The Effect of Factor Xa Inhibition with Rivaroxaban on the Pathology of Sickle Cell Disease

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Principal Investigator

Kenneth I. Ataga, M.D.
Division of Hematology/Oncology
University of North Carolina
CB# 7305, 170 Manning Drive
Chapel Hill, NC 27599-7305
Telephone: (919) 966-0178
Fax: (919) 966-6735

E-mail: kataga@med.unc.edu

Co-Investigators

Nigel S. Key, MD
Division of Hematology/Oncology
University of North Carolina
Chapel Hill, NC 27599-7305
Telephone: (919) 966-0900

Rupa Redding-Lallinger, MD
Division of Hematology/Oncology
University of North Carolina
Chapel Hill, NC 27599-7305
Telephone: (919) 966-0900

Jainwen Cai, PhD
Department of Biostatistics
University of North Carolina
Chapel Hill, NC 27599-7305
Telephone: (919) 966-0900

Study Coordinator

Susan Jones, R.N.
UNC Comprehensive Sickle Cell Program
CB# 7305, 170 Manning Drive
Chapel Hill, NC 27599
Telephone: (919) 966-6876

Rivaroxaban in Sickle Cell Disease

SYNOPSIS

Title of the Protocol: The effect of factor Xa inhibition, with rivaroxaban, on the pathology of sickle cell disease
Overview: As a result of the presence of macrovascular thrombotic complications, as well as the biochemical evidence of ongoing coagulation activation, sickle cell disease (SCD) is often referred to as a "hypercoagulable state." SCD is characterized by increased tissue factor expression, increased levels of plasma markers of thrombin generation and fibrinolysis, increased platelet activation and decreased levels of natural anticoagulant proteins. Despite the abundant laboratory evidence of hypercoagulability observed in these patients, it still remains uncertain whether the observed coagulation activation contributes to the vascular occlusive episodes that characterize SCD. However, recent studies suggest that certain SCD-related complications, including thrombotic stroke, may be associated with coagulation activation. In addition, inhibition of tissue factor abrogated the activation of coagulation and attenuated inflammation and endothelial cell injury as demonstrated by reduced plasma levels of IL-6, serum amyloid P, soluble vascular cell adhesion molecule-1 and decreased levels of myeloperoxidase in the lungs of sickle cell mice, suggesting a cross-talk between coagulation and inflammation in SCD. The treatment options for SCD remain limited. Although the low molecular weight heparin, tinzaparin, has been reported to significantly reduce the duration of acute pain episodes, it remains uncertain whether this effect is due to its anticoagulant or anti-adhesive actions. In this study, we will evaluate the efficacy and safety of rivaroxaban in SCD. If the data support the hypothesis that rivaroxaban is effective and safe in this setting, we plan on carrying out adequately powered studies to more definitively evaluate its safety and efficacy in the treatment and/or prevention of selected SCD-related complications.
Intervention: We will conduct a randomized, double-blind, placebo-controlled, crossover trial of rivaroxaban (n = 34). After baseline assessments are performed, subjects will receive rivaroxaban 20 mg/day or placebo for 4 weeks, separated by a 2 week washout phase. At the end of the washout period, a repeat history and examination and laboratory studies will be performed which will serve as a baseline for the second treatment phase, and patients will receive either rivaroxaban or placebo, depending on their first treatment administration.
IND Holder: Kenneth I. Ataga
Specific Aims: A) To evaluate the effects of rivaroxaban on plasma markers of coagulation activation, inflammation and markers of endothelial cell activation in SCD patients during the non-crisis, steady state. B) To evaluate the effects of rivaroxaban on microvascular blood flow in SCD patients during the non-crisis, steady state. C) To evaluate the safety of rivaroxaban in patients with SCD
Hypotheses/Estimates: Our primary hypothesis is that inhibition of factor Xa with rivaroxaban will reduce inflammation, coagulation and endothelial cell activation in patients with SCD.

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Criteria for Evaluation:

Efficacy: Primary Endpoints: *Soluble vascular cell adhesion molecule-1 (VCAM-1) and interleukin-6 (IL-6):* Plasma levels of soluble VCAM-1 and IL-6 will be obtained at Baseline, and then at weeks 2 and 4 during both Treatment Phases. The primary efficacy measure will be a comparison of the difference between the measurements following 4 weeks of treatment with rivaroxaban/placebo and baseline measurements.

Exploratory Endpoints: *Markers of Coagulation Activation, Endothelial Activation, and Inflammation:* We will measure plasma levels of markers of coagulation activation (thrombin-antithrombin [TAT] complexes and D-dimer), other markers of endothelial activation (soluble ICAM) and other markers of inflammation (high sensitivity CRP, MPO, IL-2, IL-8, TNFα, sPLA2), at Baseline and then at weeks 2 and 4 during the Treatment Phases.

Microvascular blood flow: We will analyze microvascular blood flow using laser Doppler velocimetry (LDV) assessments of post-occlusive reactive hyperemia (PORH). Measurements will be obtained at baseline and at 4 weeks during the Treatment Phases.

Safety Assessment: We will evaluate patients for treatment-related complications for the duration of the study. Major bleeding complications (any bleeding episode into critical sites, e.g. intracranial bleed, decrease in hemoglobin concentration of at least 2g/dL from baseline or any prolonged bleeding that requires a blood transfusion), and other clinically relevant non-major or trivial bleeding will be recorded. We will also evaluate for episodes of SCD-related events, including acute pain episodes during scheduled follow up visits

Study Design: This is a randomized, double-blind, placebo-controlled, crossover study of rivaroxaban to evaluate the efficacy and safety of rivaroxaban in SCD. It will be divided into a Screening/Baseline phase, Treatment phase, and Follow-up phase.

Study Population: Thirty two patients with SCD (HbSS or HbSβ^0 thalassemia) between the ages of 18 and 65 who meet the eligibility criteria and provide consent to participate in the study, will be randomized in this crossover trial.

Clinical and Laboratory Evaluations: The Screening/Baseline Phase will occur within 28 days of study drug administration and will include: informed consent, a history and physical examination, and clinical laboratory tests including: a complete blood count, routine coagulation studies, routine chemistries to assess liver and renal function chest x-ray, brain MRI/MRA scan and serum or urine pregnancy test (if female, and of child-bearing capacity). During the Treatment Phase, we will also obtain a complete blood count, routine chemistries to assess liver and renal function, and pregnancy test (if female); and prior to the second treatment phase, a repeat history and examination and laboratory studies will be performed, which will serve as a baseline for the second treatment phase. Finally, measurements of microvascular blood flow (assessed non-invasively using laser Doppler velocimetry), endothelial activation (soluble VCAM, soluble ICAM), coagulation activation (TAT and D-dimer) and markers of inflammation (IL-6, high sensitivity CRP, MPO, IL-2, IL-8, TNFα, sPLA2) will be obtained at specified time points during the Baseline, Treatment and Washout/Follow-up Phases. The Follow-up Phase will consist of safety assessments performed four weeks after the final dose of study treatment.

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Sample Size: In a 2x2 crossover design, assuming a correlation of 0.65 for the pre- and post-treatment measures, to detect a difference of 0.245 in the change of log(sVCAM) and 0.475 in the change of log(IL-6) between the rivaroxaban and placebo groups requires a minimum sample size of 15 per sequence (a total sample size of 30) at 80% power and $\alpha = 0.05$. Assuming a 10% dropout rate, a total of 34 patients is required for this study (17 in each sequence).
Randomization: 1:1 randomization to receive rivaroxaban or placebo.

Rivaroxaban in Sickle Cell Disease

BACKGROUND, SIGNIFICANCE AND RATIONALE

Sickle cell disease (SCD) affects approximately 100, 000 individuals in the United States. The polymerization of deoxygenated sickle hemoglobin is the primary event in the pathogenesis of SCD {Bunn HF, 1997}. SCD is also associated with additional pathologic processes, including inflammation, increased oxidative stress, endothelial activation and dysfunction, stasis, reperfusion injury and activation of coagulation. These processes are highly interconnected and contribute to the complex pathophysiology of SCD{Frenette, 2007; Hebbel, 2009}.

The coagulation cascade can be divided into the extrinsic, intrinsic and common pathways {Mackman, 2007}. The extrinsic pathway consists of the transmembrane receptor tissue factor (TF) and plasma factor VII/VIIa (FVII/FVIIa). TF binds FVII/FVIIa with a high affinity and forms a TF:FVIIa complex that activates FX. The intrinsic pathway is an alternative way to activate coagulation via FXIIa and FXIa. It amplifies the generation of FXa via the FVIIIa/FIXa intrinsic tenase complex. Finally, the common pathway consists of the proteases, FXa and thrombin which cleaves fibrinogen to fibrin. Importantly, coagulation and inflammation are interconnected in many diseases and protease activated receptors (PARs) mediate this cross-talk {Mackman, 2007}. PAR-1 is activated by various proteases, including thrombin and FXa, whereas PAR-2 is activated by FXa and FVIIa as well as other proteases {Coughlin, 2000}{Riewald, 2001}{Camerer, 2000}{Rao, 2005}. Patients with SCD demonstrate elevated levels of whole blood TF procoagulant activity that correlates with increased expression of TF antigen on monocytes {Key, 1998; Setty, }. Furthermore, we and others have shown that these patients have increased plasma levels of various markers of activation of coagulation, such as thrombin-antithrombin complexes (TAT), prothrombin fragment F1.2 (F1.2) and D-dimers {Ataga, 2007}. In mouse models of SCD, TF expression is increased in the endothelium of the lung microvasculature and in circulating monocytes {Solovey, 2004}.In addition, TF expression is increased by hypoxia/re-oxygenation {Solovey, 2004}.

Despite the fact that SCD patients are hypercoagulable, little is known about the contribution of coagulation to the pathology of SCD. Thrombosis appears to play an important role in certain complications of SCD. Large vessel narrowing with superimposed thrombosis is the most common cause of thrombotic stroke in SCD patients. Our recent observation of the association between D-dimer levels and a history of stroke suggests that coagulation activation may contribute to the pathophysiology of thrombotic stroke in SCD {Ataga, 2012}. Both old and new thrombi are found in the pulmonary vasculature of patients with lung disease (2) and an *in-situ* thrombotic arteriopathy is observed in patients with PHT. Furthermore, published retrospective studies based on discharge diagnoses suggest that both pulmonary embolism and pregnancy-related venous thromboembolism appear to occur more commonly in SCD patients than in appropriate control patients {Novelli et al}.

Several clinical studies have analyzed the effect of different anticoagulants, including warfarin, heparin or acenocoumarol, on acute pain crisis in sickle cell patients. These studies have demonstrated modest effects at best {Ataga, 2007}. However, most of these studies were performed on small numbers of patients and used pain crisis as the only clinical endpoint. Notably, the only adequately powered, appropriately designed (i.e. placebo-controlled) study to examine the effect of anticoagulation (low molecular weight heparin, tinzaparin, for 7 days) in SCD showed a positive result, with a reduction in the duration of pain crisis and hospital stay{Qari, 2007}. However, it remains uncertain whether the observed clinical effect is due to the anticoagulant or anti-adhesive action of the drug. We contend that the contribution of the hypercoagulable state to

the pathophysiology of SCD has yet to be investigated adequately in clinical studies using new anticoagulant agents.

Hypothesis: Inhibition of FXa will reduce coagulation, inflammation, and endothelial cell activation and improve microvascular blood flow in patients with SCD. We will perform a proof-of-concept study using the FXa inhibitor, rivaxoxaban, to: (i) evaluate the effects of rivaroxaban on coagulation, inflammation and markers of EC activation in SCD patients during the non-crisis, steady state; (ii) determine the effects of rivaroxaban on microvascular blood flow in SCD patients during the non-crisis, steady state; and (iii) assess the safety of rivaroxaban in patients with SCD.

Rivaxoxaban

Rivaroxaban is an oral direct factor Xa inhibitor that selectively blocks the active site of factor Xa. In addition, it inhibits prothrombinase activity, clot-associated factor Xa, and thrombin generation. This mechanism is unique to small, direct inhibitors because factor Xa that is incorporated in the prothrombinase complex is protected from inhibition by antithrombin and by antithrombin-dependent anticoagulants. Rivaroxaban is metabolized in the liver through oxidative and hydrolytic processes catalyzed by cytochrome P450 (CYP) 3A4/5 and 2J2. It is also a substrate for the P-glycoprotein efflux transporter protein. Approximately 66% of rivaroxaban is excreted in the kidneys (36% as unchanged drug), and the remainder is excreted in the feces as unchanged drug. In healthy, white men between 19 and 45 years, the administration of a single dose of rivaroxaban (5 to 80 mg) resulted in a maximum factor Xa inhibition of 20% to 80% within 1 to 4 hours after administration. After administration of the drug, maximum concentration was attained in 2 hours and the half-life was between 6 and 7 hours. Rivaroxaban prolonged the prothrombin time, activated partial thromboplastin time, and HepTest (a low-molecular-weight heparin [LMWH] activity assay), but had no effect on thrombin or antithrombin activity. In a study with multiple doses of rivaroxaban (5 mg once or twice daily to 30 mg twice daily) administered to healthy men aged 20 to 45 years, the maximum concentration was reached in 2 to 4 hours and maximum factor Xa inhibition ranged from 22% (5-mg dose) to 68% (30-mg dose). Based on data from a multiple-dose study, rivaroxaban has predictable, dose-proportional pharmacokinetic and pharmacodynamic properties. The AUC of rivaroxaban is increased by 50% and the half-life prolonged to between 11 and 13 hours in elderly patients compared with younger patients.

Moderate renal impairment (creatinine clearance [CrCl] of 30–49 mL/min) and increased age lead to slight increases in rivaroxaban exposure. A rivaroxaban dose of 15 mg daily in patients with CrCl of 30 to 49 mL/min achieves a maximum concentration similar to that observed with a dose of 20 mg daily in patients with normal renal function. The increases in the AUC of inhibition of factor Xa activity are 50%, 86%, and 100% for mild, moderate, and severe renal impairment, respectively. In patients being treated for nonvalvular atrial fibrillation, it is recommended to reduce the dose of rivaroxaban to 15 mg once daily if the CrCl is 15 to 50 mL/min. It is recommended that the use of rivaroxaban be avoided in patients with moderate (Child-Pugh B) and severe (Child-Pugh C) hepatic impairment or with any hepatic disease associated with a coagulopathy. Drugs that are combined p-glycoprotein and CYP3A4 inhibitors, including “azole” antifungals and HIV protease inhibitors, result in increases in rivaroxaban exposure and factor Xa inhibition. Drugs that are combined p-glycoprotein inducers and strong CYP3A4 inducers such as rifampicin (which cause 50% and 22% decreases in AUC and maximum concentration, respectively), phenytoin, carbamazepine, and St. John's wort

should also be avoided with rivaroxaban administration. The pharmacokinetic and pharmacodynamic properties of rivaroxaban are not affected by the co-administration of aspirin, naproxen, ranitidine, omeprazole, or aluminum-magnesium hydroxide. Rivaroxaban exposure is reported to be lower after meals and the presence of food delayed the time to maximum concentration, but increased both the maximum plasma concentration and AUC at doses greater than 10 mg. It is recommended to take 15- and 20-mg tablets of rivaroxaban with the evening meal. The absolute bioavailability of the 10 mg rivaroxaban tablet is not affected by food and this dose can be taken either with or without food.

The RECORD (Regulation of Coagulation in Orthopedic Surgery to Prevent Deep Venous Thrombosis and Pulmonary Embolism) trials evaluated the efficacy and tolerability of rivaroxaban for venous thromboembolism (VTE) prophylaxis after elective total hip and total knee replacements. RECORD 1 compared the rates of VTE and death for rivaroxaban 10 mg given orally once daily for 35 days to enoxaparin 40 mg given subcutaneously once daily for 35 days after elective total hip arthroplasty (THA). The primary efficacy outcome (composite of any DVT, nonfatal pulmonary embolism, or death from any cause up to 36 days after the procedure) occurred in 0.8% of patients treated with rivaroxaban [13/1537] vs. 3.4 % of patients treated with enoxaparin [50/1492] in the per-protocol population [absolute risk reduction (ARR), 2.6%; 95% CI, 1.5%–3.6%]), which met the pre-specified non-inferiority requirements. Rivaroxaban was superior to enoxaparin in the modified intent-to-treat group, with 1.1% (18/1595) of rivaroxaban treated patients having 1 of the primary efficacy events compared with 3.7% (58/1558) of enoxaparin-treated patients (ARR, 2.6%; 95% CI, 1.5%–3.7%). Six of 2209 patients (0.3%) assigned to receive rivaroxaban compared with 2 of 2224 patients (0.1%) assigned to receive enoxaparin had a major bleeding event during the on-treatment follow-up ($p = 0.18$), although one of the 6 bleeding events in the rivaroxaban group occurred during the procedure and before the patient received the first dose of drug. The rate of clinically relevant bleeding appeared to be higher in the rivaroxaban group (2.9% vs 2.4%), as was the combination of major and clinically relevant bleeding (3.2% vs 2.5%). The rates of non–bleeding-related adverse events were similar, and there was no difference in the prevalence of liver enzyme elevations. With extended anticoagulation therapy (31–39 days) with rivaroxaban and shorter-duration anticoagulation therapy (10–14 days) with enoxaparin (RECORD 2), there were fewer VTE and deaths after elective total hip arthroplasty in the extended duration anticoagulation group (i.e, rivaroxaban 10 mg administered orally once daily for a mean of 33–34 days) (2.0% [17/864]) compared with that in the shorter-duration anticoagulation group (ie, enoxaparin 40 mg administered subcutaneously once daily for a mean of 12–13 days) (9.3% [81/869]) (AAR, 7.4%, 95% CI, 5.2–9.4; $P < 0.0001$). The incidence of major bleeding was similar between both treatment 2 groups, but there were more cases of non-major bleeding events (6.5% vs. 5.5%) and clinically relevant bleeding (3.3% vs 2.7%) in the rivaroxaban group. Serious, non–bleeding-related adverse event rates were similar between the 2 treatment groups (1.1% with rivaroxaban vs 1.4% with enoxaparin) as were rates of ALT elevations >3 × ULN (0.5% with rivaroxaban vs. 0.6% with enoxaparin). RECORD 3 demonstrated that a 10- to 14-day course of rivaroxaban 10 mg daily after elective total knee replacement provided a greater reduction in risk for the composite efficacy outcome (defined as DVT, PE, or death occurring 13–17 days after surgery) than the equivalent duration of enoxaparin 40 mg given subcutaneously once daily (9.6% [79/824] vs 18.9% [166/878]; $P < 0.001$). As in RECORD 1 and RECORD 2, there was no difference between the 2 treatment groups in major bleeding rates (0.6% and 0.5% for rivaroxaban and enoxaparin, respectively; $P = 0.77$). However, the rate of clinically relevant, non-major bleeding

was higher in the rivaroxaban arm (2.7%) compared with the rate in the enoxaparin-treated group (2.3%). In addition, drug-related adverse event rates were similar (12% with rivaroxaban vs. 13% with enoxaparin). In the RECORD 4 trial, a 10 to 14-day duration of rivaroxaban 10 mg given orally once daily was found to be non-inferior to a similar duration of enoxaparin 30 mg given subcutaneously every 12 hours in the intent-to-treat population (6.9% [67/965] vs. 10.1% [97/959]; ARR, 3.19%; 95% CI, 0.71–5.67; $P = 0.0118$) and superior to enoxaparin in the per-protocol population (6.7% [58/864] vs. 9.3% [82/878]; AAR, 2.71%; 95% CI, 0.17–5.25; $P = 0.0362$) after elective total knee replacement. There were no significant differences in major bleeding events (0.7% [10/1526] vs 0.5% [4/1508]; $P = 0.1096$) between rivaroxaban and enoxaparin, although p values were not reported for clinically relevant, non-major bleeding events (2.6% vs 2.0%) and drug-related adverse events (20.3% vs 19.6%). In a pooled analysis of the RECORD 1 to 4 trials, the primary outcome of the composite of symptomatic VTE and all-cause mortality occurred in 29/6183 (0.5%) of rivaroxaban-treated patients and 60/6200 (1.0%) in enoxaparin-treated patients (odds ratio [OR], 0.48; 95% CI, 0.30–0.76; $P = 0.001$). There were numerically more bleeding events in the 6183 rivaroxaban-treated patients compared with the 6200 enoxaparin-treated patients, but the differences were not statistically significant. A meta-analysis of 8 randomized control trials of 15,586 patients who underwent elective total hip arthroplasty and total knee replacement showed that rivaroxaban lowered VTE events and all-cause mortality by an additional 44% (relative risk [RR], 0.56; 95% CI, 0.39–0.80) compared with enoxaparin, without a statistically significant difference in major or clinically relevant bleeding.

The ROCKET-AF (Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism) trial was a double-blind study that compared rivaroxaban 20 mg daily with warfarin (international normalized ratio [INR], 2.0 – 3.0). In the intent-to-treat group, 2.1% of rivaroxaban-treated patients versus 2.4% of warfarin-treated patients experienced a primary event (HR, 0.88; 95% CI, 0.74–1.03; $P < 0.001$ for noninferiority, $P = 0.12$ for superiority). There was no difference in the prevalence of major and clinically relevant non-major bleeding or major bleeding. Rivaroxaban was associated with significantly decreased incidence of intracranial hemorrhage (0.5% vs 0.7% per year; HR, 0.67; 95% CI, 0.47–0.93; $P = 0.02$), whereas the frequency of major gastrointestinal bleeding was more common in rivaroxaban-treated patients (3.2% vs 2.2%; absolute increase 1%; $P < 0.001$).

The EINSTEIN program provides efficacy and tolerability data for rivaroxaban use for VTE treatment. The EINSTEIN program consists of 3 randomized trials: the acute DVT treatment trial, the acute PE trial, and the study of extended-duration treatment for DVT and PE. Rivaroxaban was non-inferior to standard therapy with 36 of the 1731 patients (2.1%) in the rivaroxaban group and 51 of the 1718 patients (3.0%) having a confirmed recurrence of symptomatic VTE (defined as the composite of DVT and fatal or non-fatal PE) (HR, 0.68; 95% CI, 0.44–1.04; $P < 0.001$ for non-inferiority). The prevalence of the tolerability outcome was similar, with 8.1% of patients in both arms having a major or non-major, clinically relevant bleeding event (HR, 0.97; 95% CI, 0.76–1.22; $P = 0.77$). Numerically there were fewer major bleeding events in the rivaroxaban group, but this difference was not statistically significant (0.8% vs 1.2%; HR, 0.65; 95% CI, 0.33–1.30; $P = 0.21$). Overall, the rate of adverse clinical events, defined as the occurrence of a VTE or major bleeding event, was lower in the rivaroxaban treatment group (2.9% vs 4.2%; HR, 0.67; 95% CI, 0.47–0.85). The EINSTEIN-PE results showed rivaroxaban to be non-inferior to standard treatment in reducing the risk for recurrent VTE (2.1% [50/2419] for rivaroxaban vs. 1.8% [44/2413] for standard therapy; HR, 1.12; 95% CI, 0.75–1.68; $P = 0.003$). Moreover, the

occurrence of the principal tolerability outcome was similar between the 2 treatment groups (10.3% [249/2419] with rivaroxaban vs 11.4% [274/2413] with standard therapy; HR, 0.90; 95% CI, 0.76–1.07; $P = 0.23$). There were fewer major bleeding events in the rivaroxaban arm (1.1% vs 2.2%; HR, 0.49; 95% CI, 0.31–0.79; $P = 0.003$) and the rates of non-bleeding adverse events were similar in the 2 treatment groups. The EINSTEIN Continued Treatment Study aimed to determine the potential benefits of prolonging anticoagulant therapy by using rivaroxaban for an additional 6 to 12 months. This double-blind, placebo-controlled, superiority study showed that an additional 6 to 12 months of anticoagulation with rivaroxaban resulted in an 82% reduction in VTE recurrence (1.3% [8/602] with rivaroxaban vs. 7.1% [42/594] with placebo; HR, 0.18, 95% CI, 0.09–0.39; $P < 0.001$), but also caused a 5-fold increase in major and clinically relevant non-major bleeding events (6% [36/598] with rivaroxaban vs 1.2% [7/590] with placebo; HR, 5.19, 95% CI, 2.3–11.7; $P < 0.001$). There were 4 major bleeding events in the rivaroxaban group and none in the placebo group ($P = 0.11$).

PRELIMINARY DATA

Inhibition of TF reduces inflammation in $BERK^{BM}$ and TW^{SS} mice.

We analyzed plasma levels of various cytokines and chemokines (GM-CSF, IFN-γ, IL-10, IL-12p70, IL-13, IL-17, IL-1α, IL-2, IL-4, IL-5, IL-6, MCP-1, KC, MIP-2, TNF-α and VEGF) in sickle cell mice using a multiplex cytokine assay. Interestingly, IL-6 was the only cytokine that was significantly increased in both BERK mice or $BERK^{BM}$ compared to controls (data not shown). The elevated level of IL-6 in BERK or $BERK^{BM}$ mice was confirmed by ELISA. In addition, BERK mice also demonstrated increased plasma levels of IL-18 (not shown). We also found that BERK mice had elevated plasma levels of sVCAM-1, sICAM-1 and sE-selectin, which are markers of endothelial injury, as well as serum amyloid protein (SAP), which is a major acute phase protein in mice. To determine if TF contributes to the inflammatory response in SCD, these inflammatory markers were measured in WT^{BM} and $BERK^{BM}$ mice that were treated with either a rat anti-mouse TF monoclonal inhibitory antibody, 1H1 or control IgG. Inhibition of TF significantly reduced plasma levels of IL-6, SAP and sVCAM-1 but did not attenuate the increased plasma levels of IL-18, sICAM-1 and sE-selectin. Similar effects were observed in Townes mice treated with the anti-TF antibody.

Blocking TF attenuates neutrophil infiltration/activation into the lungs of $BERK^{BM}$ mice.

In SCD, chronic ischemia leads to pathological changes in multiple organs, including the lung, liver and kidney{Paszty, 1997}. Recent studies have demonstrated that neutrophils contribute to these proceses {Polanowska-Grabowska,; Wallace, 2009}. We observed infiltration of neutrophils in the lungs and livers but not kidneys of BERK mice compared to WT controls. Consistent with this observation, levels of MPO, a marker of neutrophil activation, were also increased in the lungs and livers of BERK and $BERK^{BM}$ mice. Interestingly, 1H1 significantly reduced levels of MPO in the lungs but not livers in $BERK^{BM}$ mice. Furthermore, increased levels of MPO in lung and liver were associated with increased expression of the chemokines MCP-1 and KC in $BERK^{BM}$ mice compared to WT^{BM} mice. Here again, 1H1 treatment significantly attenuated expression of both chemokines in the lung but not in the liver. The high level of TF in the lung compared to the liver may explain these different results (data not shown).

Endothelial cell-specific deletion of TF has no effect on activation of coagulation but reduces plasma levels of IL-6 in sickle cell mice.

To investigate the role of TF expressed by ECs in the activation of coagulation in a mouse model of SCD, we generated sickle mice with an EC-specific deletion of the TF gene. We have recently demonstrated that $TF^{flox/flox}$,Tie-2 Cre^{+} mice have almost complete deletion of the TF gene in both lung EC and hematopoietic cells{Pawlinski, 2012}. To generate sickle and control mice with EC-specific deletion of TF and normal TF expression in hematopoietic cells, we transplanted these mice with bone marrow from BERK or WT mice. $TF^{flox/flox}$ mice were used as controls. Interestingly, deletion of TF in ECs did not affect plasma TAT levels but significantly reduced IL-6 levels. However, the increased plasma levels of IL-18, SAP and markers of EC activation, as well as increased levels of MPO in the lung and liver, were not affected by EC-specific deletion of TF in sickle cell mice. These data indicate that EC TF contributes only to IL-6 expression, whereas other cellular sources of TF contribute to inflammation and EC activation.

*Contribution of PAR-1 and PAR-2 expressed on non-hematopoietic cells to the pathology of SCD***.**

We hypothesized that TF-dependent generation of coagulation proteases contributes to inflammation and EC activation by activation of PAR-1 and PAR-2. To generate sickle cell mice lacking PAR-1 or PAR-2 expression in all non-hematopoietic cells, we transplanted $PAR\text{-}1^{-/-}$ or $PAR\text{-}2^{-/-}$ mice with bone marrow from sickle cell mice. PAR-1 deficiency in non-hematopoietic cells significantly reduced plasma levels of sVCAM-1 but not IL-6 or SAP. In contrast, PAR-2 deficiency in non-hematopoietic cells had no effect on plasma levels of sVCAM-1 but significantly reduced plasma levels of IL-6 and SAP. These data indicate that PAR-1 signaling contributes to endothelial cell activation whereas PAR-2 promotes inflammation in sickle cell mice.

Inhibition of thrombin does not decrease endothelial cell activation or inflammation.

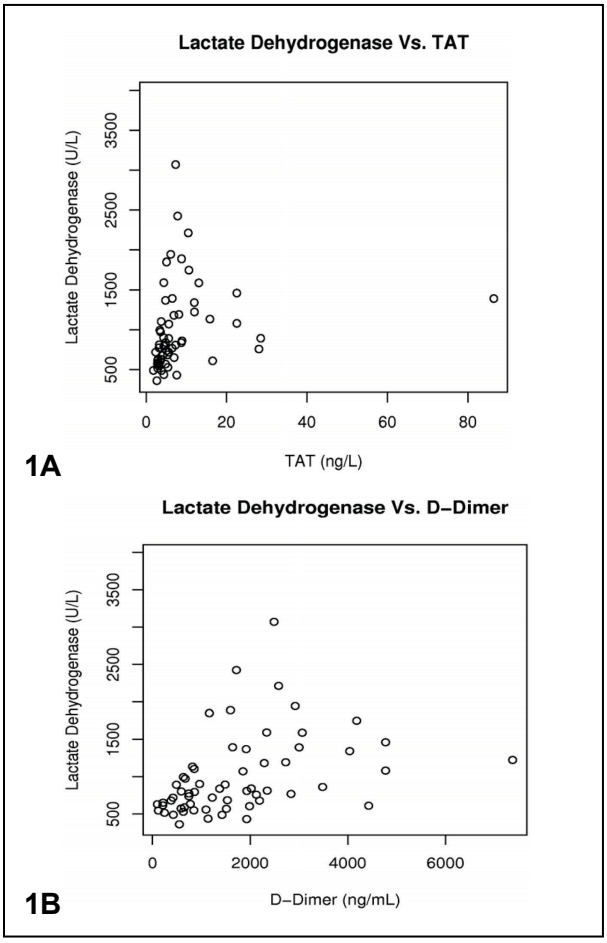
Next, we investigated the effect of thrombin inhibition with dabigatran treatment on activation of coagulation, inflammation and endothelial cell activation in sickle cell mice. We fed mice a chow containing 10 mg of dabigatran per gram of food. Plasma levels of dabigatran in these anticoagulated mice are similar to the levels observed in humans receiving a dose of dabigatran of 150 mg bid (J. Van Ryn, unpublished data). Inhibition of thrombin with dabigatran significantly increased aPTT (from 26.6±1.2 to 76.2±8.5) in WT and from 25.1±1.3 to 70.1±10.7 seconds in BERK mice) and reduced plasma TAT levels in sickle cell mice. However, thrombin inhibition had no effect on plasma levels of IL-6, SAP or sVCAM-1. These data suggest either the dose of dabigatran used is insufficient to block local thrombin activation of PAR-1 or other proteases are activating PAR-1 in the mice. This result strongly suggests that inhibition of the clotting system upstream of thrombin, i.e. at TF:FVIa, FXa or FXIa, is more likely to reduce inflammation and EC activation.

Inhibition of FXIa reduces plasma TAT levels in sickle cell mice.

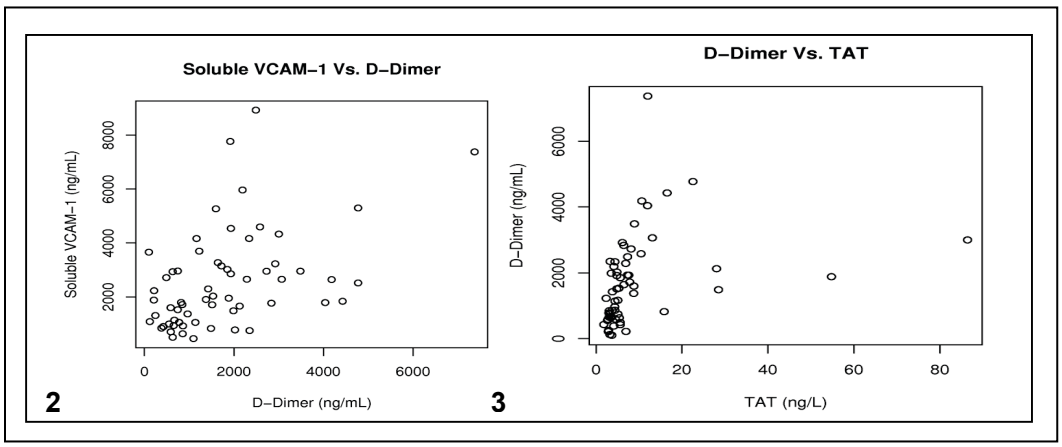
To determine if the intrinsic coagulation pathway contributes to the hypercoagulable state in SCD, we treated sickle cell mice with an antibody called 14E11 (kindly provided by Dr. A. Gruber) that blocks activation of the intrinsic coagulation pathway by inhibiting FXIa generation. We observed that 24 hours after injection of 14E11 (4 mg/kg) the aPTT was significantly increased and plasma TAT was significantly reduced in BERK mice. We did not measure other parameters due to the short time period of this experiment (1 day). The mechanism of FXIa activation is not known and will not be pursued in this proposal. It is possible that polyphosphates released from activated platelets may activate FXII {Smith, 2006}{Muller, 2009}.

Measurements of coagulation activation, EC activation and cytokine expression in sickle cell patients.

Our group has had a long-term interest in coagulation activation in patients with SCD{Ataga, 2008 #292; Ataga, 2007 #272; Key, 1998 #269}. In a study of 76 patients with SCD and 6 healthy, control subjects of African descent, SCD patients had significantly higher D-dimer levels than control subjects (1251.5 ng/mL [FEU] *vs.* 318 ng/mL [FEU]; p = 0.02) {Ataga, 2008}. The median values of TAT and F1+2 levels were also higher in SCD patients, although the differences were not statistically significant.



In another study of 64 SCD patients, we observed a correlation between TAT and lactate dehydrogenase (r = 0.57, p < 0.0001) (Figure 1A), with borderline correlations between TAT and total bilirubin (r = 0.25; p = 0.054), indirect bilirubin (r = 0.26, p = 0.051), and hemoglobin (r = - 0.24; p = 0.071) {Ataga, 2012}. TAT was also correlated with the absolute monocyte count (r = 0.27; p = 0.035) and NT-proBNP (r = 0.35; p = 0.005). Similarly, D-dimer was correlated with lactate dehydrogenase (r = 0.56; p < 0.0001) (Figure 1B), indirect bilirubin (r = 0.26; p = 0.048), hemoglobin (r = - 0.32; p= 0.012), NT proBNP (r = 0.42; p < 0.0001), with borderline correlations with absolute monocyte count (r = 0.23; p = 0.074). Both TAT (r = 0.37; p = 0.004) and D-dimer (r = 0.49; p < 0.0001) (Figure 2) were correlated with soluble VCAM-1. When the analyses were limited to only patients with SS/SD/Sβ^0 thalassemia, there was a significant correlation between TAT and lactate dehydrogenase (r = 0.62, p < 0.0001), indirect bilirubin (r = 0.296, p = 0.044), and NT-proBNP (r = 0.32, p = 0.026), with borderline correlation with total bilirubin (r = 0.28, p = 0.053) and absolute monocyte count (r = 0.25, p = 0.089). Similarly, we observed significant correlations between D-dimer and lactate dehydrogenase (r = 0.60, p < 0.0001), hemoglobin (r = - 0.29, p = 0.039), and fetal hemoglobin (r = - 0.31, p = 0.035), NT-proBNP (r = 0.49, p < 0.0001), with borderline correlations with platelet count (r = - 0.28, p = 0.053), total bilirubin (r = 0.24, p = 0.093) and indirect bilirubin (r = 0.26, p = 0.07). Finally, we observed a significant correlation between TAT and D-dimer (r = 0.66; p<0.0001) (Figure 3). However, no correlations were observed between TAT and soluble CD40 ligand (r = 0.016, p = 0.91) or between TAT and MPTF procoagulant activity (r = 0.064, p = 0.63). Similarly, no correlations were observed between D-dimer and soluble CD40 ligand (r = −0.17; p = 0.19) or between D-dimer and MPTF procoagulant activity (r = 0.11, p = 0.40).



*Plasma markers of coagulation activation are associated with clinical complications in SCD***.**

The level of TAT was significantly higher in patients with a history of retinopathy compared to those without this complication (6.05 ng/L; IQR, 4.91, 10.98 ng/L vs. 4.52 ng/L; IQR, 3.22, 7.33 ng/L, p= 0.023). In addition, the level of TAT was lower in patients on hydroxyurea therapy compared to those not on such therapy (4.39 ng/L; IQR, 3.25, 8.77 ng/L vs. 6.5 ng/L; IQR, 5.08, 8.76 ng/L, p =0.044). D-dimer appeared to be associated with a history of stroke (3003.1 ng/mL [FEU]; IQR, 1513, 3067 ng/mL [FEU] vs. 1399.5 ng/mL [FEU]; 648.8, 2217 ng/mL [FEU], p = 0.062), although the difference was not statistically significant. When those patients with a measurable tricuspid regurgitant jet velocity were evaluated (N = 43), there was no significant correlation between D-dimer and tricuspid regurgitant jet velocity (r =0.25, p = 0.10). However, when the analyses were limited to patients with SS/SD/Sβ^0 thalassemia, we observed associations between D-dimer and a history of thrombotic stroke (3035.2 ng/mL [FEU]; IQR 2559, 3494 vs. 1269.6 ng/mL [FEU]; IQR 688.4, 2347, p = 0.049), TAT and history of retinopathy (7.145 ng/L; IQR 5.225, 12.01vs. 4.465 ng/L; IQR 3.215, 7.332, p= 0.018). There appeared to be a correlation between tricuspid regurgitant jet velocity and D-dimer (r = 0.3, p =0.067), although this was not statistically significant. Although no significant correlation was observed between plasma markers of thrombin generation and echocardiography-derived TRV, TRV may not be particularly sensitive for the diagnosis of PHT {Parent, 2011}. As right heart catheterizations were not obtained in all of the study subjects it is uncertain how many patients truly have PHT. However, as NT-proBNP is a surrogate for PHT {Machado, JAMA; Ataga, 2006}, the correlation between NT-proBNP and both D-dimer and TAT (see #3 above) suggests a likely association between these markers of thrombin generation and RHC-diagnosed PHT.

*Correlation of inflammatory cytokines with echocardiography-defined pulmonary hypertension***.**

Levels of selected inflammatory cytokines were measured in 56 HbSS patients to evaluate their association with echocardiography-defined PHT {Ataga, 2008}. Patients with echocardiography-defined PHT had consistently higher median levels of IL-6 (4.7 ng/mL *vs.* 2.9 ng/mL; p = 0.06), interleukin-8 (5.4 ng/mL *vs.* 2.7 ng/mL; p = 0.07) and interleukin-10 (0.57 ng/mL *vs.* 0.35 ng/mL; p = 0.10) compared with HbSS patients without PHT, although the differences were only of borderline statistical significance (Table 1).

Table 1

Analyte	PHT (N=19) Median (25^{th}, 75^{th})	No PHT (N=34) Median (25^{th}, 75^{th})	p value
Interleukin-6 (pg/mL)	4.7 (2.7, 7.8)	2.9 (1.5, 6.5)	0.06
Interleukin-8 (pg/mL)	5.4 (2.4, 15.5)	2.7 (0, 7.9)	0.07
Interleukin-10 (pg/mL)	0.57 (0.25, 1.2)	0.35 (0.03, 0.82)	0.10
Interferon-γ (pg/mL)	31.3 (0, 85.7)	13.3 (0, 71.4)	0.76
Tumor necrosis factor-α	1.9 (0.7.8)	3.3 (0.98, 21.9)	0.25

Laser Doppler velocimetry in SCD.

We conducted a proof-of-principle study to determine whether pentosan polysulfate sodium (PPS) improves baseline blood flow in the microvasculature of patients with SCD {Kutlar, 2012}. PPS has structural and functional similarities to, but considerably less anticoagulant activity than heparin, as well as potent blocking activity against cell adhesion to P-selectin. At the time that this double-blind, placebo-controlled, randomized, multi-center study was discontinued, 19 of 41 enrolled patients had been randomized to the active PPS arm, 14 had received PPS, nine had received PPS for at least eight weeks, and two of the nine disclosed they had not taken their study drug reliably. In the seven evaluable patients, PPS was safe and non-toxic when given for 8 to 12 weeks, and there was a highly significant reduction in plasma soluble VCAM-1 from pretreatment levels ($p \leq 0.015$ at 2 wk; $p < 0.001$ at 8 wk). There also was a non-significant trend toward improved blood flow measured by laser Doppler velocimetry (LDV) in these same seven patients, but no evidence of improved soluble VCAM-1 levels or blood flow in the placebo group. This study demonstrates our experience with the conduct of studies evaluating microvascular blood flow with laser Doppler velocimetry. Dr. Neil Matsui will serve as a consultant in this application and will be responsible for interpreting the LDV test results.

Anticoagulation with warfarin is safe in SCD.

In a pilot study, we showed that low-intensity anticoagulation with warfarin (INR of 1.5 – 2.0) for 12 months appears safe in appropriately selected patients (unpublished data). There were no bleeding episodes during the course of the study. In addition, the median baseline INR in the 4 patients in the placebo arm was 1.1 (range: 1.0 to 1.1) and following treatment was 1.1 (range: 1.0 to 1.2). The median baseline INR for the 3 patients in the low-intensity anticoagulation arm was 1.1 (range: 1.0 to 1.1) and following treatment was 1.7 (range: 1.4 to 1.9). Following treatment, patients on low-intensity anticoagulation had greater reductions in TAT ($M_{baseline}$= 4 µg/L, M_{12mth}= 2 µg/L) and D-dimers levels ($M_{baseline}$= 971 ng/mL, M_{12mth}= 513 ng/mL) compared to patients on placebo who experienced relatively no change in their levels (TAT $M_{baseline}$= 5.5 µg/L and M_{12mth}= 5.0 µg/L; D-dimers $M_{baseline}$= 1050 ng/mL, M_{12mth}= 871 ng/mL). Finally,

despite concerns of the risk of bleeding in SCD patients, our clinical experience suggests that appropriately selected patients with SCD on standard intensity anticoagulation do not appear to have an increased risk of bleeding.

RESEARCH DESIGN AND METHODS

We will conduct a randomized, double-blind, placebo-controlled, crossover study to evaluate the efficacy and safety of rivaroxaban in SCD. We have selected this agent based on our preliminary data in sickle mice showing that 'upstream' TF inhibition achieves the desired effects on inflammation and endothelial activation, but that 'downstream' thrombin inhibition does not. The study will consist of three phases: 1) *Screening/Baseline*; 2) *Treatment*; and 3) *Follow-up*. The Screening/Baseline Phase will occur within 28 days of study drug administration and will include: informed consent, a history and physical examination, and clinical laboratory tests including: a complete blood count, routine coagulation studies, routine chemistries, chest x-ray, and serum or urine pregnancy test (if female, and of child-bearing capacity). The sequence of drug administration will be generated via block permuted randomization procedure. The 4-week Treatment Phase will consist of drug administration, efficacy assessments and safety assessments (including pregnancy testing in females of child-bearing capacity). After the first treatment period, there will be a washout period of 14 ± 3 days. In the second treatment phase, patients will be crossed over to receive rivaroxaban or placebo, depending on their initial treatment assignment. The total duration of the Treatment phase (including washout) will be 10 weeks. During the Follow-up Phase at week 12, patients will obtain their final study evaluation. Table 2 provides a detailed schedule of assessments for the study period.

Treatment: Subjects will receive rivaroxaban 20 mg/day (the approved dose to prevent stroke and systemic embolism in patients with non-valvular atrial fibrilation) and placebo for 4 weeks each, separated by a 2-week washout phase. Pill counting will be used to assess compliance. PT/INR will be monitored by an unblinded investigator to assess compliance with no other involvement in the study). If major bleeding or other serious adverse event occurs (see Appendix 2), the study drug will be discontinued and the patient followed until resolution of the problem. Subjects that discontinue the study early will not be replaced.

Eligibility Criteria:

Inclusion Criteria:

a) 18 to 65 years of age;
b) HbSS or HbSβ0 thalassemia; serum creatinine ≤ 1.0 mg/dL men) or 1.2 mg/dl (women);
c) ALT ≤ 2 times upper limits of normal;
d) platelet count ≥ 150,000 cu/mm;
e) normal baseline PT/INR and aPTT;
f) be in the non-crisis, "steady state" at enrollment with no severe pain episodes during the preceding 4 weeks;
g) ability to understand the requirements of the study and be willing to give informed consent;
h) women of childbearing age must be practicing an adequate method of contraception;
i) if on hydroxyurea, must be on a stable dose for at least 3 months. The dose of hydroxyurea will not be adjusted during the study duration except for safety reasons.

Exclusion Criteria:

a) hypersensitivity to any component of rivaroxaban;
b) history of major GI bleeding or bleeding diathesis;
c) baseline Hb < 5.5 g/dL;
d) history of clinically overt stroke;
e) brain MRI/MRA scan with evidence of Moya Moya;
f) pregnant or breastfeeding;
g) active liver disease or ALT > 3 times upper limit of normal;
h) on chronic anticoagulant, NSAID or statin therapy;
i) history of metastatic cancer;
j) current alcohol abuse;
k) on a chronic transfusion program or any blood transfusion in the prior 3 months;
l) ingested any investigational drugs within the past 4 weeks
m) use of CYP3A4/P-glycoprotein inducers such as carbamazepine, phenytoin, rifampin, and St John's wort;
n) use of CYP3A4/P- glycoprotein inhibitors such as ketoconazole, indinavir/ritonavir, itraconazole, lopinavir/ritonavir, ritonavir, and conivaptan

Assessments: *Activation of coagulation.:* Plasma levels of TAT and D-dimer measured using a commercially available ELISA kit at baseline, and at 2 and 4 weeks following initiation of study drug

Endothelial cell activation: Plasma levels of soluble VCAM-1 and soluble ICAM-1 measured using a commercially available ELISA kit at baseline, and at 2 and 4 weeks following initiation of study drug

Inflammatory markers: Plasma levels of IL-6 (commercially available ELISA), high sensitivity CRP, MPO, IL-2, IL-8, TNFα, sPLA2 (multiple cytokine assay using Luminex MAP technology; UNC core facility) at baseline, and at 2 and 4 weeks following initiation of study drug

Microvascular blood flow: We will analyze microvascular blood flow using laser Doppler velocimetry (LDV) (Perimed, PF5001, Stockholm, Sweden) assessments of post-occlusive reactive hyperemia (PORH). Measurements will be obtained at baseline and at 4 weeks following initiation of therapy.

Safety Assessment: We will evaluate patients for major bleeding complications (any bleeding episode into critical sites, e.g. intracranial bleed, decrease in hemoglobin concentration of at least 2g/dL from baseline or any prolonged bleeding that requires a blood transfusion) and other clinically relevant non-major or trivial bleeding. We will also evaluate for episodes of SCD-related events during scheduled follow-up visits, by employing a systems review, physical examination, vital signs, and routine laboratory tests.

Termination Criteria: Treatment with study drug will be discontinued at any time if the patient: a) withdraws consent; b) develops a major bleeding episode (defined as any bleeding episode into critical sites, e.g. intracranial bleed, decrease in hemoglobin concentration of at least 2g/dL from baseline or any prolonged bleeding that requires a blood transfusion); or if, c) in the judgment of the investigator, continuation of the study drug will be hazardous to the patient. The UNC DSMB will review the protocol following the occurrence of the 1st treatment-related serious adverse event. The study will be terminated at the occurrence of treatment-related serious adverse events, including major bleeding, in up to 3 study participants.

STATISTICAL METHODS

Normally distributed variables will be presented with means and SD, while summary of variables not normally distributed will be presented with medians and ranges or geometric means and SD as appropriate. The main study objective is to examine the difference between the measurements at baseline and following 4 weeks of treatment. We will evaluate the effect of rivaroxaban on the outcome measures by performing a comparison of treatment by visit using paired t-tests subsequently followed by crossover ANOVA model testing to assess the carry-over effect and treatment effect. In addition, when outcome measures are not normally distributed, we will perform appropriate nonparametric tests. The effect of rivaroxaban on various plasma markers and microvascular blood flow will be estimated using a multiple linear regression model. While the study is adequately powered to evaluate the effect of rivaroxaban on soluble VCAM-1 and IL-6, analysis of the effect of rivaroxaban on the other indicated endpoints are exploratory.

Sample Size Justification: The primary outcome measures are sVCAM-1 and IL-6. Based on our preliminary data in SCD patients, log (sVCAM) and log(IL-6) are normally distributed with SD of 0.49 and 0.95, respectively. In a 2x2 crossover design, assuming a correlation of 0.65 for the pre- and post-treatment measures, to detect a difference of 0.245 in the change of log(sVCAM) and 0.475 in the change of log(IL-6) between the rivaroxaban and placebo groups requires a minimum sample size of 15 per sequence (a total sample size of 30) at 80% power and $\alpha = 0.05$. Assuming a 10% dropout rate, a total of 34 patients is required for this study (17 in each sequence).

Data Management: A standardized set of data collection forms will be designed using the RED Cap system and a secure database will be created. All data will be stored under password protection on a secured server. A data manager will handle all data entry and database security. This individual will work closely with the statistician on database design and quality assurance procedures.

Timeline: We anticipate beginning recruitment in year 2, following studies on rivaroxaban in sickle mice. Recruitment for this clinical study is expected to take up to 36 months. The remaining few months will be dedicated to completing the studies, data analysis and publication.

HUMAN SUBJECTS

Protection of Human Subjects

1. Human Subjects Involvement and Characteristics: This study seeks to evaluate the efficacy and safety of the factor Xa inhibitor, rivaroxaban, in SCD. Thirty two patients with SCD (HbSS or $S\beta^0$ thalassemia) that meet the eligibility criteria will be enrolled in a randomized, double-blind, placebo-controlled, crossover trial. At the time of enrollment, all study subjects will be in the non-crisis, steady state. Informed consent will be obtained according to IRB approved procedure. Patients will be closely monitored for adverse events following randomization to the study drug. The primary goal of this study is to evaluate the effect of rivaroxaban on plasma levels of soluble VCAM and IL-6. Furthermore, we will evaluate plasma other markers of endothelial activation (soluble ICAM) and other inflammatory markers (high sensitivity CRP, MPO, IL-2, IL-8, TNFα, sPLA2), coagulation activation (TAT and D-dimer),

assessment of microvascular blood flow using laser Doppler velocimetry and safety as described in the application.

2. Sources of Research Material: Enrollment and study participation will involve the collection of a full range of clinical data, including medical history. We will obtain clinical and laboratory data as well as assessments of microvascular blood flow at specified intervals during the course of the studies as described in the research plan.

3. Plans for Recruitment: Patients will be recruited from amongst the patients that routinely receive their care at the UNC Comprehensive Sickle Cell Program. Dr Ataga and/or one of the co-investigators will discuss the studies with all eligible patients. Interested patients will be provided with a copy of the consent form(s), and the study coordinator or PI (or a co-investigator) will carefully go over the consent with each candidate before he/ she signs. We will strive to ensure that all patients understand the nature of the study and all their questions will be answered. The original consent form will be placed in the study files, in a secure, locked cabinet. Potential subjects will be contacted during regularly scheduled clinic visits, but may be contacted at home by study personnel if they are known to meet the eligibility criteria. The PI and study nurse involved in the study are regular staff of the program. Samples obtained from patients specifically for these studies will be collected as approved by the IRB. This is anticipated to occur during specified study visits.

4. Potential Risks: The study entails physical risk associated with venipuncture to obtain blood samples; administration of rivaroxaban; and pneumatic occlusion cuff placed around the arm to a suprasystolic pressure (~ 200 mmHg) during the assessment of microvascular blood flow. The most significant risks of blood tests are discomfort and bruising. There is still limited clinical experience with the use of rivaroxaban in the general population, and even less so in SCD. However, published studies indicate that the most common complications of rivaroxaban are bleeding, bruising, peripheral edema, dizziness, headache, pyrexia, fatigue, syncope, pruritus, rash, blister, diarrhea, thrombocytopenia, extremity pain, and back pain. Pneumatic occlusion of the arm to a suprasystolic pressure is uncomfortable. However, this technique has been safely performed in SCD patients. Patients will be monitored very closely for any laboratory or clinical complications during the course of the studies.

5. Procedures for Minimizing Risks: We will do everything possible to minimize the risks associated with participating in the studies. We will ensure there are no contraindications to receiving rivaroxaban. This study will be monitored by a DSMB, which will review all reports to determine if there is a safety concern. Finally, we have established data safety and monitoring procedures as described below.

Risks to Privacy: All clinical information, including personal identifiers obtained on research subjects will be maintained in a locked and secured file drawer with access limited to clinical staff. All data will be coded to protect patient confidentiality. We will make all effort to maintain patient confidentiality.

6. Potential Benefits of the Proposed Research to the Subjects and others: The available treatments in SCD remain limited. This study will help to define the contribution of coagulation activation to the pathophysiology of SCD.

7. Importance of the Knowledge to be Gained: Despite our improving understanding of the pathophysiology of SCD, treatment options for several SCD-related complications remain quite limited. This proposal aims to increase our understanding of the contribution of coagulation

activation to the pathophysiology of SCD. The risks to patients from participating in this study are modest and are reasonable in relation to the potential benefits and the importance of knowledge to be gained.

8. Data and Safety Monitoring Plan: All SAEs will be reported to the IRB and the DSMB. In addition, after 5 patients and subsequently every 6 months, all of the available safety data will be presented by treatment group to the DSMB in a confidential report. The DSMB will review and determine if there is a safety concern. The DSMB will review the study after the occurrence of the first treatment-associated SAE. The study will be terminated at the occurrence of 3 treatment-related serious adverse events, including bleeding complications. The statistician who conducts the randomization and generates the tables for the DSMB will be an 'independent statistician' not otherwise involved in the study and will not be responsible for the data analysis at the completion of the study.

The UNC TraCS DSMB is a committee that has been established within the UNC School of Medicine. The membership is appointed by the Dean (or his designee), and is available to review any clinical trial that is being carried out by a UNC investigator. Most multi-center clinical trials funded by the NIH or by industry have national data and safety monitoring boards organized by the sponsoring organization. However, single- or dual-site clinical trials such as the study proposed in this application depend on a local DSMB such as the one based here at UNC. The current makeup includes a chair (Dr. Ross Simpson), an ethicist, an epidemiologist/biostatistician, and several clinical researchers. In addition, *ad hoc* members are added to the DSMB when need for scientific expertise arises.

9. Inclusion of Women and Minorities: Based on the results of our studies in this patient population to date, we anticipate that enrolled subjects will include 50-55% women. We have achieved this in the past without special efforts to recruit women, and this reflects the gender distribution of our patient population. In addition, due to the nature of the disease to be studied (sickle cell disease), over 95% of patients will identify themselves as African-American. A small number identifying themselves as Hispanic or Native American may be available; such patients typically comprise less than 2% of our clinic patients with this disease.

10. Inclusion of Children: As rivaroxaban is not yet approved in children, no patients less than 18 years will be evaluated in this study.

Rivaroxaban in Sickle Cell Disease

	Screening[1]	Treatment 1			2 Week Washout	Treatment 2			F/U
Assessment/Event	Day -28 – -1	BL[1]	Wk 2	Wk 4		BL	Wk 2	Wk 4	Wk 6
Informed consent	X								
Medical history	X								
Vital Signs	X	X	X	X		X	X	X	X
Physical exam	X	X				X			X
MRI/MRA	X								
Hematology[2]	X	X				X			X
Chemistries[3]	X	X				X			X
Coags[4]	X	X				X			X
Chest X-ray	X								
Serum/urine pregnancy test[5]		X	X	X		X	X	X	
Biomarkers		X	X	X		X	X	X	
Laser doppler velocitometry		X		X		X		X	
Randomization		X							
Study drug initiation		X				X			
Adverse event probe		X	X	X		X	X	X	X
Record concomitant medications	X	X				X			X

[1] Screening assessments and baseline assessments for Treatment Phase 1 may be combined if eligibility can be established before treatment is initiated
[2] CBC with differential and reticulocyte count [3] BUN, creatinine, albumin, total and direct bilirubin, ALT, AST, LD, AlkPhos, GGT [4] PT/INR and aPTT