Rivaroxaban versus Warfarin in the evaluation of progression of coronary calcium

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Brief synopsis of the proposed research

Current oral anti-coagulation for atrial fibrillation is most commonly performed with warfarin. Warfarin is a vitamin K antagonist that has been shown in non-randomized trials to increase vascular calcification. Increased vascular calcification has been tied to increased cardiovascular events (CVE). This study will randomize patients currently taking warfarin to either continue on warfarin or be switched to rivaroxaban. Rivaroxaban is an oral anti-coagulant that works by inhibiting Factor Xa, and has no interaction with vitamin K. This study is a randomized, open label study that will randomize 120 patients and have them undergo blood tests and a calcium scan at baseline, and again after 12 months. Patients will be seen quarterly for examinations, safety checks and supply of rivaroxaban, as well as follow up INR testing for warfarin.

1. Introduction:

Vitamin K-antagonists (VKA) are the most widely used anti-thrombotic drugs with substantial efficacy in reducing risk of arterial and venous thrombosis. Several lines of evidence indicate, however, that VKA inhibit not only post-translational activation of vitamin K-dependent coagulation factors but also synthesis of functional extra-hepatic vitamin K-dependent proteins thereby eliciting undesired side-effects. Vascular calcification is one of the recently revealed side-effects of VKA. Vascular calcification is an actively regulated process involving vascular cells and a number of vitamin K-dependent proteins.

2. Background Information

Coronary artery calcium (CAC) is strongly associated with atherosclerotic burden and predicts coronary heart disease (CHD) events and mortality. Progression of CAC has also been shown to independently be associated with increased CVD events and all-cause mortality. Warfarin has recently been shown to not only increase calcification, but markers of atherosclerotic plaque in both animals and humans.

Rivaroxaban has been shown to reduce the risk of stroke significantly and to be non-inferior to warfarin. However, the rate of myocardial infarction was 9% lower in the rivaroxaban group than in the warfarin group in the Rocket AF study, and similarly the myocardial infarction rate was 12% lower in the apixaban group in the Aristotle study, but the difference was not significant in either trial. The potential of long term benefit by avoiding VKA therapy may be much greater for a CV event reduction. The study proposed will evaluate markers of CAC progression and atherosclerosis development, including adverse plaque characteristics such as positive remodeling and stenosis severity, which have long term outcome data supporting that slowing these processes will be associated with lower CV events.

CAC and Progression

Multiple studies have been done to demonstrate the feasiblity of measuring CAC change at 12 months. Initial studies suggested that treatment with statins was associated with slower progression of CAC. Callister et al.[i] studied 149 patients. Patients underwent a baseline and follow-up EBT scan at 12 months and serial LDL cholesterol measurements were obtained.

Progression of calcium volume score was seen in all untreated patients (n = 44; mean LDL 147 ± 22 mg/dl) and averaged 52 ± 36% per year. In contrast, the mean yearly calcium volume score change for all treated patients (n = 105; mean LDL 114 ± 23 mg/dl) was 5 ± 28% ($p < 0.001$ vs. untreated patients). Among the treated patients, 65 individuals attained a LDL level <120 mg/dl (mean LDL 100 ± 17 mg/dl) and showed a net regression of their calcium volume score (-7 ± 22%). In patients who received HMG-CoA reductase inhibitors but maintained an average LDL >120 mg/dl (mean LDL 139 ± 18 mg/dl), the mean yearly calcium volume score progression was 25 ± 22%. Similar one year studies by Budoff et al.,[ii] and Achenbach et al.,[iii] showed that statin therapy is associated with slowing of progression of coronary calcium.

The BELLES trial[iv] was a prospective randomized study of 615 postmenopausal and dyslipidemic women randomized after initial CAC scan to atorvastatin 80 mg/day or pravastatin 40 mg/day. A second scan was repeated in 12 months. The attained mean LDL cholesterol level was significantly lower with atorvastatin (94 mg/dl) than pravastatin (129 mg/dl). Nonetheless, the median relative change in the calcium volume score was not different between the two treatment arms (15.1% and 14.3% for atorvastatin and pravastatin, respectively, p = NS).

Another large scale clinical trial using CAC at one year intervals was the Treat-to-Goal Study—a randomized, multicenter clinical trial—comparing the calcium-free, non-absorbable polymer sevelamer to traditional calcium-based phosphate binders.[v] Study endpoints included serum levels of phosphorus, calcium, intact parathyroid hormone (PTH), and lipids, as well as change in calcification of the coronary arteries and thoracic aorta quantified by CT. Two hundred adult hemodialysis patients, who had received hemodialysis for a median of 3 years prior to study entry, were randomized in 15 medical centers in Europe and the United States. During the study period, phosphate binders were adjusted to maintain serum phosphorus levels between 3.0 and 5.0 mg/dL, serum calcium levels between 8.5 and 10.5 mg/dL, and serum PTH levels between 150 to 300 pg/mL. EBT was performed at the start of the study, and after 6 and 12 months of treatment. In spite of a similar control of serum phosphorus and calcium, coronary and aortic calcification progressed significantly in the calcium-treated patients while there was no statistically significant change from baseline in the sevelamer group. At one year follow-up, the median relative change in coronary and aorta scores were 25% and 28% and 6% and 5% in the calcium and sevelamer group, respectively ($p = 0.02$ for all intergroup comparisons).

One recent study by Fitch et al[vi] evaluated a 12 month, randomized, placebo controlled trial to investigate lifestyle modification (LSM) and metformin, alone and in combination was conducted among HIVinfected patients with metabolic syndrome. The primary endpoint was change in CAC score. Fasting lipids, insulin and measures of cardiorespiratory fitness were assessed. 50 subjects (age 47 ± 1 yr) were randomized, 76% were male, 48% White, 30% African American, and 18% Hispanic. Metformin-treated subjects demonstrated significantly less progression of CAC over 12 months (-1 ± 2 vs. 33 ± 17, P=0.004), less progression in calcified plaque volume (-0.4 ± 1.9 vs. 27.6 ± 13.8 mm3, P=0.008), improved HOMA-IR (-0.1 ± 0.4 vs. 1.1 ± 0.4, P=0.05) and RANKL/OPG ratio (-0.0002 ± 0.0002 vs. 0.0003 ± 0.0002, P=0.02) compared to placebo. Subjects randomized to LSM vs. no LSM showed significant improvement in HDL (3 ± 1 vs. -1 ± 1 mg/dl, P=0.03), and CRP (-1.57 ± 0.70 vs. 0.08 ± 0.45

mg/L, P=0.05). For CAC, the net effect of metformin (vs. placebo) controlling for LSM randomization was -42 and the net effect of LSM (vs. no LSM) controlling for metformin randomization was -24.

Three randomized 1 year studies were performed by Budoff's group, evaluating change in CAC over 12 months in relatively small cohorts under the influence of different formulations of Aged Garlic Extract (AGE). Budoff et al. (2004)[vii] showed that in 23 patients with known coronary artery disease (CAD) or high risk patients (Framingham Risk > 20% over 10 years), over one year, AGE reduced the progression of CAC compared to placebo (7.5 +/- 9.4% versus 22.2 +/- 18.5%, respectively) . Similarly, Budoff et al. (2009)[viii] showed significant results in 65 intermediate risk patients (age 60 +/- 9 years) with Framingham risk of 10 – 20 % and baseline CAC > 30. In this study, AGE was administered with vitamin B12, folic acid, vitamin B6 and L-arginine. In one year follow-up the CAC progression was significantly lower in the treatment group versus placebo (6.8% versus 26.5%, p = 0.005). Lastly, Zeb et al. (2012)[ix] enrolled 65 asymptomatic intermediate risk (as defined by baseline CAC > 10) males, aged 55 +/- 6 years, and treated them with AGE plus co-enzyme Q10 (CoQ10) versus placebo. At 1 year, mean CAC progression was significantly lower in treatment group versus placebo (32 +/- 6 versus 58 +/- 8, p = 0.01).

CAC and Events

Coronary artery calcium (CAC) is strongly associated with atherosclerotic burden and predicts coronary heart disease (CHD) events and mortality (1-4). CAC scanning has been proposed as a measure to track CHD progression and the effects of risk factor modification on atherosclerosis (5-6). Multiple studies suggests that CAC progression is associated with CHD events (7-8). Recently, follow-up based on a large registry of subjects receiving serial CT scans showed progression of CAC to be strongly associated with total mortality (9). We have previously demonstrated[9] in a prospective evaluation of 4,609 patients that, after adjusting for baseline score, age, sex, and time between scans, CAC progression (mean progression was 247 in this group) was associated with a 3.34 fold risk of all-cause mortality (HR 3.34; 95% CI: 2.65 to 4.21; p < 0.0001). *The MESA study also demonstrated that CAC progression was strongly associated with increased CV events(10), following 5682 persons over 7.6 years.* Among participants with baseline CAC, those with annual progression of $\geq$300 units had adjusted HR's of 3.8 (1.5-9.6) for total and 6.3 (1.9-21.5) for hard CHD compared to those without progression.

Warfarin and CAC Progression.

Vitamin K-antagonists (VKA) are the most widely used anti-thrombotic drugs with substantial efficacy in reducing risk of arterial and venous thrombosis. Several lines of evidence indicate, however, that VKA inhibit not only post-translational activation of vitamin K-dependent coagulation factors but also synthesis of functional extra-hepatic vitamin K-dependent proteins thereby eliciting undesired side-effects. Vascular calcification is one of the recently revealed

side-effects of VKA. Vascular calcification is an actively regulated process involving vascular cells and a number of vitamin K-dependent proteins. In experimental animal models as well as humans, VKA have been shown to promote medial elastocalcinosis. Mechanistic understanding of vascular calcification is essential to improve VKA-based treatments of both thrombotic disorders and atherosclerosis.

Vascular calcification is a marker of increased cardiovascular morbidity and mortality.(11) Matrix Gla protein (MGP) is an important inhibitor of calcification.(12-15) In animal studies, where carboxylation of MGP was blocked by vitamin K antagonists, excessive calcifications of the arteries were found.(16) In humans, calcification of the coronary arteries (CAC) and heart valves is increased in patients on vitamin K antagonists, whereas intake of vitamin K is associated with less progression of CaC.(17-20) VKA use has been associated with increased vascular calcification, which may impart long term risk. Several studies have demonstrated the contribution of VKA use to coronary artery calcification progression in AF patients. Rennenberg et al (21) selected 19 patients younger than 55 years who had no other cardiovascular risk factors and who had used coumarins for more than 10 years, and compared these to 18 matched healthy controls. The odds ratio for calcification in patients versus controls was 8.5 (95% confidence interval [CI] 2.01-35.95). Coumarin use and MGP were associated with calcification, even after adjusting for other risk factors. They concluded that long-term use of coumarins is associated with enhanced extracoronary vascular calcification, possibly through the inhibition of MGP carboxylation.

A prospective study using coronary calcium scans (22) was performed in 157 AF patients without significant cardiovascular disease (108 males; mean age 57 ± 9 years). The duration of VKA treatment varied between 6 and 143 months (mean 46 months). No significant differences in clinical characteristics were found between patients on VKA treatment and non-anticoagulated patients. However, median coronary artery calcium scores differed significantly between patients without and patients with VKA treatment [0, inter-quartile range (IQR) 0-40, vs. 29, IQR 0-184; $P = 0.001$]. Mean coronary calcium scores increased with the duration of VKA use (no VKA: 53 ± 115, 6-60 months on VKA: 90 ± 167, and >60 months on VKA: 236 ± 278; $P < 0.001$). Multivariable logistic regression analysis revealed that age and VKA treatment were significantly related to increased coronary calcium score. Age and VKA treatment were independently related to increased coronary calcium score.

As vascular calcification is considered an independent risk factor for plaque instability, Schurgers (23) investigated the effect of VKA on coronary calcification in patients and on calcification of atherosclerotic plaques in the ApoE(-/-) model of atherosclerosis. A total of 266 patients (133 VKA users and 133 gender and Framingham Risk Score matched non-VKA users) underwent 64-slice MDCT to assess the degree of coronary artery disease (CAD). VKA-users developed significantly more calcified coronary plaques as compared to non-VKA users. ApoE(-/-) mice (10 weeks) received a Western type diet (WTD) for 12 weeks, after which mice were fed a WTD supplemented with vitamin K(1) (VK(1), 1.5 mg/g) or vitamin K(1) and warfarin (VK(1)&W; 1.5 mg/g & 3.0 mg/g) for 1 or 4 weeks, after which mice were sacrificed. Warfarin significantly increased frequency and extent of vascular calcification. Also, plaque calcification comprised microcalcification of the intimal layer. Furthermore, warfarin treatment decreased

plaque expression of calcification regulatory protein carboxylated matrix Gla-protein, increased apoptosis and, surprisingly outward plaque remodeling.

In this study, VKA use was associated with coronary artery plaque calcification in patients with suspected CAD and causes changes in plaque morphology with features of plaque vulnerability in ApoE(-/-) mice. These findings underscore the need for alternative anticoagulants that do not interfere with the vitamin K cycle.

Rate of Progression of CAC

Almost all randomized studies of coronary artery calcification progression were 1 year duration. Annualized studies have been performed with statins, ACE inhibitors, calcium channel blockers, garlic therapy, phosphate binders among others, all studies with 1 year duration.

Relationship of Coronary Artery Calcium and Unstable Plaque

The exact contribution of coronary artery calcification to the stability of atherosclerotic plaques is not clearly defined. Some studies describe that plaque calcification initially destabilizes a plaque by providing areas of interface between high- and low density where a plaque is more prone to rupture.(24) Plaques may also rupture because of physical stress exerted by calcified nodules.(25,26) However, calcification may also represent beneficial scaffolding which could be seen as protective by strengthening atherosclerotic plaque prone to rupture. But still then enhancing the process of calcification by VKA may promote vasomotor dysfunction in the coronary arterial tree.

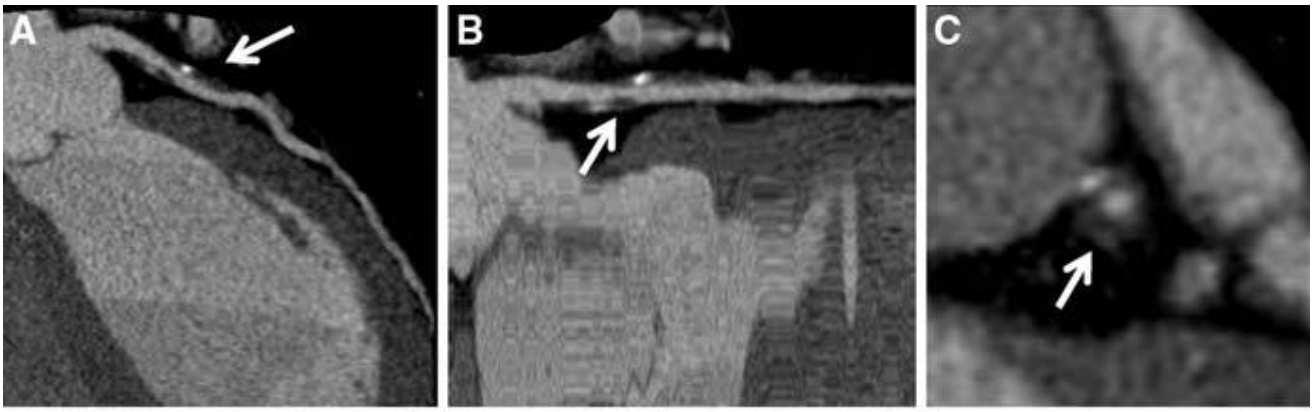
Rivaroxaban

Rivaroxaban is a novel oral direct factor Xa inhibitor that has been shown to reduce the risk of stroke in a similar population in comparison with warfarin. The rate of myocardial infarction was 9% lower in the rivaroxaban group than in the warfarin group inthe Rocket AF study and similarly the myocardial infarction rate was 12% lower in the apixaban group in Aristotle, but the difference was not significant in either trial. The potential of long term benefit by avoiding VKA therapy may be much greater for a CV event reduction. The study proposed will evaluate markers of CAC progression and atherosclerosis development, which have long term outcome data supporting that slowing these processes will be associated with lower CV events.

CCTA

C- Coronary Plaque Volume/Composition via Computed Tomography Angiography: (0 and 52 weeks)

Coronary plaque volume/composition will be measured using multi-detector computed tomography. Recent studies demonstrated the computed tomography angiography can accurate measure plaque characteristics including volume and composition with high reproducibility. Furthermore, they provide evidence that CT angiography is feasible and diagnostic utilizing radiation doses as low as 2 mSv without compromise of image quality, potentially removing one of the biggest barriers to more wide spread implementation of cardiac CT and empowers serial coronary plaque volume assessment with CT angiography (figure 1).



Multislice computed tomography is a non-invasive technique that can easily detect CAD at its earliest stages reflected by the presence and severity of coronary artery calcification. Minor lesions in the main coronary arteries could be a sign of advanced vascular disease in the microvasculature which may lead to important ischaemia, possibly even influencing the AF substrate. A recent meta-analysis among 30 prospective studies revealed that the presence of calcifications is associated with a three- to four-fold higher risk for mortality and cardiovascular events.(27)

CCTA is a novel non-invasive imaging test with high diagnostic performance for detection and quantification of anatomic stenosis compared to invasive coronary angiography (ICA) and intravascular ultrasound (IVUS). Beyond stenosis severity, CCTA also permits anatomic quantification of numerous other atherosclerotic plaque characteristics (APCs) (28, 29). These APCs include additional measures of stenosis severity (% diameter stenosis, % area stenosis, minimum luminal diameter [MLD], minimum luminal area [MLA]), plaque composition (non-calcified, calcified, "mixed"; lowest attenuation density; "spotty" calcification); plaque remodeling (positive, negative, intermediate), plaque burden (volume); and plaque location (proximal versus distal; relation to bifurcation). APCs have been described—both by intravascular methods and in studies with CCTA—to exhibit clinical import for plaque stability; and permit discrimination of patients experiencing stable angina versus acute coronary syndromes. We have performed and reported investigations demonstrating the unique value of plaque composition, low attenuation plaque, and coronary arterial remodeling for the identification of patients that exhibit myocardial ischemia and adverse cardiovascular outcome.

Further, we have published several papers related to the diagnostic performance of CCTA for volumetric quantification of coronary artery plaque volume, composition and other APCs; as well as has determined the utility of automated computational-based methods for coronary artery plaque characterization and quantification (30,32). To determine reliability of CT APC evaluation, we identified a high diagnostic accuracy of measured plaque volume by CCTA versus IVUS in 36 patients. In a separate study of 30 patients who underwent 2 CT scans within 200 days the number of plaques, and measured total, calcified plaque (CP) and non-calcified plaque (NCP) volume. Intra-, inter-, and inter-scan agreement for segments with no plaque was 100%. Intraobserver-interscan correlations of total, CP, and NCP volumes ($r=0.93$-0.97, $p<0.001$ for all), as well as interobserver-interscan correlations were also very good ($r=0.81$-0.96, P values<0.001).

To enhance CT APC evaluation efficiency, reliability and accuracy, our team has developed the first automated computer software algorithm (AutoPlaq) validated against IVUS that enables

accurate and rapid quantification of NCP and CP from CT. In 20 patients undergoing CT and IVUS, the software demonstrated excellent correlation to IVUS for NCP ($r=0.94$, $p<0.001$) and expert core lab measures for plaque volume ($r=0.92$, $p<0.001$), with no significant mean differences. Importantly, diagnostic time for the automated method was <10% of manual measurements. Reliability of the automated method was high compared to expert readers for CP and NCP volumes ($R=0.88$ and 0.94, $p<0.001$ for both) and plaque composition ($R=0.90$, $p<0.0001$). Our results are in keeping with a recent meta-analysis demonstrating high correlation of CT versus IVUS for cross-sectional area, plaque area, area stenosis, and volume. We have further corroborated these findings—and have demonstrated the robustness of CCTA (both by manual as well as automated methods—for the volumetric quantification of coronary atherosclerosis and arterial wall features.

To date, however, the longitudinal changes of coronary artery and plaque characteristics—particularly as they relate to warfarin use—has not been examined. This proposal encourages a comprehensive approach to identifying all aspects of coronary atherosclerosis progression related to medications that are vital to prevent strokes in persons with afib. These CT measures are active in the coronary artery disease pathway, providing quantitative measures of not only luminal stenosis severity, but plaque volume, plaque composition, plaque distribution, plaque length, and arterial remodeling. This proposal will significantly extend prior studies performed by our laboratory and others by examining the nature of coronary atherosclerosis progression, regression and stabilization under the influence of two different therapies. The feasibility and practicability of this study is rooted in the longstanding collaboration of investigators with unparalleled expertise, as well as the numerous preliminary studies performed at the investigators' institution.

Study Rationale

The OVERALL HYPOTHESIS of this study is that use of rivaroxaban will slow progression of vascular calcification and coronary atherosclerosis as compared to warfarin therapy in patients with AFIB.

Study Objectives

Objective 1: To demonstrate if treatment with rivaroxaban therapy, as compared to warfarin therapy, will slow the progression of calcified plaque, based upon the CAC score.

Objective 2: To evaluate if treatment with rivaroxaban therapy, as compared to warfarin therapy, will modify the progression, regression and stabilization of coronary atherosclerosis. Modifications will include differences in plaque volume, composition and arterial remodeling; as well as new atherosclerosis formation.

3. ETHICAL CONSIDERATIONS

The Investigator will follow all appropriate requirements, including Good Clinical Practice (GCP), International Conference on Harmonization (ICH), Code of Federal Regulations (CFR).
The study will be conducted by qualified personnel and be approved by the local. IRB/IEC.

4. INVESTIGATIONAL PLAN AND STUDY DESIGN

We propose a randomized, open label trial, to compare Rivaroxaban 20 mg oral once daily with food to warfarin (target international normalized ratio, 2.0 to 3.0) in patients with non-valvular atrial fibrillation. The primary endpoint will be CAC progression and key secondary objectives will be progression of total plaque, and development of new vulnerable plaques. Patients will be educated to maintain a low cholesterol diet through education to patients as well as medication compliance.

Study Design:

a. Design. The study will be a single-center, randomized, open label comparison of Rivaroxaban **20 mg once daily with food** compared to warfarin therapy (INR target 2-3) for 52 weeks on coronary calcium, coronary plaque composition and volume. One hundred ten eligible subjects will be enrolled using a 1:1 ratio. The sample size in this study is based on exploratory considerations.

b. Randomization and Stratification. Subjects who meet the eligibility criteria will be randomly assigned to receive rivaroxaban or warfarin

c. Endpoint Measures

Primary - Rate of change in coronary calcium

2.Secondary Endpoints

1. To assess incident plaque rates and quantitative changes in different plaque types in patients randomized to warfarin vs. rivaroxaban using CTA, 12 months after an initial evaluation.
2. Evaluation of plaque progression rates and incident plaque rates in subjects treated with warfarin vs. rivaroxaban. In that context, we will:
    a. determine whether subjects treated with rivaroxaban display slower rates of progression compared to warfarin treated subjects, after control for all CV risk factors and demographics

The study design calls for the enrollment of 120 eligible subjects of diverse ethnic population. Given dropout rate, at least 100 patients should be available for final analysis at the conclusion of 52 week follow-up. Baseline examination will include the results of their demographic, coronary risk factor, lab tests, coronary calcium, as well as coronary plaque volume/composition. All participants will be educated on a low-cholesterol diet at entry to the study. Baseline information regarding risk factors for atherosclerotic cardiovascular disease (cigarette smoking status, systemic hypertension, family history of premature atherosclerosis, menopausal and hormone replacement status in women, sedentary lifestyle, current medications, chest pain questionnaire and measures of obesity) will be determined. After randomization, participants will return quarterly (3, 6, 9 and 12 months to assess compliance with medication, and receive an additional supply of medicine. At 52 weeks follow up, coronary calcium and coronary plaque volume/composition will be measured by readers blinded to the randomization and clinical activities, who are not aware of study-group assignments

Patient Recruitment:

Eligible patients will have atrial fibrillation at enrollment or two or more episodes of atrial fibrillation, as documented by electrocardiography, at least 2 weeks apart in the 12 months before enrollment.
Patients randomized to warfarin will continue their monthly study visits focusing on control of the INR to warfarin clinic, and research study visits every 3 months to include an assessment of clinical outcomes and adverse events. For any patient who is lost to follow-up or withdraws consent, attempts will be made to determine vital status at the end of the trial.

TIMELINE:Recruitment will take place over 6 months, follow up for the next 12 months and closeout for 3 months. Entire study duration will conclude in less than 2 years.

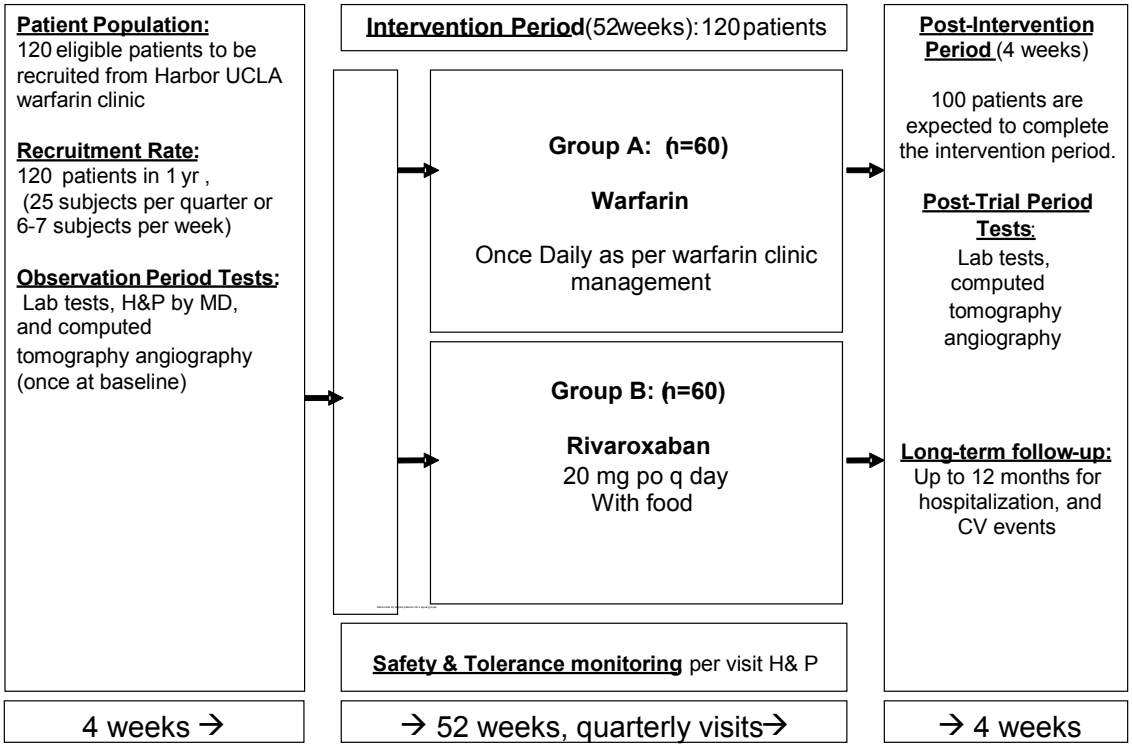


Figure 2.

Day	*What you do*
Day 1	• *You will have routine blood tests done and check your kidney function if needed.* • *You will have a CT scan (without contrast), and a CT angiogram (with contrast) (If you are a female who is able to have children, you will have a urine pregnancy test done before these tests.)* • *For Group 1, continue taking warfarin.*

	• *For Group 2, begin taking assigned study drug once a day for the next 12 months. Keep taking until the end of study, unless told to stop by the study doctor or his staff.*
3 Months	• *For Group 2, get new supply of study drug* • *For both groups, we will ask you about changes in your health and medications and about visits to the doctor, emergency room or other outpatient clinic (for example, urgent care center).*
6 Months	• *For Group 2, get a new supply of study drug* • *For both groups, we will take blood for routine blood tests.* • *For both groups, we will asked you about changes in your health and medications and about visits to the doctor, emergency room or other outpatient clinic (for example, urgent care center).*
9 Months	• *For Group 2, get new supply of study drug* • *For both groups, we will ask you about changes in your health and medications and about visits to the doctor, emergency room or other outpatient clinic (for example, urgent care center).*
12 Months	• *We will take blood for routine blood tests,* • *You will undergo a CT scan (without contrast) and CT angiogram (CT scan with contrast). (If you are a female who is able to have children, you will have a urine pregnancy test done before these tests.)* • *You are done – the study is complete.*

Study Endpoints

Events will be recorded. Stroke will be defined as a focal neurologic deficit, from a nontraumatic cause, lasting at least 24 hours and categorized as ischemic (with or without hemorrhagic transformation), hemorrhagic, or of uncertain type (in the case of patients who do not undergo brain imaging or in whom an autopsy is not performed). The rate of myocardial infarction, hospitalization for angina and revascularization will be recorded. The primary safety outcome will be major bleeding, defined, according to the ISTH criteria, as clinically overt bleeding accompanied by a decrease in the hemoglobin level of at least 2 g per deciliter or transfusion of at least 2 units of packed red cells, occurring at a critical site, or resulting in death. Additional safety outcome will be clinically relevant nonmajor bleeding, defined as clinically overt bleeding that does not satisfy the criteria for major bleeding and that led to hospital admission, physician-guided medical or surgical treatment, or a change in antithrombotic therapy. Other safety measures will include any bleeding, other adverse events, and liver-function abnormalities.

The primary and secondary efficacy and safety measures will be adjudicated on the basis of prespecified criteria by a clinical-events committee whose members will be blinded to the study-group assignments.

B. Laboratory Measurements (0 , 6 and 12 months)

All participants will be instructed to abstain from smoking, alcohol, and food intake, eg, overnight fasting or at least 10 hours before sample drawing. Venous blood samples of all patients will be drawn after admission in a fasting state. Routine blood specimens will be shipped to our

central clinical laboratory to be measure uniformly in a highly standardized center. Blood samples, including CBC, Matrix Gla protein, and INR will be assessed at every visit.

Cardiac multislice computed tomography data acquisition and analysis

In all patients, a prospective non-enhanced coronary calcium scan will be performed with a 64-slice MSCT scanner (GE 64; GE Healthcare, Milwaukee, WI). For quantitative assessment of coronary artery calcification, the Agatston score will be calculated, using a 3 mm CT slice thickness and a detection threshold of ≥130 HU involving ≥1 mm2 area/lesion (3 pixels). Cardiac CT angiography will be performed using a collimation of 64 × 0.625 mm and a rotation time of 0.4 s. The tube current will be 400–770 mA (depending on body weight), at 100-120 kV. Contrast material at a flow rate of 5.0 mL/s was administered in the antecubital vein, with volumes depending on the total scan time (60–80 mL). In the absence of contraindications, patients with a heart rate ≥60 b.p.m. will be administered 50–100 mg metoprolol oral and up to 40 mg metoprolol intravenous. Interpretation will be by expert reading by an experienced cardiologist and a radiologist blinded to all clinical data. Image analysis will be performed on dedicated workstations for post-processing and evaluation.

5. ELIGIBILITY-INCLUSION/EXCLUSION

Key Inclusion Criteria:

1. Eligible patients with atrial fibrillation at enrollment or two or more episodes of atrial fibrillation or flutter, as documented by electrocardiography, at least 2 weeks apart in the 12 months before enrollment
2. Age 18-84
3. On anticoagulation for 6 months prior to enrollment at a stable dose.
4. Willingness to participate in the study and ability to sign informed consent
5. Minimum CAC score of 10

Key Exclusion Criteria:

1. Atrial fibrillation due to a reversible cause, moderate or severe mitral stenosis, or conditions other than atrial fibrillation that require anticoagulation (e.g., a prosthetic heart valve)
2. Prior rivaroxaban use.
3. A need for aspirin at a dose of >165 mg a day or for both aspirin and clopidogrel,
4. Renal insufficiency (serum creatinine level of 12.5 mg per deciliter or calculated creatinine clearance of <50 ml per minute).
5. Serious bleeding event in the previous 6 months or a high risk of bleeding (eg, active peptic ulcer disease, a platelet count of <100,000/mm3 or hemoglobin level of <10 g/dL, stroke within the previous 10 days, documented hemorrhagic tendencies, or blood dyscrasias)
6. Weight in excess of 325 pounds
7. Resting hypotension (systolic blood pressure of <90mmHg) or resting hypertension (systolic blood pressure of >170mmHg or diastolic blood pressure of >110 mmHg).
8. History of active malignancy requiring concurrent chemotherapy.
9. Any unstable medical, psychiatric, or substance abuse disorder that in the opinion of the principal investigator is likely to affect the subject's ability to complete the study.
10. Known allergy to iodinated contrast material

11. Pregnancy or breast feeding

6. TREATMENT

Patients will be randomized to either stay on warfarin at current doses, managed by the warfarin clinic at Harbor-UCLA with target INR of 2-3 and come in for quarterly visits with INR checks and documented safety; or Rivaroxaban 20 mg once daily.

7. PRODUCT INFORMATION

Drug ordering and accountability:

Janssen is supplying study drug, and will provide enough medication for the entire study to the research pharmacy. The Research Pharmacy at Los Angeles Biomedical Research Institute will be responsible for packaging and distributing the medications.

The research pharmacy will be responsible for ensuring that the investigational product is stored under the appropriate environmental conditions (temperature, light, and humidity).

Product Description and Dosage Form

SAMPLE PRODUCT INFORMATION TABLE:

Product Description:

Product Description and Dosage Form	Potency	Primary Packaging (Label Type	Appearance	Storage Conditions (per label)
Rivaroxaban pill	20 mg	30 per bottle	pills	15-25ºC

Administration and handling

Important Food Effect Information
The 15 mg and 20 mg XARELTO tablets should be taken with food. In the nonvalvular atrial fibrillation efficacy study XARELTO was taken with the evening meal.

For those randomized to Xarelto from Warfarin:

From warfarin to XARELTO, discontinue warfarin and start XARELTO as soon as the International Normalized Ratio (INR) is below 3.0 to avoid periods of inadequate anticoagulation.
..
Dosing in Nonvalvular Atrial Fibrillation

For patients with creatinine clearance (CrCl) >50 mL/min, the recommended dose of XARELTO is 20 mg taken orally once daily with the evening meal. For patients with CrCl 15 to 50 mL/min, the recommended dose is 15 mg once daily with the evening meal

In case of Missed Dose
If a dose of XARELTO is not taken at the scheduled time, administer the dose as soon as possible on the same day as follows:
• For patients receiving 20 mg or 15 mg once daily: The patient should take the missed XARELTO dose immediately.

At Study Conclusion (for those randomized to Xarelto:

Switching from XARELTO to Warfarin - No clinical trial data are available to guide converting patients from XARELTO to warfarin. XARELTO affects INR, so INR measurements made during coadministration with warfarin may not be useful for determining the appropriate dose of warfarin. Our approach will be to discontinue XARELTO and begin both a parenteral anticoagulant and warfarin at the time the next dose of XARELTO would have been taken

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Standard Safety Laboratory Panel

Hematology
• Hemoglobin
• Hematocrit
• Red blood cell count
• White blood cell count and differential
• Platelet count
Serum chemistry
• Aspartate Aminotransferase (AST, SGOT)
• Alanine Aminotransferase (ALT, SGPT)
• Alkaline Phosphatase (reflex to GGTP)
• Creatine Kinase (Creatine Phosphokinase) (CK) (CPK) (reflex to troponin)
• Total Bilirubin
• Uric Acid
• Blood Urea Nitrogen (BUN)
• Electrolytes - sodium, potassium, chloride, Total Protein, Albumin
• Serum Creatinine (Scr)

EGFR will be estimated by the method of **Cockroft-Gault**:
Cockcroft-Gault CrCl = (140-age) * (Wt in kg) * (0.85 if female) / (72 * Cr)
The eGFR is expressed in mL/min/1.73m2

• Females only: urine HCG pregnancy test (HCG minimum sensitivity of 25 IU/L). If a urine HCG test is positive, patient will be excluded

Data to be Collected: Routine demographic and cardiovascular clinical data have been collected for all patients in the study. For CAC, we will collect Agatston and Volume scores, number of lesions and density for each plaque. For CCTA-specific variables, we will quantify and characterize the following APCs by both manual and automated plaque characterization methods:

- Coronary artery plaque volume
- Non-calcified plaque volume
- Calcified plaque volume
- Plaque location
- Plaque length
- Plaque distribution
- Arterial remodeling
- Low attenuation plaque (Hounsfield unit density <30 HU)
- Spotty calcifications

2b1a.Overview: In a single session at baseline and followup, after phlebotomy and physical examination, each subject will receive sequential non-contrast followed by contrast scans. The entire scanning procedure will take approximately 30 minutes.

Screening. Subjects will be screened for contraindications for beta-blockers and nitroglycerin (PDE5 inhibitors). The desired heart rate for adequate image quality is ≤ 70 bpm. If the heart rate is above this level, a beta-blocker will be prescribed. If a beta blocker is contraindicated, a calcium channel blocker will be given instead.
2b1b.Noncontrast scan: This will be performed to measure CAC. Patients will be positioned within the scanner with ECG leads, and a rhythm strip will be viewed. After a scout film is obtained, standard calcium scoring imaging will be performed at the end of inspiration.
2b1c.Contrast Scan. One minute prior to the CT angiogram acquisition, sublingual nitroglycerin will be administered. Iodinated X-ray contrast will then be injected. When significant heart rate variability exists, retrospective imaging will be utilized. Breath hold acquisition will be performed over approximately 20 seconds. After the study, patients will be observed for at least 10 minutes.
2b1d.Image reconstruction: Cross-sectional cardiac CT images will be reconstructed using prospective ECG triggering from 65 to 85% by 5% increments, and transmitted to the reading site.
2b1e.Reading Center: The Reading Center for CTA will be based at our Academic-based Medical facility. The Center will coordinate and oversee the collection of the data, store backup copies of the data, read each scan, send the results to the DCC and maintain quality control.
2b2.Coronary Artery Calcification (CAC)
CAC will be calculated from the non-contrast images using standard methods and scores will be reported using the Agatston method as elsewhere described (33). A total CAC score will be determined by summing the individual lesion scores from each of 4 anatomic sites (left main, left anterior descending, circumflex, and right coronary). A volume score, independent of density, is also calculated using a standard algorithm. A single experienced reader, blinded to subject identity and randomization, will interpret all the scans using commercially available software.

2b3. CT Angiography (CTA)
2b3a.Imaging Analysis. The resulting thin CT sections will be transferred to a dedicated workstation for image analysis. Images will be post-processed in an independent workstation (GE Advanced Workstation®) by the reader independently, during a dynamic reading process. Curved maximum intensity projection (MIP) multiplanar reconstructions will be performed for each coronary artery segment at the end-diastolic frame or the frame with the least motion artifact. Based on the curved MIP, the reader will identify an area of abnormality and determine the point of minimum luminal diameter within the abnormal area. Multiplanar reformatting will also be used to generate cross-sectional images of coronary segments using semiautomated software (AW 4.6, GE Healthcare, Milwaukee WI). This yields a vessel centerline using the full-width-half-maximum standard method to delineate the contrast-filled vessel, as well as automatic maximum and minimum diameters at any cross-section along the vessel centerline. A cross-sectional 5mm MIP image will be reconstructed at this location and semi-automatic software will be used to outline the intimal surface providing cross-sectional vessel area. In cases where coronary segments are normal, the most proximal cross-sectional image will be used for analysis. CT values will be measured in the most visible images such as axial source or multiplanar images of the long axis at each site of the coronary arteries. For determining image quality, both qualitative and quantitative analysis will be performed. Each segment will be classified as poor, acceptable, good or excellent quality regarding the presence of motion artifact, which is defined as any discontinuation or blurring of the sharply defined vessel contour.

2b3b.Plaque evaluation. Plaque will be evaluated from both axial source images and multiplanar reconstruction images of the long axis at each site of the coronary arteries. CP will be defined as any structure with a density greater than the contrast-enhanced lumen that could be visualized separately from the lumen, which could be assigned to the coronary artery wall, and that could be identified in at least two independent planes. NCP will be defined as any discernible structure that could be clearly assignable to the vessel wall, which had a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective tissue, and that could be identified in at least two independent planes (1). Each coronary segment (15 AHA standardized coronary segments) will be classified as either normal, containing NCP, containing MP with either predominantly NCP (<50% of plaque area occupied by calcium) or containing CP. Reviewer will independently evaluate the contrast-enhanced scans by assessment of the axial slices of multiplanar reformations and of three thin-slab maximum intensity projections. Percentage of coronary arterial stenosis will be estimated on a cross-sectional image obtained from reconstruction of standardized coronary segments. Stenosis will be evaluated in a scale of 0-4: 0 (normal segment without stenosis or plaque present), 1 (1% to 29% stenosis), 2 (30% to 49% stenosis), 3 (50-69% stenosis), and 4 (≥70% stenosis). Segments with diameter <1.5 mm will be graded as small and therefore unable to evaluate for stenosis. Segment Stenosis Score" (SSS) will be determined by summing the number of evaluable coronary segments with individual stenosis scores (maximum score = 60 (score of 4 for all 15 segments).
2b3c.Plaque Composition. The presence of noncalcified atherosclerotic plaque tissue will be defined as any discernible structure in the coronary artery wall with a CT density less than the contrast enhanced coronary lumen but greater than the surrounding connective tissue. Measurements will be obtained from manually traced regions of interests encompassing the noncalcified plaque proportions.
2b3d.Plaque Quantification. The calculation of the coronary plaque burden and volume will be performed on axial cross sectional images in a blinded fashion. The plaque area of each coronary plaque visualized in at least 2 adjacent slices (reconstructed slice thickness 0.6 mm) will be determined on all affected slices, and plaque volume will be assessed by multiplying the area by the slice thickness. Summing the total plaque per segment will be done, and compared with the subsequent scan in a blinded manner. Plaque burden will be reported in both a semi-quantitative and a quantitative (volumetric) manner; a plaque burden score will be developed to semi-quantitate the plaque in each participant. The AHA 15 segment model of the coronary arteries and visual semi-quantification of coronary artery calcific and non-calcific plaque will be used. Each plaque will be multiplied by 1 for small plaque volume, 2 for medium plaque volume and 3 for large plaque volume. "Plaque Burden Score" (PBS) will be determined by summing the number of evaluable coronary segments with individual plaque scores (maximum score = 45 (score of 3 for all 15 segments). The "Percent Plaque Score" (%PS) will be calculated by (number of segments with plaque / total number of evaluable segments) x 100. Both plaque burden score and percent plaque score will be evaluated as independent variables and compared to other measures in the study, as aforementioned. The protocol for quantitative plaque assessment (volume, in mm^3) is fully described elsewhere (2). For each lesion identified, minimal lumen diameter and minimal lumen area will be determined as elsewhere described.

9. RISK OF RIVAROXABAN

Potential Discomforts, Side Effects, and Risks Associated with Rivaroxaban

The following terms are used to describe how often side effects have been seen in patients:

- Very common: it affects 1 in 10 or more subjects (10% or more)
- Common: it affects between 1 and 10 in 100 subjects (between 1% and 10%)
- Uncommon: affects between 1 and 10 in 1,000 subjects (between 0.1% and 1%)
- Rare: it affects between 1 and 10 in 10,000 subjects (less than 0.1%)

COMMON: Seen in 1% (1 in 100 people) to 10% (1 in 10 people)

- Internal bleedings which may occur in stomach and intestines may result in a low blood count (anemia) or decrease in blood pressure (hypotension). Other common bleeding sites include eye bleeds, bleeding from gums, nose bleeds, dark urine, heavy menstrual bleedings, hematomas (collection of blood) on the skin and small bleeds under the skin, coughing up blood, bleedings from wounds and after surgical procedures.
- Abdominal and stomach pains
- Upset stomach
- Nausea
- Constipation
- Diarrhea
- Vomiting
- Fever
- Swelling (edema; swelling caused by a fluid build-up in the body)
- Decreased general strength and energy
- Bruises
- Some enzyme levels in blood may increase (such as liver enzymes called transaminases)
- Pain in limbs (arm or leg pain)
- Dizziness (feeling dizzy)
- Headache

- Kidney problems*
- Itching
- Rash (skin rash)

UNCOMMON: Seen in 0.1 % (1 in 1,000 people) to 1% (1 in 100 people):

- Increased platelet count (increased number of platelets in your blood)
- Increase heart rate
- Dry mouth
- Feeling unwell
- Allergic reactions and skin allergic reactions, like swelling of your face, skin or your limbs (angioedema, allergic edema)
- Wound oozing

- Some enzyme levels in blood may increase (i.e. lipase, amylase, alkaline phosphatase, lactate dehydrogenase, gamma-glutamyl transpeptidase and bilirubin**)**
- Bleeding into your joints (Intraarticular bleeding or Hemarthrosis)
- Brain bleeding or intracranial bleeding (especially in patients with high blood pressure and the elderly)
- Fainting (syncope)
- Hives (urticaria)

RARE: Seen in less than 0.1% (less than 1 in 1,000 people):

- Localized swelling
- Yellow skin (jaundice; liver problems that causes yellowing of the skin or eyes)
- Blood collection outside an arterial wall (vascular pseudoaneurysm)
- Elevation of bilirubin in the blood (break down blood product), with or without an elevation of a liver enzyme called alanine aminotransferase
- Hematomas (collection of blood) or bleeds in the muscle tissue

*this was observed after major orthopedic surgery of the lower limbs

10. ADVERSE EVENT REPORTING

I. Definitions

a. Adverse Event (AE)

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non- investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH]).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

b. Adverse Events of Special Interest

Events that Janssen Scientific Affairs is actively monitoring as a result of a previously identified signal (even if non-serious).

The following are considered as events of special interest, and should be reported as a serious AE:

- Suspected severe toxic effect on the bone marrow, such as severe thrombocytopenia (platelet count less than 50,000/µL), severe neutropenia (white blood cell count less than 500/µL), pancytopenia, aplastic anemia
- Suspected severe hypersensitivity reaction (eg, anaphylaxis, angioedema, severe urticaria, bronchospasm, etc.)
- Severe skin reactions such as Stevens-Johnson Syndrome
- Suspected severe liver injury

c. <u>Individual Case Safety Report (ICSR)</u>

A valid ICSR must contain the four minimum criteria required to meet regulatory reporting requirements.

- an identifiable subject (not disclosing the subject's name and address)
- an identifiable reporter (investigational site)
- a Janssen medicinal product
- an adverse event, outcome, or certain special situation

The minimum information required is:

- suspected Janssen product (doses, indication)
- date of therapy (start and end date, if available)
- batch or lot number, if available
- subject details (subject ID and country)
- gender
- age at AE onset
- reporter ID
- adverse event detail (AE verbatim in English), onset date, relatedness, causality, action taken, outcome, (if available)
- Janssen protocol ID

d. <u>Product Quality Complaint (PQC)</u>

A product quality compliant is related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a subject. Lot and batch numbers are of high significance and need to be collected whenever available.

Examples of PQC include but not limited to:

- Functional Problem: e.g., altered delivery rate in a controlled release product
- Physical Defect: e.g. abnormal odor, broken or crushed tablets/capsules
- Potential Dosing Device Malfunction: e.g., autoinjector button not working, needle detaching from syringe

- Suspected Contamination
- Suspected Counterfeit.

e. <u>Serious Adverse Event (SAE)</u>

A serious adverse event based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is medically important*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

NOTE: DEATH FOR ANY REASON SHOULD BE REPORTED AS A SERIOUS ADVERSE EVENT.

For reports of hospitalization, it is the sign, symptom or diagnosis which led to the hospitalization that is the serious event for which details must be provided.

Any event requiring in-patient hospital admission (or the prolongation of hospitalization) must be reported as an SAE. Events that do not meet the criteria for SAE reporting are:

- Reasons described in the Protocol, e.g. drug administration, Protocol-required testing
- Social reasons, e.g. overnight stay because of distance between home and hospital
- Surgery or procedure planned and documented prior to entry into the Study.

Any event requiring hospitalization or prolongation of hospitalization that occurs during the study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study. [Note: Hospitalizations that were planned before the start of data collection and where the underlying condition for which the hospitalization was planned has not worsened will not be

considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.]

The cause of death of a subject in a study within 30 days of the last dose of study drug, whether or not the event is expected or associated with the study drug, is considered a serious adverse event.

f. Unlisted (Unexpected) Adverse Event/Reference Safety Information

An adverse event is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For a medicinal product(s) with a marketing authorization, the expectedness of an adverse event will be determined by whether or not it is listed in the applicable product information.

II. <u>Special Situations</u>

Safety events of interest for a Janssen medicinal product that require expediting reporting and/or safety evaluation include, but are not limited to:

- Drug exposure during pregnancy (maternal and paternal)
- Overdose of a Janssen medicinal product
- Exposure to a Janssen medicinal product from breastfeeding
- Suspected abuse/misuse of a Janssen medicinal product
- Inadvertent or accidental exposure to a Janssen medicinal product
- Any failure of expected pharmacological action (i.e., lack of effect) of a Janssen medicinal product
- Medication error involving a Janssen medicinal product (with or without patient exposure to the Janssen medicinal product, e.g., name confusion)
- Suspected transmission of any infectious agent via a medicinal
- Unexpected therapeutic or clinical benefit from use of a Janssen medicinal product

These safety events may not meet the definition of an adverse event; however, they are treated in the same manner as adverse events. Special situations should be recorded on the Adverse Event page of the CRF.

Any special situation that meets the criteria of a serious adverse event should be recorded on a Serious Adverse Event Report Form and be reported to Janssen Scientific Affairs **<u>within 24 hours of becoming aware of the event.</u>**

III. Pregnancy

All initial reports of pregnancy must be reported to Janssen Scientific Affairs by the Sponsor Investigator **<u>within 24 hours of their awareness of the event</u>** using the Serious Adverse Event Form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported using the Serious Adverse Event Form.

Any subject who becomes pregnant during the study must be promptly withdrawn from the study and discontinue further study treatment.

Because the effect of the Janssen medicinal product on sperm is unknown, pregnancies in partners of male subjects exposed to a Janssen medicinal product will be reported by the Sponsor Investigator **within 24 hours of their awareness of the event** using the Serious Adverse Event Form. Depending on local legislation this may require prior consent of the partner.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

11. Reporting Procedures for Adverse Events and Pregnancies [and/or Pregnancies in Partners]

All adverse events, whether serious or non-serious, related or not related, special situations, pregnancy exposures and/or pregnancies in partners following exposure to a Janssen medicinal product are to be documented by the investigator and recorded in the CRF and in the subject's source records. Investigators must record in the CRF their opinion concerning the relationship of the adverse event to a Janssen medicinal product.

All (serious and non-serious) adverse events reported for a Janssen medicinal product should be followed-up in accordance with clinical practice.

All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

All serious adverse events, pregnancy exposures and/or pregnancies in partners for Janssen medicinal products under study should be reported directly by the Sponsor Investigator, **within 24 hours of becoming aware,** to Janssen Scientific Affairs using the Janssen Scientific Affairs Serious Adverse Event Report Form. In the event the study is blinded, the Sponsor Investigator will submit an unblinded SAE or pregnancy exposure report to Janssen Scientific Affairs.

All follow-up information for serious adverse events that are not resolved at the end of the study or by the time of patient withdrawal must be reported directly by the Sponsor Investigator, **within 24 hours becoming aware**, to Janssen Scientific Affairs using the Janssen Scientific Affair's Serious Adverse Event Report Form.

Product Quality Complaints for Janssen Medicinal Products

A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of patients, investigators, and Janssen Scientific Affairs, and are mandated by regulatory agencies worldwide. Janssen Scientific Affairs has established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information. Lot and/or Batch #s shall be collected or any reports of failure of expected pharmacological action (i.e., lack of effect).

All initial PQCs involving a Janssen product under study must be reported to Janssen Scientific Affairs by the Sponsor Investigator **within 24 hours after being made aware of the event.**

If the defect for a Janssen product under study is combined with either a serious adverse event or non-serious adverse event, the Sponsor Investigator must report the PQC to Janssen Scientific Affairs according to the serious adverse event reporting timelines. A sample of the suspected product should be maintained for further investigation if requested by Janssen Scientific Affairs.

Maintenance of Safety Information
All safety data should be maintained in a clinical database in a retrievable format. The Institution and Sponsor Investigator shall provide all adverse events, both serious and non-serious, in report format. However, in certain circumstances more frequent provision of safety data may be necessary, e.g. to fulfill a regulatory request, and as such the data shall be made available within a reasonable timeframe at Janssen Scientific Affair's request.

Transmission Methods:
The following methods are acceptable for transmission of safety information to Janssen Scientific Affairs:

- Electronically via Janssen SECURE Email service (preferred)
- For business continuity purposes, if SECURE Email is non-functional:
    - Facsimile (fax), receipt of which is evidenced in a successful fax transmission report
- Telephone (if fax is non-functional).

Please use the contact information and process information provided by Janssen Scientific Affairs.

Procedures for Reporting Adverse Events (AE), Serious Adverse Events (SAE), Pregnancy, and Product Quality Complaints (PQC) to Janssen Scientific Affairs

A. AEs, SAEs, Special Situations and Pregnancy Reporting.
The Institution and the Sponsor Investigator will transmit SAEs and Special Situations in a form provided by Janssen Scientific Affairs in accordance with Section VIII Transmission methods, in English **within 24-hours** of becoming aware of the event(s).

All available clinical information relevant to the evaluation of a related SAE or Special Situation is required.

- The Institution and/or Sponsor Investigator are responsible for ensuring that these cases are complete and if not are promptly followed-up. A safety report is not considered complete until all clinical details needed to interpret the case are received. Reporting of follow-up information should follow the same timeline as initial reports.

- Copies of any and all relevant correspondences with regulatory authorities and ethics committees regarding any and all serious adverse events, irrespective of association with the Janssen Product under study, are to be provided to Janssen Scientific Affairs using a transmission method in Section VIII **within 24 hours of such report or correspondence being sent to applicable health authorities.**

B. PQC Reporting
The Institution and the Sponsor Investigator will report any suspected PQC to the Janssen contact within 24 hours of becoming aware of the complaint. The product should be quarantined immediately and if possible, take a picture.

Reconciliation of SAEs
At a minimum, on a quarterly basis and at the end of the Study, Janssen Scientific Affairs will provide to the Institution and/or Sponsor Investigator, a listing of all SAEs reported to Janssen Scientific Affairs. The Sponsor Investigator will review this listing and provide any discrepancies to Janssen Scientific Affairs.

Upon request, Institution and/or Sponsor Investigator shall provide Janssen Scientific Affairs with a summary list of all SAEs, and AEs of Special Interest and Special Reporting Situation reports to date, for reconciliation purposes.

Dissemination of Safety Information from Janssen Scientific Affairs to Institution/Sponsor Investigator Sponsor Investigator will be responsible for submitting IND safety reports for the Study Product to Institution's IRB in accordance with Federal regulations 21 CFR 312.66.

Janssen Scientific Affairs agrees to provide to the Sponsor Investigator IND safety reports for the Study Product as they become available until all subjects in the Protocol have completed their last Study visit according to the Protocol (i.e. Last Subject Last Visit has occurred).

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Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a nonserious or serious AE, as appropriate, and reported accordingly.

12. **Data Safety Monitoring Board (DSMB)** A DSMB consisting of individuals with expertise in each of the efficacy areas (anti-coagulation therapy, atrial fibrillation, and cardiac CT), as well as in anti-coagulation trials and statistics will be established. The PI will prepare interim reports to the DSMB on a regular basis, every 6 months or more often as it deems appropriate. Reports that include general study information such as accrual, dropout, and ineligibility rates and other performance parameters, will be available to the study leadership and will be discussed with them at open sessions. Data relating to safety and efficacy that are presented by treatment arm will be considered highly confidential and will be available only to the DSMB and the statistician preparing the reports, and will be discussed at closed sessions of the DSMB. The DSMB will have an initial meeting prior to the opening of the study to review the report format and content proposed by the PI and determine if they are satisfactory or need to be modified. Interim reports to the DSMB will focus on analyses of the safety data -- primarily bleeding -- but other safety-related outcomes, such as CT related adverse effects, will also be reviewed in an unblinded fashion. The DSMB will decide on the basis of these data and the stopping boundaries whether to recommend modifying or stopping the trial and will make recommendations to the sponsor and the study leadership. A copy of the DSMB minutes and reports will be provided to Janssen as well.

13. STATISTICAL CONSIDERATIONS

Statistical analysis

All analyses will be performed on a full analysis set basis (those who stay in the trial to the follow up scan). For the primary analysis, ITT (defined as all those randomized into the study) will be used. Continuous variables will be expressed as mean and standard deviation; categorical variables expressed as absolute numbers and percentages. Baseline variables will be compared with an independent t-test (two tailed) after performing Levene's test for equality of variances in all normally distributed continuous variables and the Mann–Whitney test (two-tailed) in all not normally distributed variables. Categorical variables will be tested with two-sided Fisher's exact test. An analysis of variance (ANOVA) model with treatment as the main effect will be used to compare the treatment effect of rivaroxaban and warfarin treatment

strategies. For all measures, the analysis of covariance (ANCOVA) model controlling for baseline demographics and baseline CACS values will be performed. Treatment groups also will be compared with respect to incidence rates of adverse events, laboratory abnormalities, bleeding rates, and concomitant medication use. Measurements and change from baseline in coronary artery calcium scores, vital signs (blood pressure and pulse rate), continuous laboratory variables and compliance values will be summarized using descriptive statistics (mean, standard deviation, minimum, median and maximum) by treatment groups and visit. If the interaction of treatment by baseline laboratory values or CACS is significant at the 0.10 level or below, the nature of the interaction will be further explored. Spearman's rank correlation coefficient will be used for testing correlation between the mean Agatston score. All parameters showing a significant univariate relation with an increased coronary Agatston score and those representing a plausible mechanism in terms of calcification [age, VKA duration, left atrium (LA) diameter, statins, angiotensin-converting enzyme (ACE)-inhibitors] will be included as covariates in a logistic regression model (retention level set at 0.1), odds ratios and 95% confidence intervals (CIs) calculated, and results checked for interaction.

Statistical analysis will be performed with SPSS statistical software (SPSS, Inc. release 18.0) and statistical significance assumed for $P < 0.05$. The primary and secondary efficacy analyses will include all patients who undergo randomization (intention-to-treat population) and include all events from the time of randomization until the end of the study. The analyses of bleeding events will include all patients who receive at least one dose of a study drug and included all events from the time the first dose of a study drug was received until 2 days after the last dose is received.

Sample Size

Sample Size Estimation. Sample sizes for each efficacy area have been calculated assuming performance of two-sided 0.05 level tests and 90% power for the primary endpoint and >85% power for the secondary endpoint to detect differences considered clinically meaningful and plausible based on prior studies. We have based sample sizes on at least 90% power in all end points because false negatives could lead to abandoning further study of a treatment that could have a major positive impact on myocardial infarction in this population. The primary endpoint is progression of atherosclerosis after 1 year of treatment with warfarin compared to rivaroxaban as measured by the annualized relative change in the coronary calcium score, as assessed by the Agatston score. The primary endpoint will be the percentage change in CAC from the baseline, which was calculated as follows: (CAC score at 1 year - CAC score at baseline/CAC score at baseline) / 100. From multiple 1 year randomized studies in Dr. Budoff's lab, the mean annualized relative change in the Agatston calcium score was 27.2±9.5% and on pilot-treatment group, we observed an average change of 7.5 ±9. 4%. Given a 19.7 % annual change difference (27.2% vs. 7.5%) between two groups was observed from primary data, the trial requires 100 in total (50 patients per arm) to attain power of 90% to detect the effect size according to a two-tailed two-sample t-test at a significance level 0.05 (see figure). If we consider the secondary

endpoint (noncalcified plaque volume change), we will have >85% power without drop-off at a two-tailed significance level 0.05 with 120 subjects.

The secondary endpoint is noncalcified plaque volume, using cardiac CTA. Cardiac CTA has been used successfully by Budoff to measure progression of atherosclerosis (30-32). In one relevant study, the mean annualized relative change in the non-calcified plaque volume among 62 subjects given statin was -0.037 with a standard deviation of 0.351. This secondary comparison has power of 0.80 to detect an effect size of 0.5, the mean difference in the annualized relative change in the non-calcified plaque volume between the rivaroxaban and warfarin, according to a two sample t-test at a two-tailed significance level 0.05.

STUDY SUMMARY:

This study will represent a comprehensive evaluation of the atherosclerotic changes over time on both rivaroxaban and warfarin.

COMPETING STUDIES:

A 348 person study comparing rivaroxaban to OAT has been listed on ClinicalTrials.gov. This study will evaluate CAC, Echocardiography, Intima Media Thickness of carotid artery (IMT) and Flow Mediated Vasodilatation (FMD), Electrocardiography (ECG) in respect to both open label randomization of OAT and rivaroxaban. It has some similar features to our study, but also some significant differences. We are using CT angiography to be much more precise with both calcification and atherosclerosis measures and are not undertaking a broad based 'test all' method to see if there are differences. There are no suggesting that rivaroxaban will affect endothelial function, carotid intima-media thickness, diastolic dysfunction or electrocardiography any differently than OAT. We are focusing on the known benefits of rivaroxaban with the most precise measures of atherosclerosis and calcification known. Our study, being of smaller and more directed scope, will complete significantly earlier than the study on ClinicalTrials.gov.

A second study is a 2 month study evaluating the calcification score by scanography, measuring the rate of coronary calcification and the arterial site of calcification at the level of lower limbs between an inhibitor of the FXa activity by oral way versus a vitamin K antagonist. There is no study ever done that shows we can see changes in vascular calcification in 2 months, the shortest study ever done was 6 months, using a measurement tool that is more accurate and quantitative than scenography. Furthermore the study on ClinicalTrials.gov is riddled with errors and mis-statements, such as "20 mg once daily for patients with growth factor > 49 ml per minute and 15 mg rivaroxaban once daily for patients with growth factor of 15 to 49 ml." Note the errors of use of 'growth factor' instead of 'GFR'. I have little confidence that this "VICTORIA" Study will yield any useful information so this study is ongoing to decidedly answer the question.

CONCLUSIONS: We conclude that there is a growing need for better understanding of the effects of anticoagulants on vascular calcification and atherosclerosis. Other proposed

studies will not answer the question in the methods directed by our study design. This study will add considerable knowledge to the potential effects of rivaroxaban and VKA in the progression of both CAC and CT angiographic plaque. A successful 1 year pilot study could lead to a larger outcome study evaluating the long term CV benefits of avoiding VKA therapy in persons needing anticoagulation. If successful, the work in this proposal will provide the rationale for a prospective randomized CV outcome study.

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