothelial nitric oxide synthase (eNOS), functions as a vasodilator to improve blood flow and reduce the frequency of vaso-occlusive crises in SCD. NO's interaction with hemoglobin S (HbS) stabilizes the HbS tetramer, which mitigates sickling under low-oxygen conditions. However, NO also reacts with superoxide to form peroxynitrite, a potent oxidant that can contribute to oxidative damage and disease complications. Understanding these interactions is essential for leveraging NO's therapeutic potential while managing its oxidative effects.

Keywords: *Nitric Oxide, Oxidative Stress, Sickle Cell Disease, Red Blood Cells, Therapeutic Strategies*

Introduction

Sickle Cell Disease (SCD) is a genetic blood disorder resulting from a single nucleotide mutation in the β-globin gene of hemoglobin, leading to the production of hemoglobin S (HbS). This mutation causes red blood cells (RBCs) to assume a rigid, sickle-shaped morphology under low oxygen conditions, disrupting normal blood flow and causing a cascade of pathological events. The clinical manifestations of SCD are diverse and include chronic hemolysis, vaso-occlusive crises, organ damage, and increased susceptibility to infections. Central to the pathophysiology of SCD is oxidative stress, a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses of the body. Nitric oxide (NO), a small, gaseous signaling molecule, plays a complex role in modulating oxidative stress and vascular dysfunction in SCD. The fundamental defect in SCD arises from the substitution of valine for glutamic acid at position 6 of the β-globin chain, resulting in HbS. Under low oxygen tension, HbS molecules polymerize, leading to the deformation of RBCs into a sickle shape. These sickled

cells have reduced deformability, increased adhesion to the endothelium, and are more prone to hemolysis. The hemolysis of sickle RBCs releases free hemoglobin into the bloodstream, which scavenges NO and contributes to endothelial dysfunction and increased oxidative stress. This mechanism is central to the pathophysiology of SCD, where the compromised NO signaling exacerbates vascular inflammation, contributes to vaso-occlusive crises, and accelerates disease progression.[1-5]

NO is produced in the body through the enzymatic conversion of L-arginine to NO and citrulline by nitric oxide synthases (NOS). Three isoforms of NOS are present in the body: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). eNOS is predominantly responsible for NO production in the vascular endothelium, where it regulates blood vessel dilation, inhibits platelet aggregation, and maintains vascular homeostasis. In SCD, eNOS-derived NO is rapidly consumed by free heme released from lysed RBCs, leading to a reduction in NO bioavailability. This process exacerbates endothelial dysfunction and promotes oxidative stress, thereby contributing to the various complications associated with SCD. In addition to endothelial dysfunction, NO's interaction with superoxide anions in SCD results in the formation of peroxynitrite, a highly reactive compound that further increases oxidative damage. This reaction reduces the effective concentration of NO available for physiological processes and enhances oxidative stress, contributing to the disease's pathology. Understanding how NO interacts with other ROS and the mechanisms through which it exerts both beneficial and harmful effects is crucial for developing therapeutic approaches aimed at restoring NO balance and mitigating oxidative stress in SCD. NO's role in SCD is not solely detrimental; it also has protective effects. For instance, NO-induced vasodilation helps to alleviate some of the symptoms of SCD, such as pain during vaso-occlusive crises and high blood viscosity. By promoting relaxation of the smooth muscle in blood vessels, NO facilitates improved blood flow and reduces the risk of vaso-occlusive episodes. Additionally, NO has anti-inflammatory and anti-platelet effects that contribute to vascular health. These dual roles of NO in SCD highlight the complexity of NO's interactions in the disease and underscore the potential for therapeutic interventions that can modulate NO levels to achieve beneficial outcomes.[6-15]

The therapeutic potential of targeting NO pathways in SCD is an area of active research. NO donors, such as nitroglycerin and sodium nitroprusside, have been explored for their ability to release NO and improve vascular function in SCD patients. Clinical studies have shown that NO donors can enhance blood flow, reduce the frequency of vaso-occlusive crises, and provide symptomatic relief. L-arginine supplementation, which provides the substrate for NO production, has also been investigated for its potential to boost NO levels and reduce disease symptoms. These therapeutic approaches offer promising avenues for managing SCD by addressing the imbalance between NO and ROS. Recent advancements in SCD research have also focused on innovative strategies to enhance NO signaling. This includes the development of new NO donors with improved safety profiles and efficacy, as well as exploring gene therapies to increase NO production. For example, gene therapies aimed at correcting the β-globin mutation or enhancing eNOS activity represent exciting future directions for SCD treatment. These novel approaches aim to restore NO balance, reduce oxidative stress, and ultimately improve patient outcomes. The exploration of NO's role in SCD has led to a deeper understanding of the disease's pathophysiology

and has opened new avenues for therapeutic interventions. By targeting the NO pathway, researchers aim to develop treatments that can mitigate oxidative stress, improve vascular function, and reduce the frequency and severity of vaso-occlusive crises. The dual nature of NO as both a harmful and protective molecule underscores the need for a balanced approach in therapeutic development.[16-20]

The Nitric Oxide Signaling Pathway

The nitric oxide (NO) signaling pathway is a critical regulatory mechanism in various physiological processes, including vascular tone regulation, neurotransmission, and immune responses. In the context of Sickle Cell Disease (SCD), NO plays a complex role, both modulating oxidative stress and influencing disease pathology. Understanding the NO signaling pathway provides insights into how NO affects SCD and offers potential therapeutic targets for managing the disease. NO is synthesized from the amino acid L-arginine through a reaction catalyzed by nitric oxide synthases (NOS), a family of enzymes consisting of three main isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). eNOS is primarily found in the endothelial cells lining blood vessels, where it plays a key role in regulating vascular tone and blood flow. nNOS is located in neurons and contributes to neurotransmission and synaptic plasticity, while iNOS is expressed in response to inflammatory stimuli and is responsible for the production of high levels of NO in immune responses. Each NOS isoform uses L-arginine as a substrate to produce NO and citrulline, a process that also requires cofactors such as tetrahydrobiopterin (BH4) and oxygen. Once synthesized, NO diffuses across cell membranes due to its gaseous nature and interacts with target molecules. The primary mechanism through which NO exerts its effects is the activation of soluble guanylate cyclase (sGC), an enzyme that converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). cGMP acts as a secondary messenger in NO signaling, leading to various physiological effects such as smooth muscle relaxation, vasodilation, and modulation of platelet aggregation. NO also interacts with other cellular targets, including proteins involved in oxidative stress and cellular signaling pathways.[21-25]

In SCD, the bioactivity of NO is significantly affected by its reaction with reactive oxygen species (ROS), particularly superoxide anions. Under normal conditions, NO and superoxide can react to form peroxynitrite ($ONOO^-$), a potent oxidant that can further contribute to oxidative stress. Peroxynitrite is capable of causing nitration of tyrosine residues in proteins, leading to functional modifications and damage. The increased formation of peroxynitrite in SCD exacerbates oxidative damage to cellular components, including lipids, proteins, and DNA. Thus, the balance between NO and ROS is critical for maintaining cellular homeostasis and preventing excessive oxidative damage. In the endothelium, NO produced by eNOS plays a pivotal role in regulating vascular tone and blood flow. NO induces vasodilation by increasing the levels of cGMP within smooth muscle cells, leading to the relaxation of vascular smooth muscle and a reduction in blood pressure. In SCD, the decreased bioavailability of NO due to its scavenging by free heme from lysed sickle cells leads to endothelial dysfunction. This dysfunction is characterized by reduced vasodilation, increased inflammation, and a higher risk of vascular complications, including vaso-occlusive crises. In SCD, the vascular endothelium is significantly affected by the reduced NO

bioavailability. This reduction contributes to the development of vaso-occlusive crises and other vascular complications. NO's role in promoting vasodilation and inhibiting platelet aggregation is impaired, leading to increased blood viscosity and enhanced risk of occlusive events. Therapeutic strategies aimed at restoring NO levels or mimicking its effects are being explored to manage these complications and improve patient outcomes.[26-30]

NO plays a key role in regulating platelet function by inhibiting platelet aggregation and adhesion to the endothelium. In SCD, the reduced NO availability leads to increased platelet activation, contributing to the formation of thrombi and vaso-occlusive events. The therapeutic use of NO donors or drugs that enhance NO signaling aims to counteract these effects and prevent thrombotic complications in SCD patients. NO has both pro-inflammatory and anti-inflammatory effects depending on its concentration and the presence of other signaling molecules. In SCD, chronic inflammation exacerbates oxidative stress and disease symptoms. The role of NO in modulating inflammation involves complex interactions with other cytokines and signaling pathways. Targeting these interactions could offer new approaches for managing inflammation and related complications in SCD. Hemolysis in SCD leads to the release of free hemoglobin into the bloodstream, which scavenges NO and reduces its bioavailability. This interaction not only diminishes NO's protective effects but also promotes the formation of oxidative stress by increasing ROS levels. Understanding this relationship is crucial for developing therapies that address hemolysis and restore NO balance in SCD patients. Therapeutic strategies to enhance NO bioavailability in SCD include the use of NO donors, L-arginine supplementation, and agents that reduce oxidative stress. NO donors such as nitroglycerin and sodium nitroprusside, as well as L-arginine supplements, aim to increase NO levels and counteract the adverse effects of reduced NO bioavailability. These approaches are being tested in clinical trials to evaluate their efficacy in reducing SCD symptoms and improving patient outcomes.[31-35]

Nitric Oxide and Red Blood Cell Function in Sickle Cell Disease

Nitric oxide (NO) is a versatile signaling molecule with profound effects on vascular function and cellular health. In Sickle Cell Disease (SCD), the role of NO is particularly complex due to the interplay between NO signaling and the pathological processes associated with sickled red blood cells (RBCs). The interaction between NO and RBC function in SCD is crucial for understanding disease mechanisms and developing effective therapeutic strategies. This section explores the multifaceted relationship between NO and RBC function in SCD, highlighting the effects of NO on RBC properties, the implications for disease progression, and potential therapeutic approaches. NO is synthesized from L-arginine by nitric oxide synthases (NOS), with endothelial NOS (eNOS) being the primary source of NO in the blood vessels. Under normal conditions, NO produced by eNOS diffuses into the surrounding RBCs and regulates various cellular functions. NO plays a role in maintaining RBC deformability, which is essential for their passage through microcirculatory networks. In SCD, the decreased bioavailability of NO due to its scavenging by free hemoglobin released from lysed sickle cells leads to impaired RBC function, contributing to disease pathology. RBC deformability is a critical property that allows cells to traverse narrow capillaries and microvascular networks. NO affects RBC deformability by modulating the function of the RBC membrane cytoskeleton. In SCD, the sickling process causes RBCs to adopt a rigid, sickle shape,

impairing their ability to flow through the microvasculature. NO enhances RBC deformability by stimulating the production of cGMP, which leads to relaxation of the RBC membrane cytoskeleton. However, in SCD, the scavenging of NO by free hemoglobin reduces cGMP levels and impairs RBC deformability, exacerbating the disease's microvascular complications.[36-40]

RBC adhesion to the endothelium is a key factor in the pathogenesis of vaso-occlusive crises in SCD. NO normally inhibits RBC adhesion by reducing the expression of adhesion molecules on the endothelial surface. In SCD, the reduced NO bioavailability leads to increased RBC adhesion to the endothelium, contributing to vaso-occlusive events. The interaction between NO and endothelial cells modulates the expression of adhesion molecules, such as P-selectin and VCAM-1, which are crucial in the pathogenesis of vaso-occlusive crises. In SCD, the chronic hemolysis of sickle cells releases free hemoglobin into the bloodstream, which scavenges NO, leading to reduced NO bioavailability. This reduction exacerbates oxidative stress and contributes to endothelial dysfunction. The increased hemolysis in SCD patients leads to a chronic state of low NO levels, further aggravating the disease's vascular complications. Understanding this dynamic is essential for developing therapeutic strategies that target the hemolysis process to restore NO levels and mitigate disease symptoms. NO affects the gas exchange properties of RBCs by influencing the affinity of hemoglobin for oxygen and carbon dioxide. Under normal conditions, NO helps regulate the release of oxygen from hemoglobin to the tissues. In SCD, the reduced NO levels lead to alterations in hemoglobin-oxygen affinity and impaired oxygen delivery to tissues. The interplay between NO and hemoglobin in SCD highlights the importance of maintaining NO levels for optimal gas exchange and tissue oxygenation.[41-45]

The pathophysiology of SCD is significantly influenced by the interplay between NO and various disease processes. NO modulates vasodilation, RBC deformability, and platelet aggregation, all of which are altered in SCD. The decreased NO bioavailability due to hemolysis and oxidative stress contributes to the disease's clinical manifestations, including vaso-occlusive crises and chronic organ damage. Therapeutic approaches targeting NO pathways include the use of NO donors, which release NO in a controlled manner to restore NO levels and counteract disease symptoms. NO donors such as nitroglycerin and sodium nitroprusside have been tested in clinical trials for their ability to improve RBC deformability, reduce oxidative stress, and alleviate symptoms of SCD. These therapies aim to enhance NO bioavailability and provide symptomatic relief for patients. L-arginine supplementation is another therapeutic approach aimed at increasing NO production in SCD patients. L-arginine serves as a substrate for NOS, and its supplementation has been explored for its potential to boost NO levels and improve disease outcomes. Clinical studies have investigated the efficacy of L-arginine in reducing vaso-occlusive crises, improving RBC function, and enhancing overall patient health. The effects of NO on various complications of SCD, including vaso-occlusive crises, pain management, and organ damage, are areas of ongoing research. NO's role in modulating these complications offers potential for developing novel therapeutic strategies aimed at managing the complex manifestations of SCD. Understanding how NO affects these aspects of the disease is essential for improving patient care and developing effective treatments.[46-50]

Impact of Nitric Oxide on Vascular Health in Sickle Cell Disease

Nitric oxide (NO) is a key mediator of vascular health, influencing various physiological processes including vasodilation, platelet aggregation, and endothelial function. In Sickle Cell Disease (SCD), the role of NO is profoundly altered due to the interplay between NO signaling and the pathological features of the disease. NO is a potent vasodilator that regulates vascular tone by stimulating the production of cyclic guanosine monophosphate (cGMP) in smooth muscle cells. This signaling cascade results in relaxation of the smooth muscle, leading to vasodilation and increased blood flow. In SCD, NO-induced vasodilation is impaired due to the reduced bioavailability of NO. Free hemoglobin released from lysed sickle cells scavenges NO, diminishing its effects on vascular smooth muscle and contributing to endothelial dysfunction. The reduction in NO bioavailability exacerbates the risk of vaso-occlusive crises and other vascular complications associated with SCD. Endothelial cells play a critical role in maintaining vascular health by regulating vascular tone, permeability, and leukocyte adhesion. In SCD, endothelial dysfunction is a hallmark feature driven by decreased NO bioavailability. Reduced NO levels lead to increased expression of adhesion molecules such as P-selectin and VCAM-1, which promote the adhesion of sickle cells and leukocytes to the endothelium. This adhesion contributes to the formation of vaso-occlusive events and the progression of vascular damage.[51-55]

NO plays a crucial role in inhibiting platelet aggregation and thrombus formation. In SCD, the decreased NO bioavailability leads to increased platelet activation and aggregation, which contributes to thrombotic complications and vaso-occlusive crises. By modulating platelet function, NO helps maintain vascular integrity and prevent pathological clot formation. The reduction in NO levels in SCD patients exacerbates these thrombotic events and increases the risk of vascular occlusion. Inflammation plays a significant role in the pathophysiology of SCD, and NO is involved in both pro-inflammatory and anti-inflammatory responses. In SCD, low NO levels contribute to chronic inflammation by promoting the expression of inflammatory cytokines and adhesion molecules. This inflammatory environment exacerbates vascular damage and disease progression. NO's role in modulating inflammation highlights the importance of maintaining NO levels for controlling inflammatory processes and managing SCD. eNOS is responsible for the production of NO in endothelial cells. In SCD, the activity of eNOS is impaired due to decreased availability of its cofactors and substrates, such as L-arginine and tetrahydrobiopterin (BH4). The dysfunction of eNOS leads to reduced NO production and contributes to endothelial dysfunction. Strategies aimed at enhancing eNOS activity or restoring eNOS function are being explored as potential therapeutic interventions for improving vascular health in SCD.[56-60]

NO interacts with reactive oxygen species (ROS) to form peroxynitrite, a potent oxidant that can further exacerbate oxidative stress in SCD. Peroxynitrite formation is increased in SCD due to elevated levels of ROS and reduced NO bioavailability. This oxidative stress contributes to the pathogenesis of SCD by causing damage to lipids, proteins, and DNA, leading to endothelial dysfunction and vascular damage. Balancing NO and ROS levels is crucial for maintaining vascular health in SCD. Complications of SCD, such as vaso-occlusive crises, acute chest syndrome, and stroke, are influenced by the availability and activity of NO. In SCD, reduced NO levels contribute to the development and severity of these complications. Therapeutic approaches aimed at increasing NO availability or mimicking NO's effects could potentially alleviate these complications and improve patient outcomes. Clinical trials exploring the use of NO donors, such

as nitroglycerin and sodium nitroprusside, are investigating their efficacy in improving vascular health in SCD patients. These trials aim to assess whether increasing NO levels can reduce vaso-occlusive crises, improve endothelial function, and enhance overall patient health. The outcomes of these trials could lead to new therapeutic strategies for managing SCD and mitigating its vascular complications. L-arginine supplementation is a therapeutic approach aimed at enhancing NO production in SCD. Clinical studies have investigated whether L-arginine can improve NO levels, reduce vascular complications, and alleviate symptoms in SCD patients. The potential benefits of L-arginine supplementation include improved vasodilation, reduced inflammation, and enhanced overall vascular health.[61-65]

Nitric Oxide and Oxidative Stress in Sickle Cell Disease

Nitric oxide (NO) is a key mediator of vascular health, influencing various physiological processes including vasodilation, platelet aggregation, and endothelial function. In Sickle Cell Disease (SCD), the role of NO is profoundly altered due to the interplay between NO signaling and the pathological features of the disease. NO is a potent vasodilator that regulates vascular tone by stimulating the production of cyclic guanosine monophosphate (cGMP) in smooth muscle cells. This signaling cascade results in relaxation of the smooth muscle, leading to vasodilation and increased blood flow. In SCD, NO-induced vasodilation is impaired due to the reduced bioavailability of NO. Free hemoglobin released from lysed sickle cells scavenges NO, diminishing its effects on vascular smooth muscle and contributing to endothelial dysfunction. The reduction in NO bioavailability exacerbates the risk of vaso-occlusive crises and other vascular complications associated with SCD. Endothelial cells play a critical role in maintaining vascular health by regulating vascular tone, permeability, and leukocyte adhesion. In SCD, endothelial dysfunction is a hallmark feature driven by decreased NO bioavailability. Reduced NO levels lead to increased expression of adhesion molecules such as P-selectin and VCAM-1, which promote the adhesion of sickle cells and leukocytes to the endothelium. This adhesion contributes to the formation of vaso-occlusive events and the progression of vascular damage. NO plays a crucial role in inhibiting platelet aggregation and thrombus formation. In SCD, the decreased NO bioavailability leads to increased platelet activation and aggregation, which contributes to thrombotic complications and vaso-occlusive crises. By modulating platelet function, NO helps maintain vascular integrity and prevent pathological clot formation. The reduction in NO levels in SCD patients exacerbates these thrombotic events and increases the risk of vascular occlusion.[66-70]

Inflammation plays a significant role in the pathophysiology of SCD, and NO is involved in both pro-inflammatory and anti-inflammatory responses. In SCD, low NO levels contribute to chronic inflammation by promoting the expression of inflammatory cytokines and adhesion molecules. This inflammatory environment exacerbates vascular damage and disease progression. NO's role in modulating inflammation highlights the importance of maintaining NO levels for controlling inflammatory processes and managing SCD. eNOS is responsible for the production of NO in endothelial cells. In SCD, the activity of eNOS is impaired due to decreased availability of its cofactors and substrates, such as L-arginine and tetrahydrobiopterin (BH4). The dysfunction of eNOS leads to reduced NO production and contributes to endothelial dysfunction. Strategies aimed

at enhancing eNOS activity or restoring eNOS function are being explored as potential therapeutic interventions for improving vascular health in SCD. NO interacts with reactive oxygen species (ROS) to form peroxynitrite, a potent oxidant that can further exacerbate oxidative stress in SCD. Peroxynitrite formation is increased in SCD due to elevated levels of ROS and reduced NO bioavailability. This oxidative stress contributes to the pathogenesis of SCD by causing damage to lipids, proteins, and DNA, leading to endothelial dysfunction and vascular damage. Balancing NO and ROS levels is crucial for maintaining vascular health in SCD.[71]

Complications of SCD, such as vaso-occlusive crises, acute chest syndrome, and stroke, are influenced by the availability and activity of NO. In SCD, reduced NO levels contribute to the development and severity of these complications. Therapeutic approaches aimed at increasing NO availability or mimicking NO's effects could potentially alleviate these complications and improve patient outcomes. Understanding the relationship between NO and SCD complications is essential for developing effective therapies. Clinical trials exploring the use of NO donors, such as nitroglycerin and sodium nitroprusside, are investigating their efficacy in improving vascular health in SCD patients. These trials aim to assess whether increasing NO levels can reduce vaso-occlusive crises, improve endothelial function, and enhance overall patient health. The outcomes of these trials could lead to new therapeutic strategies for managing SCD and mitigating its vascular complications. L-arginine supplementation is a therapeutic approach aimed at enhancing NO production in SCD. Clinical studies have investigated whether L-arginine can improve NO levels, reduce vascular complications, and alleviate symptoms in SCD patients. The potential benefits of L-arginine supplementation include improved vasodilation, reduced inflammation, and enhanced overall vascular health.[72]

Therapeutic Potential of Nitric Oxide Donors

Nitric oxide (NO) is a critical signaling molecule with diverse physiological effects, including vasodilation, anti-inflammatory actions, and modulation of platelet aggregation. In Sickle Cell Disease (SCD), NO deficiency contributes to disease pathophysiology through mechanisms such as reduced vasodilation, increased oxidative stress, and endothelial dysfunction. Nitric oxide donors, which release NO into the bloodstream, represent a promising therapeutic approach for managing SCD. NO donors are compounds that release NO either spontaneously or through enzymatic reactions. The therapeutic effects of NO donors are primarily mediated through the following mechanisms: NO donors increase NO availability, leading to the relaxation of vascular smooth muscle cells and subsequent vasodilation. This action helps reduce vascular tone, decrease blood pressure, and improve blood flow. NO donors exhibit anti-inflammatory properties by inhibiting the expression of pro-inflammatory cytokines and adhesion molecules, thereby reducing inflammation in the vascular endothelium. NO donors prevent platelet aggregation and thrombus formation, which is beneficial in conditions like SCD where abnormal clotting is a significant problem. NO donors can also act as antioxidants by neutralizing reactive oxygen species (ROS) and reducing oxidative stress, which is heightened in SCD. NO donors have been explored for their potential to manage various aspects of SCD, including vaso-occlusive crises, pulmonary hypertension, and endothelial dysfunction. NO donors help alleviate vaso-occlusive crises by promoting vasodilation and improving blood flow. Clinical studies have shown that NO donors

can reduce the frequency and severity of these painful episodes. NO donors are used to manage pulmonary hypertension, a common complication of SCD. By dilating pulmonary vasculature, NO donors help reduce pulmonary arterial pressure and improve respiratory function.[73]

NO donors improve endothelial function by increasing NO bioavailability, which helps restore normal vascular responses and reduce complications associated with endothelial dysfunction. Different types of NO donors are available, each with varying mechanisms of NO release and therapeutic profiles. Key NO donors used in SCD include: A classic NO donor that releases NO through enzymatic breakdown. It has been used to manage acute vaso-occlusive crises and pulmonary hypertension in SCD patients. A potent NO donor used primarily in acute settings for severe hypertension and vaso-occlusive crises. It releases NO in a controlled manner and can be used in emergency situations. A NO donor that provides prolonged NO release, used for chronic management of SCD complications such as pulmonary hypertension. A common side effect due to the vasodilatory effects of NO donors. Excessive NO donor use can lead to significant drops in blood pressure. Prolonged use of some NO donors can result in elevated levels of methemoglobin, which impairs oxygen delivery. A standard therapy for SCD that increases NO levels by increasing the availability of L-arginine. Combining hydroxyurea with NO donors could have synergistic effects. Combining NO donors with antioxidants can reduce oxidative stress and enhance therapeutic efficacy.[71]

Emerging Therapies Targeting Nitric Oxide Pathways

Sickle Cell Disease (SCD) is a chronic genetic disorder characterized by abnormal hemoglobin that distorts red blood cells into a sickle shape. This deformation causes blockages in blood vessels, leading to pain, organ damage, and a host of severe complications. One of the central players in the pathology of SCD is nitric oxide (NO), a versatile signaling molecule with vasodilatory, anti-inflammatory, and antioxidative properties. Recent advancements in research have highlighted innovative therapies that target NO pathways, offering new hope for improving the management of SCD. This narrative explores these emerging therapies, shedding light on their mechanisms, clinical potential, and the future of SCD treatment. Nitric oxide is a signaling molecule with critical roles in vascular function, including the regulation of blood flow, prevention of platelet aggregation, and modulation of inflammation. In SCD, NO deficiency exacerbates disease complications by promoting vaso-occlusive crises, endothelial dysfunction, and increased oxidative stress. Therapeutic strategies aimed at restoring NO levels or mimicking its effects are being developed to address these issues and improve patient outcomes. Recent research has introduced several new NO donors designed to deliver NO in a controlled and sustained manner, offering potential advantages over traditional therapies. Traditional nitrate esters, like nitroglycerin, have been used to treat cardiovascular conditions for decades. However, new forms of organic nitrate esters are being explored for their extended release and enhanced efficacy. These compounds can provide long-lasting NO delivery, which is beneficial for managing chronic symptoms of SCD such as vaso-occlusive crises. Isosorbide Mononitrate (ISMN) is a new-generation nitrate with a longer duration of action compared to nitroglycerin. Research shows that ISMN effectively dilates blood vessels and alleviates SCD symptoms by increasing NO bioavailability.[71-72]

Inorganic nitrites and nitrates have emerged as promising NO donors. These compounds are metabolized to produce NO, which can help restore vascular function and reduce complications associated with SCD. Sodium Nitrite is being studied for its ability to convert to NO in the body, providing a novel approach for SCD treatment. Clinical trials are examining its effectiveness in reducing vaso-occlusive events and managing pulmonary hypertension. L-arginine is the precursor to NO synthesis, and increasing its availability can enhance NO production. This therapeutic approach is based on the premise that supplementing L-arginine may help manage SCD symptoms by boosting NO levels. Oral L-arginine supplements are being tested for their ability to enhance NO synthesis and improve clinical outcomes in SCD patients. Studies have shown that L-arginine supplementation can reduce the frequency of vaso-occlusive crises and improve overall patient well-being. Combining L-arginine with other treatments, such as NO donors or antioxidants, is an emerging strategy to amplify therapeutic effects and reduce SCD complications. Innovative drug delivery systems that release NO in a controlled manner are under development. These hydrogels can be used for localized NO delivery, potentially offering targeted treatment for vascular complications in SCD. NO-releasing hydrogels are designed to provide a controlled release of NO over extended periods. This approach aims to deliver therapeutic doses of NO directly to affected tissues or blood vessels, reducing systemic side effects and improving therapeutic efficacy. Gene therapy is an innovative field exploring the potential of modifying genes to enhance NO production or restore normal hemoglobin function in SCD patients. Gene editing technologies like CRISPR/Cas9 are being investigated for their potential to correct genetic defects in SCD or enhance NO synthesis. These approaches aim to address the root causes of SCD and offer long-term therapeutic solutions. Gene therapy approaches targeting the genetic basis of SCD, such as introducing functional hemoglobin genes or correcting defective hemoglobin genes, are also being developed. These therapies have the potential to cure SCD by addressing the genetic root of the disease. Combining NO-based therapies with existing treatments for SCD offers a promising approach to enhance therapeutic outcomes. Combining NO donors with hydroxyurea, which increases endogenous NO production, is being explored to maximize therapeutic benefits for SCD patients. This combination aims to address multiple aspects of SCD pathology simultaneously.[71-73]

The Role of L-Arginine in Sickle Cell Disease

Sickle Cell Disease (SCD) is a genetic disorder characterized by the production of abnormal hemoglobin, which causes red blood cells to become rigid and sickle-shaped. This abnormality leads to vaso-occlusive crises, chronic pain, and severe complications affecting multiple organs. Central to the pathology of SCD is a deficiency in nitric oxide (NO), a vital molecule that regulates blood vessel dilation, inhibits platelet aggregation, and maintains vascular health. Emerging research highlights the therapeutic potential of L-arginine, an amino acid that serves as the primary substrate for NO production. L-arginine is an amino acid that plays a crucial role in the synthesis of nitric oxide. In the body, L-arginine is converted into NO by the enzyme nitric oxide synthase (NOS). NO then acts as a signaling molecule that regulates various physiological processes, including vasodilation, immune response, and neurotransmission. The conversion of L-arginine to NO involves a biochemical reaction facilitated by NOS, which produces NO from L-arginine and oxygen. This NO then diffuses into smooth muscle cells of blood vessels, promoting relaxation

and vasodilation. NO's primary role in the cardiovascular system includes relaxing vascular smooth muscles, thereby dilating blood vessels and improving blood flow. In addition, NO inhibits platelet aggregation and has anti-inflammatory effects. In the context of SCD, the production of NO is impaired, which exacerbates the disease's complications.[70]

One of the hallmark features of SCD is vaso-occlusive crises, where sickled red blood cells obstruct blood flow in capillaries and small blood vessels. NO normally helps to relax blood vessels and improve blood flow. A lack of NO exacerbates the tendency of red blood cells to clump together, leading to further vessel blockage and pain. NO also maintains the health of endothelial cells lining the blood vessels. In SCD, reduced NO availability leads to endothelial dysfunction, which promotes inflammation, platelet activation, and further vaso-occlusive events. NO acts as an antioxidant by neutralizing reactive oxygen species (ROS). In SCD, the lack of NO contributes to increased oxidative stress, which further damages cells and tissues. Given the critical role of L-arginine in NO production, supplementing with L-arginine has been explored as a potential therapy for SCD. This approach aims to boost NO levels and counteract some of the disease's most debilitating effects. Supplementation with L-arginine can help increase NO production, which may reduce the frequency and severity of vaso-occlusive crises. Clinical studies have demonstrated that L-arginine supplements can improve blood flow and alleviate symptoms associated with these painful episodes. L-arginine supplementation has been shown to improve endothelial function by increasing NO availability. This effect helps to restore normal blood vessel function, reduce inflammation, and prevent complications associated with endothelial dysfunction. By increasing NO production, L-arginine can help counteract oxidative stress. This reduction in oxidative damage can alleviate some of the cellular and tissue damage seen in SCD.[72-73]

Conclusion

L-arginine emerges as a beacon of hope in the management of Sickle Cell Disease (SCD), a condition long marked by the painful and debilitating effects of compromised nitric oxide (NO) signaling. At its core, L-arginine acts as a substrate for NO synthesis, a molecule crucial for maintaining vascular health and combating the myriad complications of SCD. The deficiency of NO in SCD patients exacerbates vaso-occlusive crises, endothelial dysfunction, and oxidative stress. By supplementing L-arginine, we can potentially restore NO levels, offering a novel approach to mitigate these complications. The clinical studies reviewed highlight that L-arginine supplementation can reduce the frequency of vaso-occlusive crises, improve endothelial function, and decrease oxidative stress, demonstrating tangible benefits for SCD patients. In addition to these immediate therapeutic effects, L-arginine's role in SCD management is multifaceted. It not only alleviates acute symptoms but also holds promise for long-term disease management.

References

1. Ata F, Rahhal A, Malkawi L, Iqbal P, Khamees I, Alhiyari M, Yousaf Z, Qasim H, Alshurafa A, Sardar S, Javed S. Genotypic and phenotypic composition of sickle cell disease in the Arab population-a systematic review. Pharmacogenomics and Personalized Medicine. 2023:133-144.

2. Obeagu EI, Ochei KC, Nwachukwu BN, Nchuma BO. Sickle cell anaemia: a review. Scholars Journal of Applied Medical Sciences. 2015;3(6B):224422-52.
3. Obeagu EI. Erythropoeitin in Sickle Cell Anaemia: A Review. International Journal of Research Studies in Medical and Health Sciences. 2020;5(2):22-28.
4. Obeagu EI. Sickle Cell Anaemia: Haemolysis and Anemia. Int. J. Curr. Res. Chem. Pharm. Sci. 2018;5(10):20-21.
5. Obeagu EI, Muhimbura E, Kagenderezo BP, Uwakwe OS, Nakyeyune S, Obeagu GU. An Update on Interferon Gamma and C Reactive Proteins in Sickle Cell Anaemia Crisis. J Biomed Sci. 2022;11(10):84.
6. Obeagu EI, Ogunnaya FU, Obeagu GU, Ndidi AC. Sickle cell anaemia: a gestational enigma. European Journal of Biomedical and Pharmaceutical Sciences. 2023;10((9): 72-75
7. Obeagu EI. An update on micro RNA in sickle cell disease. Int J Adv Res Biol Sci. 2018; 5:157-158.
8. Obeagu EI, Babar Q. Covid-19 and Sickle Cell Anemia: Susceptibility and Severity. J. Clinical and Laboratory Research. 2021;3(5):2768-2487.
9. Obeagu EI. Depression in Sickle Cell Anemia: An Overlooked Battle. Int. J. Curr. Res. Chem. Pharm. Sci. 2023;10(10):41-.
10. Nur E, Biemond BJ, Otten HM, Brandjes DP, Schnog JJ, CURAMA Study Group. Oxidative stress in sickle cell disease; pathophysiology and potential implications for disease management. American journal of hematology. 2011;86(6):484-849.
11. Obeagu EI, Obeagu GU. Evaluation of Hematological Parameters of Sickle Cell Anemia Patients with Osteomyelitis in A Tertiary Hospital in Enugu, Nigeria. Journal of Clinical and Laboratory Research.2023;6(1):2768-2487.
12. Obeagu EI, Dahir FS, Francisca U, Vandu C, Obeagu GU. Hyperthyroidism in sickle cell anaemia. Int. J. Adv. Res. Biol. Sci. 2023;10(3):81-89.
13. Njar VE, Ogunnaya FU, Obeagu EI. Knowledge And Prevalence of The Sickle Cell Trait Among Undergraduate Students Of The University Of Calabar. Prevalence.;5(100):0-5.
14. Swem CA, Ukaejiofo EO, Obeagu EI, Eluke B. Expression of micro RNA 144 in sickle cell disease. Int. J. Curr. Res. Med. Sci. 2018;4(3):26-32.
15. Vona R, Sposi NM, Mattia L, Gambardella L, Straface E, Pietraforte D. Sickle cell disease: role of oxidative stress and antioxidant therapy. Antioxidants. 2021;10(2):296.
16. Wang Q, Zennadi R. The role of RBC oxidative stress in sickle cell disease: from the molecular basis to pathologic implications. Antioxidants. 2021;10(10):1608.
17. Obeagu EI. Sickle cell anaemia: Historical perspective, Pathophysiology and Clinical manifestations. Int. J. Curr. Res. Chem. Pharm. Sci. 2018;5(11):13-15.
18. Obeagu EI, Obeagu GU. Sickle Cell Anaemia in Pregnancy: A Review. International Research in Medical and Health Sciences. 2023;6(2):10-13.
19. Obeagu EI, Mohamod AH. An update on Iron deficiency anaemia among children with congenital heart disease. Int. J. Curr. Res. Chem. Pharm. Sci. 2023;10(4):45-48.
20. Edward U, Osuorji VC, Nnodim J, Obeagu EI. Evaluationof Trace Elements in Sickle Cell Anaemia Patients Attending Imo State Specialist Hospital, Owerri. Madonna University journal of Medicine and Health Sciences ISSN: 2814-3035. 2022;2(1):218-234.

21. Umar MI, Aliyu F, Abdullahi MI, Aliyu MN, Isyaku I, Aisha BB, Sadiq RU, Shariff MI, Obeagu EI. Assessment Of Factors Precipitating Sickle Cell Crises Among Under 5-Years Children Attending Sickle Cell Clinic of Murtala Muhammad Specialist Hospital, Kano. blood.;11:16.
22. Obeagu EI. Vaso-occlusion and adhesion molecules in sickle cells disease. Int J Curr Res Med Sci. 2018;4(11):33-35.
23. Ifeanyi OE, Stella EI, Favour AA. Antioxidants in the Management of Sickle Cell Anaemia. Int J Hematol Blood Disord, 2018; 3. Available from: https://symbiosisonlinepublishing. com/hematology/hema tology25. php. 2018.
24. Buhari HA, Ahmad AS, Obeagu EI. Current Advances in the Diagnosis and Treatment of Sickle Cell Anaemia. APPLIED SCIENCES (NIJBAS). 2023;4(1).
25. Nnodim J, Uche U, Ifeoma U, Chidozie N, Ifeanyi O, Oluchi AA. Hepcidin and erythropoietin level in sickle cell disease. British Journal of Medicine and Medical Research. 2015;8(3):261-265.
26. Obeagu EI. BURDEN OF CHRONIC OSTEOMYLITIS: REVIEW OF ASSOCIATIED FACTORS. Madonna University journal of Medicine and Health Sciences. 2023;3(1):1-6.
27. Aloh GS, Obeagu EI, Okoroiwu IL, Odo CE, Chibunna OM, Kanu SN, Elemchukwu Q, Okpara KE, Ugwu GU. Antioxidant-Mediated Heinz Bodies Levels of Sickle Erythrocytes under Drug-Induced Oxidative Stress. European Journal of Biomedical and Pharmaceutical sciences. 2015;2(1):502-507.
28. Hernansanz-Agustín P, Enríquez JA. Generation of reactive oxygen species by mitochondria. Antioxidants. 2021;10(3):415.
29. Obeagu EI, Obeagu GU. Sickle Cell Anaemia in Pregnancy: A Review. International Research in Medical and Health Sciences. 2023; 6 (2): 10-13.
30. Obeagu EI, Ogbuabor BN, Ikechukwu OA, Chude CN. Haematological parameters among sickle cell anemia patients' state and haemoglobin genotype AA individuals at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. International Journal of Current Microbiology and Applied Sciences. 2014;3(3):1000-1005.
31. Ifeanyi OE, Nwakaego OB, Angela IO, Nwakaego CC. Haematological parameters among sickle cell anaemia... Emmanuel Ifeanyi1, et al. pdf• Obeagu. Int. J. Curr. Microbiol. App. Sci. 2014;3(3):1000-1005.
32. Obeagu EI, Opoku D, Obeagu GU. Burden of nutritional anaemia in Africa: A Review. Int. J. Adv. Res. Biol. Sci. 2023;10(2):160-163.
33. Ifeanyi E. Erythropoietin (Epo) Level in Sickle Cell Anaemia (HbSS) With Falciparum Malaria Infection in University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. PARIPEX - INDIAN JOURNAL OF RESEARCH, 2015; 4(6): 258-259
34. Ifeanyi OE, Nwakaego OB, Angela IO, Nwakaego CC. Haematological parameters among sickle cell anaemia patients in steady state and haemoglobin genotype AA individuals at Michael Okpara, University of Agriculture, Umudike, Abia State, Nigeria. Int. J. Curr. Microbiol. App. Sci. 2014;3(3):1000-1005.
35. Ifeanyi OE, Stanley MC, Nwakaego OB. Comparative analysis of some haematological parameters in sickle cell patients in steady and crisis state at michael okpara University of

agriculture, Umudike, Abia state, Nigeria. Int. J. Curr. Microbiol. App. Sci. 2014;3(3):1046-1050.
36. Ifeanyi EO, Uzoma GO. Malaria and The Sickle Cell Trait: Conferring Selective Protective Advantage to Malaria. J Clin Med Res. 2020; 2:1-4.
37. Obeagu EI, Obeagu GU. Oxidative Damage and Vascular Complications in Sickle Cell Anemia: A Review. Elite Journal of Haematology, 2024; 2 (3).:58-66.
38. Obeagu EI, Obeagu GU. Addressing Myths and Stigmas: Breaking Barriers in Adolescent Sickle Cell Disease Education. Elite Journal of Health Science. 2024;2(2):7-15.
39. Obeagu EI, Obeagu GU. Implications of climatic change on sickle cell anemia: A review. Medicine. 2024;103(6):e37127.
40. Orrico F, Laurance S, Lopez AC, Lefevre SD, Thomson L, Möller MN, Ostuni MA. Oxidative stress in healthy and pathological red blood cells. Biomolecules. 2023;13(8):1262.
41. Vona R, Sposi NM, Mattia L, Gambardella L, Straface E, Pietraforte D. Sickle cell disease: role of oxidative stress and antioxidant therapy. Antioxidants. 2021;10(2):296.
42. Obeagu EI. Chromium VI: A Silent Aggressor in Sickle Cell Anemia Pathophysiology. Elite Journal of Haematology, 2024; 2 (3).:81-95.
43. Obeagu EI. Maximizing longevity: erythropoietin's impact on sickle cell anemia survival rates. Annals of Medicine and Surgery. 2024:10-97.
44. Obeagu EI, Ubosi NI, Obeagu GU, Egba SI, Bluth MH. Understanding apoptosis in sickle cell anemia patients: Mechanisms and implications. Medicine. 2024 Jan 12;103(2):e36898.
45. Obeagu EI, Ayogu EE, Anyanwu CN, Obeagu GU. Drug-Drug Interactions in the Management of Coexisting Sickle Cell Anemia and Diabetes. Elite Journal of Health Science. 2024;2(2):1-9.
46. Obeagu EI, Obeagu GU. Dual Management: Diabetes and Sickle Cell Anemia in Patient Care. Elite Journal of Medicine. 2024;2(1):47-56.
47. Obeagu EI, Obeagu GU, Hauwa BA. Optimizing Maternal Health: Addressing Hemolysis in Pregnant Women with Sickle Cell Anemia. Journal home page: http://www. journalijiar. com.;12(01).
48. Obeagu EI, Obeagu GU. Synergistic Care Approaches: Integrating Diabetes and Sickle Cell Anemia Management. Elite Journal of Scientific Research and Review. 2024;2(1):51-64.
49. Obeagu EI, Obeagu GU. Improving Outcomes: Integrated Strategies for Diabetes and Sickle Cell Anemia. Int. J. Curr. Res. Chem. Pharm. Sci. 2024;11(2):20-9.
50. Obeagu EI, Obeagu GU. The Role of Parents: Strengthening Adolescent Education for Sickle Cell Disease Prevention. Elite Journal of Public Health. 2024;2(1):15-21.
51. Obeagu EI, Obeagu GU. Hemolysis Challenges for Pregnant Women with Sickle Cell Anemia: A Review. Elite Journal of Haematology, 2024; 2 (3).:67-80.
52. Obeagu EI, Obeagu GU. Overcoming Hurdles: Anemia Management in Malaria-Affected Childhood. Elite Journal of Laboratory Medicine. 2024;2(1):59-69.
53. Obeagu EI, Ubosi NI, Obeagu GU, Egba SI, Bluth MH. Understanding apoptosis in sickle cell anemia patients: Mechanisms and implications. Medicine (Baltimore). 2024 ;103(2):e36898. doi: 10.1097/MD.0000000000036898. PMID: 38215146; PMCID: PMC10783340.

54. Obeagu EI. Maximizing longevity: erythropoietin's impact on sickle cell anaemia survival rates. Ann Med Surg (Lond). 2024;86(3):1570-1574. doi: 10.1097/MS9.0000000000001763. PMID: 38463100; PMCID: PMC10923353.
55. Obeagu EI, Obeagu GU. Malnutrition in sickle cell anemia: Prevalence, impact, and interventions: A Review. Medicine (Baltimore). 2024;103(20):e38164. doi: 10.1097/MD.0000000000038164. PMID: 38758879; PMCID: PMC11098235.
56. Obeagu EI, Obeagu GU. Management of diabetes mellitus patients with sickle cell anemia: Challenges and therapeutic approaches. Medicine (Baltimore). 2024;103(17):e37941. doi: 10.1097/MD.0000000000037941. PMID: 38669382; PMCID: PMC11049766.
57. Obeagu EI, Obeagu GU, Akinleye CA, Igwe MC. Nosocomial infections in sickle cell anemia patients: Prevention through multi-disciplinary approach: A review. Medicine (Baltimore). 2023;102(48):e36462. doi: 10.1097/MD.0000000000036462. PMID: 38050205; PMCID: PMC10695528.
58. Dilli PP, Obeagu E, Tamale A, Ajugwo A, Pius T, Makeri D. Update on the practice of premarital screening for sickle cell traits in Africa: a systematic review and meta-analysis. BMC Public Health. 2024 May 31;24(1):1467. doi: 10.1186/s12889-024-19001-y. PMID: 38822327; PMCID: PMC11143629.
59. Obeagu EI, Obeagu GU. Managing gastrointestinal challenges: Diarrhea in sickle cell anemia. Medicine (Baltimore). 2024;103(18):e38075. doi: 10.1097/MD.0000000000038075. PMID: 38701274; PMCID: PMC11062666.
60. Obeagu EI, Obeagu GU. Implications of climatic change on sickle cell anemia: A review. Medicine (Baltimore). 2024;103(6):e37127. doi: 10.1097/MD.0000000000037127. PMID: 38335412; PMCID: PMC10860944.
61. Obeagu EI. Eosinophilic dialogues: A molecular exploration of sickle cell anemia severity. Annals of Medicine and Surgery. 2024:10-97.
62. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. European Journal of Medicinal Chemistry. 2021; 209:112891.
63. Rushworth GF, Megson IL. Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits. Pharmacology & therapeutics. 2014;141(2):150-159.
64. Park SH, Bao G. CRISPR/Cas9 gene editing for curing sickle cell disease. Transfusion and Apheresis Science. 2021;60(1):103060.
65. Strouse JJ, Heeney MM. Hydroxyurea for the treatment of sickle cell disease: efficacy, barriers, toxicity, and management in children. Pediatric blood & cancer. 2012;59(2):365-371.
66. Sadaf A, Quinn CT. L-glutamine for sickle cell disease: Knight or pawn? Experimental Biology and Medicine. 2020;245(2):146-54.
67. Chou ST, Fasano RM. Management of patients with sickle cell disease using transfusion therapy: guidelines and complications. Hematology/Oncology Clinics. 2016;30(3):591-608.
68. Leonard A, Tisdale J, Abraham A. Curative options for sickle cell disease: haploidentical stem cell transplantation or gene therapy? British journal of haematology. 2020;189(3):408-423.

69. Silva DG, Junior EB, De Almeida EA, Bonini-Domingos CR. Oxidative stress in sickle cell disease: an overview of erythrocyte redox metabolism and current antioxidant therapeutic strategies. Free Radical Biology and Medicine. 2013; 65:1101-1109.
70. Ahmed SG, Ibrahim UA. A compendium of pathophysiologic basis of etiologic risk factors for painful vaso-occlusive crisis in sickle cell disease. Nigerian Journal of Basic and Clinical Sciences. 2017;14(2):57-77.
71. Lakkakula BV, Sahoo R, Verma H, Lakkakula S. Pain management issues as part of the comprehensive care of patients with sickle cell disease. Pain Management Nursing. 2018;19(6):558-572.
72. Brandow AM, Liem RI. Advances in the diagnosis and treatment of sickle cell disease. Journal of Hematology & Oncology. 2022;15(1):20.
73. Hoban MD, Orkin SH, Bauer DE. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. Blood, The Journal of the American Society of Hematology. 2016;127(7):839-848.