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NIH Principal Investigator: Arun S. Shet, MD, Ph.D.

Sickle Cell Branch, NHLBI
Building 10-CRC, Rm 6N240B
10 Center Dr,
Bethesda, MD 20892
Phone: 301-827-6808
E-mail: arun.shet@nih.gov

Investigational Agents:

Drug Name:	Isoquercetin
IND Number:	150896
Sponsor:	NHLBI
Manufacturer:	Querces AG

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

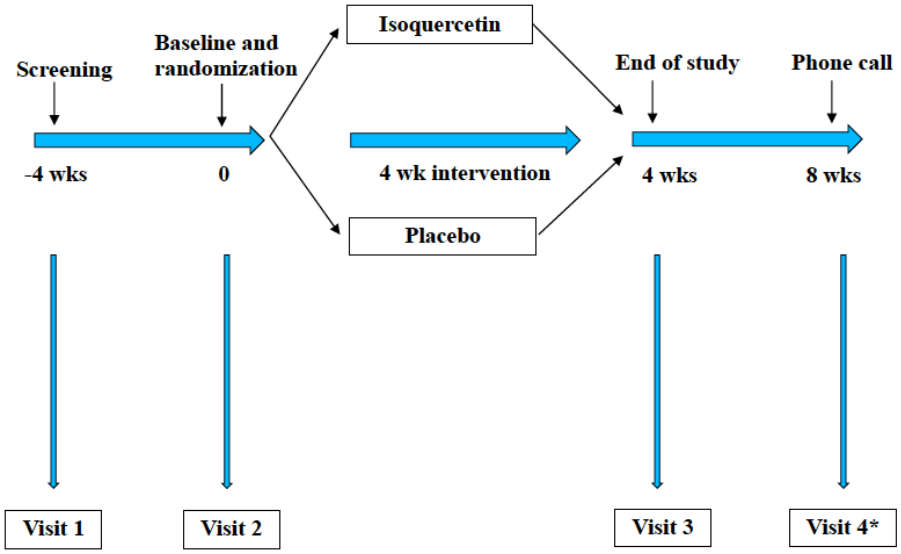
1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Study to Evaluate the Effects of fixed dose Flavonoid Isoquercetin on thrombo-inflammatory biomarkers in subjects with stable Sickle Cell Disease
Study Description:	Similar to cancer, sickle cell disease is associated with an acquired hypercoagulable state and exhibits a high prevalence of incident and recurrent venous thromboembolism (VTE). Elevated levels of the procoagulant protein tissue factor and its activator, protein disulfide isomerase (PDI) in humans with sickle cell disease (SCD) suggest a causal role for thrombogenesis. In cancer patients, pharmacological inhibition of plasma PDI with Isoquercetin (IQ) led to a reduction in VTE biomarkers and VTE recurrence. These findings provide support to test the hypothesis that Isoquercetin in sickle cell disease would diminish thrombo-inflammatory VTE biomarkers and attenuate the associated hypercoagulable state.
Objectives:	1) To measure the effect of Isoquercetin, an oral inhibitor of vascular PDI, on plasma soluble P-selectin levels in subjects with stable SCD. 2) To assess the clinical safety and tolerability of 1000 mg IQ in subjects with stable SCD. 3) To determine the effect of IQ on markers of hypercoagulability (plasma PDI, circulating tissue factor positive extracellular vesicles, plasma tissue factor procoagulant activity and plasma thrombin generation) in subjects with stable sickle cell disease.
Endpoints:	The primary outcome will be the change in plasma soluble P-selectin level comparing the baseline versus IQ or placebo. Secondary outcomes are to: 1) Compare baseline and end of study plasma PDI activity 2) Compare baseline and end of study plasma tissue factor positive extracellular vesicle number 3) Compare baseline and end of study plasma tissue factor procoagulant activity 4) Compare baseline and end of study inflammation and coagulation parameters 5) Compare baseline and end of study contemporary biomarkers of vascular function and atherothrombosis 6) Assess safety/tolerability and adherence to oral IQ
Study Population:	Up to 46 male and female subjects of all races between the ages of 18-70 years with SCD who live locally will be enrolled.

Phase:	II
Description of Sites/Facilities	Enrollment and study visits will take place at the NIH Clinical Center.
Enrolling Participants:	Subjects with sickle cell disease
Description of Study Intervention:	Isoquercetin 1000 mg or placebo once daily by mouth for 28 days.
Study Duration:	2 years
Participant Duration:	5-9 weeks

1.2 SCHEMA



* Safety visit if applicable

1.3 SCHEDULE OF ACTIVITIES (SOA)

EVALUATION AND ASSESSMENTS	Screening (A)	Baseline	End of Study	Phone Call (B)
PROTOCOL TIMEPOINT VISITS	1	2	3	4
Days		D0	D28	D56
Window +/- days	-90Days	-28Days	+ 7Days	± 7Days
Evaluations & Consent				
Consent to study	x			
Review Inclusion/Exclusion Criteria	x	x		
Review of Medical History	x	x	x	
Procedures & Assessments				
Establish Baseline Symptoms		x		
Physical Examination	x	x	x	
Review of Medications	x	x	x	
Vital Signs with Weight (kg)	x	x	x	
Review Of Adverse Events		x	x	x
24-hour Dietary Recall	x	x	x	
Peripheral Arterial Tonometry (PAT) (ENDO-PAT)		x[4]	x[4]	
NIRS (Near Infrared Spectometry)		x[4]	x[4]	
CHEMISTRY LABS				
Female, Pregnancy Test, Beta HCG (blood or urine)	x[1]	x[1]	x[1]	
Acute Care Panel	x	x	x	
Mineral Panel	x	x	x	
Fasting Glucose	x			
Hepatic Panel	x	x	x	
Total Protein	x	x	x	
Creatinine Kinase	x	x	x	
Uric Acid	x	x	x	
C-Reactive Protein (hs-CRP)		x	x	
B-type natriuretic peptide (P-BNP)		x	x	
Lactate Dehydrogenase	x	x	x	
Calculated Creatinine Clearance	x	x	x	
Viral Serology: HIV, hepaititis B and C (Serum HBsAg, anti-HBc, anti-HBs, anti-HCV, anti-HIV)	x			
HEMATOLOGY LABS				
CBC with differential	x	x	x	
Hemoglobin Electrophoresis	x	x	x	
D-Dimer	x	x	x	
Coagulation Screen (PT, PTT, INR, Fibrinogen)	x	x	x	
Reticulocyte Count	x	x	x	
Direct Antiglobulin Screen		x	x	
Research Samples (Up to 30 mL)				
Biochemical Studies (3 ml 3.8% Sodium Citrate)		x	x	
Coagulation Studies (9 ml 3.8% Sodium Citrate)		x	x	
Red Blood Cell Studies (6 ml EDTA tube)		x	x	
Platelet Function Test (6 ml 3.8% Sodium Citrate)		x	x	

Leukocytes isolation for gene expression and RNAseq (3 ml 3.8% Sodium Citrate)		**x**	**x**	
Study Supplement Medication				
Study Supplement Randonization		**x**		
Study Supplement, Diary and Electronic Pill Dispenser Dispensed		**x[3]**		
Study Supplement Compliance			**x[2]**	
Study Supplement, Diary and Electronic Pill Dispenser Returned			**x**	

A = Screening tests will be done at the Screening Visit unless any of the tests have been performed under another NIH protocol within 90 days prior to day 0 of study drug.
B. Any adverse event requiring a clinic visit during that safety follow up period will be managed as clinically indicated
x[1] = for females of child bearing potential
x[2] = subject must be greater than or equal to 75% to be considered compliant
x[3] = counsel subjects to maintain a stable diet and not start any new diet or active SCD treatment for the duration of the study (Pill Dispenser is optional).
x[4] = ENDO-PAT and NIRS testing are optional and completion will be based on availability of service and participant willingness.

2 INTRODUCTION

2.1 STUDY RATIONALE

Sickle Cell Disease (SCD) and cancer are chronic conditions that share an acquired hypercoagulable state which clinically manifests as venous thromboembolic disease and contributes to mortality 1. Moreover, both disorders share a similar increased risk for bleeding upon exposure to short and long term anticoagulation, the standard treatment for venous thromboembolism 2,3. Scientific evidence supports the notion that inflammation perturbs endothelial and leukocyte homeostasis and upregulates tissue factor (TF) and P-selectin expression, two major contributors to thrombo-inflammatory pathobiology in SCD 4-6. Endothelial and platelet injury in SCD also likely contributes to elevated plasma protein disulfide isomerase (PDI) levels possibly explaining higher PDI concentrations on the surface of sickle RBCs compared to normal red blood cells (RBCs) 7. PDI, a vascular thiol isomerase released during endothelial/platelet injury stimulates thrombin production by generating platelet factor Va in humans, and upon vascular injury in mice, facilitates fibrin deposition 8-10. PDI mediates these effects through oxidation of the Cys 186-Cys 209 bond and allosteric activation of TF, which decrypts TF pro coagulant activity 11,12. Decryption of circulating TF pro coagulant activity by plasma PDI possibly explains TF driven activation of coagulation in SCD. Consequently, increased TF pro coagulant activity triggers activation of intravascular coagulation which combined with venous stasis/hypoxia provokes venous thromboembolism (VTE).

Since SCD patients have increased blood borne tissue factor, sustained thrombin generation and demonstrate features of platelet activation, plasma PDI inhibition, by restoring post-translational regulatory control of TF, might attenuate the associated hypercoagulable state. Such an approach is rationalized by observations of elevated plasma PDI activity SCD mice that when exposed to a pharmacologic PDI inhibitor demonstrated reduced vaso-occlusive crisis and microvascular thrombosis 13. In a phase II trial of patients with active cancer, the flavonoid Isoquercetin (IQ) (Querces AG, Switzerland)) robustly inhibited plasma PDI activity and favorably reduced soluble P-selectin, a biomarker predictive of VTE development 14. Besides, none of these patients experienced any increased risk of bleeding typically observed with exposure to warfarin or non-vitamin K oral anticoagulants 2,3. The salutatory benefits of lowering soluble P-selectin

in SCD patients are both reduced inflammation and TF expression. Therefore, our overall hypothesis is that therapeutic intervention with IQ would lower soluble P-selectin and simultaneously decrease the prothrombotic effects of TF without a concomitantly increased risk for bleeding. Evaluating the effects of IQ-mediated PDI inhibition on blood borne TF procoagulant activity and plasma soluble P-selectin will provide important insight into the pathophysiologic basis of the hypercoagulable state in SCD.

2.2 BACKGROUND

Sickle Cell Disease (SCD) is an inherited monogenic hemoglobin disorder caused by a mutation in the gene encoding the β globin subunit of adult hemoglobin (HbA) resulting in a substitution of valine for glutamic acid at position 6 and thus producing hemoglobin S (HbS) 15. When deoxygenated, HbS polymerizes, rendering the red cell rigid, viscous, and abnormally adherent to the capillary endothelium. This impedes blood flow in the microcirculation, causing ischemia and microinfarcts that lead to painful crises, cerebrovascular stroke, renal impairment, retinopathy and other end-organ damage. The current scientific literature currently recognizes the contribution of an acquired hypercoagulable state in SCD to vascular pathobiology, chronic organ dysfunction, and mortality.

Sickle Cell Disease

Although sickling disorders occur predominantly in individuals of African descent, the disease is truly global with prevalence in the Mediterranean, Middle East, parts of India, the Caribbean, and South and Central America 16. Worldwide, it is estimated that more than 300,000 children are born with SCD each year 16. However, due to population migration, the clinical management of SCD is now routine medical practice in the United States 17,18. In the US, SCD affects an estimated 100,000 Americans (the vast majority of whom report African descent), and about one in 365 African-American newborns 19. An additional three million individuals heterozygous for the rs334 mutation (HbAS) possess the sickle cell trait (SCT) 20.

Frequent attacks of vaso-occlusive crises (VOC), chronic pain, and end organ damage result in recurrent hospitalization, morbidity and increased health system utilization 21. Premature mortality in SCD is also high, with an age of death estimated to be 33.4 years for SCD males and 36.9 years for SCD females in 2005 20, owing largely to an incompletely characterized small and large vessel "vasculopathy" that affects the heart, lungs, liver, and kidneys 22. Amongst clinical presentations accounting for mortality are severe VOC, acute chest syndrome (ACS), pulmonary hypertension (PH), VTE, asthma, and heart failure 23-27. Approximately 10% of SCD patients have PH, which puts them at a higher two year mortality compared to SCD patients without PH 28-30. A variable proportion of these patients have chronic thromboembolic pulmonary hypertension (CTEPH) as the primary causative factor of their PH 30. SCD patients have a 12% cumulative incidence of VTE, either deep venous thrombosis (DVT) or pulmonary embolism (PE), putting them at a 2 – 3 fold higher mortality compared to patients without VTE 26,31. Associated pulmonary thrombosis is observed in up to 20% cases of ACS, the latter being associated with high mortality 32.

Widespread clinical use of disease modifying therapies such as prophylactic antibiotics in children, hydroxyurea to increase fetal hemoglobin, and simple blood transfusion, coupled with greater acceptance of stem cell transplantation, have led to improved patient survival over the past two decades [33-35]. As a consequence, there is a growing population of older adults with SCD likely to be exposed to age related increased VTE risk [36,37], in addition to the already high risk of VTE in these patients related to their underlying SCD. Even individuals with heterozygous SCT appear to have a higher risk for VTE, specifically pulmonary thrombosis [38,39]. Since African Americans have a higher risk for VTE compared to other ethnic groups in the US [40], it remains to be determined whether this increased risk is solely attributable to HbS. Increased VTE among SCD patients and among individuals with SCT makes studies of venous thrombosis a clinical research priority.

Epidemiology of Venous Thromboembolism in Sickle Cell Disease

The clinical course of SCD is highly variable yet inexorable vascular organ dysfunction stemming from chronic vasculopathy is a hallmark of disease and eventually occurs in most SCD patients. Chronic hemolysis and recurrent vaso-occlusive crises resulting in tissue damage likely play a causal role. The damage inflicted to the vasculature by pathological adherence of sickle red cells provokes inflammation, vasoconstriction, oxidative damage, and a prothrombotic state. Vasculopathy is therefore a key driver of many of the organ complications of SCD.

Thrombotic arterial vasculopathy is historically well recognized in SCD particularly in the context of stroke [41,42], but an appreciation of the role of thrombotic venous vasculopathy in SCD is more recent [1,43]. Clinically, this is manifest as high cumulative VTE incidence ~12% by age 40 which is substantially earlier than in the general population. The more severe genotypes (HbSS and HbSβ^0) and/or patients hospitalized > 3 times a year with a pain crisis are at greatest risk. Short and long term VTE complications include higher mortality, chronic thromboembolic pulmonary hypertension, post phlebitic syndrome, and venous leg ulceration and increased mortality [44,45]. ACS has been shown to be coincident with pulmonary venous thrombosis in up to 20% cases, prompting exploration of the therapeutic benefits of heparin (NCT02580773) [32,46]. VTE incidence is 3 fold higher even among 15-30 year young SCD patients, comparable to the excess thrombosis risk in carriers of the Factor V Leiden mutation, a well-studied inherited prothrombotic state with an increased risk for early VTE [31]. The frequent incidence and recurrence of VTE in SCD patients [2,26,31] suggests a “high risk” prothrombotic state similar in risk to that associated with active cancer [1,47].

Current consensus recommendations from large clinical trials following a first episode of VTE in the general population support anticoagulation treatment for a minimum of 3 months [48]. The risk of VTE recurrence following the discontinuation of anticoagulation, is well recognized [45]. Owing to the complex risk/benefit ratio based on weighing the risk of increased bleeding versus recurrent thromboembolism, extension of anticoagulation beyond the 3-month period in a patient with first VTE is individualized. Amongst individuals with unprovoked VTE or in those with a known hypercoagulable state such as active cancer, the consensus is to extend the duration of anticoagulation indefinitely [49]. Notably, SCD patients were largely excluded from studies that led to these conclusions. There is a lack of robust, externally validated VTE recurrence and/or bleeding scores that might help guide long-term treatment decisions in SCD [1,43,48,49]. The high cumulative incidence of recurrent VTE in SCD (14% at 1 year and over 25 % at 5 years) provide

a strong clinical rationale for extended duration anticoagulation as secondary prophylaxis 2. However, the exact duration for which anticoagulation must be extended is uncertain and the increased risk of bleeding noted in SCD patients while on anticoagulation warrants caution 2. Yet, in the absence of robust evidence, following an initial episode of VTE, indefinitely extended anticoagulation remains a practical choice for many SCD patients and their physicians 1.

There is widespread evidence for coagulation system activation is SCD patients both during VOC and between VOC, while in their usual state of health, i.e., in "steady state" 1. Correlating these biomarkers with clinical evidence for VTE suggests a distinct overlap between SCD and inherited prothrombotic states such as Factor V Leiden. Unfortunately, the absence of clinical or laboratory tests predictive of VTE incidence/recurrence coupled with a higher than average bleeding risk in SCD associated with exposure to traditional anticoagulants like warfarin or agents that target factor X and factor II presents challenges. Novel therapies providing antithrombotic effects without increasing the risk of bleeding are therefore highly desirable. As an example, targeting coagulation factors XI to prevent surgically-provoked VTE in the general population is as efficacious as heparin but is associated with a lower risk of bleeding 50. Targeting thrombo-inflammatory pathways, that are disproportionately activated in SCD patients for e.g., the TF pathway, are highly relevant from this perspective.

Sickle Erythrocytes, Endothelial Inflammation and Thrombosis

Hb-S polymerization, hemolytic anemia, and impaired microcirculatory blood flow from acute vaso-occlusion are central to SCD pathophysiology 51,52. The primary pathophysiological event in SCD is abnormal polymerization of sickle hemoglobin with deoxygenation, which results in structural and functional abnormalities of the red blood cell (RBC). HbS polymerization alters RBC membrane stability, increases RBC-dependent cellular interactions, causes hemolysis, and reduces sickle RBC lifespan 53. Besides, the associated membrane abnormalities permit the loss of ions and water and result in the formation of dense dehydrated RBCs 54. These abnormal RBCs apparently play a central role in disease pathophysiology, contributing to the two principle causal mediators of disease pathology, VOC that mediates ischemia-reperfusion (IR) injury and intravascular hemoglobin release 51,55,56.

The nature of VOC events in the microcirculation described above are complex. Sickle RBCs are unable to easily traverse small capillaries, enhancing the likelihood of adherent interactions between RBCs and the postcapillary endothelial surface via RBC adhesion molecules such as CD36 and integrin a4b1. Frequent sickling and unsickling leads to release of heme loaded RBC derived vesicles 57 that adhere to the endothelium, promote vascular stasis, alter rheology and trigger vascular occlusion in various organ microcirculatory beds 58. Although the events surrounding VOC are only partially understood, its consequence, ischemia/reperfusion injury, is a profoundly proinflammatory 55,59-61.

Free hemoglobin released during hemolysis promotes inflammation by scavenging vascular nitric oxide, activating toll like receptor 4 (TLR4) on both endothelial cells and leucocytes and directly inducing adhesive (VCAM-1, ICAM, and E-selectin) and procoagulant (TF) molecules 5,6,62-64. Moreover, intracellular molecules released during tissue damage e.g., high mobility group box-1 (HMGB1) protein are capable of mediating thrombotic vascular pathobiology in

both SCD and VTE 65-68. Intravascular heme stimulates neutrophils to release extracellular traps in SCD 69 which can further favor thrombosis 70. Heme-bound iron stimulates expression of placental growth factor in erythroid cells 71. This promotes TLR4 signaling in endothelial cells and macrophages, activating NF-k B and further triggers vaso-occlusion through Weibel-Palade body degranulation and adhesion molecule expression in SCD 71,72. Sickled RBCs themselves may also directly stimulate thrombosis, based on recent observations of the association between sickled RBCs and fibrin in loosely compacted venous clots 73.

In summary, Hb-S polymerization results in vascular inflammation that can serve as an important stimulus for TF-driven activation of coagulation and increased thrombogenic risk. Venous thrombosis in SCD is likely mediated by known and unrecognized mechanisms, highlighting the need for additional research to understand key thrombo-inflammatory processes that drive vasculopathy.

Tissue Factor, hypercoagulability and thrombosis in SCD

Overview of the hemostatic system and sickle cell disease

The formation, growth and breakdown of venous thrombi in the vasculature is always in a state of flux between thrombogenic stimuli and protective antithrombotic mechanisms. First identified by Virchow , the triad of thrombogenic stimuli are i) stasis, ii) activation of plasmatic coagulation factors, and iii) endothelial or vessel wall damage, are all present in patients with SCD (Figure 1) 37,74-76. The protective antithrombotic mechanisms preventing coagulation include i) inactivation of activated coagulation factors by circulating inhibitors, ii) clearance of coagulation factors and soluble fibrin polymer complexes by mononuclear phagocytes and hepatocytes, iii) lysis of fibrin by the fibrinolytic system in plasma and endothelial cells. These pathways have almost all been shown to favor thrombosis in SCD patients, as reviewed below 77-79.

Figure 1 Virchows triad in Sickle Cell Disease

Increased tissue factor antigen and pro coagulant activity

TF, the principal trigger of coagulation *in vivo*, has procoagulant and proinflammatory effects by virtue of its ability to signal both intra and extracellularly 80. Although normally separated from contact with the plasma coagulation proteins (FVIIa) by an intact endothelial layer, there is a growing appreciation of the contribution of "blood borne" TF to thrombosis. In SCD patients, blood borne TF is localized to endothelial cells 5, leucocytes 64 and extracellular vesicles (EVs) derived from these cells 81. The number of circulating blood cells and EVs exposing TF increases during an acute SCD crisis when compared to the steady state 5·81. In parallel with cellular and EV TF antigen expression, whole blood TF procoagulant activity is elevated among SCD patients providing a biologically plausible connection between intravascular hemolysis and coagulation 81-84. TF pathway-mediated factor Xa generation and subsequently thrombin (IIa) formation results in intravascular coagulation and eventual formation of an insoluble fibrin. The central role of TF pathway-mediated thrombin generation and fibrin deposition provides the scientific rationale for targeting TF to reduce pathological thrombosis.

Biochemical markers of Factor Xa and thrombin

Thrombin plays dual pro- and anti-inflammatory roles but is amongst the most potent of biological stimulatory molecules for endothelial cells and platelets. Plasma thrombin (evidenced by elevated prothrombin fragment F1.2 [PF1.2], thrombin-antithrombin complexes (TAT), fibrinopeptide A, and fragment E levels) is elevated in SCD patients during acute crises and when patients are in their steady state 1. Increased thrombin generation is also suggested indirectly by elevated factor VII turnover, enhanced platelet activation, and reduced anti-thrombin (AT), proteins S and protein C levels 1. Proinflammatory effects of thrombin result in endothelial release of vasoactive substance von Willebrand Factor (vWF), prostacyclins, plasminogen activator and platelet activating factor, along with increased expression of endothelial adhesion molecule and P-selectin expression, that could contribute to or worsen vaso-occlusion. The prothrombotic cycle is completed by thrombin mediated platelet activation and subsequent platelet release reactions that accelerate and propagate coagulation.

Evidence for Hemostatic perturbation from murine models of SCD

Limitations notwithstanding, murine models provide insights into the complex vascular pathobiology of SCD highlighting in particular the involvement of red cell sickling, inflammation, adhesive events, coagulation, vascular stasis, reperfusion injury, deficient nitric oxide (NO) bioavailability and oxidative biochemistry 85-90. Clearly, the proinflammatory SCD milieu through distinctive yet intersecting pathways cooperatively recruits coagulation molecules (TF and thrombin), cellular components of blood (leucocytes, endothelial cells, and platelets) and extracellular vesicles to promote stasis, thrombosis and vasculopathy 58·91·92. That TF is a key player in this pathophysiology is apparent from studies in which mice engineered to express low TF levels (1%) and transplanted with HbSS bone marrow stem cells demonstrated a reduction in vascular inflammation, endothelial cell injury, and cellular stasis 93·94. Moreover, its capacity to incite organ specific thrombotic pathophysiology is demonstrated by higher endothelial luminal surface TF expression in pulmonary veins of Berkeley SS mice unrelated to dietary fat intake 95. Subjecting such mice to transient hypoxia followed by reoxygenation

upregulates pulmonary vein TF expression above the baseline, a phenomenon reversible by lovastatin 95. Since thrombo-inflammatory pathology in SCD appears to be mediated by abnormal TF expression and procoagulant activity, lowering TF expression or reducing its procoagulant activity would be an attractive strategy to pursue to prevent VTE in SCD patients.

Endothelial – Leukocyte – Platelet mediated inflammation in SCD

Recurrent adhesive events and VOC leave the cellular components of blood and vascular endothelium in a state of constant activation. Between crises, when patients report themselves to be in "steady state", their baseline biochemical inflammation markers remain abnormal supporting the notion of a persistent chronic inflammatory state. Activation of vascular endothelial cells itself seems necessary but not sufficient to mediate vaso-occlusion. Sickle erythrocytes are key contributors to the occlusion of smaller vessels whereas in post capillary venules, it is possible that activated, adherent leukocytes are likely drivers of vaso-occlusion 96. Additional factors favoring a prothrombotic state include decreased thrombomodulin, TF pathway inhibitor, and increased release of von Willebrand factor. Lastly, in addition to inducing sickling, hypoxia promotes development of a highly prothrombotic proangiogenic microvascular environment favoring fibrin deposition, stasis and ultimately vascular thrombus formation 97,98.

P-selectin found in storage granules of resting endothelial cells and platelets is rapidly transferred to the cell membrane during inflammation and cellular activation. Endothelial surface P-selectin expression mediates abnormal rolling and static adhesion of sickle erythrocytes *in vitro* 99-101 and prompt adhesion of sickle erythrocytes to vascular endothelial cells followed by vascular occlusion in transgenic mice with SCD 90,101-103. Importantly, SCD mice deficient in P- and E-selectin demonstrate defects in vessel wall leukocyte recruitment and are protected from vaso-occlusion 101,102,104. In transgenic mice expressing human HbS, adherence of sickle erythrocytes and leukocyte to the endothelium is substantially reduced by pharmacological P-selectin blockade 101,105. P-selectin also drives TLR-4/heme dependent complement mediated endothelial attack that contributes to vessel wall inflammation in several hemolytic disorders including SCD 106.

In humans, P-selectin shedding from vascular cells is increased in cardiovascular disease, sepsis, and cancer and its levels in some situations are predictive of venous thromboembolic events 107. In a prospective study of 687 cancer patients followed for a median of 415 days, the risk of VTE was 2.6 (95% CI, 1.4-4.8) times higher among those with levels higher than the 75th percentile compared with cancer patients with levels below the upper quartile 108. The cumulative probability of developing VTE 6 months after study inclusion was 11.9% in patients with elevated soluble P-selectin levels and 3.7% in those with lower levels 108. Soluble P-selectin levels predict VTE recurrence risk but whether they have causal relevance in venous thrombosis remains to be elucidated 107,109. Besides, while soluble P-selectin levels are elevated in SCD patients 110, no studies have evaluated their utility in predicting VTE. Selectins mediate adhesive cell-cell interactions and in SCD patients favor the formation of hetero-cellular aggregates that alter vascular rheology 111,112. A clinical trial of an anti P-selectin antibody targeting the P-selectin/PSGL-1 interaction reduced sickle cell crisis rates by 45.3% compared with placebo, simultaneously prolonging the time to the first and second crises 4. As a

consequence, a phase 3 study of this agent is ongoing (NCT03814746) and several other selectin antagonists are in advanced phase clinical trials (NCT02433158).

Regulatory control of Tissue Factor

TF is the principal initiator of coagulation *in vivo* 80. TF binding to plasma FVIIa, leads to formation of a TF–FVIIa complex that triggers the coagulation cascade by activating both FIX and FX. Thus, in the interest of preventing catastrophic intravascular coagulation, the genetic and posttranslational regulation of TF is tightly controlled 113,114. In the healthy state, TF expression is limited primarily to perivascular and epithelial cells, but inflammatory conditions induce TF expression in circulating monocytes or vascular endothelial cells, by activation of TF gene transcription. In sepsis, monocyte TF expression leads to disseminated intravascular coagulation whereas surface TF expression by tumor endothelium and subsequent release of TF^+ vesicles favors venous thromboembolism 115,116. Irrespective of its cellular source and whether it is induced or expressed constitutively, most cell surface expressed TF is in an 'encrypted' inactive confirmation and demonstrates little or no pro-coagulant activity 114.

Current literature supports the notion that low levels of TF are measurable in blood, so called "blood-borne TF" 80,117. However, blood-borne TF is either fully encrypted or inactivated by plasma inhibitors, i.e., tissue factor pathway inhibitor (TFPI). Among several proposed mechanisms to explain TF decryption, externalization of phosphatidyl serine (PS) to the outer leaflet of plasma membrane and oxidation of a TF disulfide bond appear most plausible 118,119. In support of the former are *in vitro* studies that require TF association with phospholipids for expression of its procoagulant activity, with anionic phospholipids, such as PS, in the phospholipid mixture markedly increasing that activity 118,120. Due to their high surface PS TF^+ EVs do not appear to require decryption to exhibit pro coagulant activity.

The observations that cell surface expressed TF contained free thiols, and that cellular activation with ionomycin or HgCl2 depleted free thiols in TF led to the suggestion that Cys186-Cys209 disulfide bond formation changes the conformation of TF facilitating the binding of substrates FIX and FX 121. Support for this hypothesis is the observation that mutagenesis of Cys186 and Cys209 residues which prevents the formation of a Cys186-Cys209 disulfide bond, severely impairs TF pro coagulant activity in several cell types 11,122. Biochemical studies using purified placental TF show that reduction of the allosteric disulfide bond in TF does not affect TF binding to FVIIa but results in a complete loss of TF co-factor function 123.

Oxidation of an allosteric disulfide bond of TF to regulate TF pro coagulant activity is likely achieved by disulfide regulatory switching 119,124, a function exhibited by protein disulfide isomerase (PDI, see section 2.7) 119,125. PDI associated with the extracellular domain of TF on the surface of keratinocytes maintains TF in a low pro-coagulant state, supporting its role as a regulator of TF 11. In vivo studies using a murine injury induced thrombosis model revealed that inhibition of PDI released by adherent platelets and disrupted vessel wall cells at the injury site decreased TF triggered fibrin deposition 12. In another murine vascular thrombosis model, time-dependent increases in PDI were observed in vessel thrombi and PDI inhibition (using either bacitracin or a function-blocking mAb) abrogated thrombus/fibrin formation 10,126. These studies suggest that PDI plays a key role in regulating TF mediated thrombin generation at the site of vascular injury.

Vascular Protein Disulfide Isomerase: thrombosis and tissue factor regulation

Thiol isomerases contain a thioredoxin-like domain and function as reductases, oxidases, or isomerases. The archetypical and most abundant thiol isomerase PDI, is a 57 kDa thiol oxidoreductase isolated mainly from cellular endoplasmic reticulum (ER) where it possesses chaperone and protein folding functions. PDI is also located at the surface of the plasma membranes of platelets, endothelial cells and the vessel wall where it can act on extracellular substrates involved in thrombosis, specifically integrins 125,127. Vascular wall injury prompts PDI release from disrupted endothelium and bound platelets. PDI at the cell surface promotes thrombosis via disulfide modification of covalently-bound substrates, specifically thrombospondin, vitronectin and TF decryption 125,128,129. PDI's role in thrombus formation has become evident from several murine vascular thrombosis models 12,126. The hypothesis that plasma PDI acts on intravascular coagulation protein substrates to prime thrombus formation following vessel injury or activation is therefore consistent with current observations. Global inhibition of vascular thiol isomerases interferes with this "priming" of molecules involved in coagulation and blocks thrombus formation in animal models.

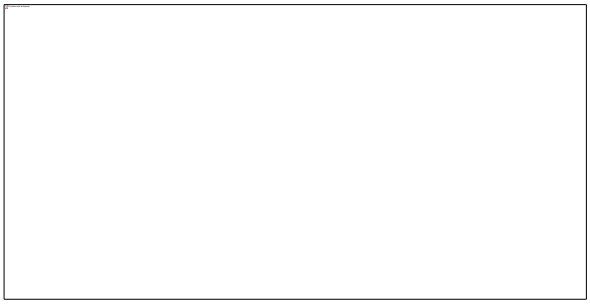


Figure 2. Surface PDI in RBCs membranes. Figure taken from Prado et al. 2013.

By immunoblotting, cell surface associated PDI on sickle RBCs is present in higher concentrations compared to control RBCs (Figure 2) 7. We independently confirmed these data in our laboratory (results not shown). It is therefore possible that plasma PDI activity in SCD is cell surface associated, a location where it could promote thrombosis via disulfide modification and activation of blood borne tissue factor. The origin of RBC surface PDI in SCD is uncertain, but is very likely transferred from endothelial cells, following inflammation and injury (see section 2.3). Further support for PDI as a therapeutic target in SCD comes from the observation that plasma PDI activity is elevated in Townes SS mice 7,13. Pretreatment of these mice with a flavonoid inhibitor of plasma PDI activity reduced TNF alpha mediated vascular-occlusion and laser vascular injury mediated thrombosis 13.

In contrast to the abundance of literature on intracellular thiol isomerases, very little information is available on plasma vascular thiol isomerases in humans. Plasma PDI activity is measurable in healthy individuals (median=330 pg/mL) with low intra-individual variability over time 130. Despite a lack of clarity pertaining to the repertoire of vascular thiol isomerases that participate in thrombosis, trials of agents that target these molecules in humans are ongoing. However, safety features of the flavonoid agents in human clinical trials to date 9,14 suggest that these are valid candidates with which to modulate the thrombo-inflammatory axis in SCD. Evaluating PDI

inhibition with Isoquercetin as a means to prevent thrombosis in SCD is a novel therapeutic approach.

Isoquercetin inhibits PDI and lowers P-selectin

Flavonoids quercetins are present in food (fruit and vegetables), and epidemiologic studies of diets rich in these substances point to potential cardiovascular benefits, specifically decreased mortality secondary to myocardial infarction 131. Quercetin a small molecule flavonoid inhibits the enzymatic activity of PDI by occupying its substrate-binding pocket 132. Structure-activity relationship assays showed that all quercetins tested that possessed a glycoside at the third position on the C ring inhibited PDI, including IQ and quercetin-3-rutinoside 133,134. IQ (also known as quercetin-3-glucoside), has a similar structure and PDI inhibitory activity to quercetin but demonstrates superior bioavailability in humans134. In healthy volunteers, within 2 hours of administration of 1,000 mg oral IQ, reduced plasma PDI activity by 38% compared with activity observed in pre IQ ingestion plasma (see figure 3 panel A) 9. Peak plasma IQ concentration correlated with peak plasma PDI inhibition when Isoquercetin concentrations were above 4 µM (see figure 3 panel B), achieving a median PDI inhibition of 0.69 U/ml 9. In cancer patients, 2 months of daily 1000 mg oral IQ exposure resulted in a median change in PDI inhibitory activity of >70 % and a median 58 % reduction in soluble P-selectin 14.

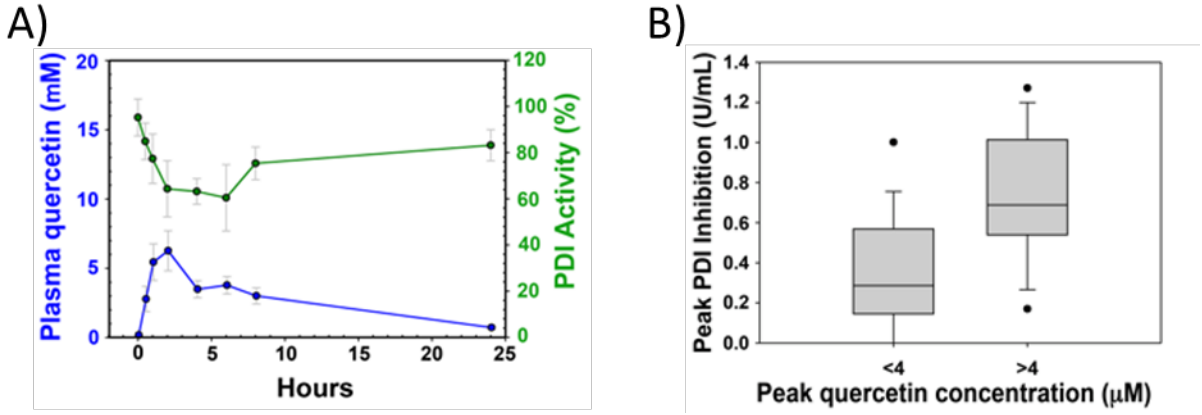


Figure 3: Isoquercetin inhibits PDI activity in plasma. A) Comparison of PDI inhibition and quercetin levels following administration of Isoquercetin to healthy adults. B) Box-and-whisker plot of peak PDI inhibition at peak quercetin concentrations above or below 4 µM. Figures taken from Stopa *et al* 2017.

Preliminary Data:

The primary endpoint of this study is to evaluate the effect of IQ on soluble P-selectin, a plasma biomarker that predicts future risk of VTE. Our cross-sectional observations (AS Shet unpublished data) and published studies demonstrate elevated soluble P-selectin in SCD patients when compared to ethnic matched healthy controls 135,136. Moreover, limited data is available on the variability of soluble P-selectin in SCD patients sampled over different time points. We therefore determined the variability of soluble P-selectin using stored plasma obtained from SCD subjects enrolled on protocol 17-H-0056. Subjects enrolled in this study were sampled at baseline during "steady state" when they self-reported to be in their usual state of health and were at least 8 weeks

remote from experiencing an acute painful crisis. Samples were also collected at the end of study when patients were at least 8 weeks remote from experiencing an acute painful crisis or blood transfusion. Blood samples obtained by routine phlebotomy were anticoagulated in sodium citrate containing tubes and processed within 2 hours of collection to prepare platelet-free-plasma in a two-step centrifugation technique. Supernatant plasma thus obtained was stored at −80°C until analysis using a commercially available ELISA method to detect soluble P-selectin.

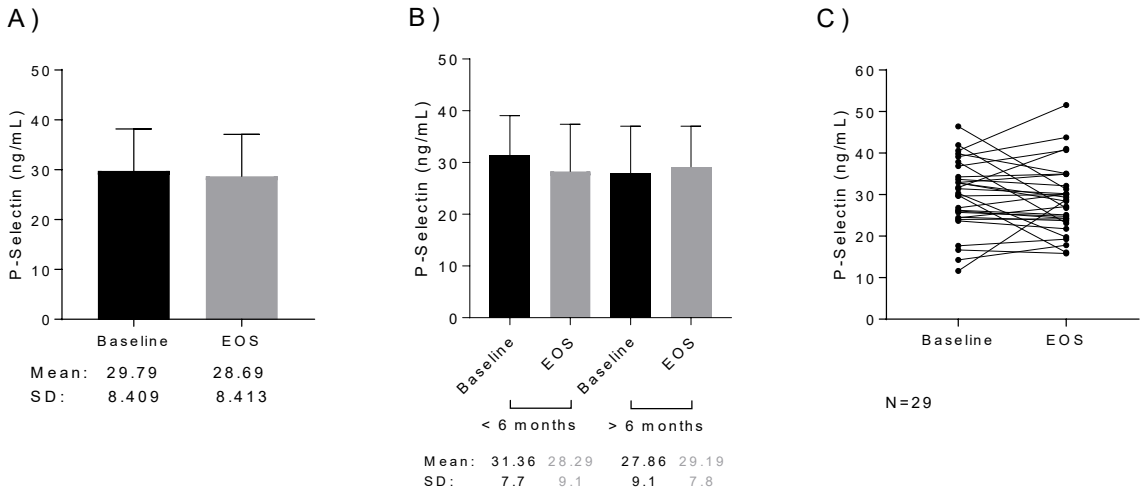


Figure 4. A) P-Selectin measurements at Baseline and End of Study (EOS) stages in patients with SCD, B) P-selectin measurement comparison between two different groups of SCD patients: < 6 months between Baseline and EOS sampling (n=16) and >6 months between Baseline and EOS sampling (n=13) C) Paired test between the amount of P-Selectin at Baseline and EOS sampling. No significant differences were observed, Wilcoxon test, *P* value > 0.05, N=29.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

Quercetin is commonly consumed by humans as it is naturally present in a wide variety of fruits and vegetables, especially apples and onions. According to Querces Pharmaceuticals AG documentation, the background consumption of quercetin from naturally occurring sources is 5.9 mg/p/d (0.1 mg/kg bw/d) and 14.7 mg/p/d (0.25 mg/kg bw/d) at the mean and 90th percentile, respectively, for the total U.S. population, with a maximum intake value of 258 mg/p/d (4.3 mg/kg bw/d).

There is extensive literature on the beneficial effects of Quercetin in animal models, and multiple lines of non-clinical data suggest that it should be well tolerated in human subjects. Moreover, published and unpublished information support the safety of quercetin including absorption, distribution, metabolism, and elimination (ADME) studies; acute, subchronic, and chronic toxicity studies; carcinogenicity, genotoxicity, and reproductive/developmental toxicity studies; pharmacokinetic studies; and finally, human clinical and epidemiological studies. From all of the available toxicology studies in multiple species, there is an apparent lack of significant adverse systemic toxicity. The overall lack of *in vivo* toxicity of quercetin is consistent with its known metabolic fate; specifically, its extensive microbial degradation in the gastrointestinal tract of both animals and humans, followed by methylation, oxidation and conjugative metabolic processes that occur in the liver and kidneys and eventual elimination through the bile, feces, and urine. A two-year toxicity and carcinogenicity study in rats by the National Toxicology Program (NTP) was pivotal in establishing safety of this agent and the no observed adverse effect level for this study was approximately 2200 mg/kg bw/d (highest dose tested) [137].

Published human clinical studies, including a study with levels up to 1000 mg/d (16 mg/kg bw/d) for 12 weeks, showed that quercetin supplementation was safe and did not majorly influence on several measures of oxidative stress and antioxidant capacity [138]. The weight-of-the-evidence from toxicological safety studies, human clinical studies corroborating epidemiological studies, together with recent human pharmacokinetic studies, demonstrates that quercetin is safe for intended use as a supplement. Based on the information provided by Querces AG, as well as other information available to FDA, the agency accepted the conclusion that quercetin is generally recognized as safe (GRAS) under the intended conditions of use.

Isoquercetin (IQ), a flavonoid quercetin, has also completed clinical trials in healthy individuals and cancer patients [9,14]. Adverse effects reported in these trials include gastrointestinal effects such as nausea, reflux, constipation, diarrhea and rare reports of headache and mild tingling of the extremities. In the Phase 2 study conducted in cancer patients, two patients had epistaxis and mild bruising (both grade 1) that were possibly related to IQ [14]. There has been no grade 3 and grade 4 toxicity associated with IQ treatment in either of these two published human studies.

In summary, careful analysis of the available information on IQ does not reveal any potential serious toxicity that would preclude its use in SCD subjects. Given the safety and tolerability of IQ in published human studies[9,138], and the favorable experience with IQ in a published Phase

II study in cancer patients14 discussed in Background section, we anticipate that testing an identical dose of IQ to that currently investigated in other active clinical studies would not pose any undue risk in SCD patients.

The only tests and procedures done under this study are blood draws, peripheral arterial tonometry and urine collection. The individual study procedure risks are listed below.

Peripheral Arterial Tonometry (PAT) (ENDO-PAT 2000, Itamar Medical):

The use of peripheral arterial tonometry is not associated with any known adverse effects. After the procedure, the patient may experience bruising due to microcapillary bursts in their arm – these marks should disappear within a week.

Phlebotomy: Standard precautions for obtaining human blood samples will be taken. Transient discomfort and minor bruising may occur at the phlebotomy site. Vasovagal symptoms can occur during blood drawing. Blood samples will be obtained by venipuncture. The quantities of blood to be drawn for research purposes will be less than 550
ml, which is consistent with the CC policy as provided in Medical Administrative Series (MAS) 95-9 (revised 5/29/12): for adults, no more than 10.5 mL/kg/ or 550 mL (whichever is smaller) will be drawn for research purposes over any 8 week period.

Once blood samples will be drawn, the samples designated for platelet free plasma (PFP) preparation (Biochemicals studies, coagulation studies, contact pathway), and platelet aggregation assays should be processed within 2 hours after phlebotomy. The samples designated for red blood cell studies and leukocytes isolationcan be processed greater than 2 hours after phlebotomy. .

Urine Collection: There are no known risks to those collections.

Near infrared spectroscopy (NIRS): In general, commercial NIR tissue oximetry devices that use similar light energy levels have been cleared by the FDA and are classified as "minimal risk." The current research DOS instrument is a type of NIR oximetry instrument that employs comparable levels of NIR light to conventional FDA-cleared clinical instruments. Although DOS has advanced capabilities for quantitative tissue measurements that make it a research tool, the safety/effectiveness of the device is NOT being evaluated in this study nor will the data generated be used to support an FDA application for research or a marketing permit.

There are no risks associated with the use of near infrared light as it has been described in this protocol. The amount of optical energy is too low to cause appreciable tissue heating. Temporary pain or paresthesias may occur during the approximately 3-5 minute blood pressure cuff inflation. The vascular occlusion challenge may also result in redness or minor bruising of the limb due to cuff inflation. This occlusion is a standard test that is performed in many hemodynamic assessments, such as in the case of peripheral vascular disease. At the NIH Clinical Center, previous studies (including 07-H-0196 and 12-H-0101) have used 5-minute brachial artery occlusions in combination with non-invasive imaging modalities without reporting significant safety issues. Subjects will be monitored closely for any concerning signs and symptoms during the occlusion challenge, and if there are any concerns for safety or if subjects experience significant discomfort, the occlusions will be released immediately.

2.3.2 Known Potential Benefits

In this supplement study, the prospect of direct benefit is modest. Clinical benefit relates to a lowered risk for thrombogenesis if soluble P-selectin and tissue factor activity are reduced as hypothesized. These benefits are more likely to be seen in those patients with VTE or are at greatest risk for developing VTE.

2.3.3 Assessment of Potential Risks and Benefits

The risks of the study supplement and the minor procedures included in this protocol are minimal and justified by the potential benefit of expanding our understanding of how flavonoid anti-oxidants may play a role in the thrombotic vascular pathobiology of SCD and at the same time employing strategies to minimize the bleeding risk posed by traditional anticoagulation treatments. This supplement has been employed at the same dose proposed in this study without any reported adverse effects.

3 OBJECTIVES AND ENDPOINTS

Table:1

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Evaluate the effect of IQ on thrombo-inflammatory markers	The primary outcome will be the change in the plasma soluble P-selectin level comparing the baseline to IQ response.	IQ blunts soluble P-selectin and SCD subjects exhibit elevated soluble P-selectin levels, which is a consistent inflammatory signature of this disease.
Exploratory		
Explore whether other thrombo-inflammatory biomarkers linked to steady state SCD are affected by administration of IQ.	1) Compare baseline and end of study plasma protein disulfide isomerase activity 2) Quantify baseline and end of study plasma tissue factor positive extracellular vesicle number 3) Compare baseline and end of study tissue factor procoagulant activity 4) Compare baseline and end of study platelet thrombin generation 5) Compare baseline and end of study inflammatory cytokines 6) Compare baseline and end of study plasma D-Dimer, TAT and PF1.2 7) Compare contemporary biomarkers of vascular function and atherothrombosis obtained at baseline and end of study 8) Assess safety and tolerability of IQ 9) Assess adherence to oral IQ	The primary driver of hypercoagulability appears to be endothelial and monocyte derived tissue factor in SCD, however, other cells (platelets and sRBCs) are linked to thrombo-inflammatory signals by the innate immune system and complement that can trigger proinflammatory pathways. We will explore the effect of IQ on modulating the intersecting pathways of inflammation and coagulation as exploratory end points. Simultaneously, we will evaluate safety of intervention and adherence.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This study is a prospective, randomized, double-blind, parallel group, placebo-controlled study where the thrombo-inflammatory profile of steady state SCD subjects will be studied at baseline and following 4 weeks of treatment with IQ or placebo. The allocation of IQ: placebo will be 1:1.

The screening visit will be used to determine initial eligibility to the protocol. Final subject eligibility will be confirmed on day 0 just prior to receipt of IQ or placebo. day 0 will be adjusted if any changes to patient eligibility occur after screening. If there is a change in eligibility and the subject will not meet the requirements within 90 days of the screening visit, the subject will be removed from study and may rescreen if/when eligibility criteria is met.

All subjects who have completed screening and have met all inclusion criteria and do not have any exclusion criteria on Day 0 of the start of the study drug will receive 28 days + 7days of IQ or placebo. Research parameters at the end of the study will be compared to baseline to determine the effects of this intervention. Research blood samples and atherothrombotic risk measures to monitor primary and exploratory study end points will be obtained during baseline after enrollment (Day 0, visit #2) and after 4 weeks exposure to IQ/placebo (D28, visit #3).

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SCD patients will be defined as being in the "steady state" if they report clinical symptoms to be their baseline and are at least 60 days remote from an acute "crisis". Adapting from the Multicenter Study of Hydroxyurea trial 139, a crisis will consist of "a visit to a medical facility for acute pain that has no evident cause other than SCD, for which the patient requires hospital attention, either as in- or out-patient, and is treated with parenteral narcotics". Patients receiving hydroxyurea as disease modifying therapy must have been on the same dose for at least 90 days to be defined as "stable".

To adhere to the intention-to-treat principle, for every patient that is randomized, we will attempt to obtain 28-day endpoint measurements despite occurrence of expected and/or unexpected events due to the underlying disease that may influence biomarker end points. Therefore, in the event of their occurrence, clinical SCD related complications will be handled as described below:

- Study participants experiencing "acute pain crisis" < 7 days of initiating drug or placebo will be instructed to discontinue their study drug. These participants will not resume study drug but the research team will attempt to obtain their 28-day endpoint measurement.

  Study participants experiencing stroke or acute chest syndrome requiring exchange transfusion during the 28-day study drug treatment window will discontinue study drug. These participants will not resume study drug but the research team will attempt to obtain their 28-day endpoint measurement.
- Study participants experiencing an "acute pain crisis" between 7 - 28 days of treatment with either drug or placebo will continue treatment and complete end of study activities according to the protocol.

- Study participants experiencing VTE or an acute febrile illness (fever 101°F, requiring admission to the hospital and IV antibiotic treatment) and/or require simple transfusion during the 28-day study drug will continue study drug treatment and complete end of study activities according to the protocol.

- At any time, the investigator may suspend the study medication for reasons related to safety or tolerability.

- For patients who are unable to provide a 28-day end of study measurement see section 9.4.6.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This is a pilot phase II study and the number of subjects was chosen to provide good statistical power to detect a 25-30% reduction in plasma soluble P-selectin, a clinically meaningful thrombo-prophylactic benefit, similar to that observed in cancer patients (see power analysis in section 9). Such an effect would simultaneously offer the potential to explore the effects of PDI inhibition on post-translational regulatory control of TF. A parallel group double blind placebo-controlled study would be the most effective study design in which to enroll and complete the subject group in the proposed protocol with minimal risk for study withdrawal due to frequent acute painful crises.

4.3 JUSTIFICATION FOR DOSE

Investigators at Harvard University, Massachusetts previously reported that oral administration of 1000 mg IQ once daily to healthy adults, was well tolerated, readily absorbed, and detectable in human plasma [the measured peak plasma quercetin concentration (9.2 μM) exceeded its IC50 for inhibition of PDI by IQ *in vitro* (2.5 ± 0.4 μM)]9. The same group of investigators recently published a phase II clinical trial of IQ at two doses (500 mg and 1000 mg once daily) to evaluate its effects on preventing thrombosis in cancer patients14. In this study, IQ (1000 mg/day) achieved optimal plasma PDI inhibition (73.3% median change in PDI inhibitory activity) and lowered soluble P-selectin levels (-58% % median change, P <0.001), with no detectable toxicity. However, the 500-mg dose failed to achieve a significant reduction in soluble P-selectin in cancer patients (-0.3%, P=0.26)14. Taken together, these data suggest that 1000 mg of IQ once daily is an optimal starting point to evaluate restoration of perturbed thrombo-inflammatory processes in SCD patients.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

For enrollment onto the active phase of the study (IQ supplement vs placebo), subjects must meet all of the following criteria during the screening period (visit #1) which can last from 0-28 days prior to start of study intervention:

5.1.1 Unequivocal diagnosis of sickle cell anemia (HbSS or HbSß^{0}thal or HbSß$^+$thal or HbSC) confirmed by hemoglobin electrophoresis performed on patients at least 90 days after a blood transfusion if previously transfused, or DNA genotyping.

5.1.2 Age 18-70 years old

5.1.3 Steady state SCD (no acute vaso-occlusive crisis within 60 days of D0 of the study) and if on HU therapy, on an optimized dose for at least 30 days. For those newly initiated on HU therapy, the dose should be unchanged for at least 90 days

5.1.4 Be willing to comply with all study procedures for the duration of the study.

5.1.5 Have provided signed written informed consent prior to performing any study procedure, including screening procedures.

5.2 EXCLUSION CRITERIA

Subjects who meet any of the following criteria during screening will not receive the study intervention and will be counted toward study accrual. Screen failures will not be included in the analysis for statistical purposes:

5.2.1 SCD with a recent VOC (<60 days from D0 of study).

5.2.2 SCD with history of recent blood transfusion (<60 days from D0 of study) or exchange transfusion (<90 days from D0 of study).

5.2.3 SCD with a recent VTE (within 90 days of diagnosis of either DVT, PE or both).

5.2.4 Any patient receiving crizanlizumab therapy for SCD or that has received crizanlizumab within the past 30 days of D0 of study.

5.2.5 Have a significant medical condition that confers an unacceptable risk to participating in the study, and/or that could confound the interpretation of the study data. Such significant medical conditions include, but are not limited to the following:

a. History of recent (within 3 months prior to signing informed consent) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke.

b. Active infection requiring the use of parenteral antimicrobial agents or Grade ≥3 in severity (per National Cancer Institute Common Terminology Criteria for Adverse Events v5.0) within 2 months prior to the first dose of study drug.

c. Active viral infection as evidenced by testing positive for hepatitis B surface antigen or hepatitis C virus (HCV) antibody (Ab) with signs of active hepatitis B or C virus infection. If the subject is positive for HCV Ab, a reverse transcriptase-polymerase chain reaction test will be conducted. Subjects with hepatitis C may be rescreened after receiving appropriate hepatitis C treatment.

d. Testing positive for human immunodeficiency virus (HIV) 1 or 2 Ab with evidence for ongoing active infection (i.e., CD4 count <400/μL and viral load >100,000 copies/mL) on antiretroviral therapy.

e. Active acute inflammatory disorders rheumatoid arthritis or systemic lupus erythematosus on disease modifying therapy.

f. Diabetes mellitus judged to be under poor control by the Investigator evidenced by a single fasting sugar value >250 gm/dl or requiring >3 antidiabetic agents, including insulin (all insulins are considered 1 agent); use of insulin per se is not exclusionary.

g. History of any primary malignancy, with the exception of curatively treated nonmelanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent, no known active disease present, and no treatment administered during the last 3 years.

h. Any injury or medical condition that, in the judgement of the Investigator.would prevent the subject from participating in the study

5.2.6 Have a prior bone marrow or stem cell transplant.

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

Vulnerable subjects will not be included in this study.

5.4 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Refrain from making major changes in dietary intake or physical activity during the 6 weeks of active study participation.

5.5 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention. These subjects will sign consent and will receive a study ID.

Individuals who do not meet criteria for participation based on an abnormal clinical history or laboratory test result may be rescreened if warranted, with assignment of a new participant ID number from the initial screening.

5.6 STRATEGIES FOR RECRUITMENT AND RETENTION

Subjects with steady state sickle cell disease will be recruited from the sickle cell clinic at the NIH CC. We will also recruit subjects through NIH using traditional recruitment methods such as referrals from other protocols, outside physician referral and self-referral.

The study may opt to use the following strategies for recruitment of patients:

- ClinicalTrials.gov website
- Clinical Center Research Studies ("Search the Studies") website
- Sickle Cell Anemia Research Fund website
- National Heart, Lung and Blood Institute (NHLBI) patient recruitment website
- Twitter messages and chats with study investigators
- Facebook Posts
- Google AdWords
- Use of Clinical Center Office of Patient Recruitment services including creation and distribution of study flyers and information through pre-existing recruitment avenues such as the NIH recruitment listserv.

The DIR Patient Recruitment Office (PRO) will work with study investigators to ensure accrual goals are being met. All recruitment materials and tools will use IRB-approved language and information to include standard recruitment contacts.

We expect to screen up to 60 subjects to enroll 46 eligible study participants.

5.6.1 Costs

There will be no anticipated costs that the subject will be responsible for.

5.6.2 Compensation

Subjects will be compensated for some of the procedures that are performed at the NIH since they may not provide direct benefit to the subject. They will be compensated by check or direct deposit at the end of the study or at the time of study withdrawal for the procedures completed. Payment will typically be received within 2 months of the last study visit.

Table : 2

Procedures	**Inconvenience Units**	**Compensation per procedure**	**Frequency**	**Total Compensation**
Outpatient visit ($20 first hour, $10 each additional hour)	4 hrs	$50	3	$150
Medical History and physical	2.5	$35	3	$105
Research Blood Draw	6	$60	2	$120
Labs	5	$50	2	$100
ENDO-PAT (optional)	1	$50	2	$100
NIRS (optional)	1	$50	2	$100
24-hour Dietary Recall	1	$15	3	$45
Phone call	1	$10	1	$10
Compliance incentive ($200) All				$200
		Total Compensation:		$ 930

Reimbursement for local travel will be provided. We will reimburse for car mileage up to 30 miles each way. We will also reimburse for train and bus.

Reimbursement for food and lodging will not be provided.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS(S) ADMINISTRATION

6.1.1 Study Intervention Description

The study will use the dietary supplement IQ or a placebo capsule. IQ is a derivative of the commercially available form of flavonoid Quercetins, small-molecule inhibitors of PDI that block thrombus formation. Considering that the clinical investigation is designed to study the relationship between a dietary supplement's effect on structure or function in humans or to characterize the mechanism by which a dietary supplement acts to maintain such structure or function, this study would not need to be conducted under an IND. However, the IQ formulation proposed for use is not marketed in the United States and is manufactured overseas by Querces AG. Moreover, the dose proposed for the clinical investigation (1000 mg) is higher than the dose for which it is marketed as a dietary supplement (250 mg). Thus, this study will be conducted under an IND.

6.1.2 Dosing and Administration

Subjects will take four capsules by mouth (250 mg IQ or identically formulated placebo) once daily for a total of 4 weeks (+ up to 7 days if needed).

6.1.3 Dose Escalation

N/A

6.1.4 Dose Limiting Toxicity

N/A

6.1.5 Dose Modifications

IQ is not expected to result in any protocol-related SAEs. Therefore, no dose modifications are planned.

6.1.6 Drug Administration

Subjects will take four capsules by mouth (250 mg IQ or identically formulated placebo) once daily for a total of 28 days (+7 days if needed). Capsules must be taken whole and may be taken with or without food. The capsules should be taken at a fixed time in the morning (e.g. 9.00AM according to subject convenience). If a dose is missed and it is more than 6 hours before the next dose is due, then the dose can be taken. If a dose is missed and it is less than 6 hours before the dose is due, then the dose should be skipped.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

Both IQ and placebo will be obtained from Querces AG. Product will be stored and dispensed by the NIH CC Pharmacy. Unused product will be returned to the clinical center and destroyed.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Common name: Isoquercetin
Product name: Isoquercetin
Chemical name: Quercetin-3-glucoside
Daily dose: 1000 mg x 4 wk
Route of administration: oral
Dosing instructions: 1000 mg (4 capsules of 250 mg) once daily
Supply: Supplements will be obtained from Querces AG.
Toxicology: None known
Drug Interactions: None known

The capsules will be put into individual bottles, labeled and dispensed by the NIH Clinical Center pharmacy. The label will include at a minimum the study ID and instructions for taking the supplement or placebo.

Each 250-mg capsule contains ascorbic acid (62 mg) and niacin (5 mg) to prevent oxidation during storage and has previously been shown to not influence IQ pharmacokinetics.

The placebo will be manufactured and supplied by Querces AG to match the active supplement and additives.

6.2.3 Product Storage and Stability

IQ and placebo will be stored in the pharmacy at room temperature in light restricted containers.

6.2.4 Preparation

N/A

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Patients will be randomized in a block randomization scheme with block sizes of 2 and 4 randomly generated by an unblinded study statistician who is not the primary statistician for the study. In this double-blind study, the clinical study team and primary statistician will be blinded to the randomization assignments until enrollment is complete and the assays performed for assessment of the primary endpoint. Isoquercetin and placebo will be supplied in identical formulations to allow blinded administration.

The investigator will not be provided with randomization codes. The codes will be maintained with the NIH Pharmacy, which has the functionality to allow the investigator the break the blinded allocation for an individual subject.

Under normal circumstances, the blind should not be broken until completion of the study or if the Independent Data Safety Committee Board (DSMB) makes a recommendation for unblinding that is accepted by the PI.

6.4 STUDY INTERVENTION ADHERENCE

Study capsule adherence will be monitored using patient report in the form of a capsule diary and capsule counts performed by the research team during Visit 3.

6.5 CONCOMITANT THERAPY

Excepting folic acid, subjects should not take any other multivitamins or supplements for the duration of the study.

It is recommended patients take folic acid, at least 1mg PO daily while taking the study supplement, IQ. For patients in which folic acid is not part of their SCD management, a 30-day supply of 1 mg of folic acid will be prescribed, to be taken once daily. Upon study enrolment and randomization, subjects will initiate folic acid with IQ and discontinue it at the end of the study. Subject will be asked if they were adherent to folic acid but a formal pill count will not be performed.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

The supplement has a good safety record and we do not expect any significant adverse events. A DSMB will evaluate safety trial data based on the below triggers and if warranted, may recommend modification or even termination of this protocol. Stopping rules follow a Bayesian design. The stopping rule is designed to halt enrollment for further evaluation of study data by the safety committee and determination whether enrollment can continue, or the study should be modified or terminated. A Bayesian posterior probability will be calculated to determine the likelihood the probability of having a qualifying adverse event is 25% or more.

Qualifying AEs/SAEs for the stopping rule will be counted only in the Isoquercetin arm (and not in the placebo arm), will be monitored for reaching the stopping boundary by the unblinded statistician, and are any of the following three types of events:

- Any Grade ≥3 treatment related SAE (these include acute painful crises that are probably related to the intervention),
- Grade 4 bleeding, related to Isoquercetin ,
- Any non-hematological AE Grade ≥ 3 related to IQ

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

1) Subject taking less than 75% of their capsules
2) Subject found to be pregnant or wishes to breastfeed during the study will automatically be withdrawn
3) Subject found to be taking or electing to take multivitamins or supplements while on study supplement, Isoquercetin. This does not include folic acid.
4) If subject no longer wishes to participate
5) Per PI discretion, for development of a SCD related complication that does not permit study participation.

Prior to removal from study, any subject who has started the study intervention should complete a safety assessment phone call. If a subject requests withdrawal early for any reason, we will make every effort to have them complete an unscheduled safety assessment phone call and a

clinic visit if indicated. Subjects experiencing side effects during the 28-day Isoquercetin/placebo treatment may have a clinic visit during the study.

The reason for participant discontinuation or withdrawal from the study will be recorded in the source document. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will be counted toward the accrual ceiling of 46. Randomization will continue until either the accrual ceiling of 46 is reached.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for the scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit to another day within 7 days of the scheduled visit, and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study. If the visit cannot be rescheduled within the 7 day timeframe, then the patient will be considered lost to follow-up.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 attempts at contact) These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

Screening activities performed prior to obtaining informed consent
Minimal risk activities that may be performed before the subject has signed a consent include the following:
• Email, written, in person or telephone communications with prospective subjects
• Review of existing medical records to include H&P, laboratory studies, etc.

Once the research team has identified a potential subject for the study, the subject will be asked to come to the NIH. The study team will consent the subject, allowing time for the subjects to ask questions and make a voluntary decision.

Once the subject signs the consent, the following tests will be done at the Screening Visit unless any of the tests have been performed under another NIH protocol 90 day prior to signing the consent (unless otherwise stated).

Screening Visit:

- History and physical exam including prior transfusion history
- CBC with differential
- Reticulocyte count
- Acute care panel, Mineral panel, Hepatic panel, total protein, creatinine kinase, uric acid, lactate dehydrogenase
- Calculated creatinine clearance
- Serum HBsAg, anti-HBc, anti-HBs, anti-HCV, anti-HIV
- Coagulation screen (PT/INR, PTT, fibrinogen, D-dimer)
- Pregnancy test (serum or urine) for female subjects and use of barrier methods of contraception for male subjects
- Hemoglobin electrophoresis

Up to 60 subject will sign consent to be screened with the goal of enrolling 46 subjects eligible to participate in the interventional portion of the study.

8.2 STUDY EVALUATION PROCECURES

Study Procedures will be conducted per table in section 1.3 (SOA) and are described below. The effectiveness of IQ in reducing P-selectin levels will be assessed as described in section 9.

Physical examinaton and medication history:

A physical examination, review of medication history, and review of available laboratory tests may be completed on each subject. Medical records obtained under other protocols may also be reviewed. Vital signs and biophysical data (blood pressure, heart rate, respiratory rate, temperature, height, and weight) may also be collected at each visit. These measurements will be made per NIH Clinical Center policies.

Optional exploratory studies of peripheral arterial tonometry and/or NIRS will based on service availability and may be conducted in patients that agree to this procedure.

Peripheral Arterial Tonometry (PAT) (ENDO-PAT 2000, Itamar Medical):

PAT is an FDA-approved noninvasive technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave activity with pneumatic probes, which provides a measure of endothelial function, similar to that of brachial artery ultrasound (BAUS) assessment of flow mediated dilation. It is claimed that this method is highly automated, consistent, and operator independent.

The PAT finger probe consists of a thimble-shaped sensor cap that imparts a uniform pressure fields and exhibits a clamp-like effect on the entire surface of the distal phalanx and measures pulsatile volume changes. PAT applies a significant counter pressure (70 mmHg) on the digit to avoid distal venous distention, thereby inhibiting venous pooling and blood stasis, which could otherwise induce a veno-arteriolar reflex vasoconstrictor response.

Near Infrared Spectroscopy (NIRS):a.Additional non-invasive studies relating to muscle physiology, tissue oxygenation and blood flowmay also be assessed using near infrared spectroscopy (NIRS) methodologies. The red to nearinfrared (NIR) part of the electromagnetic spectrum (600 to 1000 nm) allows photons to penetrate a few centimeters below the surface of the skin. These photons are non-ionizing and do not induce local heat. There are numerous FDA cleared clinical instruments in wide use that employ NIR light for non-invasive muscle and brain tissue oximetry. Quantitative optical spectroscopy in the NIR allows for safe, non-invasive measurements of the concentrations of blood, water, and lipids in tissues.Caliper measurements are used to measure the thickness of the skin to approximate tissue depth. We will use a number of different NIRS technologies to precisely and accurately characterize tissue metabolism, composition, and perfusion in subjects with SCD. These technologies, used in combination with non-invasive perturbations to evaluate vascular health (vascular occlusion challenge and respiratory challenge), are described below:

b. Diffuse Optical Spectroscopic Imaging (DOSI): DOSI is a progression on continuous-wave NIRS techniques that utilizes a frequency-modulated light source that allows for quantitative measurement of blood, water, and lipid concentrations in tissues. DOSI requires more advanced hardware than traditional NIRS techniques but is similarly safe and non-invasive.

c. pocketNIRS: This is a portable NIRS device developed by Hamamatsu Photonics in conjunction with the Tromberg lab. pocketNIRS consists of a control unit and 2 probes with 3 light emitting diodes (740nm, 800nm, 850nm) and can be controlled via cell phone. It measures changes in oxyhemoglobin, deoxyhemoglobin and total hemoglobin and also gives absolute measurement of tissue saturation.

d. Laser Speckle Imaging (LSI): (LSI is a NIRS technology that is sensitive to flow. LSI consists of a coherent non-collimated light source (typically 785 nm) and a camera that images the illuminated tissue area. When biological tissue is illuminated with coherent light, an interference pattern will be formed at the detector, known as a speckle pattern. Blood flow speed affects the pattern captured by the camera and characterizing these changes in the pattern allows for a measurement of blood flow in the skin microvasculature.

e. Affixed Transmission Speckle Analysis (ATSA) (trade name: FlowMet-R): FlowMet is the contact, transmission based equivalent of LSI. Within this device, the source and detector are on opposite sides of the finger. Light that shines through the finger or toe is picked up by the camera on the other side. Blood flow speed affects the pattern captured by the camera. Characterizing these changes in the pattern allows for a measurement of arterial blood flow within the finger or toe.

f. Diffuse correlation spectroscopy (DCS): DCS uses a 785 nm coherent light source with a detector some distance away. DCS measures the temporal fluctuations of near-infrared (NIR) light to measure flow in the microvasculature at a single point.

g. PeriFlux 6000 for microcirculatory blood flow assessments: The PeriFlux System 6000 device will be used for measuring microcirculatory blood flow and oxygenation in the limb and poses a

non-significant risk. Diffuse reflectance spectroscopy (DRS) and laser Doppler flowmetry (LDF) have been successfully integrated enabling accurate measurements of blood flow and oxygenation in the microcirculation. A dedicated fiber-optic probe that integrates the DRS and LDF modalities is required as well as an external light source.

h. Vascular Occlusion Challenge: Subjects will not have any active role in this challenge. A standard blood pressure cuff will be placed upon the limb (arm or leg) to be studied. Subjects may be asked to lie on a gurney or reclining chair for comfort and to keep the occluded limb stable. A DOS probe will be placed on the limb, and continuous measurements using the NIRS methodologies described above will be taken throughout the challenge. Additional optical probes may be placed on other tissue sites, such as the forehead or non-occluded limb. The FlowMet may be used on one of the fingertip/toe in order to monitor blood flow. If the subject does not tolerate limb cuff inflation, we may instead switch to performing a vascular occlusion challeng on the finger using a finger-sized cuff instead.

i. Respiratory Challenge: Subjects will be asked to a) pace their breathing according to a specific metronome-controlled rate or b) hold their breath for as long as they are able. Participants may be asked to lie on a gurney or reclining chair for comfort and to aid in reducing motion artifacts in data. An optical probe will be placed on at least one tissue site and will collect data continuously during paced breathing. To better understand how muscle oxygenation and blood volume changes during this challenge, additional optical probes may be placed over the top of other tissue sites (e.g. shin bone, abdominal adipose tissue, forehead, etc.). Data from these additional locations does not increase the risk to participants and only adds more physiological context to data from muscle locations.

8.2.2 Biospecimen Evaluations

Biological specimen collection and laboratory evaluations. In addition to the clinical blood samples, subject will have research bloods drawn within the blood withdrawal volume limits established by the Clinical Center. RBC structure and functional studies, Flow cytometry, Platelet studies, RNAseq, Immunoblotting, and cytokine profiling may be performed.

Table :3

Table of samples to collect at baseline and end of study (2 visits)				
Test/assay	**Volume blood (approx.)**	**Type of tube**	**Collection point (+/- 72hrs)**	**Location of specimen analysis**
Routine* • Hepatic, Mineral and Acute care panel • LDH, T. protein, CK, uric acid • C-reactive protein • B-type natriuretic peptide • Beta HCG (Female only)	4 mL	LtGrnPSTGel (GLT) tube	Baseline (D0) and end of study D28)	Clinical Lab
Routine* • CBC • Reticulocyte count • HPLC for Hb electrophoresis • Direct antiglobulin test (DAT)	9ml (3 tubes)	EDTA tube	Baseline (D0) and end of study D28)	Clinical Lab
Routine* • PT • PTT • INR • Fibrinogen • D-dimer	3ml	3.8% Sodium Citrate	Baseline (D0) and end of study D28)	Clinical Lab
Biochemical studies** • Soluble P-selectin • Plasma PDI activity assay Protein Carbonylation state (Western Blot)	3 ml	3.8% Sodium Citrate	Baseline (D0) and end of study D28)	NHLBI Sickle Cell Branch, Sickle Thrombosis Lab
Coagulation studies** • Microparticles/EVs • Prothrombin fragment F1.2, Thrombin anti-thrombin complexes (TAT)	3 ml (1 tube)	3.8% Sodium Citrate	Baseline (D0) and end of study D28)	NHLBI Sickle Cell Branch, Sickle Thrombosis Lab

Red Blood Cell studies** • Deformability • Oxygenscan • Aggregation • ROS production • PS and PDI exposure and distribution (FACS)	6 ml (2 tubes)	EDTA tube	Baseline (D0) and end of study D28)	NHLBI Sickle Cell Branch, Sickle Thrombosis Lab
Platelet function tests** • Studies of platelet aggregation and platelet dependent thrombin generation	6 ml (2 tubes)	3.8% Sodium Citrate	Baseline (D0) and end of study D28)	NHLBI Sickle Cell Branch, Sickle Thrombosis Lab
Leukocytes isolation for gene expression and RNAseq** • Monocyte TF gene expression • Tissue factor RNAseq • Complement variants • P-selectin variants	5 ml (1 tube)	EDTA	Baseline (D0) and end of study D28)	NHLBI Sickle Cell Branch, Sickle Thrombosis Lab
Contact Pathway	6 ml (2 tubes)	3.8% Sodium Citrate + Corn Trypsin Inhibitor (CTI)	Baseline (D0) and end of study D28)	NHLBI Sickle Cell Branch, Sickle Thrombosis Lab

*Tests necessary for routine care of patient with sickle cell disease.

**Research blood samples to be collected at baseline during study enrollment and during follow up.

8.2.3 Correlative Studies for Research/Pharmacokinetic Studies

N/A

8.2.4 Samples for Genetic/Genomic Analysis

The mononuclear cell fraction will be isolated and stored for gene expression and RNAseq specifically looking at tissue factor expression in monocytes and gene variants involving thrombo-inflammatory pathways (P-selectin and the complement).

8.3 SAFETY AND OTHER ASSESSMENTS

Physical examination, laboratory tests and adverse event review will be assessed at baseline and at 4 weeks.

Safety, tolerability and adherence assessments will be performed at baseline and at 4 weeks and whenever clinically indicated.

Counseling: Subjects should maintain a stable diet and not start any new diet or active SCD treatment for the duration of the study.

24-hour Dietary Recall: At three timepoints throughout the study, trained nutrition staff from the NIH CC will collect a 24-hour recall to reflect dietary intake from the prior day with nutrition staff entering information directly into Nutrition Data Systems for Research software (NDSR, University of Minnesota, Minneapolis, MN) to obtain nutrient and dietary quercetin intake data.

Participants experiencing adverse events after the start of IQ/placebo up until 4 weeks after discontinuation, may have a clinic visit for assessment and will be managed as clinically indicated. All patients will received a phone call study visit 4 weeks after IQ/placebo discontinuation to assess for AEs.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

Severity of Event

This study will utilize the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) for toxicity and adverse event reporting. A copy of the CTCAE v5.0 can be downloaded from the https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs will be recorded, verified, and followed until satisfactory resolution or stabilization. In the event of any treatment related SAEs, enrollment will be suspended until discussed with the IRB and Clinical Director.

8.4.4 Grading and attribution of adverse events

Severity definitions found in the CTCAE v5.0 will be used for grading the severity (intensity) of AEs:

1) **Mild**: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2) **Moderate:** Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*
3) **Severe:** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4) **Life- threatening:** Life-threatening consequences; urgent intervention indicated.
5) **Death:** Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

8.4.5 Relationship to Study Intervention

- All adverse events (AEs) must have their relationship to study intervention assessed by the investigator or designee who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.
- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge).

- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.4.6 Expectedness

The PI or designee will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described in the Investigator's Brochure for IQ.

We expect some vasovagal symptoms during blood draws (expected frequency 50%) and transient bruising at the site of blood draws (expected frequency 50%). Approximately 20% of SCD patients may experience a vaso-occlusive pain crisis "acute crisis" as part of their underlying disease, thereby resulting in hospitalization and reporting of SAEs.

8.4.7 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

Investigators will assess the occurrence of AEs and SAEs at all patient evaluation time points during the study. Grade 2 and above adverse events plus grade 2 laboratory abnoramlitites associated with clinical signs or symptoms whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, clinically significant laboratory test, or other means will be recorded on the appropriate CRF.Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date of resolution/stabilization of the event.AEs collected for the purpose of the study will be documented appropriately regardless of relationship to the IND agent. AEs will be followed to adequate resolution or stabilization or until V4 (phone call).

The following AEs will not be captured in the research database:

- Grade 1 adverse events

- Grade 2 laboratory abnormalities not associated with clinical signs or symptoms.

SCD is a chronic disease associated with recurrent acute painful crises and this information will be documented in the medical record. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI or designee will record all reportable events (those entered in the research database with **start dates** occurring any time after the study intervention has started until 7 days (for non-serious AEs or SAEs) after the last day of administration of the study supplement. At each study visit, the team member will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization or until V4 (phone call).

8.4.8 Adverse Event Reporting

Grading and attribution of AEs captured in the database will be determined by the Principal Investigator or Associate Investigator's designated on the delegation log.

8.4.9 Serious Adverse Event Reporting

The study investigator will report to the sponsor any SAE occurring after the start of intervention, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or that the participant is stable.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting using a MedWatch Report (FDA Form 3500).

Reports to the IRB and CD

Expedited Reporting

Events requiring expedited reporting will be submitted to the IRB per Policy 801 "Reporting Research Events".

Reports to the IRB at the time of Continuing Review:

The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.

Reports to the CD:

The PI or designee will refer to NHLBI DIR Policy to determine CD reporting requirements and timelines.

Reports to the DSMB

The NHLBI DSMB as the independent data safety monitoring commitee will review and approve the protocol before the enrollment. SAEs that meet the definition of an Unanticipated Problem will be submitted to the DSMB within 7 days of the investigator being notified of the event. DSMB will have access to safety data upon request any time. The monitoring plan will be developed and approved by the DSMB before enrollment.

8.4.10 Events of Special Interest

N/A

8.4.11 Reporting of Pregnancy

In the event a subject becomes pregnant while on study, this event will be reported to the IRB and Clinical Director as a protocol deviation. Monitoring of the pregnancy thru chart review (per HIPPA guidelines) will continue until conclusion of the pregnancy, and then subject will be taken off study.

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

8.5.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESIS

Treatment with IQ will blunt systemic inflammation and coagulation activation in sickle cell disease patients.

Primary Endpoint(s): The primary outcome will be the change in the plasma soluble P-selectin comparing the baseline to IQ response after 28 days in patients with SCD.

9.2 SAMPLE SIZE DETERMINATION

This is a study designed to assess the effect of IQ on plasma soluble P-selectin in subjects with stable SCD at baseline. The mean baseline soluble P-selectin measurement in a cross-sectional study of SCD patients (n = 29) in steady state was 30 ng/ml with standard deviation of 8.4 (see preliminary data Figure 4). Follow-up measurements from the same individuals had mean values of 29 ng/ml and standard deviation of 8.4 with intra-person correlation of 0.60 for the two measurements. The proposed analysis is an analysis of covariance model with follow-up P-selectin measurement (i.e. post-treatment measurement) as the dependent variable with baseline measurement and treatment assignment as the covariates. A 25% reduction in P-selectin (i.e. an 7.25 ng/ml decline from an average value of 29 ng/ml) is the hypothesized treatment effect for IQ proposed to achieve a clinically meaningful reduction in thrombosis risk. Under these conditions a total of 40 respondents (20 per arm) is needed to obtain power of 90%. Based on these considerations we request an accrual ceiling to the study intervention portion of the study of 46 individuals to compensate for loss to follow-up or inaccurate sample size assumptions.

In the table below, we present power calculations for various scenarios. With 20 subjects on IQ and 20 on placebo, the study will have >90% power to detect a difference of 7.25 ng/ml in the mean baseline to 4-week changes between the two study groups. Furthermore, even with only 34 subjects (17 in each arm), the study will have >85% power to detect a mean change of 7.25 ng/ml. Therefore, we request approval to enroll up to a total of 46 subjects to: 1) account for potential diluting effects of possible treatment non-compliance, and/or study dropouts, 2) provide a buffer against possibly inaccurate sample size assumptions, and 3) provide power to test our hypothesis in the subgroup of per-protocol patients who avoid crises that may distort their P-selectin and other measurements (see section 9.3).

Table 4

Analysis Method	Anticipated P-selectin reduction	Power	Required Sample Size per arm
ANCOVA	25%	80%	15
ANCOVA	25%	85%	17
ANCOVA	25%	90%	20

9.3 POPULATIONS FOR ANALYSES

The primary analysis will be conducted among patients with SCD that are randomized. The occurrence of the following clinical events that are related to the underlying disease biology may confound study outcomes. In particular, occurrence of acute crises can profoundly alter inflammation, coagulation biology and affect P-selectin measurement. Therefore, of particular interest is the subgroup of steady state SCD patients that complete the 28-day study treatment period without experiencing an acute crisis. A subgroup analysis of the primary and key secondary endpoints will be conducted on this subset using the same analytic techniques.

9.3.1 Evaluable for toxicity

All patients randomized will be evaluable for toxicity from the time of their first treatment with study supplement or placebo until 28 days after completion of the end of study visit.

9.3.2 Evaluable for objective response

N/A

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

Please refer to sections 9.4.2, 9.4.3 and 9.4.6 for the general approach to statistical analysis.

9.4.2 Analysis of the Primary Endpoints

The primary outcome will be the change in the soluble P-selectin level comparing baseline levels versus their respective response to IQ or placebo among randomized SCD patients. The proposed statistical method is an analysis of covariance model with follow-up P-selectin measurement (i.e. post-treatment measurement) as the dependent variable with baseline measurement and treatment assignment as the covariates. Significance in this pilot phase II study will be evaluated using a two-sided test with alpha level of 0.05.

9.4.3 Analysis of the Secondary Endpoint(s)

The following endpoints are exploratory in nature:

- Compare baseline and end of study plasma protein disulfide isomerase activity
- Quantify baseline and end of study plasma tissue factor positive extracellular vesicle number
- Compare baseline and end of study tissue factor procoagulant activity
- Compare baseline and end of study platelet thrombin generation
- Compare baseline and end of study inflammatory cytokines
- Compare baseline and end of study plasma D-Dimer, TAT and PF1.2
- Compare baseline and end of study contemporary biomarkers of vascular endothelial function, specifically reactive hyperemia index.
- Assess safety and tolerability of IQ
- Assess adherence to oral IQ

Baseline and end of study measures of secondary end points will be similarly conducted using ANCOVA or Wilcoxon's rank sum test, if assumptions of normality are not warranted. We will explore statistical correlation between PDI activity, tissue factor EVs, tissue factor procoagulant activity and soluble P-selectin levels using Spearman's or Pearson's correlation coefficients.

Changes in hemoglobin, leukocytes, reticulocytes, platelets, LDH, Total and direct bilirubin, C-reactive protein, inflammatory cytokine panel and coagulation parameters from baseline after exposure to 28 days of Isoquercetin will be summarized.

9.4.4 Safety Analyses

The planned analyses will also include descriptive statistics on the incidence and severity of adverse events. The proportions of treatment related serious adverse events (TRSAEs) will be summarized using sample proportions and confidence intervals for binomial distributions. Safety will be summarized using descriptive statistics. Summaries will be produced for all treatment-emergent adverse events (TEAEs), related TEAEs (those considered by the Investigator as related to study treatment), SAEs, TEAEs leading to treatment discontinuation, and TEAEs Grade ≥3 in severity. Individual subject listings will be provided for any deaths, SAEs, TEAEs leading to interruption and TEAEs leading to treatment discontinuation.

Stopping rule

When the Bayesian posterior probability reaches 75% or higher, it will trigger a meeting of the DMSC. The prior distribution for the probability of a qualifying adverse event is given by a beta distribution with parameters a = 1.25 and b = 3.25. For stopping rule purposes, qualifying AEs/SAEs will only be counted in the Isoquercetin arm. Monitoring for crossing the boundary and reporting to the DSMB, if necessary, will be done by the unblinded statistician.

The Table below summarizes the threshold numbers for the resulting boundary in the Isoquercetin arm, which would lead to a meeting of the safety committee to evaluate stopping or

modifying the study due to an excess number of qualifying AEs/SAEs. AEs will be counted for anyone randomized into the Isoquercetin arm regardless of whether they are withdrawn or lost to follow-up.

Table 5: Threshold numbers for resulting boundary

Number of subjects in the study	Hold enrollment if the number of subjects who have developed a qualifying AE/SAE is
≤3	2
≤7	3
≤10	4
≤14	5
≤17	6
≤21	7
≤23	8

We investigated the performance of the above stopping rule through simulation. In each simulation we generated a set of 23 independent Bernoulli trials; each representing an Isoquercetin-receiving patient with probability *p* for having a qualifying AE/SAE. For each simulation we determined if the stopping boundary would have been reached with consideration for halting or suspending the study. We conducted this simulation 100,000 times for each different true value of probability *p* and recorded the average number of Isoquercetin-receiving patients treated (it may be less than 23 if the study was stopped early) and the average number of qualifying AEs/SAEs observed. In addition, we show the proportion of the 100,000 simulations in which the stopping boundary was met. The table below summarizes the performance of the stopping rule under a number of different values for the qualifying AE/SAE probability *p*.

Table 6: Performance of the Stopping Rule under multiple scenarios for the qualifying AE/SAE Probability *p*

Probability of a qualifying AE/SAE	0.15	0.20	0.25	0.30	0.40
Proportion of Stopped Studies	14%	28%	45%	63%	89%
Average number of subjects	20.7	18.7	16.2	13.5	8.9
Average number of qualifying AEs/SAEs	3.1	3.7	4.0	4.1	3.5

9.4.5 Baseline Descriptive Statistics

For clinical laboratory values, and vital signs both actual values and changes from baseline will be summarized by visit using summary statistics.

Given the short nature of the trial and safety profile of the drug, few patients are expected to drop out or otherwise produce missing data. Individuals with missing data will be tabulated and reasons for missing data will be collected where possible and presented. If randomized subjects have missing data in either arm, then the primary analysis will employ a multiple imputation procedure that will be developed without knowledge of the treatment assignments (beyond assessing the percentage of missing outcomes in each arm). In these circumstances the analysis utilizing only available information would be secondary.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

Informed consent will be conducted following OHSRP Policy 301- Informed Consent.
An IRB-approved consent form will be provided to the participant electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved platforms). The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the participant in a private setting.
The participant will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member

will be available to answer any questions.

A signed and dated informed consent document will be obtained by any investigator authorized to consent (See Key Study Personnel Page) prior to entry onto the study. Consent may be obtained with required signatures on the hard copy of the consent or on the electronic document.

When a document that is in electronic format is used for obtaining consent, this study may use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures. During the consent process, participants and investigators may view the same approved consent document simultaneously when participant is being consented in person at the Clinical Center or both may view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture (remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.
Whether hard copy or electronic, both the investigator and the participant will sign the document with a hand signature using a pen (if using hard copy), finger, stylus, or mouse (if electronic). When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator. Whether in person or remotely, the privacy of the participant will be maintained. Finally, the fully signed informed consent document will be stored in the electronic medical record, and the participant will receive a copy of the signed informed
consent document.

10.1.2 Consent for minors when they reach the age of majority

N/A

10.1.3 Telephone consent

N/A

10.1.4 Participation of Subjects who are/become Decisionally Impaired

N/A

10.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements

- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB.

10.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the NIH for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored in a research database in conformity with NHLBI DIR policy. Individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the research staff will be secured and password protected.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

We may share specimens and data with other researchers for future use.

Following analyses of biospecimens for primary research purposes as described in the protocol, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB approval. Biospecimens may be destroyed only when permitted by the clinical director and approved by the IRB. Any future research use of identifiable biospecimens not defined in the research protocol will occur only after IRB review and approval.

10.5 SAFETY OVERSIGHT

Safety oversight will be under the direction of a DSMB composed of individuals with the appropriate expertise. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet semiannually or annually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to National Institutes of Health staff.

10.6 CLINICAL MONITORING

The monitoring of this study will be conducted by clinical research associates (CRAs)/monitors employed by an independent contract organization working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP) and applicable guidelines (ICH-GCP) are being followed. Monitoring will be conducted according to the OCD schedule. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the site monitors, and the NHLBI staff for confirmation of the study data.

10.7 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.8 DATA HANDLING AND RECORD KEEPING

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data and clinical laboratory data will be entered into CTDB, a 21 CFR Part 11-compliant data capture system. Clinical data will be entered directly from the source documents.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, or as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the NHLBI Clinical Director.

10.9 PROTOCOL DEVIATIONS

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH Institutional Review Board as per Policy 801.

NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 PUBLICATION AND DATA SHARING POLICY

10.10.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-

reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 3 years after the completion of the primary endpoint by contacting Dr. Arun Shet at the NHLBI

10.10.2 Genomic Data Sharing Plan

N/A

10.11 COLLABORATIVE AGREEMENTS

10.11.1 Agreement Type

A Clinical Material Supply Agreement between NHLBI and Querces AG for the conduct of this study is being executed.

10.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NHLBI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

ACS	Acute Chest Syndrome
ADME	Absorption, Distribution, Metabolism, and Elimination
AE	Adverse Event
AT	Anti-Thrombin
BAUS	Brachial Artery Ultrasound
BNP	B-type natriuretic peptide
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
COC	Certificate of Confidentiality

CRF	Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
CTEPH	chronic thromboembolic pulmonary hypertension
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DSMB	Data Safety Monitoring Board
DSMC	Data Safety and Monitoring Committee
DVT	Deep Venous Thrombosis
eCRF	Electronic Case Report Forms
ELISA	Enzyme-Linked Immunosorbent Assay
EOS	End of Study
ER	Endoplasmic Reticulum
EVs	Extracellular Vesicles
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GRAS	Generally Recognized As Safe
HbS	Hemoglobin S
HCG	Human Chorionic Gonadotrophins
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HMGB1	h\High Mobility Broup Box-1
HU	Hydroxy Urea
ICH	International Conference on Harmonization
IND	Investigational New Drug Application
IND	Investigational New Drug
IQ	Isoquercetin
IRB	Institutional Review Board
MAS	Medical Administrative Series
NCT	National Clinical Trial
NHLBI	National Heart, Lung and Blood Institute
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NIRS	Near Infrared Spectroscopy
NO	Nitric Oxide
NTP	National Toxicology Program
OHRP	Office for Human Research Protections
PAT	Peripheral Arterial Tonometry
PDI	protein disulfide isomerase
PI	Principal Investigator
PRO	Patient Recruitment Office
PTT	Partial Thromboplastin Time
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAE	Serious Adverse Event
SCD	Sickle Cell Disease

SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
SOPs	Standard Operating Procedures
TAT	Thrombin-Antithrombin Complexes
TEAEs	Treatment-Emergent Adverse Events
TF	Tissue Factor
TLR4	Toll Like Receptor 4
UP	Unanticipated Problem
US	United States
VOC	Vaso-Occlusive Crises
VTE	Venous Thromboembolism
vWF	von Willebrand Factor

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