Protocol:

Clarification of Optimal Anticoagulation Through Genetics (COAG):

A RANDOMIZED, MULTICENTER, DOUBLE-BLIND CLINICAL TRIAL TO EVALUATE EFFICACY IN THE USE OF CLINICAL PLUS GENETIC INFORMATION TO GUIDE WARFARIN THERAPY INITIATION AND IMPROVE ANTICOAGULATION CONTROL FOR PATIENTS

VERSION 1.3
Effective Date: October 3, 2012

Sponsored by:	National Institutes of Health (NIH) National Heart, Lung, and Blood Institute (NHLBI) 6705 Rockledge Drive One Rockledge Centre Bethesda, MD 20892
Prepared By:	University of Pennsylvania School of Medicine Center for Clinical Epidemiology and Biostatistics (CCEB) Clinical Trial Coordinating Center (CTCC) 3535 Market Street Suite 560 Philadelphia, PA 19104

COAG Protocol Summary

Study Title & Description	Clarification of Optimal Anticoagulation Through Genetics (COAG): A Randomized, Multicenter, Double-Blind Clinical Trial to Evaluate Efficacy in the Use of Clinical Plus Genetic Information to Guide Warfarin Therapy Initiation and Improve Anticoagulation Control for Patients
Sponsor	National Institutes of Health (NIH), National Heart, Lung, and Blood Institute (NHLBI)
Pharmaceutical and Other Collaborators	Bristol-Myers Squibb, The Critical Path Institute, Osmetech, AutoGenomics,Inc.
Agent	Warfarin (Coumadin®)
Design & Sample Size	2-arm randomized clinical trial design – initial dosing guided by genetic and clinical information (genotype-guided dosing) *OR* initial dosing guided by clinical information only (clinical-guided dosing). 1,022 patients - 511 genotype guided dosing / 511 clinical guided dosing Sample size estimates assume estimated drop-out rate of 10% after randomization Analysis of the primary outcome will be by intention-to-treat.
Power & Effect Size	The sample size of 1,022 will provide 80% power for the primary cohort analysis to detect an absolute difference of 5% in PTTR between groups and to detect an absolute difference of 9.15% between groups in the primary subgroup analysis.
Population	Patients starting anticoagulation therapy for the first time at in-patient and out-patient levels of care
Inclusion Criteria	Age $\geq$18 years, Expected duration warfarin therapy at least 1 month, Target INR 2-3
Dose Regimen	Dose day 1-3 according to dose initiation algorithm; dose day 4-5 according to dose revision algorithm and INR. After day 5, dose titrated according to INR.
Treatment Duration	4 weeks blinded study phase and 20 weeks follow-up period
Primary Endpoint	Percentage of time participants spend within the therapeutic INR (PTTR) during the first four weeks of therapy
Primary Objective	Compare efficacy of two dosing strategies with respect to the time spent within the therapeutic INR Rang (PTTR) during the first 4 weeks of therapy
Interim Analysis	DSMB request for a conditional power analysis when approximately 600 patients complete primary endpoint at 28 days.
Target Accrual	1, 022 patients
Rate of Accrual	Two (2) patients per site per month
Total Clinical Sites	18 (U.S.A.)
Trial Initiation Date	September , 2009
Accrual Completion	April 30, 2013 and study completion July 31, 2013
ClinicalTrials.Gov Registration Number & Title Link	▪ http://www.ClinicalTrials.gov ▪ NCT00839657 – Clarification of Optimal Anticoagulation Through Genetics • http://coagstudy.org

PROTOCOL MODIFICATIONS

Protocol Version 1.3 includes the following Modifications: #14, #15, #16, #17, #18, #19, #20

Modification #1: **Figure 8.1- Table 8.1: Dosing Algorithm During Dose Algorithm Intervention Period - pages 32-33**
Added clarification text to table contents for Day 4 INR 2.5-3.0; >3.0 and for Day 5 INR 2.9-3.0; >3.0. Added clarification text to explanation of dose revision on days 4 and 5.

Modification #2: **Section 9.3 – Table 9.1: Dose Titration After 5-Day Intervention Period – pages 43-44**
Added clarification text to table contents for INR 3.01-3.39. Added clarification text to weekly dosing

Modification #3: **Section 11.1.2.1 – Method of Data Collection – page 48**
In first sentence of this section, added text: "and at 3 and 6 months" and corrected term "research nurses" to "research coordinators".
Removed erroneous text: "*Data on medication use that may alter warfarin's effect will be collected using state-of-the-art strategies to obtain the most complete drug histories possible: first open-ended queries about drug ingestion; then indication-specific questions about the drugs of interest (e.g., infections, arrhythmias, or fevers); and last, referring to medication lists and a photocard displaying pictures of each of the drugs. Each of these methods can dramatically increase recall*"
Added corrected text: "*Information on medication use will be collected at each study visit during the first 30 days and at 3 and 6 months. At baseline, subjects will be asked to report their current medications. At each subsequent visit during the first four weeks, subjects will be asked if they started or stopped use of any medications. Current medication use then will be collected at 3 and 6 months.*"

Modification #4: **Section 11.1.3 – Sample Collection for Storage and Analysis in Central Laboratory - page 48**
Removed erroneous text: "*Sites also will collect sodium citrate anticoagulated blood, and have it spun so that platelet poor plasma can be frozen for coagulation factor assays later.*"

Modification #5: **Section 11.3.1 –Missing Data – page 51**
Corrected text of first sentence to clarify main missing data problem.
Removed erroneous text: "*The main missing data problem will be a result of missing protocol-specified visits, which in turn causes missing INR values that are needed to compute the primary outfome of PTTR. As such, missing data in INR results is defined as the situation where an INR is expected by protocol but is missing due to a missed visit. The use of linear interpolation of INR for the PTTR will be the primary approach to missing data.*"

Modification #6: **Section 11.3.4 – Genotyping for Pharmacogenomic Dosing - page 52-53**
Corrected Text in first paragraph on number of reference samples from 5 to 10.
Removed erroneous phrase: "*and the same platform as the clinical site*". Inserted corrected phrase: "*and one of the platforms approved for use at the clinical site*".
Corrected text in Ongoing Quality Monitoring After Certification to clarify the quality monitoring plan

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Modification #7: Section 11.3.5 – Quality Control Calibration of INR – page 53-54
Removed erroneous text in criteria (4): *"Two blinded control samples must be run on the machine prior to enrolling patients to ensure accuracy of the INR measurements. This set of tests will be repeated at the frequency recommended by the manufacturer".*
Added corrected text in criteria (4): *"Quality control procedures, including INR testing with liquid controls, if required, will be performed at intervals recommended by the instrument manufacturer".*
Added clarification text: *"Due to reports of decreased accuracy for POC INR results >4.0, POC INRs exceeding 4.0 will be confirmed by obtaining a plasma INR from a citrated whole blood sample at the same encounter. The plasma INR result should be used for warfarin dose adjustments."*
Frequency of Measurement: Removed erroneous text *"There is a standard of visit frequency where INR will be measured."*
Added corrected text: *"There is a sequence of visit windows where INR should be measured"*

Modification #8: Section 12.2.2 Out of Range INR Values – page 56
Corrected text to clarify reporting of out of range INR values.

Modification #9:Section 13.3.1 – Primary Outcome – Table13.3 – page 66
Added Text below Table 13.3 to clarify alpha allocation for the subgroup.

Modification #10:Section 13.4 – Interim Analyses – page 70
Added clarifying text to first sentence, paragraph 3: *"for assessing the need for a sample size adjustment based on an underestimate of the variance of PTTR"*

Modification #11:Section 13.5 – Changed title from *Missing Data* to *Computation of PTTR*– page 71
Removed erroneous text first paragraph: "*protocol specified visits, which in turn causes missing INR values that are needed to compute the primary outcome of PTTR.*"
Inserted corrected text: *"INR values within protocol specified windows needed to compute the primary outcome of PTTR."*
Removed erroneous text: *"As such, missingness in INRs is defined as the situation where an INR is expected by protocol but is missing due to a missed visit".*

Modification #12: Section 14.3 Steering Committee – page 74
Added Benjamin French, PhD to CTCC Members
Added Suzanne Goldberg, MSN,RN to NHLBI Members
Removed Eleanor Schron, PhD from NHLBI Members

Modification #13: Appendix A: Informed Consent Template – pages 8 & 9
Section: Will confidential health information be collected as part of this study?
Paragraph 2-Added the following clarifying text: *"Authority to Collect Information: The authority to collect this information is under 42 USC [National Heart, Lung, and Blood Institute (NHLBI) – 42 USC 285b]."*
Paragraph 5- sentence 1:
Changed text from: *"To help us protect your privacy, a Certificate of Confidentiality from the National Institutes of Health will be obtained."*
Changed text to: *"To help us protect your privacy, a Certificate of Confidentiality from the National Institutes of Health has been obtained."*

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Modification #14: Section 5 (page 24) Section 14 (page 80-Figure 14.1); Informed Consent Template
Changed text from 12 clinical sites to 18 clinical sites (United States of America).

Modification #15: Section 7.5 (page 29) Follow-up Period & Informed Consent Template
Added text to clarify follow-up period for patients enrolled after February 2013.

Modification #16: Section 13.3 Sample Size and Power (page 68); Informed Consent Template
Changed text of sample size from 1,238 to 1,022
Added Section 13.3.3 (page 75) text to explain statistical power implications

Modification #17: Section 13.4 Interim Analysis (page 77)
Replaced existing text on Interim Analysis to conform with Amendment II.

Modification #18: Section 14.3 Steering Committee (page 80)
Added Dihua Xu, PhD to NHLBI Members
Removed Jungnam Joo, PhD from NHLBI Members
Removed Dina N. Paltoo, PhD, MPH from NHLBI Members

Modification #19: Section 13.5 Computation of PTTR (page 78)
Clarification of method for calculating the PTTR for patients with temporary discontinuation of warfarin.
Revised Table 13.10 -Approaches to dealing with missing INRs

Modification #20: Section 10.1 Visit Schedule (page 52)
Removed erroneous text (paragraph 3) "Discontinuation of warfarin will terminate participation for patients in the study because, after this, their warfarin will often be restarted by clinicians outside of the study (e.g., surgeons at the hospital where patients are being treated). Most importantly, the trial would no longer be testing the effect of genotype-guided initiation dosing."
Inserted corrected text (paragraph 3) "Permanent discontinuation of warfarin will terminate collection of INRs for patients in the study, but they will continue to be followed for other outcome data. Temporary holds of warfarin will be treated as in Table 13.10.

PROTOCOL AMENDMENTS

Protocol Version 1.3 includes Amendment #2

Protocol Amendment #1: **Section 6.1 Inclusion Criteria-item #4 - page 24**

Changed text from: "Expected duration of warfarin therapy of at least 3 months"
Changed text to: "Expected duration of warfarin therapy of at least 1 month"

Protocol Amendment #2: **Section 13.3.3 Statistical Power Implications- page 75**

The protocol was approved for amendment on September 16, 2012 (recommended by the COAG DSMB and approved by the NHLBI Director), to reduce the required sample size from 1,238 to 1,022. The justification for this change as well as modifications to the several parts of the Protocol that were impacted, are provided below.

Background and Rationale:
Given current rates of recruitment (described in subsequent sections), the inability to procure additional funds for the COAG trial, and the very conservative estimates used for the study's sample size calculations (effectively inflating the sample size), the coordinating center has been asked to re-assess the sample size requirements for the trial and to balance those requirements against available financial resources.

Original Sample Size Calculations: In the original sample size calculations, we considered the distribution of CYP2C9 and VKORC1 variants and estimated whether genotype-guided dosing is equally effective across groups defined by the total number of CYP2C9 and VKORC1 variants. Based on a subgroup analysis from a single randomized trial of 200 participants (CoumaGen), we hypothesized that certain genotypes would not benefit from genotype-guided dosing, most likely because their predicted dose from genotype-guided dosing algorithms would not meaningfully differ from that predicted by clinical-guided algorithms. We based sample size estimates on the comparison of the percent time in therapeutic INR range (PTTR) between the genotype-guided and clinical-guided dosing groups. Specifically, we assumed that participants who possess a single genetic variant (in either CYP2C9 or VKORC1) would not benefit from clinical-guided dosing because this subgroup did not appear to benefit in CoumaGen (based on a post-hoc analysis). Based on this assumption and an assumption that there would be a 15% relative difference in PTTR between the genotype-guided and clinical-guided dosing groups in the subgroup with 0 or >1 variants (again based on the post-hoc analysis in CoumGen), the absolute difference in PTTR between genotype-guided and clinical-guided arms would be 5.49%. This estimate was consistent with the a priori clinically meaningful difference of between 5% and 10%.

It is important to note that sample size and power estimates are dependent only on this clinically meaningful absolute difference in the PTTR between the two study groups. Regardless of whether assumptions regarding the differential effectiveness of genotype-guided dosing across subgroups defined by number of genetic variants are correct, we can calculate the sample size and power required to detect a clinically meaningful difference. For example, if the proportion of participants with a single genetic variant is higher than initially estimated (e.g., 50% versus 40%) and all other assumptions regarding the effect of the intervention are correct, then the absolute difference in the PTTR would be 4.6%. This difference is below our a priori threshold for a clinically relevant difference and for which the study was never designed to detect. On the other hand, if our assumption regarding the lack of benefit in the subgroup with a single genetic variant is incorrect and there is indeed some benefit in this subgroup (e.g., due to the day 4/5 dose-revision algorithm) such that this subgroup has a 2.5% relative benefit, then the absolute difference in PTTR would be 5.5%, even if 50% of participants have a single variant. Therefore, knowing the percent of participants with a single genetic variant in the study will not

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allow us to better estimate sample size and power. Most importantly, relying on this knowledge could lead to an incorrect estimate of the actual effect of the intervention and a faulty decision on the target sample size to maintain adequate power. Therefore, we base our forecasts for sample size and power on the single reliable parameter: the a priori desired detectable difference in PTTR of 5−10%.

To determine the level of significance (α) for the statistical test of PTTR between the genotype guided and clinical-guided dosing groups, we considered an alpha-allocation approach. In this approach, a portion (α_A) of the overall level of significance is used to test the comparison in the overall cohort; the remaining portion (α_S) is used to test the comparison in a pre-defined primary subgroup.The alpha-allocation approach facilitates a traditional primary analysis to assess a statistically significant difference between the treatment groups, as well as a predefined primary subgroup analysis that is not relegated to a secondary analysis. We defined the primary subgroup based on participants whose predicted initial dose employing the genetic and clinical dose-initiation algorithms differs by ≥ 1.0mg, a factor known at the time of randomization. We posited that the subgroup of participants with a larger difference between the predicted initial doses should have a larger separation in PTTR between the two groups. We assumed that a clinically relevant absolute difference to detect in the primary subgroup is 9.15%, from a PTTR of 61% to 70.15%, reflecting a 15% relative difference (as done for the overall cohort analysis). We selected a type-I error rate of $\alpha = 0.05$ and fixed $\alpha_A = 0.04$ for the overall cohort analysis. The correlation between the two tests will be obtained under the null hypothesis when the size of the primary subgroup is known. The correlation will then be incorporated to obtain $\alpha_S > \alpha - \alpha_A$ given that α_A is fixed.

Projections for Accrual: In planning for a target enrollment and completion date, we use a conservative assumption that, with all 17 clinical centers actively enrolling (achieved as of May 2012), 34 new participants will be recruited each month (assuming a rate of 2.0 participants per center per month, lower than the previous estimate of 3.0 participants per center per month). We believe that this estimate is reasonable because, over the last three months, even without all 17 clinical centers enrolling and with new centers just beginning to enroll, we averaged 25 participants per month (or 1.52 participants per center per month). However, we also make a 'worst-case' assumption that recruitment will not increase above 1.5 participants per center per month, even though we have two centers that have not yet contributed to our estimates and four others that only recently began recruiting. Given these estimates, the achievable sample sizes are shown in the table below.

Sample size projections for various enrollment end dates and accrual rates

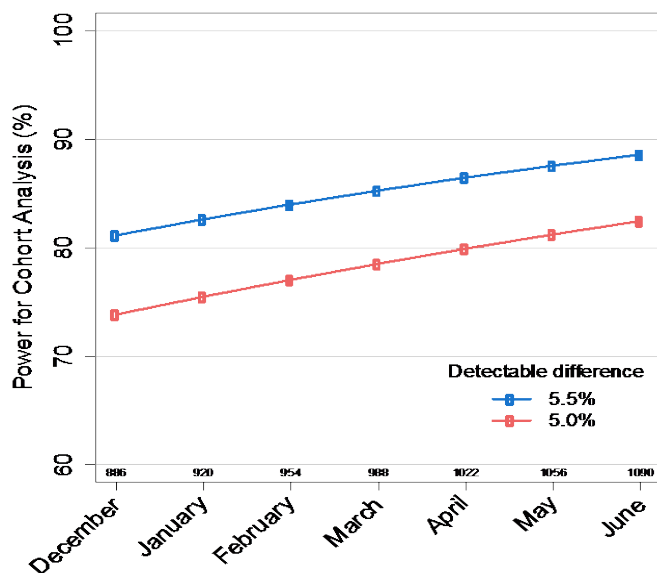|	Enrollment end date	Estimated sample size (2.0/center/month)	'Worst-case' sample size (1.5/center/month)
Current	31 December 2012	886	818
Scenario 1	30 April 2013	1022	920
Scenario 2	31 May 2013	1056	945
Scenario 3	30 June 2013	1090	971

The original goal for completion of accrual was 31 December 2012. As demonstrated below, this could lead to an underpowered study. Given this, the coordinating center proposed that the accrual period be extended through 30 April 2013 (Scenario 1 in the table above) with a target sample size of 1022 in order to maintain at least 80% power to detect the pre-specified 5.5% absolute difference in PTTR for the primary outcome (see *Projections for Statistical Power* below). In making this recommendation, we assumed that:

- The extension of accrual, follow-up, and all coordinating center activities can be done with the allocated resources (i.e., no additional funding is needed);

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- The performance regarding accrual and loss to follow-up for the primary endpoint remains at the current level or better;
- The study contracts are extended to accommodate the above changes, with follow-up of study participants through 31 July 2013, and an extension for the coordinating center to allow for data cleanup and analyses (through 31 January 2014).

Projections for Statistical Power: Power of at least 80% should be achieved for both the overall cohort analysis and the primary subgroup analysis. The power assessment for the full cohort (α_A = 0.04) through June 2013 is given in the figure below, assuming: a detectable difference of 5.0%, the lower bound for a clinically relevant detectable absolute difference in PTTR (red line); and 5.5%, the difference in PTTR targeted in the original protocol (blue line). The standard deviation of PTTR is assumed to be 25%. Sample sizes are provided on the horizontal axis.
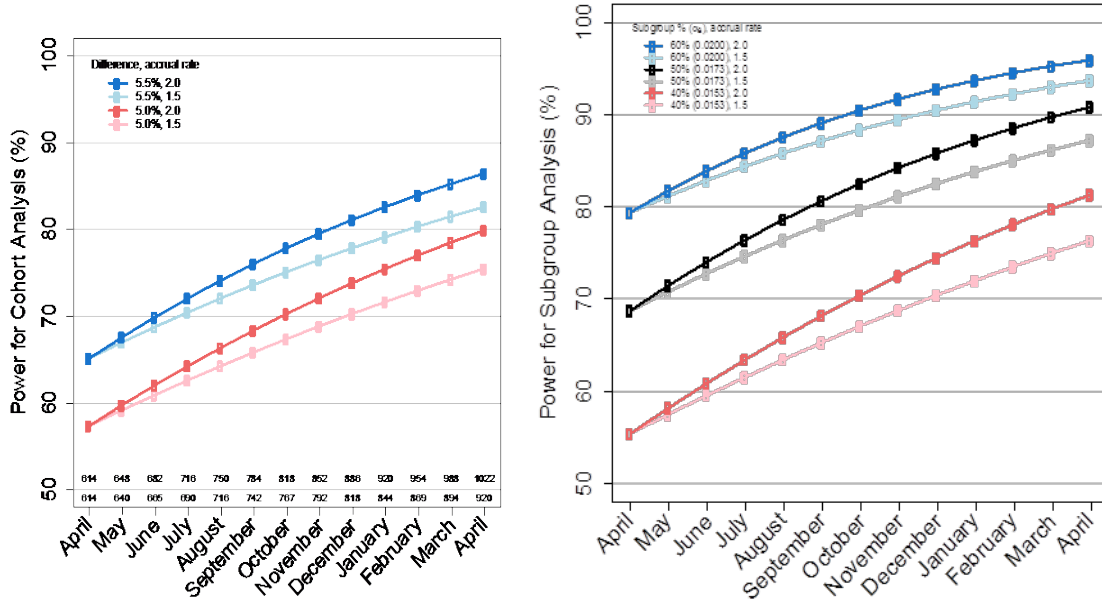

It is seen that by the end of April 2013, after a sample size of 1022 is achieved, there will be at least 80% power for the overall cohort analysis to detect an absolute difference of at least 5% in PTTR between groups.

We also examined power under assumptions of 'worst-case' recruitment through April 2013 (see figures below), assuming: detectable differences of 5.0% and 5.5% for the overall cohort analysis (left panel); and a detectable difference of 9.15% for the primary subgroup analyses (right panel) with different subgroup sizes (60%, 50%, and 40% for the proportion with ≥ ± 1.0mg/day predicted initial dose difference between the clinical and the genetic algorithms). The standard deviation of PTTR is assumed to be 25%. In each panel, the accrual rate is either assumed to be the 'best estimate' of 2.0 participants per center per month (represented in darker color) or that obtained under the 'worst-case' scenario of 1.5 participants per center per month (represented in lighter color). Corresponding sample sizes for the overall cohort analysis (left panel) are provided on the horizontal axis ('best estimate' on top; 'worst case' on bottom).

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Statistical power for overall cohort analysis (left) and primary subgroup analysis (right) through April 2013 under different accrual rates

It is seen that by the end of April 2013, after a sample size of 1022 is achieved, there will be at least 80% power for the primary cohort analysis to detect an absolute difference of 5% in PTTR between groups and to detect an absolute difference of 9.15% between groups in the primary subgroup analysis. Even under the 'worst-case' scenario, we still will be able to detect an absolute difference of 5.5% in PTTR for the overall cohort analysis and 9.15% for the primary subgroup analysis if the size of the subgroup is > 40%.

List of Abbreviations

AC	Anticoagulation
ACAD	Atherothrombosis and Coronary Artery Disease
AE	Adverse Event
ASPS	Ancillary/Substudy Proposals Subcommittee
CAP	College of American Pathologists
CL	Central Laboratory
CLIA	Clinical Laboratory Improvement Amendments
COAG	Clarification of Optimal Anticoagulation through Genetics
CPT	Clinical Pharmacology and Therapeutics
CRF	Case Report Form
CROWN	Creating an Optimal Warfarin Nomogram
CTCC	Clinical Trial Coordination Center
CYP2C9	Cytochrome P-450 2 Subfamily C Polypeptide 9 Enzyme
DASS	Duke Anticoagulation Satisfaction Scale
DDC	Drug Distribution Center
DMS	Data Management System
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
DVT	Deep Vein Thrombosis
EC	Executive Committee
ECS	Endpoints Classification Subcommittee
EDTA	ethylenediaminetetraacetic acid (anticoagulant additive in blood collection tube for blood banking)
EuroQol	European harmonization measurement of health quality
FDA	Food and Drug Administration
GLM	Generalized Linear Models
GLP	Good Laboratory Practice
GS	Genotyping Subcommittee
GWA	Genome-wide Association
HIPAA	Health Insurance Portability and Accountability Act
HU12	Health Utilities Index Mark 2
ICMJE	International Committee of Medical Journal Editors
IDE	Investigation Device Exemptions
IDS	Investigational Drug Service
IMS	Interventional Management of Stroke
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
IWPC	International Warfarin Pharmacogenetics Consortium
MedRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
NHGRI	National Human Genome Research Institute
NHLBI	National Heart Lung and Blood Institute
NIH	National Institutes of Health

List of Abbreviations

NINDS	National Institute of Neurological Disorders and Stroke
NPO	(Latin: nil per os) nothing by mouth
PHI	Protected Health Information
PHS	US Public Health Service
PID	Participant Identification
POC	Point of Care
PTT	Partial thromboplastin time
PTTR	Percentage of Time in Therapeutic Range
QA	Quality Assurance
QALY	Quality Adjusted Life Years
QCS	Quality Control Subcommittee
RNA	Ribonucleic acid
S/S	Signs and Symptoms
SAE	Serious Adverse Event
SC	Steering Committee
SD	Standard Deviation
SF-36	Short-form health survey - 36 questions.
SNPs	Single Nucleotide Polymorphisms
SOB	Shortness of Breath
TE	Thromboembolism
THINRS	The Home INR Study
VKORC1	Vitamin K Epoxide Reductase Complex 1

INVESTIGATOR AGREEMENT PAGE

Clarification of Optimal Anticoagulation Through Genetics (COAG)
Randomized Clinical Trial #1
A Randomized, Multicenter, Double-Blind Clinical Trial to Evaluate Efficacy in the Use of Clinical Plus Genetic Information to Guide Warfarin Therapy Initiation and Improve Anticoagulation Control for Patients

INVESTIGATOR (S)

- I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol and will only make changes in the protocol after notifying the sponsor except when necessary to protect the safety, rights, or welfare of subjects.

- I will ensure that the requirements relating to obtaining informed consent and institutional review board (IRB) review and approval in 45 CFR 46 are met.

- I will ensure that the requirements relating to obtaining HIPAA authorization following the federal mandate for disclosure of access to data and associated privacy protection will be met.

- I agree to report to the sponsor adverse experiences that occur in the course of the investigation, and to provide annual reports and a final report in accordance with 45 CFR 46.

- I agree to maintain adequate and accurate records and to make those records available for inspection in accordance with 45 CFR 46.

- I will ensure that an IRB that complies with the requirements of 45 CFR 46 will be responsible for the initial and continuing review and approval of the clinical investigation. I also agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

- I agree to personally conduct or supervise this investigation and to ensure that all associates, colleagues, and employees assisting in the conduct of this study are informed about their obligations in meeting these commitments by providing them with copies of the protocol, any subsequent protocol amendments, and access to all information furnished by the sponsor.

Principal Investigator Signature: _____

Date: _____

Name (Please Print):_____

Institution: _____

Once signed, this original shall be maintained in the Regulatory Binder at the clinical center, with a copy faxed to the Project Manager at the DCC (215-573-6262).

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Abstract

Current dosing practices for warfarin are empiric and result in the need for frequent dose changes as the international normalized ratio (INR) gets too high or too low. As a result, patients are put at increased risk of thromboembolism, bleeding, and premature discontinuation of a highly efficacious therapy. Also, primarily because of difficulties using the drug, there is substantial underuse of warfarin in millions of patients who would benefit from anticoagulation (AC). There is clearly a need to improve warfarin management.

Although clinical research has identified clinical and genetic factors that can alter warfarin dose requirements, limited prospective clinical research has examined the utility of using clinical and genetic information to improve outcomes among a large, diverse group of patients using warfarin.

The objective of the Clarification of Optimal Anticoagulation through Genetics (COAG) trial is to conduct a 1,022 participant, multicenter, double-blind, randomized trial comparing two approaches to guiding warfarin therapy initiation: 1) initiation of warfarin therapy based on algorithms using clinical information and an individual's genotype using genes known to influence warfarin response ("genotype-guided dosing"), and 2) initiation of warfarin therapy based on algorithms using only clinical information ("clinical-guided dosing"). The study hypothesis is that the use of genetic and clinical information for selecting the dose of warfarin during the initial dosing period will lead to improvement in stability of AC relative to a strategy that incorporates only clinical information (without genetics) for initial dosing. Each study arm will include a baseline dose initiation algorithm and a dose revision algorithm applied over the first 4 to 5 doses of warfarin therapy. By comparing the two strategies in this trial, the study will be able to determine if genetic information provides added benefit above and beyond what can be gleaned simply with clinical information.

1 Study Hypothesis

The study hypothesis is that the use of genetic and clinical information for selecting the dose of warfarin during the initial dosing period will lead to improvement in stability of anticoagulation (AC) relative to a strategy that incorporates only clinical information (without genetics) for the initial dosing period.

This study is a proof-of-concept efficacy trial. Efficacy is defined as a measure of whether, under optimal application, dosing algorithms will lead to improvement in care. The trial will thus answer the question: "can the use of clinical plus genetic information lead to an improvement in anticoagulation control above and beyond the use of only clinical information during the initiation of warfarin, when applied in a uniform and optimal manner to all patients?" This is in contrast to an effectiveness trial, which would measure whether the application of dosing algorithms would lead to improvement of care in the clinical setting where factors associated with AC other than dosing may be affected by the intervention. An effectiveness trial would answer the question: "if a dosing algorithm were available and used in clinical practice, would it improve clinical outcomes, accounting for the fact that not all patients will receive standardized care, that the algorithm may not be applied in a uniform manner in all patients, and that knowing genetic information may alter other patterns of warfarin use in unpredictable ways?" These distinctions are critically important because they drive much of the design of the trial, including the need for blinding of warfarin dosing and standardization of dose titration after the initial dosing period. Because efficacy has not yet been established for genotype-guided dosing of warfarin, it is important to first test whether this approach can, indeed, improve AC outcomes under controlled conditions.

2 Introduction and Background

Warfarin sodium is one of the top 20 medications used in the US.[1] Its use will only increase as the population ages. Warfarin is highly efficacious at preventing thromboembolism (TE), a condition associated with substantial morbidity and mortality. Numerous conditions put patients at risk for TE, including atrial fibrillation, deep venous thrombosis, mechanical heart valves, and dilated cardiomyopathies. However, warfarin must be dosed properly to avoid life-threatening complications (from overdosing) and lost efficacy (from underdosing).

The impetus for identifying clinical and genetic factors that alter warfarin dose response, thus better predicting starting dose, is that warfarin dose requirements vary widely among patients and are typically identified by trial and error, putting patients at risk for complications and drug failure. Although the average maintenance dose is 4-6 mg per day, warfarin dose requirements can vary over 30-fold and warfarin has an unusually narrow therapeutic range. Current practice relies primarily on empirical dosing (i.e., giving all patients the same starting dose, regardless of clinical and genetic factors). For example, at many centers in the US, most patients are begun empirically on 5 mg/day during the "initiation phase" of warfarin on the basis of population averages,[2-4] and the dose is titrated based on response, as measured by repeated measures of the international normalized ratio (INR). Because of empiric dosing, the dose of warfarin must be changed frequently when initiating therapy in response to out-of-range INRs, and frequent (up to several times a week) monitoring is needed during this initiation phase.

The practice of empiric dosing results in improper dosing in a large number of individuals, and out-of-range INRs are extremely common early in therapy (e.g., 57%[5] to 69%[6]). These improper levels of AC provoke life-threatening bleeding and thromboembolic complications, resulting in substantial morbidity and cost.[7-9] One of the major morbidities is major bleeding complications due to over-anticoagulation. Although major bleeding is of substantial concern, even minor bleeding can lead to withdrawal of therapy,[10] thus depriving patients of the most effective, and often only, therapy available to prevent TE. Minor bleeding also leads to repeat office visits and sometimes emergency room visits. Under-AC due to improper empiric dose selection puts patients at risk for thromboembolism (TE).[11-16] In addition, among patients on warfarin who suffer a stroke, those who are under-anticoagulated at the time of the event have significantly higher morbidity and mortality compared with those with proper AC control.[17]

The direct costs associated with improper warfarin dosing include greater warfarin-related visits to emergency rooms and hospitalizations[14] and more frequent follow-up.[18] The direct medical costs of failure of AC therapy are enormous; for example, ischemic stroke (approximately 2005 US $32,635-$131,630 in the first year following the event), hemorrhagic stroke ($16,823-$50,468), non-cerebral major hemorrhage ($2,907-$8,721), and other TE events ($3,807-$11,421) such as PE and deep vein thrombosis.[19;20] Along with bleeding and TE, empiric dosing and its attendant under- and over-AC have other medical and economic consequences. Patients who have out-of-range INRs must be carefully reassessed within a short period and often require dosage changes. This generates additional clinic visits, blood tests, and potential for miscalculations of dosage requirements.[18;21] Substantial additional costs may be incurred for long-term care for stroke survivors, multiple repeat visits to re-titrate warfarin dosing,[21] and emergency room visits for high INRs and bleeds.[14] Patients whose INRs fluctuate excessively may have their warfarin discontinued,[10] depriving them of the substantial benefit of the drug. Improper AC that leads to bleeding events, even minor ones, can also result in diminished quality of life[22] and, as noted above, permanent discontinuation of warfarin.[10]

Importantly, reductions in the time that a patient is under- or over-anticoagulated have been associated with reductions in bleeding, TE, and costs.[14;23-26] These benefits are particularly relevant during the initiation phase of warfarin, when the proper dose is being determined. Because the initiation phase is a period when AC control is particularly vulnerable to dosing errors and when significant bleeding and TE can occur, efforts to improve our ability to predict warfarin maintenance dose at the start of therapy are clearly needed to enhance the safety and efficacy of the drug and to reduce the associated costs and early discontinuations.

Variability in warfarin dose-response is related to both clinical and genetic factors. Many patient and environmental factors (herein referred to as "clinical factors") that can influence warfarin response have been identified over warfarin's more than 50 years of use.[27] However, despite knowledge of these factors, a large proportion of variability in warfarin dose requirements remains, and dosing algorithms have, to date, had limited success.[28-30] One possible reason for the limitations of prior dosing algorithms is that they do not incorporate genetic factors that alter warfarin dose requirement. As a result, interest has turned to understanding genetic factors that may play a role in warfarin response.

Two genes, the cytochrome P-450 family 2 subfamily C polypeptide 9 enzyme (CYP2C9) gene and the vitamin K epoxide reductase complex 1 (VKORC1) gene, have been the focus of extensive studies.[7;9;31-56] These genes provide complementary

information: CYP2C9 is a gene related to warfarin pharmacokinetics (the effects of the body on the drug; e.g., metabolism) and VKORC1 is related to warfarin pharmacodynamics (the effects of the drug on the body).[57] From the pharmacokinetic perspective, warfarin consists of a racemic mixture of (R)- and (S)-warfarin, and these 2 forms are metabolized to the inactive metabolite by different cytochrome CYP450 enzymes. The S-form accounts for 60-70% of warfarin's overall anticoagulant activity. CYP2C9 is largely responsible for the metabolic clearance of (S)-warfarin, with S-warfarin being converted to inactive 6-hydroxy and 7-hydroxy metabolites almost exclusively by CYP2C9. To date more than 50 single nucleotide polymorphisms (SNPs) have been described in the regulatory and coding regions of the CYP2C9 gene,[58] but only two functional variants, termed CYP2C9*2 and CYP2C9*3, are relatively common and useful in dose prediction.[59] In vitro studies have confirmed that the most common allele, CYP2C9*1, yields a significantly more potent enzyme than either the CYP2C9*3 variant[60;61] or the CYP2C9*2 variant.[62] From the pharmacodynamic perspective, the most important gene identified to date in the pharmacodynamic pathway is VKORC1, the warfarin-sensitive and rate-limiting enzyme of the vitamin K cycle that recycles the epoxide and quinone form of vitamin K to the reduced non-oxidized form.[63;64] Several variants within the VKORC1 gene have been associated with altered warfarin dose requirements and haplotypes have been described that are associated with a relatively low hepatic VKORC1 mRNA expression and with lower warfarin dose requirements.[49;50;65;66] In Caucasians, one SNP, 1173C/T, was as informative as VKORC1 haplotypes for predicting warfarin dose in a Caucasian population[67] and has been shown to similarly predict dose in African Americans.[65] Although other genes have been identified that may alter warfarin response, none to date have proven useful in dose prediction.

Conceptual Framework and Experience with Dosing Algorithms. Because of the difficulties of dosing warfarin and the multifactorial nature of warfarin response, the concept of dosing algorithms that use clinical and genetic variables to improve AC management, reduce complications, and enhance efficacy has real potential. Current dosing practices are empiric and thus patient's doses must be titrated in response to supra-therapeutic and sub-therapeutic INRs in order to identify an individual's correct, stable dose. The conceptual framework of this trial is that, by choosing a dose early in the course of therapy that is more likely to be an individual's ultimately required stable dose, the degree of improper anticoagulation that is common early in therapy can be reduced. We define the initial dosing period in this trial as the first 4-5 days of therapy, thus including the original dose selection that relies on clinical and genetic variables prior to beginning therapy and a dose revision after the initial several doses that includes not only clinical and genetic variables but also the INR response to the original doses of the drug.

This proof-of-concept trial is important because, despite our current understanding of the influence of clinical factors and genetic factors on warfarin dosing, formal testing of the utility of a genetic-guided dosing strategy among a large, diverse group of patients using warfarin has not been rigorously performed. Although two small trials have recently been published, neither trial was definitive.[68;69] Nonetheless, these trials and other work have demonstrated that dosing algorithms are feasible in practice.[5;7;28;29;70] Equally importantly, the recently completed Couma-Gen trial suggested that more accurate prediction of maintenance warfarin dose could translate into better AC control.[68]

In August 2007, the US Food and Drug Administration (FDA) announced the approval of revised warfarin labeling, to explain that patients' genetic makeup may influence how

they respond to the drug and that genetic information could be used to determine initial dosing.[71] In addition, the Centers for Medicare and Medicaid Services has recently asked for public comment on pharmacogenetic testing, highlighting warfarin, because of the "relative scarcity of high-quality published evidence from outcome-related clinical trials about the clinical utility due to pharmacogenetic testing at this time."[72]

In summary, current dosing practices for warfarin are empiric and result in the need for frequent dose changes as the INR gets too high or too low. As a result, patients are put at increased risk of TE, bleeding, and premature discontinuation of a highly efficacious therapy. Also, primarily because of difficulties using the drug, there is substantial underuse of warfarin in millions of patients who would benefit from AC.[73-76] There is clearly a need to improve warfarin management.[77] In order to definitively determine if the use of clinical plus genotype-based dosing will translate into improvement of AC control, a large, rigorously controlled, randomized trial is necessary.

3 Objectives of the Study

The objective of the Clarification of Optimal Anticoagulation through Genetics (COAG) trial is to conduct a multicenter, double-blind, randomized trial comparing two approaches to guiding warfarin therapy initiation: 1) initiation of warfarin therapy based on algorithms using clinical information and an individual's genotype using genes known to influence warfarin response ("genotype-guided dosing"), and 2) initiation of warfarin therapy based on algorithms using only clinical information ("clinical-guided dosing"). Each arm will include a baseline dose initiation algorithm and a dose revision algorithm applied over the first 4-5 doses of warfarin therapy. Thus, the intervention will be applied over these first 4-5 days (the "intervention period"). Following this period, dose titration will be the same between arms. By comparing the two strategies in this trial, the study will be able to determine if genetic information provides added benefit above and beyond what can be done simply with clinical information.

3.1 Primary Objective

The primary objective of the study is to compare the two strategies with respect to the time participants spend within the therapeutic INR range (PTTR) during the first 4 weeks of therapy.

3.2 Secondary Objective

Secondary objectives of the study are to compare the two strategies with respect to the PTTR during the first 2 weeks and 3 and 6 months of therapy; other outcomes at 2 and 4 weeks and 3 and 6 months, including time to stable warfarin dosing and INR above range (>4.0); number of dose changes required; and major clinical outcomes, including major bleeds, combination of major and minor bleeds, combination of major bleeds and thromboembolic complications, cost, and quality of life.

4 Study Endpoints

4.1 Primary Endpoint

The primary outcome of this study is the percentage of time participants spend within the therapeutic INR range (PTTR) during the first four weeks of therapy. This will be calculated from the INR values using the standard method that assumes a linear change

in INR from one measurement to the next using the method of Rosendaal et al.[78] This linear interpolation method has been shown to be valid and, in the absence of high levels of missing data (e.g., ≥20% missing INR values), reproducible.[79]

Rationale for Study Endpoint. The trial is designed to improve the management of warfarin, and the PTTR is the most widely accepted measure of improved warfarin management. The rationale for using PTTR is as follows.

(1) The PTTR is a measure of the most important factors influencing safe and effective AC: over-AC and under-AC. Excessive AC and the variability in AC control are the strongest, most consistent, and often the only predictors of AC-related bleeding.[14;80-94] The risk of major bleeding increases by 80% for each one-point increase in the INR,[80;95] and begins to increase substantially at INRs above 3.[81;82;96] Randomized trials have also confirmed that a target INR of 2 to 3 is associated with a greater than 50% reduction in bleeding compared with target INRs >3, without loss of efficacy.[89-93] The degree of over-AC also is the most important risk factor for intracranial hemorrhage.[81]

Under-AC also is a significant risk factor for TE, and, if patients are under-anticoagulated, they clearly are not being offered the full benefit of the drug. A minimum level of AC must be maintained in order to preserve the benefits of warfarin therapy. The risk of TE increases as the INR falls below 2.[11;14-16] For example, the risk of stroke in patients with atrial fibrillation increases dramatically as the INR falls below 2, even at levels of 1.9.[11] Fihn et al. found that, among all patients treated with warfarin, the risk of TE increased 11-fold with PTTRs below 1.3 (estimated to correspond to an INR < ~1.9).[85] In a randomized trial comparing a target INR of 2 to 3 with a target INR of <2 in atrial fibrillation, there were significantly fewer thromboembolic events in the 2-3 INR range group.[16] Therefore, the effectiveness of warfarin will be substantially reduced by insufficient levels of AC.

Given these data, it is not surprising that one study has estimated that for each 10% increase in the time spent out of range (i.e., 1-PTTR) over approximately 2 years of follow-up, there is a 29% increase in mortality, a 10% increase in ischemic stroke, and a 12% increase in other thromboembolic events.[97]

(2) The PTTR is often the only modifiable factor that can be improved to reduce complications and costs in AC patients. Once a decision is made to put a patient on warfarin, most risk factors for bleeding or TE (e.g., age, underlying lesions) are not modifiable by the health care provider or the patient.[89]

(3) It has been clearly shown that improving AC control can reduce complications and costs.[14;98-10062-6424-26]

(4) Improving AC could have significant impact on numerous other important outcomes besides bleeding or TE, including patient satisfaction, costs, and quality of life.[101] Just as importantly, improvement in AC control could reduce permanent discontinuation of warfarin.[10]

(5) PTTR is an acceptable and commonly used measure to judge AC control across centers and practices (e.g., Verhovsek et al.,[102] Garwood et al.,[103] and Nichol et al.[104]). It has also been used to assess AC control in other interventions related to warfarin. For example, PTTR is the primary measure of AC control in The Home INR Study (THINRS), which is assessing patient self-testing of AC. Similarly, the proportion of INRs in range was used as a primary outcome measure in a meta-analysis of clinical trials of warfarin self-monitoring.[98]

(6) The PTTR is a primary outcome in completed and ongoing clinical trials of warfarin pharmacogenetic-based dosing. It was the primary outcome in the published Couma-Gen trial. It is also currently the planned primary outcome in a large multicenter European trial of warfarin pharmacogenetics set to begin next year, and it is a primary outcome measure for the ongoing warfarin pharmacogenetics trial being conducted at the Marshfield Clinic. Thus, the PTTR will allow comparability of primary outcomes across clinical trials of warfarin pharmacogenetics.

Thus, the PTTR is a valid measure of successful warfarin management, the primary aim of this study. In addition, although the COAG trial is designed as a proof-of-concept trial, the primary outcome should have the potential to be correlated with major clinical events. That is, if the PTTR does not improve in the COAG trial, there may be little rationale for conducting a much larger and more expensive study using major clinical events as the outcome measure. (Of course, if other, secondary outcomes, are positive, there may be the need for further studies.) If the trial does show benefit on the PTTR, there will be a very good rationale to then consider a larger trial with major clinical outcomes, realizing that improvement in PTTR may not ultimately translate into improved clinical outcomes.

4.2   *Secondary Endpoints*

Secondary endpoints include the following:

Occurrence of INR >4 or serious clinical event in the first 4 weeks. This endpoint was chosen because it was also utilized by Anderson[68] and because it is an endpoint more closely related to clinical outcomes. INR >4 is associated with substantial increases in bleeding risk.[96] Furthermore, INR >4 captures the effects of even short-term over-AC, which can increase risk that might not be fully captured by the PTTR. The serious clinical outcomes to be included are major bleeding and thromboembolism events, as defined below. This will be the principal, secondary outcome.

Clinically relevant, non-major bleeding. Clinically relevant non-major bleeding will be defined based on a similar definition used in the Van Gogh trial.[105]

Time to first therapeutic INR. First therapeutic INR will be defined as the first INR that is between 2 and 3. This will also be an important secondary outcome. It was not chosen as the primary outcome for several reasons. First, although genotype-based dosing is likely to improve this measure, it is also possible that an algorithm could over-dose some patients in the first several days, leading to a therapeutic INR more quickly, which would not be desirable if there is then a subsequent overshoot of the INR. Second, improving time to first therapeutic INR has not, to our knowledge, been shown to correlate with improved clinical outcomes in prior studies. Third, it is not being used as the primary outcome measure in other warfarin pharmacogenetic trials.

PTTR <60%, or INR ≥ 4 at least twice during the first 4 weeks. In order to better capture the severity of out-of-range INRs, the values of high INRs will be combined with PTTR during the first 4 weeks. A binary outcome will be defined in which an adverse event will be defined as having a PTTR <60% and/or two or more INR measurements greater than 4. This outcome simultaneously captures information regarding clinically meaningful high INR measurements and unsuccessful maintenance of a therapeutic INR over the period of observation. The threshold of 60% for PTTR is based on the fact that the mean PTTR in clinical care should be approximately 60% for the first 4 weeks.[106]

Variability in INR at 4 weeks, 3 and 6 months. This outcome measures the variation in INR over time, as measured by the standard deviation from the target INR (using 2.5 as the target) over the defined period of time, and has been used by some as a measure of AC control.[84] Variability in the first 4 weeks and 3 months are considered most likely to be impacted by the intervention.

Number of warfarin dose changes in the first 4 weeks of therapy. Anderson et al. demonstrated fewer dose changes among those receiving pharmacogenetic-based dosing versus usual care.[68] Reducing the need for dose changes and thus repeat AC clinic visits could reduce costs, reduce the potential for improper dose changes (e.g., "overshooting" or "undershooting" when making dose changes), and improve quality of life.

PTTR during the first 2 weeks, 3 months, and 6 months of therapy. This is defined using the same method as for the primary outcome but over different durations of follow-up. PTTR in the first 4 weeks and 3 months are considered most likely to be impacted by the intervention.

Time to stable warfarin dosing. Stable dosing will be defined, similar to numerous studies,[9;32;49;65;107-109] as two consecutive INR measurements in the therapeutic range without a dose change over a period of at least 1 week apart. Secondary analyses may be performed to examine other definitions used for stable dose, to allow comparability with other studies.

Rate of INRs >4 at 2 and 4 weeks and at 3 and 6 months. These outcomes measure the occurrence of more extreme levels of over-anticoagulation that are associated with substantial increases in bleeding risk,[96] and are thus highly clinically relevant.

Rate of INR <2 at 2 and 4 weeks and at 3 and 6 months. These outcomes measure the occurrence of underanticoagulation. Because of the dramatic increase in the risk of thromboembolism as the INR falls below 2,[11-16] this is a highly clinically relevant outcome. The dosing algorithms tested could reduce or increase the rate of occurrence of this outcome. For example, if a dosing algorithm tends to underestimate the initial dose needed, the algorithm could be associated with a higher rate of INRs <2 early in therapy. Even if the algorithm correctly predicts dose exactly, it is possible that starting at the correct dose (e.g., 3 mg) rather than the empiric starting dose (e.g., 5 mg) could lead to an increase in the rate of INR <2 prior to reaching steady state.

Time to bleeding at 4 weeks and 3 and 6 months. Major bleeding will be defined using standard definitions. The current plan is to use the definition used in the Italian Study on Complications of Oral Anticoagulant Therapy.[82] These criteria will be reviewed by the Endpoints Classification Subcommittee (ECS) and modified as needed. Minor bleeding will be all other bleeding events. Time to major bleeding will be evaluated at each time frame; minor bleeding will be assessed at 4 weeks and 3 months (because only major bleeding events will be collected after the first 3 months). All major bleeding events will be adjudicated by members of the ECS. Each event will be reviewed independently by 2 members of the ECS, blinded to study arm. If there is disagreement between the 2 reviewers, the reviewers will discuss the case. If there is still a lack of consensus, a third reviewer will review the results, again blinded to study arm and also blinded to the results of the first 2 reviewers; this reviewer's rating will be the final rating. This proposed procedure will be reviewed by the ECS who will make the final determination on the adjudication plan.

Time to Thromboembolism (TE). The criteria for TE will be developed by the Endpoints Classification Subcommittee (ECS). All TE events will be adjudicated by members of the ECS as described above for bleeding events. A Time to Major Bleeding or TE endpoint will also be measured.

Cost at 4 weeks, 3 months, and 6 months. The economic analysis will be based on an assessment of genetic testing cost, warfarin and warfarin management cost, other medical services use and cost, and patient preference. This will include Genetic Testing Cost based on microcosting. Microcosting is the process of observing and quantifying the person-hours, equipment, and supplies required to conduct a test or process. Observations are conducted several times after which cost estimates are developed based on salaries/benefits, equipment costs, and supply costs.

Alternative cost estimates will be derived from medical reimbursements (not charges). Warfarin Management Cost will be derived by the frequency of warfarin monitoring (and differences in the two arms will be particularly driven by non-protocol visits due to poor AC control and complications). The cost of warfarin monitoring services will be derived from the Medicare fee schedule. Alternative estimates will be derived from the literature (e.g., Menzin et al.[110]). Medical Service Use and Cost will be based on medical service use assessed by patient self-report at baseline and weekly during the first 4 weeks and then monthly. Patients will be asked to report outpatient care (warfarin dosing visits, including labs; other physician visits; and medications). They will also be asked about hospital care and emergency room visits (all, subdivided by whether or not they are warfarin/anticoagulation/bleed related); days by type of other acute care facility; and days by type of other non-acute care facility. Discharge summaries and/or bills will be obtained for any patient-reported hospitalizations. Reported medical services will be costed out by use of the Medicare fee schedule (physician services), Medicare Diagnosis Related Groups (hospital services), Medicare Clinical Diagnostic Laboratory Fee Schedule (diagnostic services), Medicare Home Health Agency cost reports (home care), and the Medicare Durable Good Fee Schedule (durable goods). We will stratify medical costs by whether they are related to warfarin and bleeding or to other health conditions. Patient Preferences will be assessed at baseline, 2 weeks, 4 weeks, 3 months, and 6 months. As recommended by the Panel on Cost-Effectiveness, we will assess the general public's preferences (by use of the HUI2) as well as patients' preferences (by use of the EuroQol feeling thermometer). The latter will be used for sensitivity analysis. Quality-adjusted life years (QALYs) will be calculated as the area under the QALY curve.

Quality of Life at 2 weeks, 4 weeks, 3 months and 6 months. The Duke Anticoagulation Satisfaction Scale (DASS) will be used to measure quality of life (including at baseline). This scale has demonstrated reasonable psychometric properties to date.[111] The DASS is a 25-item scale that measures 2 components of AC therapy: (1) negative impacts of anticoagulation (limitations, hassles, and burdens); and (2) positive impacts of anticoagulation (confidence, reassurance, satisfaction). The DASS is currently being employed to measure AC-related quality of life in a clinical trial of home-based INR monitoring.[112] In addition to the DASS, the SF-36 will be administered on the same schedule.

Although the trial will not be able to provide definitive information on the cost-effectiveness of the intervention relative to major events, measuring cost and quality of life will provide several pieces of useful information. First, it will provide information for future evaluations of the economic value of gene-directed initiation strategies. Direct measures of the cost of genetic testing, warfarin management, and medical outcomes

will be available from the trial, as will probabilities of events during a year of follow-up. At present, only broad estimates can, and are, being used to estimate and justify genetic testing. Ultimately, the trial results will provide true measures of effect and cost, and thus could be more conclusive. Second, the results will be candidates for inclusion in future meta-analyses of the economic value of such strategies. Third, to the extent that ratios of the cost per time within INR range can be interpreted, they will be available and confidence intervals for the ratio will be estimable.

5 Study Design

This is a randomized, multicenter, double-blind trial comparing two approaches to guiding warfarin therapy initiation. Participants will be recruited from up to 18 US clinical sites prior to initiating warfarin. Clinical and genotype data will be collected on all participants, who will then be randomized to one of the two study arms. The initial dosing of warfarin will be determined according to the study arm, and study investigators, clinicians, and participants will be blinded to the treatment assignment and warfarin dose for the first 4 weeks of the trial (up to the primary endpoint). Subsequently, warfarin dose will be unblinded. Dose adjustments will be based on the INR response to the warfarin dose, using a standardized dose titration protocol to minimize possible biases in the subsequent management of the participants. Clinical information, including potential environmental modifiers of dose requirements, will be collected at baseline and follow-up visits. All visits will be performed per current clinical practice (e.g., all protocol-required data will be collected during usual, clinically required visits). As part of the trial analyses, additional blood will be stored and DNA will be extracted and stored for biomarker, genome-wide association (GWA) genotyping, sequencing, and other future genomic analyses to determine genetic and biomarker predictors of large differences in pharmacogenetically- predicted vs. actual dose and additional analyses on efficacy and safety of warfarin (e.g., in meta-analyses with other trials).

6 Participant Eligibility Criteria

The study population will be drawn from patients with a variety of conditions requiring long-term anticoagulation therapy with warfarin. Through the site selection process and based on eligibility criteria and the population of patients treated with warfarin, it is expected that a large proportion of enrollees will be elderly, will include a racially and ethnically diverse population representative of the US population, and will have a variety of diseases or conditions that usually require long-term use of oral anticoagulation.

6.1 Inclusion Criteria

1. Age ≥ 18 years
2. Willingness and ability to sign informed consent
3. Able to be followed in outpatient AC clinic
4. Expected duration of warfarin therapy of at least 1 month
5. AC management for the patient will be performed in-hospital and as an outpatient by clinicians that will adhere to the study dosing algorithms and dose titration plans (discussed below)
6. Target INR 2-3

6.2 Exclusion Criteria

1. Currently taking warfarin

2. Prior warfarin therapy with known required stable dose

3. Clinician opinion that warfarin dosing needs to be adjusted for reasons not accounted for by dosing algorithm

4. Abnormal baseline INR (off warfarin), e.g., due to liver disease, antiphospholipid antibody

5. Contraindication to warfarin treatment for at least 3 months

6. Life expectancy <1 year

7. Pregnant women or child-bearing women not using medically-approved method of birth control (requires negative pregnancy test to exclude pregnancy in child-bearing women)

8. Inability to follow-up on a regular basis with anticoagulation practitioners participating in the trial

9. Any factors likely to limit adherence to warfarin. For example,

   - dementia

   - alcohol or substance abuse

   - plans to move in the next 6 months

   - history of unreliability in medication taking or appointment keeping

   - significant concerns about participation in the study from spouse, significant other, or family members

   - lack of support from primary health care provider

10. Cognitive or other causes of inability to provide informed consent or follow study procedures

11. Participating in another trial that prohibits participation in the COAG trial or planned enrollment in such a trial within the first 6 months of warfarin therapy

12. Estimated blood loss of >1000 cc requiring blood transfusions within 48 hours prior to randomization

13. Genotype (CYP2C9 or VKORC1) known to participant from prior testing

The 3-month minimum for warfarin therapy was chosen for several reasons. First, shorter duration (< 3 months, such as prophylactic therapy after hip surgery or short-term, peri-cardioversion therapy in atrial fibrillation patients) would not contribute the necessary follow-up to examine the effects of the intervention beyond a period of a few weeks. Second, a longer minimum duration would likely create a study population that is not similar to that of the AC population in the US. Most patients who require >3-6 months of warfarin therapy have atrial fibrillation. Although some patients with recurrent deep venous thrombosis or pulmonary embolism are put on long-term warfarin, and although some studies suggest that longer-term anticoagulation is of some benefit for first-time TE, not all studies have confirmed this benefit[113] and most patients with deep vein thrombosis (DVT) are treated for only 3-6 months. In addition, although patients who have recurrent TE while on warfarin may require longer therapy, they often have their

target INR increased above the 2-3 range, which would make them ineligible for the trial. Therefore, requiring only long-term warfarin therapy would substantially limit the variety of conditions that are included in the trial.

The use of antiplatelet medications such as aspirin and clopidogrel, and bridging parenteral anticoagulation (such as unfractionated heparin, low molecular weight heparin) are allowed.

6.3   Deferral Criteria

Because this study requires all patients to be enrolled at the initiation of warfarin, patients who have already received a dose of warfarin cannot be enrolled. The only reason for deferral would be if a patient is planned to start on warfarin and the initiation of therapy is delayed. These patients will remain eligible to enroll, assuming they continue to meet all inclusion/exclusion criteria at the time of warfarin initiation.

7   Patient Selection and Follow-Up

7.1   Informed Consent Procedure

Each clinical center will be responsible for ensuring that informed consent is obtained from each participant according to the guidelines of its local Institutional Review Board (IRB). Informed consent must be obtained (signed and dated by the participant or authorized representative) prior to initiation of any study-related activity. At the time of screening, written consent for the research will be obtained.

Clinical sites will prepare an informed consent form following the guidelines of their local IRB and applicable regulations for informed consent. The form must include the following elements:

- a statement that the study involves research;
- an explanation of the purpose of the research;
- expected duration of participation;
- a description of the procedures to be followed;
- identification of experimental procedures;
- a description of foreseeable risks and benefits that may reasonably be expected;
- disclosure of appropriate alternative procedures;
- a description of the protection of confidential records;
- an explanation as to whether any compensation and medical treatments are available;
- contact information for answers to pertinent research questions, questions about subjects' rights and whom to contact in the event of a research-related injury;
- a statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits, and that the subject may discontinue participation at any time without penalty or loss of benefits, to which the subject is otherwise entitled; and
- a description of planned genome-wide studies and genotype-phenotype data sharing.

See Appendix A Informed Consent Template.

Prior to signing the informed consent, the research coordinator will review the details of the consent form verbally with the participant, and answer any questions they may have concerning participation in the study. The original, signed IRB-approved consent form will be kept in the participant's study file at the clinical center and a copy of the signed consent form will be given to the participant.

7.2   Informed Consent

7.2.1   Informed Consent for DNA/Genetic Testing

Each participant will be asked to give consent to allow DNA extraction for the main study analysis and storage of DNA, biomarkers, and other related biological factors for future analysis. The consent form will clearly indicate that providing genetic data is required in order for the subject to participate in the study. Future uses anticipated as part of the trial include GWA genotyping and sequencing to determine genetic predictors of large differences in pharmacogenetically-predicted vs. actual dose. Genetic data will be de-identified and shared in accordance with NIH GWAS policies (http://grants.nih.gov/grants/gwas/index.htm), and will be used for scientific and/or commercial purposes. These samples will be stored at the Central Laboratory, as part of the COAG study. All data will be kept confidential as outlined below.

7.2.2   HIPAA Authorization

Following mandated federal HIPAA regulations and according to local IRB guidelines, the use and disclosure of the subject's protected health information (PHI) will be explained and participant authorization will be obtained. The consent and/or authorization forms will list those individuals and organizations that may have access to the participant's research data.

Other elements of authorization must include: the use of protected health information in future studies (e.g., storage of blood samples for future analyses other than that which is listed in the protocol at the time the informed consent was obtained) and the subject's right to withdraw permission and have the blood samples destroyed. Authorization will make it clear that data (e.g., studies from blood samples) that are generated and shared with outside investigators cannot be retrieved from these investigators, but once permission is withdrawn no future distributions of these data will occur.

The consent and/or authorization forms must also state that investigators will have the right to reject subjects from the research trial if written authorization is not provided.

At each clinical site, the process of subject recruitment must be reviewed and approved by the site's local IRB to help ensure that privacy protections are consistent with federal HIPAA regulations.

7.3   Participant Recruitment

Records of catchment areas and patient logs will be examined for each clinical site to determine the expected flow of patients, and realistic goals for recruitment efforts will be established. Many study participants will be hospitalized at the time of recruitment. Thus, the ability to do hospital-based recruitment will be required, along with recruitment of patients sent to the AC clinic for elective initiation of warfarin. Each clinical site will be responsible for developing a recruitment strategy that best works at that site. A model

plan may be as follows: Each site establishes a method to identify all newly hospitalized patients with an admission diagnosis that is likely to require warfarin (e.g., deep venous thrombosis, pulmonary embolism, atrial fibrillation). Patients already on warfarin will be excluded. The primary providers for each patient will be approached to determine if the patient is likely to start warfarin; if so, the provider will be asked to notify the study nurse as soon as a final decision is made. In addition, the study nurse will follow the patient throughout their hospital course to identify when a decision is made to start warfarin. Hospital pharmacies can also be used to identify any first-time orders for warfarin for these patients in case the other methods fail to identify a particular participant. The pharmacies will also be able to identify new starts of warfarin among patients not initially identified on admission (e.g., a patient who develops a DVT during a hospitalization). Some hospitals have inpatient "Anticoagulation Management Services" and/or "transition teams" that are responsible for helping with the transition from inpatient to outpatient anticoagulation; such teams will be ideal to identify study participants.

Some patients will begin warfarin as planned outpatient therapy after first being referred to an anticoagulation clinic. Such patients will be identified within each of the participating site's clinics and those in whom warfarin will be initiated at their first anticoagulation clinic visit will be contacted in advance of that appointment. If they agree to participate, informed consent and samples for genotyping will be obtained prior to the first clinic visit.

All patients will receive algorithm-guided dosing beginning with the first dose of warfarin. Although patients randomized into the genotype-guided arm may receive, as their first dose, a clinical-algorithm-based dose of warfarin if genotyping is not available prior to the first dose (see Section 8.3.1), the goal of recruitment will be to enroll as many patients as possible who have the genotyping available prior to the first dose. The recruitment strategy at each site must be developed to maximize the number of patients who can begin warfarin therapy with genotype-based dosing.

There are several potential pitfalls to recruitment. Perhaps the greatest concern is the inability to identify patients quickly enough to allow for enrollment, informed consent, and genotyping prior to the first dose of warfarin. Several strategies can address this issue: The identification of potential study patients prior to the decision to start warfarin, as discussed above, will allow patients to be identified in advance of the first dose of warfarin. Although this will require more effort on the part of study personnel, it is likely a key method to avoid the aforementioned pitfall. Another strategy to consider is to approach patients who may start warfarin and discuss the trial and obtain informed consent. Thus, as soon as a decision is made to start warfarin, the patient (after reconfirming their willingness to participate) can be randomized and genotyping can be performed. (Such an approach depends, of course, on IRB approval.) An additional method, as discussed above, is to identify new orders for warfarin through pharmacy or other systems. Warfarin is often given first in the evening in hospitalized patients (to allow for subsequent dose adjustments to be performed after the INR values return); although this will require the study nurse to obtain informed consent, collect blood samples, and have genotyping completed within one day, this may be feasible with rapid turnaround genotyping. However, such patients would have to be identified in the morning. In contrast, outpatients who are referred to AC clinics for initiation of warfarin will be known in advance from the initial referral (and identified from the AC practitioners through daily screening by study coordinators). Thus, there should be sufficient time to perform genotyping in these patients.

Another potential pitfall to recruitment is that clinicians treating patients for their clinical conditions may not believe in or trust genetic-based dosing and/or may not want to participate in a study where dose titration is standardized by protocol. Another possibility is that some clinicians may already be using genetic data for warfarin prescribing by the time the trial begins. The main method to avoid this pitfall is to recruit sites that manage warfarin therapy themselves in their AC or similar settings and that are in equipoise with respect to genetic-based prescribing. Nonetheless, should there be resistance to genotype-guided dosing among some clinicians who refer patients to the clinics, additional methods can be employed, including distribution of educational materials and individual meetings with specific clinicians to understand and try to address their concerns. Of course, if, after these efforts, an individual clinician does not want his or her patients to participate, sites would not expend the effort to recruit that clinician's patients but rather focus on other clinicians' patients.

In order to minimize early dropouts due to patients in whom, for example, house staff in the hospital start patients on warfarin but in whom attending physicians then decide against warfarin therapy the next day on rounds, attending physicians will be contacted whenever possible prior to enrolling patients.

7.3.1 Patient Flow

Patient flow throughout the study will be described in the Manual of Procedures. A representation of the patient flow is provided in Appendix B.

7.4 Participant Retention

Considerable efforts will be made to retain patients once they are enrolled, although it is expected, given the nature of the condition being treated and extensive prior experience in anticoagulation management centers, that failure of retention to the primary 4-week endpoint will be extremely rare. Retention to the 6 month follow-up might be somewhat more problematic. To ensure retention, we will include the following strategies: 1) the use of noncoercive participant payments (monthly payments for completing follow-up for patients' time and effort); 2) study newsletters; 3) calendars given to subjects at the beginning of the trial and periodically updated throughout the trial based on their therapeutic course, to inform them well in advance of upcoming visits and visit frequency/intervals; and 4) reminder phone calls prior to visits and immediately after a missed visit. In addition, patients will receive their medications for free (in order to accommodate the blinded pill process) for the first 4 weeks, which may also enhance retention.

7.5 Follow-up Period

Participants will be followed in the study for 6 months. (Except for those patients enrolled after February 2013 whose follow-up period will be for less than 6 months). To whatever extent possible, the data collection periods will coincide with clinical care for warfarin dose adjustment and maintenance. See Visit Schedule, Section 10.1.

7.6 Competing Trials

Patients in a clinical trial that prohibits them from being in the COAG trial (e.g., a trial that is randomizing patients to warfarin versus other drugs) will be ineligible for this study. Clinical sites that are participating in such studies and including many of their warfarin patients in these trials will not be eligible to be a site for the study unless they can

convincingly demonstrate that it will not interfere with their ability to adequately recruit and follow the patients to the end of the COAG trial. If such a competing study should begin after the COAG trial has started, clinical sites will have to ensure that the overlap in enrollment criteria are minimal, such that they will still have adequate numbers of patients who meet criteria for the COAG trial that do not overlap with the other trial (e.g., short-term DVT prophylaxis patients will not be eligible for the COAG trial so a trial of other drugs in these patients will not create a conflict). If there is overlap in enrollment criteria and if the other trial prohibits randomizing warfarin-treated patients in the COAG trial, then the clinical site will have to present a plan to the Steering Committee (SC) to demonstrate how they will continue to maintain adequate recruitment for the COAG trial. Any patients co-enrolled in another study would also have to receive the approval of the Clinical Trial Coordination Center (CTCC) PI and/or Medical Monitor prior to enrolling the patient in the COAG trial. If such a plan is not feasible, the clinical site may need to be dropped from the trial and, depending on the stage of the trial and the numbers recruited, an alternative site recruited.

Patients who are enrolled in the COAG trial will be prohibited from remaining in the study if they enroll in other trials that may alter their therapy (e.g., randomize them to a warfarin-alternative or alter their standardized dosing of warfarin). If such enrollment is planned, patients will be ineligible to begin the COAG trial. There may be other studies that do not prohibit enrollment or continued participation in the COAG trial. The clinical sites will have to present any potential conflicting studies in advance to the SC who will make the final decision on whether the study prohibits enrollment or continuation in the COAG trial. Should such a decision need to be made acutely in an individual patient who is already enrolled in COAG, the decision will be made by the Executive Committee.

7.6.1   Patient Confidentiality

Of major concern to most research participants is the confidentiality of information collected as part of a research study. All information will be kept strictly confidential and used for research purposes only. No identifying information will be disclosed in reports, publications or presentations. The study will apply for a Certificate of Confidentiality, which will be issued to protect the privacy of research subjects by protecting investigators and institutions from being compelled to release information that could be used to identify the research participants.

Procedures to assure confidentiality will be strictly observed. The participating clinical sites will follow standard guidelines to assure that participant confidentiality is maintained. All data will be:

- kept in confidential locked files;
- identified by participant identification number (PID) only and initials only; and
- kept separately from identifying information used for subject tracking and follow-up contacts.

The following methods will be utilized to protect the confidentiality of participant data at the CTCC and Central Laboratory:

1. Data collected at clinical sites and entered into the Clinical Trial Coordinating Center (CTCC) Data Management System (DMS) will contain a participant identification (PID) number that does not reflect any personal information.

2. Data linking a participant to a PID number will be stored locally in locked files. This information will not leave the clinical site and will be accessible to clinical site study staff only (but CTCC staff, IRB and sponsor staff may request access to patients' files during site visits).

3. Access to the DMS will be strictly controlled by the CTCC. User names and passwords will be distributed only to the appropriately trained clinical staff members. DMS access will be restricted by clinical site such that data associated with site A are not accessible from site B.

4. Specimens collected from participants and transferred to the Central Laboratory will be coded for tracking purposes but will not contain any personal identifiers.

5. The CTCC will not receive any original data for auditing or quality assurance purposes that contain any personal identifying information. Clinical site staff must completely remove or obscure these data before sending them to the CTCC.

8   Randomization and Study Arms

8.1   *Randomization Scheme*

The randomization module in the Data Management System (DMS) will assign the study arm to each participant. This module will be programmed to deliver a randomization assignment only after eligibility information is confirmed and then re-entered into the system for verification purposes.

Randomization will be stratified by participating institutions, to provide reasonable balance of study arm allocation within institutions. Randomization will also be stratified by race (African American versus non-African American) because race has been strongly associated with differential benefit of dosing algorithms, particularly with lesser benefit in African Americans,[114] and the dosing algorithms that will be used in the trial predict dose differently among African Americans versus non-African Americans (i.e., the variables used for race are coded as "African American versus non-African American"). In addition, race is strongly associated with the prevalence of variants in CYP2C9 and VKORC1[56;115] (the genes in the dosing algorithm) and any imbalances in race among arms will create imbalances in these variants; race is likely to be associated with the prevalence of other genetic variants (known and unknown) that may alter warfarin response; and race may also be a surrogate for other factors associated with warfarin response that are difficult to measure (e.g., diet and access to care[116]). Because some clinical sites will have small numbers of some racial groups and because adjustment for race will be important in all analyses, stratification of race will be important. Randomization will not be stratified into further categories of race because it is anticipated that non-African American, non-Caucasians will represent only about 5-7% of the total study population.

Blocking ensures that, within each of the clinical centers, there will be a reasonable balance of the number of patients in each treatment arm within the clinical center. With approximately 100 participants expected to be recruited within each center, it is likely that the arms will be balanced within individual centers. However, if a center is not successful in its recruitment goal, this will not be guaranteed. Thus, we will use permuted blocks with block sizes of 4 and 6, randomly chosen, which will minimize any imbalances in study arm assignment.

Online, web-based 24/7 randomization procedures will be used. This process essentially makes the complex nature of the use of stratification and blocking in the randomization process invisible to the sites. A manual process will be developed by the CTCC in the event that online access is not possible.

8.2 Trial Arms and Dose Adjustments

The trial will test whether an initial dosing strategy that uses both an initial dosing algorithm and a dose revision algorithm improves AC control. The trial will randomize patients to their initial dose using the initiation algorithm based on study arm ("randomized initial dose phase"). Following this initiation dose, a second dose adjustment will be made after 3 and/or 4 doses of warfarin using a "dose revision" algorithm in each study arm. Further dose adjustment will be the same between arms using a standardized dose adjustment protocol ("dose titration phase" described below).

8.2.1 Rationale for Initial Dosing Using Predicted Maintenance Dose

The trial will test whether an initial dosing strategy that uses an initial dosing algorithm and a dose revision algorithm that incorporate genetic information will improve AC control relative to a dose initiation and dose revision algorithm that does not use genetic information. By identifying a dose for patients that is closer to their required dose, we hypothesize that there will be fewer out-of-range INRs during the dose-finding period where a stable warfarin dose for an individual patient is not yet known. As noted earlier, the recently completed Couma-Gen trial suggested that, indeed, a more accurate prediction of maintenance warfarin dose at the start of therapy could translate into better AC control.[68;68]

The initial dose-finding period, however, is complicated by several factors. First, initial dosing in poor metabolizers of warfarin (i.e., those with CYP2C9 variants) should theoretically not be altered based on differences in metabolism. That is, consistent with pharmacokinetic principles, the initial dose of a drug should be unaffected by difference in clearance rates. Because the CYP2C9 variants lead to a longer elimination half-life, it takes longer for the drug to accumulate in plasma, which means that the INR will rise more slowly over the first couple of days if they are initiated on their target dose. Drug concentrations will also continue to rise for much longer than normal, since it will take longer to achieve steady-state drug concentrations). In addition, recent studies suggest that CYP2C9 variants have little influence in INR response in the initial week of therapy and are more important in later INR response (in contrast to VKORC1 variants, which are important at all times).[117] Therefore, dosing patients with CYP2C9 *2 or *3 variants at lower doses during the first several days of therapy may not lead to improvement in AC and could lead to under-anticoagulation. As a result, initial dosing of warfarin may be improved if a pharmacogenetic algorithm does not alter dosing for those with these CYP2C9 variants. In this trial, the first dose (only) will not be altered based on CYP2C9 variants. Thus, those with CYP2C9 variant alleles will get a slightly higher dose on the first day than they will on days 2 and 3 (i.e., they might get 4 or 5 rather than 2.5 or 3). This will cause them to have higher concentrations in the first few days, which should help blunt the delay in rise in INR (the delay that might lead to dose increases that aren't really warranted). For example, if one starts on 2.5 mg as the predicted dose and on day 4 the INR has not increased, there could be an inappropriate dose increase such that, a couple of days later, the INR has been overshot, and the dose has to be reduced again. Giving a slightly higher dose for the first dose leads to higher drug concentrations following the initial few doses (but these concentrations would not go above what the

eventual steady state will be) so that the INR begins to move more quickly, thus helping diminish the chance that the warfarin dose will be inappropriately increased on day 4 or 5. Recent studies have, indeed, taken this approach clinically with good results.[118-120]

Second, there is debate about whether initial dosing (e.g., during the first two days) should include a "loading dose" (e.g., twice the predicted dose). Several randomized trials have examined the use of 10 mg starting doses ("loading" doses) for the first two days versus 5 mg ("standard" empiric) starting doses, with mixed results.[2;121-123] Several studies have found that an initial dose of 5 mg resulted in less over-anticoagulation compared with an initial dose of 10 mg in hospitalized patients.[2;122] Although one study suggested that 10 mg starting doses resulted in more rapid achievement of therapeutic INRs compared with 5 mg starting doses without increased risk of over-AC, this study was performed in relatively young outpatients.[121] A more recent, but smaller trial, found no advantage in time to achieving two consecutive in-range INRs with the use of 10 mg versus 5 mg starting doses.[123] Based on currently available data, the American College of Chest Physicians Evidence-Based Clinical Practice Guidelines 8th Edition (2008) recommends that loading doses not be given, although acknowledges that there is room for flexibility in selecting the starting dose, with some clinicians preferring larger doses (e.g., 7.5 to 10 mg) and some scenarios (e.g., elderly patient with impaired nutrition) in which lower starting doses may be preferred.[124] Given the totality of current evidence and the debate about using loading doses, we have chosen not to use loading (i.e., two-times predicted) doses in this trial.

8.3 Dosing Interventions

Patients will be randomized to one of the two dosing intervention arms that will test the effects of algorithm-guided dosing over the first 4-5 days of therapy. The overall scheme and details of each arm are depicted in Figure 8.1 and described in detail below.

8.3.1 Genotype-guided Dosing

Algorithm Selection Criteria. An algorithm's validity in the context of the COAG trial is its ability to predict the dose of an individual patient that will provide a stable INR. The criteria for choosing an algorithm are:

(1) The algorithm has been developed on a derivation dataset and then validated separately. Ideally this would be in multiple different, independent datasets but this may not be feasible.

(2) The algorithm characteristics are favorable, including measures of the closeness of the predicted vs. the stable dose for therapeutic INR value using the mean absolute error, R^2, and the percent predicted within 1 mg.

(3) The study in which the algorithm was developed has been published in a respected peer-reviewed journal or, at least, has undergone formal external review, with no valid contradictory papers.

(4) The algorithm is clinically usable. For example, an algorithm that requires extensive, extra clinical information to be collected (such as complex dietary histories) or that requires costly, non-routine laboratory measures (e.g., protein C levels), would have to be clearly superior to other algorithms to justify their use.

(5) The algorithm has "face validity": that is, the predictors and the direction and relative size of their effect (e.g., their coefficients in the regression equation) are consistent with the many other studies that have examined dose prediction and identified relatively consistent predictors.

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                                      │
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   ┌──────────────────────────┐              ┌──────────────────────────┐
   │ Genotype-guided Dosing Arm│              │ Clinical-guided Dosing Arm│
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Day	Genotype-guided Dosing Arm		Clinical-guided Dosing Arm
	Genetics Available for 1st Dose	Genetics Not Available for 1st Dose	
1	PGx-algorithm Based Dose w/o CYP2C9	Clinical-algorithm Based Dose	Clinical-algorithm Based Dose
2	PGx Based Dose, incl CYP2C9		Clinical-algorithm Based Dose
3	PGx Based Dose, incl CYP2C9		Clinical-algorithm Based Dose
4*	Genetic Dose Revision Algorithm Dose		Clinical Dose Revision Algorithm Dose
5*	Genetic Dose Revision Algorithm Dose		Clinical Dose Revision Algorithm Dose

<u>Overview</u>. Patients in this arm will have their initial dose based on an "initiation algorithm" that uses both clinical and genetic data and the dose will then be revised after 3 and/or 4 warfarin doses (i.e., on days 4 and/or 5) using a "dose revision algorithm." The dose will be calculated by the DMS using the regression coefficients from the dosing algorithm (sites will not need to do any calculations). The clinical and genotype data will be input into the central database: The clinical information will be input by the study coordinator, who will not know the genotype information and will not have access to that information in the DMS, and the genetic information will be input by laboratory personnel who will not know the clinical information and will not have access to that information in the DMS. This information will then be used to calculate the patient's estimated maintenance dose using the dosing algorithm. This will be the patient's initial dose (details of dosing scheme follow below). The calculated dose will be determined from the clinical information entered by the Study Coordinator at each clinical site (who will be blinded to genotype, study arm, and dose) and the genotype data entered by personnel in genotype laboratory (who will be blinded to clinical information, study arm, and dose) at that clinical site. This information will then be accessed by the Investigational Drug Service (IDS) personnel who will not be blinded to dose but will be blinded to clinical information, genotype, and study arm. The IDS will then dispense the blinded warfarin to the patient. After three doses of warfarin, each patient's INR on day 4 of therapy and the doses that the patient received in the first 3 days will be input into the DMS. This information, along with clinical and genetic information already in the system, will be used to calculate a new estimated dose. If patients are unable to have an INR on day 4 (e.g., because they are outpatients and the fourth day falls on a Sunday), they will continue on their initial dosing and the dose revision algorithm (which includes a variable for the 5th day of dosing along with one for the fourth day of dosing) will be applied on day 5. Although the protocol specifies that only a day 4 or a day 5 INR will be drawn, some patients will have INRs drawn on both days. For these patients the dose revision algorithm will be applied on both of those days. Following this initial dose identification phase (i.e., from days 5 or 6 on), patients will have subsequent dose titrations based on a standardized algorithm, discussed further below (9.3). Patient flow throughout the study will be detailed in the Manual of Procedures. A representation of the patient flow diagram is provided in Appendix B.

<u>Dosing Details</u>. Dosing details for both algorithm arms are shown in Table 8.1 and discussed in detail in the text that follows.

First Dose. It is unknown whether genotype-based dosing from day 1 is necessary to benefit patients. However, it is clear that recruitment and feasibility of the trial will be severely diminished if all patients must have genotype information available prior to the first dose. This is because the nature of this trial would make it prohibitive to enroll many patients prior to their first dose, particularly hospitalized patients in whom clinicians will need to begin warfarin without delay: First, patients will have to be identified, the study described, and informed consent obtained (estimated time of at least 1 hour). Second, blood will have to be obtained, DNA extracted, and genotyping completed (even with rapid turnaround genotyping, this time period is estimated to take 4 hours). Third, because of blinding, clinical sites' investigational drug services (IDS) must be available to distribute blinded drug to patients. Most IDS units close at 5 PM. Therefore, if a potential study participant is identified after noon, it is highly likely that they would not be enrolled if genetic information were needed prior to their first dose. Furthermore, many clinicians do not decide to begin warfarin and/or write orders for the drug prior to noon (e.g., in teaching hospitals, which will make up the majority of sites in this trial, decisions about beginning warfarin often

are made on attending rounds and orders are written mid-day following those rounds). In addition, it is very likely that genotype information will not be available in clinical practice prior to the first dose of warfarin in many patients. In contrast, applying a clinical-algorithm for the first dose in these patients will eliminate the 4-hour delay for genotyping and therefore substantially increase the chance of successful enrollment and also will be easily applied in practice. Therefore, the first dose of warfarin in the genotype-arm will be based either on the genotype-algorithm-predicted dose or, if genotype information is not available at the time of first dose, the clinical-algorithm-predicted dose. The second dose would then be the genotype-predicted dose.

Figure 8.1: Dosing Scheme for Study Arms During the Intervention Period

(*See text for explanation of dose revision on days 4 and 5)

Table 8.1: Dosing Algorithm During Dose Algorithm Intervention Period*			
Day	INR	Warfarin Dose (adapted from original Crowther algorithm using 5 mg starting dose[125])	Warfarin Dose for Trial (both algorithm arms)
1		5	Initiation algorithm rounded up,[†] ignoring CYP2C9 variants (if known) for genotype-guided arm (i.e., set CYP2C9 variable to 0 in equation); use clinical algorithm for those in clinical-algorithm arm or those in genotype-guided arm in whom genotype not yet known
2	No INR per protocol		Initiation algorithm rounded up, including CYP2C9 variants hereafter [†]
If INR checked off protocol	< 1.5	5.0 mg	Initiation algorithm rounded up, including CYP2C9 variants hereafter [†]
	1.5-1.9	2.5	0.5 * Initiation algorithm, including CYP2C9 variants hereafter[‡]
	2.0-2.5	1.0-2.5	0.5 * Initiation algorithm, including CYP2C9 variants hereafter[‡]
	> 2.5	0.0	0
3	No INR per protocol		Initiation algorithm rounded up[†]
If INR checked off protocol	<1.5	5.0-10.0 mg	Initiation algorithm rounded up[†]
	1.5-1.9	2.5-5.0	0.75 * Initiation algorithm[‡]
	2.0-2.4	0-2.5	0.5 * Initiation algorithm[‡]
	2.5-3.0	0.0-2.5	0.25 * Initiation algorithm[‡]
	>3	0.0	0
4****	<1.5	10.0	Dose Revision algorithm rounded up[§]
	1.5-1.9	5.0-7.5	
	2.0-2.4	0.0-5.0	Dose Revision algorithm rounded down[ǁ]
	2.5-3.0	0.0-5.0	0.5 * Dose Revision algorithm today, then Dose Revision algorithm rounded down [**]
	>3.0	0.0	0 today, then Dose Revision algorithm rounded down [***]
5****	Not avail.		Dose Revision algorithm (from prior day)
	<1.5	10.0	Dose Revision algorithm rounded up[§]
	1.5-1.9	7.5-10.0	
	2.0-2.8	0.0-5.0	Dose Revision algorithm rounded down[ǁ]
	2.9-3.0	0.0-5.0	0.5 * Dose Revision algorithm today, then Dose Revision algorithm rounded down [**]
	> 3.0	0	0 today, then Dose Revision algorithm rounded down [***]

Table 8.1: Dosing Algorithm During Dose Algorithm Intervention Period*

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* Items in shaded boxes are per-protocol dosing for the intervention period. Items not in shaded boxes are dose changes if INRs are checked clinically, off-protocol.

† If predicted dose is >=3.0 mg, the dose will be rounded up to the nearest 1.0 mg. If predicted dose is <3.0 mg, the dose will be rounded up to the nearest 0.5 mg (e.g., 2.1 mg would be rounded to 2.5 mg rather than to an integer value).

‡ If the algorithm dose is adjusted due to INR on days 2 or 3 (e.g., 0.5 * Dose from initiation algorithm), the dose will first be calculated using this correction, and then rounded to the nearest 0.5 (if calculated dose is <3.0 mg) or 1.0 mg (if calculated dose is >=3.0 mg): (e.g., if on day 2 the INR is 1.7 and the dose initiation algorithm dose is 4.3, the dose will be 4.3 mg * 0.5 = 2.15 mg, and the day 2 dose will be rounded up to 2.0 mg; if on day 2 the INR is 1.7 and the dose initiation algorithm dose is 7.5, the dose will be 7.5 mg * 0.5 = 3.75 mg, and will be rounded to 4.0 mg.) Exact half doses above 3 mg on days 2 and 3 will be rounded up (e.g., 3.5 mg will be rounded to 4, 4.5 mg will be rounded to 5, etc.)

§ Weekly dose will be calculated from dose revision algorithm on days 4/5. If the predicted weekly dose is >=11 mg (e.g., more than 1.5 mg/day), the weekly dose will be rounded up to the nearest 1.0 mg (e.g., if weekly dose is 14.4, it will be rounded to 15 mg/week). If the predicted weekly dose is < 11 mg (e.g., less than 1.5 mg/day), the weekly dose will be rounded up to the nearest 0.5 mg (e.g., if weekly dose is 10.4 mg, it will be rounded to 10.5 mg). This convention of using half doses for weekly doses below 11 mg follows that used in the Couma-Gen trial.

‖ Weekly dose will be calculated from dose revision algorithm on days 4/5. If the predicted weekly dose is >=11 mg (e.g., more than 1.5 mg/day), the weekly dose will be rounded down to the nearest 1.0 mg (e.g., if weekly dose is 14.7, it will be rounded down to 14 mg/week). If the predicted weekly dose is < 11 mg (e.g., <= 1.5 mg/day), the weekly dose will be rounded down to the nearest 0.5 mg (e.g., if weekly dose is 10.6 mg, it will be rounded down to 10.5 mg).

** If INR is 2.5-3.0 on day 4 or 2.9-3.0 on day 5, the dose for that day (day 4 or day 5) will be 0.5* dose calculated for that day (as described in ‡ above). After that day, the weekly dose will be the weekly dose calculated by the dose revision algorithm, rounded down as in ‖ above.

*** If INR is >3.0 day 4 or day 5, the dose for that day (day 4 or day 5) will be held. After that day, the weekly dose will be the weekly dose calculated by the dose revision algorithm, rounded down as in ‖ above.

**** If INR not done on this day, dose from prior day will be used..

As discussed above, there are pharmacokinetic principles and clinical experience that support the rationale that dosing patients with CYP2C9 *2 or *3 variants at lower doses during the first day of therapy may not lead to improvement in AC and could lead to worse anticoagulation.[117] In addition, prior studies have prospectively used a dosing algorithm by setting the CYP2C9 variable to *1/*1 for the first dose in all with good results.[118;119] Therefore, the first dose in patients who have genetic information available will set the CYP2C9 variable to 0 (e.g., will not assume slowed metabolism in this group).

Second and Third Doses. For the 2nd and 3rd warfarin doses, the genotype-guided arm will receive the predicted maintenance dose from the genotype-based algorithm. There is no planned INR check on these days, but it is possible that clinicians will check an INR clinically. If so, there can be a dose adjustment for unusually high INRs (INR ≥1.5 on days 2 or 3, which are expected to be very rare) for patient safety purposes (see Table 8.1 above).

Fourth and/or Fifth Doses. After a total of 3 doses in most patients, an INR will be checked (on the morning of the 4th day of therapy). The subsequent dose will be titrated

based on the dose revision algorithms as described above. Patients who have an INR checked on both the 4th and 5th days of therapy will have the dose revision algorithm applied on both days. As shown in Table 8.1 above, the predicted dose will be rounded up if the INR is <2.0, rounded down if the INR is 2.0-2.4, cut in half if the INR is 2.5-3.0, and held if the INR is >3.0. These adjustments are consistent with the approach used by Crowther et al.[124]

If the third dose falls on a Friday (e.g., the INRs used for the dose revision algorithm fall on a Saturday or Sunday) and the participant is an outpatient, the dose revision algorithm will still be used if the patient can come in for an INR check on either Saturday or Sunday and blinded drug can be provided on that day. If the third dose falls on a Saturday, patients can have their INRs measured on Monday (day 5) as the dose revision algorithm can predict dosing after 4 doses of warfarin (see below for details of algorithm).

<u>Initial Dose Algorithm</u>. The proposed initial dose algorithm to use, based on the criteria above, has been published by Gage et al. in *Clinical Pharmacology and Therapeutics*[120] (referred to herein as the CPT genetic algorithm). This algorithm was: (1) developed on a derivation dataset and validated in a separate dataset, including validation in an independent set of patients by other investigators;[114] (2) the algorithm characteristics are favorable in the validation dataset (R^2 54%, median absolute prediction error 1.0 mg/day); (3) *Clinical Pharmacology and Therapeutics* is a well-respected, peer-reviewed journal; (4) the algorithm is clinically usable, requiring only easily obtained clinical information plus genetic information; and (5) the algorithm variables and degree and direction of their effect are consistent with other, smaller studies. Other favorable characteristics of this study are that it is relatively large (1,105 patients in the derivation dataset – which is substantially larger than all other studies that have been published to date – and 292 in the validation dataset), includes 15% African Americans, included both CYP2C9 and VKORC1 genotype, and includes a clinical-only dosing algorithm derivation as well (a required algorithm for the COAG trial, discussed below in 8.2.2).

The algorithm is as follows:

Estimated daily dose (mg/day) = exp[0.9751 − (0.3238 × VKOR3673G>A) + (0.4317 × Body Surface Area) − (0.4008 × CYP2C9*3) − (0.00745 × Age) − (0.2066 × CYP2C9*2) + (0.2029 × Target INR) − (0.2538 x Amiodarone) + (0.0922 ×Smokes) − (0.0901 × African-American race) + (0.0664 × Deep Vein Thrombosis/Pulmonary Embolism as Indication for Therapy)],

> where the SNPs are coded 0 if absent, 1 if heterozygous, and 2 if homozygous, and race is coded as 1 if African American and 0 otherwise. For this trial, target INR will be fixed at 2.5. VKOR3673G>A is also known as VKORC1 -1639 (rs9923231). Dose will be rounded as detailed in Table 8.1, above.

> Between now and the start of the trial, it is possible that other algorithms will be published. The same criteria will be applied to those algorithms and further efforts will be made to validate and compare those algorithms with the CPT algorithm in independent datasets. The results of these efforts may lead to the selection of a different algorithm.

Dose Revision Algorithm. The concept of a second algorithm, applied after 3 or 4 days of warfarin dosing, has been proposed and validated in orthopedic patients by Millican et al.[118] and Lenzini et al.[119] These studies incorporated clinical and genetic data as well as dosing data in the first 3 or 4 days of therapy and the INR response after the 3rd or 4th dose. These studies included only short-term anticoagulation and were designed to predict first therapeutic warfarin dose. However, ongoing efforts are underway to develop a similar dose revision algorithm for patients receiving longer-term AC and in whom maintenance dose is available. The preliminary dose revision algorithm is as follows:

> Estimated Daily Dose = [EXP(3.06839 – (0.53107 x ln(INR)) – (0.00752 x Age in years) – (0.22372 x VKORC1 G>A) – (0.32324 x CYP2C9*3) – (0.16755 x CYP2C9*2) + (0.25915 x BSA) + (0.27146 x Target INR) – (0.11589 x African Origin) – (0.25709 x Stroke) – (0.0991 x Diabetes) – (0.12549 x Amiodarone Use) – (0.18327 x Fluvastatin Use) + (0.01697 x $Dose_{-2}$)+ (0.02047 x $Dose_{-3}$) + (0.01156 x $Dose_{-4}$)]÷7

> where race is coded as 1 if African origin and 0 otherwise; "Dose-i" is the dose given i days before the INR measured; BSA is body surface area; stroke, amiodarone, and diabetes are 1 if yes and 0 if no. *CYP2C9*2* and *CYP2C9*3* SNPs are coded as 0 if absent, 1 if heterozygous, and 2 if homozygous. *VKORC1* is *VKORC1*–1639/3673 G>A (rs9923231) and is coded 0 (homozygous GG), 1 (heterozygous), or 2 (homozygous AA). Dose will be rounded as in Table 8.1, above.

This algorithm will also be validated and, if not yet published prior to the trial, will be, at minimum, vetted by an external review group.

8.3.2   Clinical-guided Dosing

Patients in this arm will have their initial maintenance dose predicted based on an initial dose algorithm that uses only clinical data but not genotype information, again deriving dose from regression coefficients. Criteria for choosing this algorithm are the same as those for choosing the genotype-guided algorithm. The clinical information will be input by the study coordinators into the central database, which will estimate the patient's stable dose. Study coordinators will not know if genetic information is also being used to calculate dose or if the patient is in the clinical-guided dosing arm. Following this initial

dose, and after 3 and/or 4 doses of warfarin, a clinical dose revision algorithm will be used to modify dose (similar to the genetic dose revision algorithm, but not utilizing genetic information). After the 4th or 5th day, patients will have subsequent dose adjustments based on standardized titration, similar to the genotype-guided arm as discussed in Section 8.3.1 and shown in Table 8.1.

Initial Dose Clinical Algorithm. The proposed algorithm to use, based on the criteria above, is from Gage et al. in *Clinical Pharmacology and Therapeutics*[120] (referred to herein as the CPT clinical algorithm). As discussed above: (1) this algorithm was developed on a derivation dataset and validated in a separate dataset; (2) the algorithm characteristics are favorable in the validation dataset [R^2 17%, median absolute prediction error 1.5 mg/day, which, although low, may be better than empiric 5 mg/day dosing (IWPC paper submitted)]; (3) *Clinical Pharmacology and Therapeutics* is a well-respected, peer-reviewed journal; (4) the algorithm is clinically usable, requiring only easily obtained clinical information; and (5) the algorithm variables and degree and direction of their effect are consistent with other, smaller studies. Other favorable characteristics of this study are also discussed above in 8.3.1. The algorithm is as follows:

Estimated daily dose (mg/day) = exp [0.613 + (0.425 × BSA) − (0.0075 × Age) + (0.156 × African-American race) + (0.216 × Target INR) − (0.257 × Amiodarone) + (0.108 × Smokes) + 0.0784* Deep Vein Thrombosis/Pulmonary Embolism as Indication for Therapy].

Dose will be rounded as detailed in Table 8.1, above.

The IWPC collaboration has also developed a clinical-only algorithm, developed in 4,137 patients from across 21 international sites that was validated in a random sample of 1,027 patients from the Consortium. In addition, ongoing validation of the CPT and IWPC clinical-only algorithms in independent datasets are ongoing. The results of these efforts may lead to the selection of a different algorithm.

Dose Revision Clinical Algorithm. A dose revision algorithm that uses only clinical factors, INR, and dose given in the first 3 days of therapy has been developed using the same cohort as for the genetic-based dose revision algorithm described above. This algorithm will be used in a similar manner to that for dose revision in the genetic-guided arm and will have to meet the same criteria as detailed above.

Estimated Daily Dose = [exp (2.785 – (0.79401 x ln(INR)) – (0.00565 x Age) - (0.31252 x Stroke) + (0.29008 x Target INR) – (0.16746 x Diabetes) + (0.18342 x BSA) – (0.27234 x Fluvastatin Use) – (0.1256 x Amiodarone Use) + (0.03461 x $Dose_{-2}$) + (0.03012 x $Dose_{-3}$)+ (0.02023 x $Dose_{-4}$)] ÷7

where "Dose-i" is the dose given i days before the INR measured; BSA is body surface area; stroke, amiodarone, and diabetes are 1 if yes and 0 if no. Extra time' is 0 if the patients' $dose_{-3}$ was taken after noon and their INR draw was in the morning and is 1 otherwise. Dose will be rounded as in Table 8.1, above.

8.4 Potential for New Genetic Variants or Other Biomarkers Important in Dose Prediction

As of this writing, only variants in the CYP2C9 and VKORC1 genes have been clearly demonstrated to be of importance in dosing algorithms for warfarin.[7;126;127] However, as the field of warfarin pharmacogenomics is evolving, it is possible that before randomization begins, or even during the course of randomization, new genetic variants

or even other biomarkers with significant impact on warfarin dosing will be identified. If other biomarkers or variants are identified that add useful predictive ability prior to initiating the study, consideration will be given to using a different algorithm, assuming it meets the same criteria as discussed previously. The following discussion focuses on new genetic variants because these are most likely to be discovered, but would equally apply to new biomarkers and/or new warfarin dosing algorithms that use other clinical information.

If new variants are discovered after the study has begun, there will be several important issues that must be considered in making a decision to change the genetic algorithm in the middle of the trial. These issues include: the potential to devote large resources to a trial that could be considered outdated once complete, and the design issue of changing a treatment arm in an ongoing trial with the potential for impact on both the power to detect differences as planned and the interpretation of the ensuing results. Specifically, adopting a new dosing algorithm in the middle of the trial could be interpreted as changing the intervention in a way analogous to changing from one drug to a different drug in a randomized trial of drug therapy. The decision to change the algorithm during the trial must be made on the basis of establishing the best scientific evidence for warfarin dosing and, most importantly, must ensure that the risk-benefit ratio for study participants remains optimized. A decision to change the protocol must be made on the basis of the same rigorous level of scientific evidence that one would use in the decision to change the drug therapy in a drug trial.

Process. A hierarchical approach to deal with this important issue is proposed. Most importantly, the validity and relevance of any new scientific findings that may constitute grounds to consider modification of the protocol algorithm must be carefully assessed by the Steering Committee. It is expected that attention and weight will be given to findings high on a hierarchy of evidence (in declining order) as outlined in Figure 9.1 on the following page.

There are many issues that will be taken into account in determining whether a new algorithm with different or additional genetic variants would be considered worth studying. These issues include:

a) The evidence that a new genetic algorithm will be better than the one in use for the COAG protocol will need to be very strong.

As outlined in Figure 9.1 below, the highest level of evidence will come from a rigorous randomized trial comparing the new algorithm with an alternative dosing strategy in a cohort similar to the COAG cohort (level A in Figure 9.1). If it is deemed necessary to change the dosing algorithm in the COAG trial, this would change the nature of the trial from one testing a specific algorithm to a more strategy-oriented study. That is, the trial would be testing whether genotype-guided dosing as a strategy is superior to a clinical-guided strategy. Also, the analysis of the data would have to incorporate the change in approach to understand and adjust for the change in strategy during the trial. Of course, depending on the clinical effects of the new algorithm in the other trial, such results could require consideration of termination of the COAG trial (e.g., if the new algorithm is shown to reduce major bleeding).

In the absence of a randomized comparison of the new algorithm with alternative dosing strategies, a newly developed algorithm that is validated in an independent observational study is the next and most likely level of evidence (level B in Figure 9.1). In the absence of a randomized trial, it will not be possible to definitively determine if this algorithm would perform better than the genetic algorithm in the COAG or even better than

Figure 8.2.

Scientific Evidence for New Genetic Variants

Evaluation of Evidence

A. New algorithm with additional genotypes shown superior to a comparison arm on a clinically meaningful outcome in a different randomized trial

- Highest level of evidence
- Strong consideration for COAG modification, or termination

B. New algorithm developed in observational study and validated in independent dataset

- Moderate level of evidence
- Consider first an observational study comparing new algorithm with COAG algorithm in a validation dataset

C. New algorithm developed in observational study but not yet validated in independent dataset

- Low level of evidence
- Await independent validation

D. New genetic variants identified but not yet demonstrated to be potentially useful in a dosing algorithm

- Lowest level of evidence
- Watchful waiting

alternative approaches (e.g., clinical-based algorithms, usual care). Nonetheless, careful consideration will need to be given to modifying the trial design if the external evidence supports the potential superiority of the new algorithm. This could be done by directly comparing the new algorithm with the COAG algorithm in an independent dataset using predefined metrics of model performance. Specifically, this analysis would use an independent observational dataset to compare the COAG algorithm with the alternative algorithm using an external population that is independent from both derivation datasets. This could include data being collected by investigators in the IWPC or data from an alternative dataset from non-IWPC investigators (for example, a group of investigators at

Harvard is performing an observational study of warfarin dosing, the "CReating an Optimal Warfarin Nomogram (CROWN)" study, which is anticipated to conclude in 2009[128]), or the clinical-algorithm group from the COAG trial itself if there are enough patients. These options will take into account the availability of specimens, appropriate informed consent of study participants, agreement of investigators, and adequate sample size for validation.

Metrics that would be used to compare model performance are the R^2 statistic, the percent of predicted doses within 1 mg of stable dose, and the absolute mean error of stable dose predicted by the dosing algorithm. Predicted dose would be calculated for each subject in the validation cohort using both the COAG and the comparison algorithm. Next, predicted doses would be compared with actual maintenance doses for each algorithm and the R^2 statistic calculated for each algorithm. The algorithms would also be compared with respect to the percentage of predicted doses within 1 mg/day of actual doses. Again, the difference in these percentages from the COAG model would be compared with the alternative model. Consideration must also be given to the degree of over- and under-prediction of dose. Over-prediction will be considered worse than under-prediction because over-anticoagulating a patient can lead to unavoidable life-threatening bleeding while under-anticoagulation at the start of therapy can be managed with continued intravenous or subcutaneous anticoagulants. There may not be consistency among the comparative metrics, nor a readily available evaluative measure of difference between the algorithms that marks a difference of clinical importance, beyond statistical tests of significance. Ultimately the scientific and clinical judgment of NHLBI, the SC, and the DSMB will be brought to bear to make the final decision on whether the level of evidence is sufficient to alter the trial design.

If a new algorithm is developed but not validated in an independent dataset, the level of evidence for superiority will be considered low (level C in Figure 9.1). If the available empirical results in the derivation study were both impressive and scientifically plausible, further evaluation of the new algorithm could be considered by the SC, and the NHLBI. In this case, both the validation of the new algorithm and its comparative performance to the COAG algorithm would have to be completed, using the approach described in the paragraph above.

If only new genetic variants are identified but are not yet shown to add predictive ability to a dosing algorithm (level D in Figure 9.1), the NHLBI and SC could choose to await further research to demonstrate whether or not these additional variants can contribute to a dosing algorithm through others' efforts. The algorithm above could then be applied, based on the level of evidence that emerges for a new dosing algorithm.

b) The decision on whether to make any modifications in the trial algorithm will require a change in the characterization of the genotype-guided dosing arm. Instead of the testing of a pre-defined, fixed algorithm, the arm will be considered a gene-assisted dose strategy. The distinction being made is one that allows for change in the algorithm as evidence of new and better predictive variants becomes available. Further, the protocol statistical analysis plan will need to allow for the primary analysis to incorporate stratification by time for all arms, marking the change point of the modification. Consideration would also be given to re-powering the study to address whether the new dosing algorithm itself is superior to the clinical-based dosing algorithms and/or adding in a third arm using the new dosing algorithm. These decisions would need to weigh the level of evidence, stage of the study, ethical considerations, and budgetary restraints at the time.

c) The timing of the discovery of a new algorithm will be important. Design, analysis, and practical limitations of making an algorithm change will to a large degree determine a study time frame after which modifications should not be considered.

d) In order to ensure that any new evidence is acted upon expeditiously, the CTCC will perform a systematic and regular review of the state of the literature. In brief, each in-person SC meeting will include an agenda item to review any new scientific findings that may constitute grounds for conveying genetic information to study participants or to modify the trial for the reasons discussed above (or for any other reasons). These findings will include any new research known to a member of the SC or the NIH plus a review of the literature performed by the CTCC prior to each SC meeting. In turn, the DSMB should be charged with reviewing any new scientific finding brought to its attention by the NIH. Any DSMB recommendation for action or no action will be sent to the NHLBI for a final decision. If the NHLBI determines that a change in protocol is appropriate, standard operating procedures for amendment to the protocol will be implemented, including presentation to each of the study site Institutional Review Boards and updating of the consent procedure as needed.

Regardless of whether the algorithm is modified, other genetic variants are likely to be identified during the course of the COAG trial that may be associated with warfarin dosing but that are not incorporated into a dosing algorithm. It will be important to examine the effects of these additional genes on the relative efficacy of the trial's dosing strategies. This would include adjusting for these additional genetic variants and examining for effect modification by these other variants in a regression analysis framework.

9 Administration of Study Drug

9.1 Rationale for Blinding of Study Arm and Warfarin Dose for First 4 Weeks

The goals of blinding in this and any trial include: (1) ensuring that outcomes are assessed similarly in all groups, and (2) ensuring that investigators and subjects behave similarly with respect to all aspects of post-randomization care other than the randomized intervention. It is this latter aspect that is most prone to substantial bias if this study were to be performed without full blinding. The outcome of warfarin therapy can be influenced by many factors, including not only proper dosing, but also monitoring vigilance, educational efforts by clinicians, patient adherence to therapy, patient adherence to diet, and the use of interacting medications. The primary outcome of the study (PTTR) could therefore be affected not only by warfarin dose or warfarin dose adjustments, but by other, post-randomization factors that could differ if study arms and drug dose are not blinded. These include differential dropout, protocol deviations, cross-overs (e.g., genotyping those in the non-genotype-guided arm), differences in adherence, and differences in patient care. These would be particularly problematic if the occurrence of these post-randomization factors both differed by study arm and were also related to anticoagulation control, as might be expected.

For example, patients who know or suspect that they are in the clinical arm who are having difficulty with anticoagulation early in therapy (those who might contribute the most to any differences by study arm) may be more likely to withdraw from the protocol than those in the genotyping arm because clinicians (or the participants themselves) would want to know their genotype or manage their dosing themselves. Another

potential bias is that patients on very low doses, who would be known or presumed to carry genetic variants that make them sensitive to warfarin, may be managed more carefully: they may be counseled more aggressively about dietary adherence and they may be more meticulous about avoiding interacting medications, be asked to come for more frequent INR monitoring beyond that required by protocol, and/or seek such extra monitoring. As another example, physicians could be more likely to withdraw a patient from the study if they felt that one of the algorithm-based approaches was not working and wanted to dose patients themselves (for example in a patient taking too long to reach adequate levels of anticoagulation, thus delaying hospital discharge). Patients may also be scheduled for extra study visits and be more likely to be adherent with these visits (e.g., less missed visits) if they know they are "more sensitive" to warfarin. All of these scenarios could bias the results in manners that are difficult if not impossible to measure and control.

Therefore, in the proposed trial where post-randomization differences in patient management and/or participant behaviors could differ substantially by study arm, blinding as to study arm and dose is critical if one is to examine the efficacy of the interventions. The blinding scheme avoids the potentially major biases discussed above.

There are two main reasons for the blinding to dose: to blind participants, clinicians, laboratory staff and study site investigators to study arm; and to blind participants, clinicians, and study site investigators to genotype. If the dose being prescribed is known, complete blinding of both of these parameters will not be possible.

With respect to study arm, one could reasonably guess that patients randomized to unusually low or high doses were in the genotyping arm. One can demonstrate how knowing warfarin dose will reveal study arm using, as an example, an online dosing algorithm (www.warfarindosing.org). The variables used in this model are similar to currently proposed dosing algorithms. Without blinding to dose, one cannot fully blind to study arm. Without full blinding, dropouts or crossovers (e.g., performing genotyping to guide dose in the non-genotype-guided arms) can occur differentially by study arm in a manner that is related to the risk of poor anticoagulation control (thus creating bias) as discussed above.

The other purpose for the blinding scheme is to blind participants, clinicians, and study site investigators to genotype. As noted above, dose selections of ≤3 mg/day or ≥7 mg/day will be unlikely to occur in the absence of genetic variants, given the strong effects of CYP2C9 and VKORC1 on warfarin dose requirements.[49;56;65;129] This is particularly true in typical patients, who would not have extreme clinical variables that substantially alter dose requirement (e.g., a combination of multiple interacting medications, very low weight, and elderly, as the example of the 85-year-old woman noted above demonstrates). As another example, in Caucasian patients in one warfarin cohort,[65] only about 8% would be predicted to require <4 mg/day using age, BMI, and sex as predictors while 20% would be predicted to require <4 mg/day when VKORC1 and CYP2C9 are added to the model. Thus, although knowing dose will not perfectly predict genotype, it will partially unblind those caring for study patients as to their patients' genotype (i.e., clinicians will be able to infer genotype in at least a subset of patients in the trial). This could lead to differential care and patient behavior in the subset of patients most likely to have difficulty with anticoagulation control and thus bias the study's assessment of the primary outcome.

The main problem with the potential biases inherent in an unblinded study is that they cannot be completely measured, and therefore their occurrence can never be ruled out

nor can they be accounted for in analyses. This could lead to reduced scientific and clinical acceptance of the trial results. These concerns are particularly prominent for warfarin, where many factors other than dose can influence INR response.

9.2   Method of Blinding

In order to blind participants, study personnel at clinical sites, and clinicians to warfarin dose, and thus blind to study arm and genotype, warfarin tablets will be blinded for the first 4 weeks for each study participant (i.e., up until the primary study endpoint). In order to do this and to replicate as closely as possible the usual way in which warfarin is prescribed and taken in practice, tablets of each warfarin dose unit (e.g., 2 mg, 3 mg, etc.) will be encapsulated in hard gelatin capsules, similar to that used for several prior randomized trials[130-133] and demonstrated not to alter warfarin pharmacokinetics.[133] Appendix C illustrates this scheme.

This scenario allows for centralized dose preparation by the Drug Distribution Center (DDC) at the Clinical Trial Coordinating Center (CTCC). A supply of bottles with all possible doses would be supplied by the DDC to the investigational drug service or pharmacy at each site. Specifically, the DDC will pre-package the doses into bottles and then an unblinded individual at the investigational drug service or pharmacy at each site would select the proper bottle and provide the dose determined by randomized group. The unblinded personnel at each site will confirm that the dose is the correct one in the DMS and then tear off the dose identification tag from the bottle so that, when distributed, all other study personnel will be blinded to dose. The patient would be provided with the appropriate number of doses (labeled only as warfarin study dose with an identifying number to allow the DDC to link to the patient and the drug dose), to last until the next scheduled INR check.

After 4 weeks (the primary outcome duration), clinicians will be informed of the actual dose that the patient is taking and patients will then receive their warfarin through their usual pharmaceutical outlet. Patients will still have their dose adjustments made via the standardized titration protocol until they reach maintenance dose, but continued blinding for 6 months of follow-up would be too cumbersome for patients and quite costly. Because all capsules will look the same regardless of warfarin dose, clinicians will remain blinded to study arm in patients who are still in the trial.

9.3   Dose Titration Phase

Following the intervention period (dose initiation and dose revision algorithms), patients will enter the dose titration phase. In order to make the subsequent management of participants as equivalent as possible for all participants in both arms, all dose adjustments beyond the dose intervention phase and until stable maintenance dose is reached will be based on INR measurements according to a standardized protocol. During this phase dose changes will be based on the INR measured on study-specific days, using a standardized dose-titration adjustment based on INR and applied equally between groups. The titration method to be used will be the one used in the Couma-Gen trial.[68] After 4 weeks, dosing will be unblinded. Patients will continue to follow the dose-titration algorithm by inputting INR and dose data into the DMS to identify any changes in dose needed, until each patient reaches stable dose (defined as the dose that leads to a therapeutic INR over two consecutive INR measurements, spanning a period of at least one week apart). After that, dose titration will continue to be recommended as per

the study titration algorithm, but will not require the use of the DMS. The Couma-Gen trial dose titration algorithm is shown in Table 9.1.

Table 9.1: Dose Titration After 5-Day Intervention Period*[68]	
INR 1.0-1.59	-Inquire about s/s of clotting*, and if necessary, refer to an appropriate facility for care -Immediate extra dose (average of day 4-5 dose if on day 6) -Increase weekly dose by 20% -Retest in 3-5 days (if INR not yet therapeutic), retest on next protocol specified day (if INR previously therapeutic)
INR 1.6-1.79	-Give an extra half dose today (average of days 4-5 for day 6) -Increase weekly dose by 10% -Retest in 3-5 days (if INR not yet therapeutic), retest on next protocol specified day (if INR previously therapeutic)
INR 1.8-1.99	-Increase weekly dose by 5% if patient has received at least 8 warfarin doses -Retest in 3-5 days (if INR not yet therapeutic), retest on next protocol specified day (if INR previously therapeutic)
INR 2.0-3.0	- No change in dose - During first 2 weeks of therapy, retest in 3-5 days - During weeks 3 and 4, retest in 1 week - After week 4, retest in 1 month
INR 3.01-3.39	First episode: -Retest in 3 days if INR never therapeutic, in 1 week if INR previously therapeutic Second, consecutive episode: -Decrease weekly dose by 5% -Retest in 3 days if INR never therapeutic, in 1 week if INR previously therapeutic -If prior INR was between 3.01 and 3.39 and dose has not be reduced within the previous 6 days, decrease weekly dose by 10%
INR 3.4-4.99	-Inquire about s/s bleeding**, and if necessary, refer to an appropriate facility for care -Reduce today's dose by a half if INR <4, or omits today's dose if INR ≥4. -Decrease weekly dose by 10% -Retest 3 days if INR never therapeutic, in 1 week if INR previously therapeutic
INR ≥ 5.0	-Inquire about s/s bleeding**, and if necessary, refer to an appropriate facility for care. Customize care if bleeding. -Omit 2 doses -Retest in 48 hours -When retested, if INR is between 1.8 and 3.39, decrease weekly dose by 15% and retest in 7 days (if INR never therapeutic), 14 days (if INR previously therapeutic) - When retested, if INR still >3.39, omit 2 more doses and retest in 48 hours and then repeat as above **Note: If INR > 9.0 follow special protocol (IHC guidelines)**
* If weekly dose is <11 mg/week, weekly dose will be rounded to the nearest 0.5 mg weekly dose. If weekly dose is ≥ 11 mg/week, weekly dose will be rounded to the nearest 1.0 mg weekly dose	

*S/S of Clotting: pain or swelling in the legs, SOB, chest pain, new focal weakness or numbness, slurred speech, vision changes, etc.

**S/S of Bleeding: nose bleeds, unusual bruising, dark stools, pink or bloody urine, excessive menstruation, blood in the sputum, etc.

Weekly dosing may require two different doses of warfarin on different days of the week (e.g., a 27 mg weekly dose requirement would be given as: 5 mg Mon/Wed/Fri and 3 mg Tues/Th/Sat/Sun Dosing). Weekly dosing will be rounded to the nearest integer (e.g., if the weekly dose is 35 mg/week and the dose titration calls for a 5% increase in dose, the new dose of 36.75 mg will be rounded to 37 mg/week). These dose titrations are both typical of available standardized dose adjustments and allow for titration to be standardized across arms, during the blinded phase of the trial and beyond. Each weekly dose will have a standard dosing regimen, modified based on the Couma-Gen dosing schedule, to be used during the first 4 weeks. Thus, all patients who are getting a specific dose will receive the same weekly regimen (e.g., all patients receiving 29 mg/week will receive two bottles: one with 5 mg encapsulated tablets to be taken on Monday, Wednesday, Friday, and Sunday and one with 3 mg encapsulated tablets to be taken on Tuesday, Thursday, and Saturday).

<u>Clinician Over-ride of Dose Titration in All Arms</u>. Although clinicians will not know any individual patient's current dose for the first 4 weeks of therapy, they will know the INR and will be told of the relative change in dose during the dose titration phase (e.g., the DMS would report "Based on your patient's INR, their dose will be increased by 10%"). If clinicians believe that there are reasons not to follow this recommendation (e.g., a patient has been non-adherent with therapy as a cause of a low INR and the clinician wants them to simply start back on their current dose without increasing the dose, intercurrent event/illness, etc.), they will contact the Medical Monitor to request a change (see 12.7 for details of procedures). Throughout the dose adjustment phase, participants also will be notified to contact the Study Coordinator at the site if they start any new medications or stop any current medications. If these medications interact with warfarin, the participant will return in 5-7 days for an INR check and adjustments will be made accordingly, again maintaining blinding of dosing during the first 4 weeks of therapy.

10   Study Visits

10.1   Visit Schedule

Patient visits will follow a standard visit schedule (Table 10.1) that is consistent with usual clinical protocol (i.e., additional clinic visits will not be necessary).

Table 10.1 – Visit Schedule									
Blue indicates non-discretionary data collection period.	Patient Screening	Eligibility Confirmation & Randomization (Baseline)	Week 1 (2 visits)		Week 2 (2 visits)		Week 3	Week 4	Monthly Visits
Data Collection Schedule	May be combined or separate.		Day 4	Day 7	A	B	1 X	1X	Weeks 5-24
Screening	X								
Informed Consent	X								
Eligibility [Inclusion & Exclusion Criteria]	X	X							
Randomization		X							

Table 10.1 – Visit Schedule

Blue indicates non-discretionary data collection period.	Patient Screening	Eligibility Confirmation & Randomization (Baseline)	Week 1 (2 visits)		Week 2 (2 visits)		Week 3	Week 4	Monthly Visits
Data Collection Schedule	May be combined or separate.		Day 4	Day 7	A	B	1 X	1X	Weeks 5-24
Specimen Collection		X							
Genotype		X							
Demographic Data		X							
Baseline Medical Conditions & History		X							
Baseline Environmental Modifiers**		X							
Change in Environmental Modifiers**			X	X	X	X	X	X	X
INR***			X	X	X	X	X	X	X
Dose Data***		X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X
Medication Adherence			X	X	X	X	X	X	X
Change in Medical Conditions			X	X	X	X	X	X	X
Hospitalization Discharge Summary & ICD9 Codes for Clinical Events									
Additional Data Collection Instruments (TBD)									
Health Resource Utilization		X		X	X			X	X
Quality of Life		X			X			X	X†

*May occur at any time during the schedule.
** E.g., dietary vitamin K, alcohol intake.
*** All INR and dose values including those between scheduled visits will be recorded. These INRs will be entered as they are measured.
† Recorded at 3 months and 6 months only.

Hospitalized patients will have an INR measured daily after the first 3 doses (inpatients), usually until therapeutic INR is reached (unless they go home on bridging anticoagulation therapy with, for example, low molecular weight heparin).[3] In order to complete the initial dosing algorithm adjustments (i.e., the dose revision algorithms), an INR on day 4 and/or day 5 (after 3 doses of warfarin) will be required. If the third dose falls on a Friday and the participant is an outpatient, the dose revision algorithm will still be used if the patient can come in for an INR check and blinded drug can be provided on that day. If the third dose falls on a Saturday, patients can have their INRs measured on Monday as the dose revision algorithm can predict dosing after 4 doses of warfarin.

Subsequently, INR is checked twice per week as an outpatient (typically Monday and Thursday at many clinics) for two weeks, then weekly for two weeks, until a stable dose is reached (i.e., two consecutive visits with an in-range INR and no dose changes). These visits prior to reaching a stable dose are termed the "initiation phase." For patients beginning warfarin as outpatients, the first INR will be measured after 3 doses of warfarin and subsequent monitoring is performed similar to that described above for hospitalized patients after discharge.

After steady-state dosing is reached (the computer dose adjustment program will notify the site when this phase is reached), patients enter the "maintenance phase" and thereafter are typically seen monthly unless an INR measurement is out of range, at which point dose adjustments are made and the patient is scheduled to return, typically within a week. In addition, some patients may have their warfarin discontinued temporarily (e.g., prior to a surgical procedure). Patients are typically then started back on their maintenance dose and then followed as per the initiation phase discussed above. Permanent discontinuation of warfarin will terminate collection of INRs for patients in the study, but they will continue to be followed for other outcome data. Temporary holds of warfarin will be treated as in Table 13.10.   Discontinuation in the first 4 weeks of treatment (to the primary endpoint) is expected to be extremely rare (and patients will be followed for clinical outcomes even if they discontinue therapy during the first 4 weeks).

At each study visit, patients will be interviewed and data (discussed below) will be collected.

11 Data Management, Quality Assurance, Monitoring Procedures

11.1 Data Collection and Management

11.1.1 Data to be Collected

There will be 3 broad categories of data collected (Table 11.1): 1) participant clinical (i.e., patient-specific) and environmental factors; 2) genetic and biomarker data; and 3) study outcomes. All participants will undergo a baseline interview to collect clinical and environmental data, and blood will be drawn for genotyping and storage for future genetic and biomarker studies. At each subsequent visit, participants will undergo a follow-up interview to collect clinical and environmental data that can change over time (time varying factors) and to identify study outcomes. In addition to participant interviews, study outcomes will also be ascertained by direct queries of site clinicians.

11.1.2 Clinical and Environmental Factors that can Alter Warfarin Dose Requirements

The clinical and environmental factors to be collected will be those that can alter the INR response to warfarin, increase the risk of any of the other study outcomes (e.g., bleeding, thromboembolism), and fully describe the patient population and relevant subgroups. A preliminary abbreviated table of all such factors is shown in Table 11.1 (and a complete table will be provided in the Manual of Operations).

Clinical factors include factors associated with altered warfarin dose, such as age, sex, and body surface area. This will include all factors required for either of the two warfarin dosing algorithms and those associated with warfarin response that do not make it into the dosing algorithms.

Environmental factors include interacting medications, smoking, alcohol use, and vitamin K intake. Interacting medications will include all possible interacting medications and dietary supplements that may alter the pharmacokinetics or pharmacodynamics of warfarin.[134]

Table 11.1. Clinical and Environmental Variables to be Collected*		
Clinical Factors	Hypothyroidism	Caffeine intake; smoking
Demographics	Pyrexia	Genetic and Biomarker
Age	Malabsorption syndrome	SNPs for prediction model
Sex	Diarrhea	Other genomic data
Race/Ethnicity	Indication for warfarin	Biomarker (TBD)
Education	Prior bleeding & TE history	Outcomes
Employment	Environmental Factors	PTTR at 4 weeks†
Marital status	Medications/Supplements	PTTR at 2 weeks, 3 & 6 months
Health insurance	Potentiate warfarin effects[135]	Time to therapeutic INR and to stable warfarin dose
Annual income	Inhibit warfarin effects[135]	INR >4 at 2 weeks, 3 & 6 months
Body mass index; body surface area	Other anticoagulant use (e.g., heparin)	Variability in INR
Liver disease	Vitamin K	INR <2 at 2 weeks, 3 & 6 months
Malignancy	Dietary intake	Bleeding
Hyperthyroidism	Change in vitamin K intake	Thromboembolism
Family history of VTE	Other dietary changes	Cost data
	Alcohol use	Quality of life

 * See text for details; † primary outcome

Alcohol use will be assessed in two ways, based on recent experience in a prospective cohort study of warfarin-treated patients. First, typical weekly alcohol intake will be ascertained. Second, the maximum number of drinks that a patient has on any one occasion will be recorded. Among those who drink on a regular basis, the additional data on the maximum number of drinks may add to the ability to predict warfarin dosing, consistent with theory on the effects of alcohol on enzyme induction.[114;136] Dietary vitamin K will be determined via a questionnaire asking each participant about their regular, usual intake of each of 12 high vitamin K-content foods at baseline. At baseline, participants will identify each of 12 high vitamin K-content foods[137] that they consumed in the prior week, regardless of quantity. For each item consumed in the prior week, a value of one is assigned and the total number of items is summed to obtain a score. This score also has been shown to be strongly associated with AC control[137]and may be useful for dose prediction.[40;137] We refer to it as the "recent vitamin K score." For follow-up visits, changes in recent dietary intake of vitamin K might affect the INR, although probably only if intake changes substantially from usual consumption.[138-140] Nonetheless, in order to ensure that we address this possibility, recent diet prior to each INR measurement will be collected using a method previously shown to predict AC level.[137] First, participants are asked about new diet programs or regimens, or overall changes in the amount of overall intake (eating more, less, or the same) over the week prior to the INR (or during the time period since the last INR check if the interval between visits is less than one week). A decrease in oral intake measured with these simple questions is associated with an over 3-fold increased risk of elevated INR. In addition, for each of the 12 high vitamin K-content foods discussed above, participants will be asked about any change in consumption compared with usual diet (more, the same, or less, than usual). For each item, "more" is coded as 2, "no change" as 1, and "less" as 0; the sum of all items is then used as a score reflecting change in diet (referred to as the "change in

vitamin K score").[137] Although, overall, this method does not capture small changes in diet, it has been shown to produce measurements that are strongly associated with AC control. This is not unexpected because only large, recent, and sustained vitamin K intake (at least 250 μg per day) is likely to alter the AC response to warfarin.[21;138;139;141-145] Small changes in diet are not measured, but would not affect the INR anyway.[91,93]

11.1.2.1 Method of Data Collection

Data on clinical and environmental factors will be collected from in-person interviews during the first 4 weeks and at 3 and 6 months of follow-up at each site by trained research coordinators using standardized questionnaires and data collection forms. Information on medication use will be collected at each study visit during the first 30 days and at 3 and 6 months. At baseline, subjects will be asked to report their current medications. At each subsequent visit during the first four weeks, subjects will be asked if they started or stopped use of any medications. Current medication use then will be collected at 3 and 6 months. Importantly, all data will be ascertained before subjects have their INR values revealed to the research coordinators to prevent interviewer bias. After the first 4 weeks of follow-up, data will continue to be collected as per the visit schedule, but telephone management will be allowed (e.g., patients can be interviewed by telephone) because there will no longer be a need for blinded study drug distribution and the primary outcome will have been reached. In person visits are still the preferred way of collecting data while the patient is on warfarin, at least until the 3-month visit.

11.1.3 Genetic/Biomarker Data

There will be three types of genetic and biomarker data: 1) genotype data related to warfarin dosing, which includes a) primary genotype data required for the dosing algorithms and b) data related to planned genotyping and sequencing efforts to further study warfarin dosing, and 2) other genetic and biomarker data that will be used for additional, ancillary analyses. The data unrelated to the dosing algorithms will be derived from the stored blood at the Central Laboratory (CL) and are not required for the clinical trial itself. As such, they are not discussed further in this protocol. The first (genotype data required for the dosing algorithm) will be assayed at the clinical sites in order to ensure same-day genotyping results required for this trial. However, the CL will also perform genotyping of the same genetic variants for quality assurance purposes, as discussed below. The other genotype/sequencing data to define additional genetic determinants of warfarin dosing in persons with disparate pharmacogenomic and actual doses will be assayed through the CL using approaches to be determined in the near future.

Genotyping and Biomarkers at the Central Laboratory. The CL will be responsible for: 1) extracting DNA from all participants' blood samples; 2) genotyping the variants that are used in the dosing algorithm to confirm the results of the individual centers; 3) storing DNA on all participants for future genotyping of other variants of other genes that may be important in warfarin dose response; and 4) storing blood for future biomarker (e.g., plasma vitamin K) or gene expression studies. For future studies, the CL will be responsible for developing a multiplex platform that can assay numerous genetic variants within one reaction under the guidance of the Genotyping Subcommittee (GS) and Ancillary/Substudy Proposals Subcommittee (ASPS)

Sample Collection for Storage and Analyses in Central Laboratory. Six (6) mL of whole blood will be drawn from each subject into plastic purple-top (EDTA) Vacutainer tubes and shipped via overnight service to the CL in a chilled insulated container. Samples will

be labeled with the subject's PID number and a bar code only so that laboratory personnel are blinded to the patient's identity. All samples will be tracked using the bar code and tracking system. The Genotyping Subcommittee will also consider whether state-of-the-art methods are available at the initiation of the trial to make feasible the collection of RNA for expression studies.

11.2 Monitoring Reports

11.2.1 Executive Committee

The CTCC will provide the Executive Committee reports on a weekly basis during the recruitment phase of the study. These reports will include the following information, reported by site and in total:

1. Number of participants evaluated and randomized;
2. Number of patients in the genotype-guided arm who receive genotype algorithm-based versus clinical algorithm-based dose for their first warfarin dose;
3. Number of eligible participants who refused to participate;
4. Number of ineligible participants and reasons for ineligibility;
5. Participant demographic (e.g., sex, race) information;
6. Number of completed data collection visits;
7. Number of missed visits;
8. Number of serious adverse events reported; and
9. Report of Central Laboratory quality assurance results.

The Executive Committee will evaluate monitoring reports on a weekly basis and request additional information from the CTCC and clinical site investigator as needed. The CTCC will communicate with clinical site personnel to identify and develop action plans for study-related issues.

11.2.2 Steering Committee

The CTCC will provide the Steering Committee with the reports described above as well as the following information:

1. Summary of Executive Committee decisions and actions;
2. Summary of targeted enrollment versus actual enrollment by site and in total;
3. Adverse Event report summary by site and in total; and
4. Report of Central Laboratory specimen collection, storage, and quality assurance results.

11.2.3 Data and Safety Monitoring Board

The CTCC will adhere to the guidelines established in the NHLBI Responsibilities of Data and Safety Monitoring Boards (DSMBs) appointed by the NHLBI (Revised: October 30, 2001, or a later version as appropriate).

The DSMB is responsible for safeguarding the interests of study participants, assessing the safety and efficacy of study procedures, and for monitoring the overall conduct of the study. The DSMB will consider the ethical concerns surrounding the use of genetic testing in determining warfarin dose and evaluate study-related problems.

The DSMB is an independent advisory group that reports to the Director and NHLBI, and is required to provide recommendations about starting, continuing, and stopping the

study. In addition, the DSMB will make recommendations, as appropriate, to the NHLBI about:

- Efficacy of the study intervention;
- Benefit/risk ratio of procedures and participant burden;
- Sample size estimation, in the face of evidence of inappropriate assumptions;
- Selection, recruitment, and retention of participants;
- Adherence to protocol requirements;
- Completeness, quality, and analysis of measurements;
- Amendments to the study protocol and consent forms;
- Performance of individual centers and central lab;
- Participant safety; and
- Notification of and referral for abnormal findings.

The CTCC, in its analysis and reporting responsibilities, will provide the information necessary for the DSMB to make informed assessments as stipulated above.

The NHLBI will establish a schedule for regular, semi-annual DSMB meetings. If the DSMB recommends an interim analysis, the CTCC will provide these data at the designated time. The frequency of interim analyses, if any, will be determined by the DSMB.

Routine DSMB Reports. It is expected that the substance of the routine reports will include but not be limited to:

1. Study objectives, list of primary and secondary hypotheses, and summary of the trial design parameters

2. Accrual by site and total
   a. Estimates for accrual completion
   b. Timeline for study milestones

3. Baseline data by arm and total
   a. Demographic (gender, race)
   b. Clinical characteristics
   c. CYP2C9 and VKORC1 distribution

4. Performance
   a. Participants evaluated and randomized
   b. Reasons for ineligibility
   c. Visit completion and timeliness of visits
   d. Participant status and withdrawals by severity by arm and total
   e. Data quality reports
   f. Laboratory data and quality assurance reports
   g. Number of dose overrides requested/performed by clinical site investigators
   h. Unblinding requests

5. Adverse Event Rates
   a. Overall Adverse Event Rates by severity by arm
   b. Overall Adverse Event Rates by Body System by severity by arm
   c. Serious Adverse Event Rates; Brief description by patient ID number

Maintenance of Blinding. At this time, the plan is to provide data by study arm but not to reveal which study arm is which. However, the DSMB will determine if it wishes to receive study results during the trial in a blinded or unblinded fashion and the CTCC will provide these data in the appropriate manner.

Delivery of DSMB Reports. During the protocol development process, communication and information distribution procedures will be established with the DSMB. The timing of delivery of the routine and interim reports to the DSMB prior to a scheduled meeting will be established by the Executive Committee and reviewed by the DSMB. This will take into account the need for reasonably current data at the time of the DSMB meeting, and the ability to validate the data to a reasonable degree in order to meet a specified date. The DSMB may prefer to review slightly older data that has been validated as well as the most current data for the primary endpoint and major known side effects, and will accept these data without complete editing. The sponsor and DSMB Chairperson will determine the content of the study reports and the CTCC will make study reports available to the DSMB electronically, approximately two weeks before a scheduled meeting.

11.3 Quality Assurance

11.3.1 Missing Data

The main missing data problem will be a result of missing INR values within protocol specified windows that are needed to compute the primary outcome of PTTR. Given that the follow-up time (4 weeks) for the primary outcome is not long in this study, we expect attrition to be low. Extensive efforts will be made to collect complete information on each subject enrolled in the study. The optimal approach to missing data is assiduously avoiding it. The methods discussed in 7.4 for retention (participant payments, newsletters, calendars, and reminder calls) will also be employed to minimize missed visits. Complete analytic approaches to missing data are discussed in 13.5.

11.3.2 Site and Central Laboratory Compliance

Site compliance with the protocol will be monitored throughout the trial using continuous measures of data validity and through site visits. Data validity will be monitored with the Data Validation Plan to address critical missing, inaccurate, illogical, or inconsistent values in the data submitted by the clinical sites and Central Laboratory. Rules will be developed and applied to evaluate data in a hierarchical fashion: 1) Safety and Regulatory data; 2) Eligibility data; 3) Randomization and Registration data; 4) Analysis and Outcome data; and 5) Descriptive Non-outcome data. Some rules will be implemented as online edit checks in the DMS, alerting the staff person keying the data at the clinical site at the time of entry into the web-based entry system, while others are implemented after the data are committed and are managed via the query tracking system, a component of the DMS.

Site compliance also will be monitored during the trial through site visits to each of the clinical sites to evaluate study operations (e.g., staff performance, adherence to protocol, and data management) as well as to implement corrective measures responding to lapses in data quality and patient safety. Specifically, the site visit will ensure the following: 1) the clinical center and site personnel remain in compliance with all aspects of the protocol and adhere to the study procedures outlined in the manual of operations; 2) the study data are being collected accurately and completely; and 3) the rights and well being of human subjects are protected.

A site visit will include the following activities: 1) tour of the clinical facility; 2) meeting with study personnel; 3) observation of screening, baseline and/or follow-up visits and associated medical and laboratory procedures; 4) viewing document and record storage facility; 5) reviewing selected study charts and CRFs; 6) reviewing the genotyping procedures; and 7) group meetings to assist in solving problems that have arisen during study initiation and implementation.

The CTCC also will conduct site visits to the CL at least twice over the course of the study. Overall, it is essential that the CL adheres to Good Laboratory Practice (GLP) guidelines for internal consistency and accuracy of data for analyses. As the services provided by the CL are highly specialized, an experienced lab investigator or external subject matter expert will be identified to accompany the site visit team. The following activities will occur: 1) inspection of laboratory facilities, including adequacy and security of storage space, monitoring equipment for freezer function, certification documents, hardware and software for inventory control; 2) review of current GLP, Laboratory Standard Operating Procedures, Quality Assurance and Specimen tracking procedures; 3) review process for receiving and processing a typical specimen; 4) review Quality Assurance documentation and procedures at CL that assures adherence to protocol and the Manual of Procedures (MOP) by clinical site personnel and CL staff; 5) assess methods for training/communicating with clinical centers regarding lab specimen preparation and transfer; 6) evaluate lab procedures for maintaining records, data storage, data transmission and security; 7) examine methodology for transmission of lab data and Quality Control procedures regarding the data transfer process; and 8) review methods for training and documenting competency of core lab staff.

11.3.3   Patient Adherence with Study Medication

Several methods have been used to measure adherence; however, no "gold standard" currently exists. The primary method of measuring patient adherence with warfarin will be by pill counts. Pill counts, although limited by the inability to rule out lost, discarded, or doubled doses[150-154] can be used to estimate overall adherence throughout the course of the trial. An additional method that has been shown to be more concordant with electronic measurements of adherence than patient interviews is formal questionnaires. Because of the potential importance of adherence on response to warfarin,[155] the medication compliance subscale of the Hill-Bone Compliance Scale questionnaire, which has been shown to be a fairly reliable measure,[156] or the modified Morisky scale (in press) also will be administered at each visit, providing a second measure of adherence. These methods have been chosen over alternatives for the following reasons: Serum drug level monitoring (serum warfarin levels) is impractical because it would have to be measured at each visit (requiring an extra phlebotomy blood draw because fingerstick blood for INR testing cannot be used) and is expensive. More importantly, warfarin levels only provide indirect information on drug taking (i.e., other factors can influence drug levels even in perfectly adherent patients). Patient self-report by interview (which is different than the self-administered questionnaire discussed above) is limited by inaccurate recall and intentional deception (to avoid the stigma of having to admit missing doses) and tends to overestimate adherence.[157] Electronic measuring of adherence (e.g., with electronic pill caps) is costly and adds patient burden to clinical studies.

11.3.4   Genotyping for Pharmacogenomic Dosing

<u>Certification of clinical site genotyping facilities prior to initiating patient enrollment</u>: Details of the lab certification will be determined by the study's Genotyping Subcommittee and approved by the Steering Committee. The current plan is as follows: In order to ensure that each site is obtaining equally robust results in their laboratories, each site will have to genotype 10 independent reference samples provided by the Central Laboratory prior to enrolling patients in the trial. These same samples will have been tested by the Central Laboratory using the genotyping platform that the CL will use for the trial. If the clinical site produces even one discrepant result, the CL will repeat the genotyping using a sample of the DNA extracted by the CL and one of the platforms approved for use at the clinical sites. If the CL reference and clinical methods give concordant genotypes, the clinical site will be responsible for troubleshooting the problem with assistance from the CL and must resolve all issues prior to enrolling patients in the trial. If the CL recurrently finds discordant results during site reference testing, the Genotyping Subcommittee will evaluate the suitability of that platform and choose an alternative for implementation at all affected clinical sites.

<u>Ongoing quality monitoring after certification:</u> Throughout the trial, the CL will perform genotyping on participants to ensure that each site continues to achieve accurate genotyping results. Details of the genotyping QA plan will be determined by the study's Genotyping Subcommittee and approved by the Steering Committee. The current plan is as follows: Following the certification process on 10 reference samples as described above, there will be 2 additional types of QA. First, sites will send blood samples from all patients included in the trial to the CL, which will then repeat genotyping on all samples on a weekly basis, using the CL genotyping platform. Any discordant genotype compared to the clinical test will trigger a hold on patient recruitment at that site and troubleshooting as described above for the reference samples. If the CL genotype results are entered into the DMS prior to the subject's 3rd warfarin dose, the dose will be recalculated based on the corrected genotype data if a patient was randomized to the genotype-based dosing arm. If the CL enters a corrected genotype for a patient after the dosing intervention is completed, these data will be used to identify any genotyping problems that develop during the course of the trial, allow the CL and CTCC to identify any sites that my develop inaccuracies in their genotyping and thereby resolve these problems in a timely fashion, and allow analysis of the effects of genotyping errors (if any) on the study results.   We do not believe that it is practical to confirm all genotyping in "real-time" as patients are being recruited; however, the platforms that are approved for the study should ensure extremely high quality genotyping results at the clinical sites. Secondly, the CL and clinical site laboratories will participate in a biannual external proficiency program as required by accrediting organizations and CLIA.   The CL will also periodically compile and review genotype frequencies, Hardy-Weinberg equilibrium, etc. by site and race to ensure data accuracy.

11.3.5   Quality Control Calibration of INR

<u>Calibration of INR</u>: The Quality Control Subcommittee (QCS) will have to approve all point-of-care (POC) instruments and quality assurance (QA) methods at each site. The following criteria will be applied to all sites: (1) All INR testing instruments will have to follow College of American Pathologist standards and be CLIA certified. (2) For POC testing machines (used at many clinics), the specific machine used must undergo the required QA measures specified by the manufacturer at the specified intervals. The QCS will review these manufacturer-specific requirements, and the CTCC will ensure that

each site submits the results of these QA measures on schedule and that the machines pass the QA specifications. (3) POC instruments must be self-calibrating and perform a self-test every time they are activated and a test is performed. The self-test should include: verification of adequate battery power to complete a full test, verification that the device display (e.g., LED display) is functioning properly, verification of proper cuvette temperature, verification that the sample is present and is of sufficient size to run tests, and verification that the internal timers function correctly for each test. (4) Quality control procedures, including INR testing with liquid controls, if required, will be performed at intervals recommended by the instrument manufacturer. (5) A correlation test must be performed if required by the POC device manufacturer at the intervals recommended. Patients will have venous blood drawn at the same time as their fingerstick blood is assayed on the POC machine, and the hospital or clinic laboratory will assay the INR to ensure valid POC results. (6) The hospital or clinic laboratory must meet CAP guidelines.[158] All of these QA measures will be monitored by the CTCC through the use of standardized forms and review of results by the QCS, SC, and the CTCC hematology expert.

Due to reports of decreased accuracy for POC INR results > 4.0, POC INRs exceeding 4.0 will be confirmed by obtaining a plasma INR from a citrated whole blood sample at the same encounter. The plasma INR result should be used for warfarin dose adjustments.

In addition, to ensure accuracy, it is recommended that INRs be repeated within the next 24 hours if they are drawn in the setting of supratherapeutic PTT from heparin, because heparin can elevate the INR in this setting.

There may be variability of INR measurement across different methods of measuring INR. In order to minimize this variability, the primary outpatient INR values used for analyses will be required to be done using the POC instrument at each site's Anticoagulation Clinic or the Clinic's chosen CLIA-approved laboratory for at least the first 4 weeks. Also, for each INR value measured in the study, the source of that value will be recorded so that sources of measurement variability can be examined in the study analyses. For patients who have initial INRs measured by a hospital lab followed by POC testing, the degree of change in INR from the last inpatient result to the first outpatient POC result will be compared to the degree of change in patients who have the same source of INR measurement throughout the trial (e.g., outpatients), controlling for time between INR and changes in dose.

Frequency of Measurement: There is a sequence of visit windows where INR should be measured that each site will follow to ensure uniformity of these visits and minimize bias in the estimates of PTTR (see Table 10.1, Section 10.1). The use of a standardized frequency of visits will ensure that INR data are measured at equal timing relative to the beginning of therapy and that the calculation of PTTR will rely on a similar distribution of INR measurements across subjects.

11.3.6   Data Management

The CTCC at the University of Pennsylvania will develop a data management system (DMS) for the collection, storage and management of all study data. Full details and instructions for use of the system will be provided in the MOP. This system will be developed using Oracle Corporation's suite of database applications. The DMS will provide for data entry (including data entry at the trial sites using a web portal and

immediate storage of the data in the central database), specimen tracking, data security, and reporting.

The data management team will develop a data validation plan, rule set specifications, and programming logic to implement data validation rules. The rule set will include checks for missing fields, range checks, skip pattern-logic, and inter and intra form checks.

Prior to release of the production system, the data management team will perform extensive, independent testing of the validation program functionality by entering data known to violate validation rules and determining if the errors are detected. A graphic summary of the working plan for the data management system is provided in Appendix D.

12 Safety and Adverse Events

Federal regulations obligate those who conduct clinical research to inform the sponsor of adverse events and unanticipated problems, and obligate the sponsor to ensure that the appropriate procedures are in place to support this reporting. The CTCC will oversee and manage reportable events as described below.

12.1 Definitions

Definitions are per the January 2007 Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events, OHRP Guidance. http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm.

Adverse Event (AE): Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Serious Adverse Event (SAE): Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

    (1) results in death;
    (2) is life-threatening or places the subject at immediate risk of death from the event as it occurred;
    (3) requires or prolongs hospitalization;
    (4) causes persistent or significant disability or incapacity;
    (5) results in a congenital anomaly or birth defect; or
    (6) is another condition that investigators judge to represent significant hazards.

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

    (1) unexpected (in terms of nature, severity, or frequency) given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, and the characteristics of the subject population being studied;
    (2) related or possibly related to participation in the research; possibly related means that there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in the research;

(3) suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

Unexpected Adverse Event: Any adverse event occurring in one or more subjects in a research protocol, the nature, severity, or frequency of which is not consistent with either:

(1) the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts; or

(2) the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Preexisting Condition: A preexisting condition is one that is present at the start of the study. At baseline, any clinically significant abnormality should be recorded as a preexisting condition. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

12.2 Expected Adverse Events

An expected AE is one that is known to be associated with the intervention or condition under study.

12.2.1   Associated with Warfarin Use

Reports of side effects will be evaluated for changes and severity during each clinical visit. The following side effects are associated with warfarin use:

a. Bleeding
b. Allergic reactions
c. Skin necrosis
d. Purple toes syndrome
e. Hepatic injury
f. Asthenia or paresthesias

The following are reported, less serious, side effects of using warfarin:

a. Bruising
b. Bleeding gums when brushing teeth
c. Nausea, abdominal pain, vomiting, diarrhea
d. Alopecia
e. Cold intolerance
f. Fatigue
g. Dysgeusia

12.2.2   Out-of-Range INR Values

It is expected the INR values will be out of the therapeutic range (2.0-3.0) during the dose-finding phase and periodically thereafter. Out-of –range INR values will not be reported as adverse events unless they require intervention. For example, an INR value of 6.5 in and of itself should not be reported as an adverse event unless the clinician treats this patient by administering Vitamin K, admits them to the hospital for monitoring, and/or prolongs a hospital stay because of the INR. As another example, an INR of 1.5 in and of itself will not be considered an adverse event unless a clinician treats the patient with parenteral anticoagulants (e.g., low molecular weight heparin), admits them to the hospital for monitoring, and/or prolongs a hospital stay because of the INR.

12.3   Identification of Adverse Events

All adverse events (AE) will be identified in several ways throughout the trial. First, participants will be interviewed at each AC clinic visit and queried about adverse events since the last visit using a standardized, structured interview form. This will include open-ended questions about hospitalizations and specific questions about bleeding and TE events. Second, clinicians managing patients in the trial will be queried by study personnel on a monthly basis in order to identify adverse events among patients that were not identified (e.g., bleeding that occurred, leading to discontinuation of warfarin). Third, any patient who does not return for follow-up to a scheduled clinic visit will be called by telephone to identify any adverse events that might have occurred. For all of these queries, a CRF will be completed for identified events.

The CTCC will train clinical site personnel in identification, assessment and coding procedures for adverse events and problems in order to achieve internal consistency among the sites. CTCC personnel will be available to assist clinical site personnel in the documentation required to appropriately describe and report events.

The data management system (DMS) will be utilized to manage the capture, reporting and analysis of adverse events and serious adverse events (SAE). Events will be coded by the clinical sites using current MedDRA terminology and will be available for assessment and review by the CTCC upon data entry.

12.4   Classifying Adverse Events

Adverse Events will be classified as to severity, expectedness, and potential relatedness to the study intervention and participation. The AE classification will determine the reporting requirements. The following categories will be used to describe AE severity:

12.4.1   Severity

Severity	Seriousness
1. Mild AE; did not require treatment	Not serious
2. Moderate AE; resolved with treatment	Not serious
3. Severe AE; inability to perform normal activities; required professional medical attention	SAE if required or prolonged hospitalization
4. Life-threatening or permanently disabling	SAE
5. Fatal AE	SAE

12.4.2   Expectedness

Events will be assessed with regard to expectedness meaning not anticipated based on current knowledge as described in the protocol, investigator brochure, product insert or label.

Unexpected: Event is not consistent with information about the condition under study or intervention in the protocol, consent form, product brochure or investigator brochure.

Expected: Event that is known to be associated with the intervention or condition under study.

12.4.3   Relatedness

The potential event relationship to the study intervention is described below.

Definite: event is clearly related to the intervention
Probable: event is likely related to the intervention
Possible: event may be related to the intervention
Unrelated: event is clearly not related to the intervention

12.5   Reporting Adverse Events and Unanticipated Problems

The clinical site is responsible for reporting SAEs to the CTCC within 24 hours of first knowledge of the event. The CTCC will facilitate the timely reporting and updates to regulatory authorities and NHLBI. The Manual of Procedures will specify the steps involved in recording all adverse events; however, SAEs will be reported as follows:

a. Serious and unanticipated AEs that are fatal or life-threatening must be reported within 7 days to the local IRB and the NHLBI (through the CTCC).
b. All SAEs that are also unanticipated problems must be reported within 7 days to the local IRB and NHLBI.
c. Those events that don't meet criteria a. or b. above, but are either unanticipated problems or SAEs must be reported within 2 weeks to the NHLBI (through the CTCC).
d. Any unanticipated problem that is not an SAE must be reported within 30 days to NHLBI and other participating sites for their IRB notification (through the CTCC).

Routine reporting requirements for all other categories of AEs will be the responsibility of the CTCC. The CTCC will produce aggregate safety reports and distribute such reports to the appropriate parties.

12.6   Patient Safety and Unblinding

In some circumstances such as a medical emergency, a patient's dose assignment may need to be revealed. This may occur in cases when the clinical site PI thinks it is important to unblind the warfarin dose in order to medically manage the patient. In order to minimize potential risks to the participant's safety while also maintaining the integrity of the study blind, a process will be defined that involves consultation between the clinical site PI, the Investigational Drug Pharmacy, and the CTCC PI and/or the Medical

Monitor before unblinding (see also 12.7). It will be the clinical site PI's responsibility to assess issues of patient safety and communicate them to the Medical Monitor. The Medical Monitor will make the final determination regarding unblinding a particular patient and the clinical site PI will be responsible for communication with the patient.

12.7 Medical Monitor

An experienced stroke neurologist who has expertise in managing warfarin, Dr. Scott Kasner will act as the independent Medical Monitor for serious events and for managing requests for warfarin dose titration exceptions as well as unblinding requests during this study. Dr. Kasner has served in prior and ongoing stroke trials as safety monitor, endpoint adjudicator, and DSMB member.

12.7.1   Major Clinical Events:

The Medical Monitor will review all SAEs that are considered by the local investigator to be probably or definitely related to treatment allocation (or "more likely than not" in recent FDA parlance). In addition, all acute thromboembolic events and major bleeding events will be reported to and reviewed by the Medical Monitor, regardless of how the local investigator reports the relationship between the event and the study intervention.

In cases in which there is disagreement on the classification of the SAE, thromboembolic event, or major bleeding events between the local investigator and the Medical Monitor, the determination by the Medical Monitor will be used as the final classification. This is similar to the methods used for the NINDS rt-PA Stroke trial and the ongoing Interventional Management of Stroke (IMS)-III trial.

12.7.2   Warfarin Dosing Adjustments:

The Medical Monitor will be contacted by local investigators any time they are considering prescribing a warfarin dose other than that determined by the study algorithms. During the blinded first 4 weeks, these changes would represent a "change in the percentage adjustment of warfarin dose" since investigators will not know the actual dose being given. In general, such considerations should be made solely for patient safety, or when there is an issue not otherwise considered by the standard study algorithm such as patient error with prior assigned dose, prescribing or dispensing error with prior assigned dose, recently instructed not to take anything by mouth, i.e., "NPO", nonadherence, new medication addition or deletion, recent bleeding event, and recent thromboembolic event.

In these and other circumstances, a discussion between the local investigator and the Medical Monitor will ensue, and an acceptable warfarin dose will be determined based on the relevant clinical issues. All deviations from the study algorithm dosing schedule must be approved and recorded by the Medical Monitor. If a dose override is approved, the new dose will be entered into the computer system by the Medical Monitor. Since subsequent INRs and dose adjustments will refer back to the override dose, this dose needs to replace the dose assigned by protocol in the database, and specifically flagged as an override.

The frequency of dose overrides will be made available to the CTCC and the SC on a regular basis, without unblinding treatment allocation. A large number of overrides may be indicative of systematic issues that require further review. The DSMB will have full information regarding overrides and treatment allocation at their request.

12.7.3   Breaking the Blind:

If the treatment allocation needs to be unblinded for safety reasons, the Medical Monitor must be contacted to review and approve unblinding. This should be an extremely rare event in this study as treatment arms are unlikely to dictate clinical care in any way other than the initial dosing of warfarin and blinded dosing will only occur for the first 4 weeks of therapy in each patient. Every attempt will be made to keep the allocation blinded. If unblinding is deemed necessary and approved, the Medical Monitor will obtain treatment allocation from the clinical database personnel and discuss with the local investigators as necessary.

12.7.4   Other Safety or Study-related Concerns:

The Medical Monitor will be available to all investigators and coordinators for urgent concerns related to patient safety, particularly when such concerns might require interruption or cessation of the study intervention.

13   Statistical Considerations

13.1   Analytic Approaches: Primary Endpoint

Analysis of the primary outcome will be by intention-to-treat.[159] We expect few or no treatment crossovers, since the difference between the arms occurs in the initial dose period of five days and grossly abnormal INRs that might cause either discontinuation or a switch to clinic based warfarin dosing are unlikely that early in treatment. Every effort will be made to maintain the INR schedule despite inevitable variations from protocol in the initial and follow-on periods, and the outcomes will be recorded for the arm of randomization. The null hypothesis for the primary outcome is that the percent of time that participants spend within the therapeutic INR range (PTTR) during the first 4 weeks of therapy is equal between the two arms. While there is some expectation that genetic-guided initial dosing will increase PTTR, it is considered appropriate that the hypothesis of no difference be considered with a two-sided α level.

Before proceeding with inferential analyses, the data for this study will be fully described. Data will be examined for the primary outcome and all covariates to assess distributional assumptions, and balance of covariates among the study arms. Based on data from the University of Pennsylvania cohort, the statistical distribution of the PTTR will be symmetric but with a somewhat higher frequency in the tails than would be expected with a normal distribution (kurtotic). The robustness of normal sampling theory to modest variations in the underlying distribution is well known for large sample sizes, and normal theory will be used for the analysis of the primary outcome barring jarring contradictions to this assumption in the actual trial data. If such is the case, a nonparametric approach will be taken.

In this normal theory framework, we will first test for any difference between the trial arms, using a multivariate linear model. The multivariate model will be implemented where the interest is on testing the two arms using a Wald test. The assessment of the primary hypothesis of no difference between the arms will be done using a two-sided test.

Since randomization will be stratified on site and ethnicity, these variables will be included in the model for the primary analysis. The impact of the factors not included in this primary analysis would be assessed in secondary analyses. Other potential

covariates include clinical (e.g., concomitant medications, weight) and demographic (e.g., gender) factors that can be included in the secondary analyses as confounders. In order to determine which confounders to include in these secondary multivariable models, we will determine if the unadjusted regression coefficient differs from the coefficient adjusted for each potential confounder by more than 10% of the unadjusted coefficient.[160] In addition to clinical and demographic factors, additional genetic factors will also be considered in these analyses. Specifically, since CYP2C9 and VKORC1 genotypes may not be the only genetic variants that may determine the optimal warfarin dosing in population, it is possible that more such variants are identified during the current trial, or that more sophisticated genetic analyses will have been done. As such, it is important to consider allowing the ability to adjust for these additional genetic effects when comparing the primary outcomes of the different dosing methods. To control for these additional genetic factors in secondary analyses, we will enter them into the normal linear model and also consider possible interactions between them and CYP2C9 and VKORC1.

13.2   *Analytic Approaches: Subgroup and Secondary Endpoint Analyses*

For both subgroup and secondary analyses, covariates and confounders will be assessed in models as to their explanatory value as per discussion in 13.1. In addition, potential interaction terms will be included in the model (e.g., study arm by race) as discussed below.

Differences between the predicted doses computed from the genotype-guided versus clinical-guided. It is posited that among those participants with larger differences between the two algorithms with respect to the baseline predicted doses, there will be a greater effect of the intervention on the PTTR between the two arms. A difference in clinically important predicted dose between the genetic and clinical algorithms is defined as ≥ ± 1 mg/day. Thus, analysis of this subgroup will use a small part of the α of the primary analysis, as discussed below in 13.3.1). While this is a subgroup of the full cohort, it is considered part of the primary analysis.

13.2.1   Subgroup Analyses Based on Allelic Variation and Race/Ethnicity

Allelic variation. Subgroups will be defined based on allelic variation to explore whether the effect on PTTR between the two treatment arms differs across groups defined by allelic variation. Both three (zero, versus >1, versus one) and two (zero and >1, versus one) subgroups will be defined, based on the findings by Anderson et al.[68] A multivariate linear model that includes an interaction term between allelic variation and treatment arm will be used to test for a difference in mean PTTR between the two treatment arms across allelic variation groups.

Race/ethnicity. Subgroups will also be defined based on race/ethnicity to explore whether the effect on PTTR between the two treatment arms differs across groups defined by race/ethnicity. Because the Asian sample size is expected to be small at between 4-6%, with the two most populated groups being Caucasian and African American, the race breakout will be African American versus non-African American (the groups that will determine stratum in the randomization scheme). We also will examine Caucasians versus African Americans (i.e., excluding other races). A multivariate linear model that includes an interaction term between race/ethnicity and treatment arm will be used to test for a difference in mean PTTR between the two treatment arms across race/ethnicity groups.

13.2.2   Secondary Outcomes and Their Analytic Assessment

<u>INR ≥4 or serious clinical event in first 4 weeks</u>. A logistic regression model will be used to test whether the two arms are different with respect to their odds of experiencing an adverse event.

<u>PTTR <60%, or INR ≥4 at least twice during first 4 weeks</u>. The analytic approach will be the same as for the INR ≥4 or serious clinical event in the first 4 weeks outcome.

<u>PTTR at 3 and 6 months</u>. These outcomes will require analysis similar to that of the primary outcome. The primary timeframes for assessment will be 2 weeks and 3 months, as discussed previously. These measures would be cumulative over time and therefore include observations in the first 4 weeks. In addition to looking at PTTR cumulatively over time, secondary analyses should distinguish these later periods from the dose stabilization period in the first 4 weeks. For PTTR at 6 months, the expected dropout rate has been doubled to 20%. It is expected that at 3 months: (i) PTTR in the clinical arm will be higher than at 4 weeks because patients are now more stable on warfarin, and (ii) the difference among arms in PTTRs is smaller, compared to at 4 weeks, because the effects of genetics and other clinical factors (e.g., weight) on INR response will be diminished after a stable dose is determined. For these reasons, an absolute difference of 4.5% (instead of 5.49% in the case of PTTR at 4 weeks) between the genotype-guided arm and clinical arm is a more reasonable clinical difference (see section 13.3). Equivalent thinking will be applied to later PTTR comparisons.

<u>Anticoagulation status (INR >4, INR <2)</u>. A categorical outcome to indicate INR <2, 2< INR <4, and INR >4 will be defined and a global Chi-square analysis with 2 degrees of freedom will be used to assess whether any differences exist among the three outcome groups at 2 and 4 weeks and 3 and 6 months, and cumulatively over the entire study period. Further between-group analysis will follow. Additionally, a binary outcome will be defined to indicate INR <2 or INR >4 and a logistic regression model will be used to test whether the two arms are different with respect to their odds of having this event in each time period and cumulatively over the study period. The sandwich variance estimator will be used to appropriately adjust standard error estimates to account for correlation due to repeated measurements.[161] In the case of repeated measurements, naive standard error estimates that ignore correlation are incorrect and inference may be invalid.[161]

<u>Bleeding, Thromboembolism, and Time to Stable Dose</u>. For major and minor bleeds, thromboembolic complications, and time to stable warfarin dosing, we will first use the log rank test to conduct univariate analysis after producing Kaplan-Meier curves within each of the two arms. For purposes of these analyses, a 'stable dose' is defined as two consecutive INRs within range without a dose change over a period of at least 1 week. The time to stable dose is the time of the first of a sequence of these two INRs. A Cox proportional hazards model will then be used to adjust for confounders. We will test for the proportionality assumption and conduct residual analysis to verify model assumptions. Secondary analyses will also explore other definitions of stable, maintenance warfarin dose.

<u>Number of dose changes required to obtain a stable dose</u>. We expect that the number of dose changes required to obtain a stable dose will be variable among patients and differ by study arm. An ordinal outcome will be defined for the number of changes required to achieve a stable dose and a proportional odds model will be used to test whether the two arms are different with respect to their odds of receiving each number of dose changes. As a more global assessment, the median number of dose changes required to obtain a stable dose will be used to define a binary outcome, and a logistic regression

model will be used to test whether the two arms are different with respect to their odds of receiving greater than the median number of dose changes required to obtain a stable dose.

Cost and Cost-effectiveness. The perspective of the analysis will be that of society (e.g., the cost to society). While such a perspective can include both medical service use (e.g., hospitalizations) and productivity of study participants (e.g., time lost from work), we will focus our attention on medical service use and its associated cost. Because follow-up of individual patients is for 6 months only, we will not discount cost; because patients will be enrolled during more than a one-year period, all costs will be translated into dollars in the last year of the study using the medical component of the consumer price index. For costs and preferences, we will make our between-group comparison by use of generalized linear models (GLM). We will choose the family distribution (e.g., gamma or poisson) and link function (e.g., log, linear) for the GLM based on diagnostic tests. Cost-effectiveness analyses will be performed for two time horizons: a within-trial analysis that evaluates what was observed during the study follow-up in the trial and a projection analysis that evaluates the likely impacts on longer-term outcomes.[162] [163] For the within-trial analysis, we will calculate two ratios, the cost per time within therapeutic range and the cost per QALY gained. For both ratios we will calculate 95% CI for the ratios as well as cost-effectiveness acceptability curves.[164] Sensitivity analyses will be conducted to assess the impact about assumptions on price weights (i.e., unit costs) on the observed cost-effectiveness ratios.

Quality of Life measures. The DASS results as well as the SF-36 can be compared among the two arms, at 2 and 4 weeks, and at 3 and 6 months. Using analysis of covariance, the assessments can be adjusted for baseline QOL and other variables that are considered to affect the QOL measures.

13.3   Sample Size and Power

The sample size for the study is 1,022. The sample size was modified by an amendment (dated September 16, 2012) from 1,238 to 1,022. See rationale for amendment in the amendment section of the Protocol and the text explaining the power implications in Section 13.3.3.

 While we consider the sample size for the primary and secondary outcomes separately, the sample size for the primary outcome will necessarily limit the power for secondary outcomes, and the approach taken has been conservative to some degree to protect the power of the secondary outcomes. An absolute difference of about 5-10% in PTTR is believed to be a clinically meaningful detectable difference.[106;165;166] For example, the use of formalized AC Clinics has been estimated to improve PTTR by about 10%, and this benefit has been used, in part, to justify the use of such an approach as standard of care.[106;124] The use of detectable difference between 5 and 10% ensures that the study will be able to detect potentially clinically meaningful difference and allows for the possibility of variation in the parameters estimated, as discussed further below.

The sample size considerations are based on an underlying paradigm for the primary comparison of the two arms as given below:

$$PTTR_c = 0.4*73\% + 0.6 * 61\% \qquad = 65.80\% \qquad\qquad (1)$$

$$PTTR_g = 0.4*73\% + 0.6 * (61\% X 1.15) = 71.29\% \qquad\qquad (2)$$

$$\Delta (PTTR_c - PTTR_g) = -5.49\% \qquad\qquad (3)$$

where:

o PTTRc represents the PTTR in the clinical-algorithm arm and PTTRg represents the PTTR in the genotype-algorithm arm;

o a PTTR of 73% and 61% is assumed for those with a single variant and for those with no or multiple variants,[68] respectively;

o it is assumed that approximately 40% of participants will not have any benefit (possessing a single variant), producing the same PTTR regardless of their treatment assignment, thus diluting the treatment difference; these latter 2 estimates are based on Anderson et al.[68] and the IWPC (unpublished data), and while considered a good estimate, we have considered the sensitivity of the sample size calculations to possible errors in these estimates; and

o a 15% relative increase of PTTR in the pharmacogenomic-based algorithm arm is an obtainable difference from the clinical algorithm arm, for those with 0 or multiple variants, again based on Anderson et al.[68] It should be noted that the assumption that those with only 1 genetic variant will not benefit, while suggested by the Couma-Gen study, was not similarly supported in another clinical trial (using only CYP2C9) in which all patients benefited for CYP2C9-based dosing.[68;69] In that study, the effect of a dosing algorithm was similar regardless of the number of CYP2C9 variants present. Therefore, we believe that our estimates are conservative.

Further assumptions in the computation of sample size include the expected dropout rate of participants after randomization. A conservative estimate of 10% has been assumed, based on the literature and current experience of the investigators involved in the design of the study. It is expected that a large part of these dropouts will occur within a day after randomization because clinicians may decide after the first dose of warfarin not to continue therapy. (For example, when an attending physician sees a patient at a teaching hospital the morning after warfarin is started.) We will minimize this possibility by discussing the plan for warfarin treatment with the attending physician prior to enrolling patients. Sample size adjustments for dropout were made as suggested by Lachin.[167]

Another parameter to estimate was the standard deviation of the PTTR outcome. The estimates of the within-study variability of PTTR in the literature arise from myriad designs and constraints as well as differences in the definitions of outcome although there was a reasonable consistency of variability for the genetic and control arms in the studies reviewed.[5;168-172] At this time, the best estimate for the standard deviation is 25%.

13.3.1   Primary Outcome

Two options were considered for the evaluation of the primary outcome PTTR: The first, a single two-arm comparison of the clinical and genetic arms and the second, an alpha splitting approach that uses an α = 0.04 to test the comparison of the two arms in the total cohort and then uses the remaining 0.01 of α to test a subgroup of the cohort.[173;174] This would allow the assessment in the context of the primary analysis to assess the statistical significance between the arms for the entire cohort, as well as in a predefined "enriched" subgroup. The decision was made to split α, providing a full cohort analysis and a subgroup analysis, where the subgroup is defined as those participants whose predicted first dose employing the genetic and the clinical algorithm differed by 1 or more milligrams, a factor known at the time of randomization and therefore not a post-randomization selection.

Sample size to provide a full cohort (α = 0.04) and subgroup (α = 0.01) two arm comparison

FULL COHORT ANALYSIS: This split α approach[173;174] does have a down side in that the overall test is stricter than if it had been left at α = 0.05, but the sample size computations below allow for selection of sample size to account for this additional burden. Table 13.1 provides the sample size required for the full cohort analysis for various SDs and power = 80 or 90%. It is seen that a sample size of 1,140 will provide 90% power to detect the absolute difference within the full cohort of 5.49% when the SD = 25%. Taking a conservative approach to protect against errors in the estimates of PTTR standard deviation and allelic variants, as well as providing a cushion for powering subgroup analyses, a sample size of 1,238 has been chosen.

Table 13.1. Sample size estimates for the full cohort analysis, for α = 0.04 with 10% dropout.

Proportion with allelic variants = 0, >1	PTTRg-PTTRc	SD = 20%		SD = 25%		SD = 30%	
		Power = 0.8	Power = 0.9	Power = 0.8	Power = 0.9	Power = 0.8	Power = 0.9
40%	3.67%	1238	1642	1932	2564	2782	3692
50%	4.58%	792	1050	1238	1642	1782	2364
60%	5.49%	550	730	860	**1140**	**1238**	1642

For example, if the SD is at 25% and the allelic variants are actually 50% of the cohort, not 60%, then we would still have 80% power to detect an absolute difference in the overall study population of 4.58%. If the SD were higher than 25%, we would still have 80% to detect the 5.49% difference. Since the sample size is also sensitive to the distribution of the allelic variants, and the 60% is based on two concrete and well designed studies, the DSMB may want to consider monitoring of the proportion within the various allelic variant groups and consider modification of the protocol if this number appears different than the 60% assumed.

SUBGROUP ANALYSIS. This approach allocates α = 0.01 to testing of the subgroup. The subgroup will be based on the difference between predicted initial doses from the genetic and clinical algorithms. A difference is defined as one greater than ± 1 mg/day. It is posited that the subgroup of participants with larger differences between the predicted initial doses should have a greater separation in the results between the two arms. If the difference in PTTR is related to the magnitude of difference in dosing between the genetic and clinical arms, a comparison within the group with differentiable doses should generate a larger difference than that assumed for the full cohort analysis [Δ = 5.49%, Equation (3)]. We assume that the minimally important difference to detect in the enriched subgroup is 9.15% [from a 61% PTTR to a 70.15% PTTR, Equation (2) above] reflecting a 15% relative difference. The power to detect an absolute increase of 9.15%, with α = 0.01 and various SDs is given in Table13.2, for the equivalent parameters considered in Table 13.1.

Table 13.2. Power estimates for primary subgroup analysis, for α = 0.01 with 10% dropout. Total sample size = 1238.

Percentage of total sample with dose difference > ± 1 mg/day	SD = 20%	SD = 25%	SD = 30%
n = 60%	99.9%	97.2%	87.8%
n = 50%	99.5%	93.6%	79.9%
n = 40%	97.8%	86.2%	68.4%

It should be noted that the subgroup analysis α level of 0.01 is conservative. Splitting alpha so that the sum of the significance levels for two tests becomes the overall type I error rate (0.05) is a Bonferroni type correction. This is unnecessarily conservative when there is a positive correlation between the two tests. As in the group sequential method, the correlation of two tests (full cohort and subgroup) will be obtained under the null hypothesis (H_0) when the proportion of the subgroup is known. Then we will incorporate this correlation to obtain α for the subgroup analysis given α = 0.04 for the full cohort analysis while controlling the overall type I error rate. Table 13.3 below demonstrates the actual α level for the subgroup analysis under various assumed correlations between the full cohort and subgroup tests, which increases as the subgroup becomes a larger proportion of the total cohort. Thus, although we assumed a test of the subgroup at an α level of 0.01, the final α level may be higher, and the power even higher than shown in Table 13.2.

Table 13.3. The significance level for the subgroup given α = 0.04 for the full cohort analysis, and various proportions of the cohort in the subgroup accounting for the null correlation between two tests.

α for the full cohort	Proportion of the subgroup	α for the subgroup
0.04	0.3	0.0136
	0.4	0.0153
	0.5	0.0173
	0.6	0.0200
	0.7	0.0236
	0.8	0.0286

A key assumption made in calculating the correlation between the two tests is that the variance of the primary outcome for the subgroup is the same as that for the remaining subjects. Data from Couma-Gen show that the variability of the primary outcome for the subgroup may be higher; in Couma-Gen the standard deviation of PTTR for the subgroup of patients with multiple or no genetic variants was 25.2%, and 17.8% for the remaining subjects with a single genetic variant (the overall standard deviation was 22.9%). We will accommodate a differential in the standard deviation when calculating the α level for the subgroup.

Impact of empirical magnitude of differences in stable dose predictions between the clinical and genetic algorithms.

There is a concern that the predictive algorithm for the two arms may not produce sufficiently differentiable doses between the two arms, which might lead to an underestimate of the expected difference in PTTR between the two arms. Our current working model for the two arms is as follows:

PTTRc =   0.40 x 73% + 0.60 x 61%   = 65.80%
PTTRg =   0.40 x 73% + 0.60 x 61% x (1.15)   = 71.29%

If this is a reasonably correct characterization of the process, then any difference between the two arms will come from the activity in the subgroup of patients with either 0 or multiple variants (expected to exist in about 60% of the cohort) who also receive different algorithm-derived doses of warfarin. The expected relative difference in the genetic arm is 15% in those with 0 or >1 variants. If, in effect, the difference in the algorithm predictions is negligible or clinically meaningless, then the expected 15% would be diluted, making it more difficult to detect a meaningful clinical difference between the arms. In this case the genetic arm can be rewritten as:

PTTRg = 0.40 x 73% + 0.60 x 61% x [1 + (0.15 x *D*)]

> where *D* is the proportion of patients with allelic variants in whom there is, indeed, a difference in predicted dose (because, for patients in this allelic subgroup who receive the same dose of warfarin, it is reasonable to expect no difference in PTTR between the 2 study arms in this subgroup of patients).

We examined the distribution of the differences between the predicted clinical and genetic dose among groups defined by allelic variation in the IWPC cohort and calculated the difference between the rounded predicted doses. A dose difference of 0 (i.e., an absolute dose difference of < ±1 mg) was defined as a same predicted dose, and any absolute dose difference greater than or equal to 1 mg/day (i.e., $\geq \pm 1$ mg) was defined as a different predicted dose. The rationale for this cut-point is that the average initial dose is 5 mg; therefore, a 1 mg difference represents a clinically relevant 20% difference, on average. Approximately 9% of IWPC participants in the (0, >1) group received the same dose (i.e., *D* = 0.9). With this dilution of the treatment effect, in order to detect an overall effect size of 5.49% in PTTR, the effect size in the (0, >1) group would need to be 16.5%.

Table 13.4 provides power estimates for the test of the primary hypothesis for a range of "diluted" treatment effects corresponding to the parameter *D*, the proportion of participants in the (0, >1) group who receive a genetic dose different from the clinical dose. We are not highly confident in our estimate of how frequently the predicted dose will differ between the two algorithms and therefore have not taken this potential dilution effect into account in the power considerations. However, given the potential impact of the dilution effect on the sample size requirements of the study seen in Table 13.4, we have placed monitoring of this factor during the operation of the trial in the hands of the DSMB for their consideration and attention.

Table 13.4. Power estimates for two arm comparison (α = 0.04, 10% dropout, 60% either 0 or >1 variants, SD = 25%, n = 1258); *D* represents the proportion in the (0, >1) group who receive a genetic dose different from the clinical dose.		
D	"Diluted" PTTRg – PTTRc	Power
70%	3.843	65%
80%	4.392	77%
90%	**4.941**	**86%**
Undiluted treatment effect		
100%	5.490	93%

13.3.2   Secondary Comparisons

In addition to the assumptions made for the primary analyses, for the proposed secondary comparisons, assumptions have been made about the frequency of outcomes and sizes of subgroups.

SUBGROUP ANALYSES

For the subgroups defined by gender and race, the minimum detectable treatment effects for the primary PTTR outcome given 80% power, and the powers to detect a 5.49% absolute PTTR difference are given in Table 13.5. The male/female ratio (50.3% male and 49.7% female) and the expected race/ethnicity breakdown (33.8% AA and 66.2% non-AA) are based on projections from 12 clinical centers that will participate in this study. These are not our target enrollment rates; the proportion of African Americans is somewhat higher than the US population. We aim to recruit a population that reflects the racial distribution in the US. However, given the possibility that recruitment of elderly African Americans may be more challenging, we believe that having a higher number of African Americans from which to recruit up-front will enhance the likelihood of maintaining an adequate racial distribution in the actual study population.

The power to detect the same difference of 5.49% for the primary hypothesis is, as expected, less than for the primary analysis on the full cohort. However, the minimal detectable differences with 80% power are within the clinically meaningful range of 5-10%.

Table 13.5. Power and minimal detectable differences for gender and race comparisons, with α = 0.05, 10% dropout, and total study sample size of 1238.			
Subgroup definition		Power[1*]	Minimal detectable Delta[2*]
Gender	**Male**	69.3%	6.24%
	Female	68.9%	6.27%
Race	**AA**	52.4%	7.61%
	Non-AA	80.8%	5.44%

[1*]Power: Power to detect 5.49% absolute PTTR difference when SD= 25%.
[2*]Minimal detectable Delta: Minimum detectable, absolute PTTR difference with 80% power when SD = 25%.

For the subgroups defined by allelic variants, the minimum detectable treatment effects in the primary PTTR outcome given 80% power, and the powers to detect various PTTR differences are given in Table 13.6. The frequencies of the allelic variants (40% with 1 variant vs. 60% with no or multiple variants) are derived from the IWPC cohort (unpublished data). The power to detect the same difference of 5.49% for the primary hypothesis is considerably less than for the primary analysis on the full cohort, but is reasonable for a 10% difference. The minimal detectable differences with 80% power are also clinically meaningful.

Table 13.6. Power and minimal detectable differences for allelic variation comparisons, with α = 0.05, 10% dropout, power = 80% and total study sample size of 1238.

Subgroup definition		Relative Difference	TRT[2]	Power[3]	Minimal Detectable Delta
Allelic variants	**1 variant [73% PTTR, Equation (1)]**	5%	3.65%	31.0%	6.99% (9.57% relative increase)
		10%	7.30%	83.3%	
		15%	10.95%	99.2%	
	0 or >1 variants [61% PTTR, Equation (1)]	5%	3.05%	32.1%	5.71% (9.36% relative increase)
		10%	6.01%	84.9%	
		15%	9.15%	99.4%	

TRT[2]: % absolute treatment effect (relative increase in PTTR).
[3]Power: Power to detect 5%, 10% and 15% relative difference (increase) in PTTR when SD = 25%.

SECONDARY OUTCOMES

For infrequent adverse outcomes such as bleeding (3-4%/year) and thromboembolism (1%/year)[175;176] it is seen in Table 13.7 that the power to detect even large percentage differences from the clinical arm is extremely low. Only huge differences for this secondary outcome will be detected with the primary sample size of 1,238. Detecting the difference with adequate power would require tens of thousands of patients.

Table 13.7. Power to detect relative changes of 10, 15 and 25% for clinical events under various clinical-arm event rates with α = 0.05, 10% dropout, and sample size of 1238.

Treatment Effect / Control Event Rate	10%	15%	20%
5%	5.6%	8.2 %	11.6%
7.5%	6.7%	10.4%	15.5%
1 0%	7.8%	12.7%	19.6%
12.5%	8. 9%	15.1%	23.9%

Table 13.8 provides the minimal detectible differences for a given power of 80%, and various other clinical event rates. Similar to Table 13.7, this generalizes the result of

fairly low power unless an analysis of a secondary outcome has a high expected event rate. For higher frequency events, the power is more reasonable, but still modest (Table 13.9). For example, for the event defined as combined events of INR ≥4 and clinical events from Anderson,[68] the control frequency is estimated to be approximately 34% for the genetics arm, and 42% for the standard of care arm. The power to detect this difference would be about 75%.

Table 13.8. Minimum detectable treatment effect for clinical events, with 80% power with α = 0.05, 10% dropout, and sample size of 1238

Control Event Rate (including minor bleeding)	80% power	
	Absolute Reduction	Relative Reduction
5%	3.21%	64.2%
7.5%	4.03%	53.7%
10%	4.70%	47.0%
12.5%	5.27%	42.2%

Table 13.9. Power to detect 10, 15 or 20% relative reduction in an adverse event with α = 0.05, 10% dropout, and sample size of 1238

Treatment Effect / Control Event Rate	10%	15%	20%
35%	21.6%	42.7%	66.5%
40%	25.6%	50.3%	75.2%
45%	30.1%	58.2%	82.8%
50%	35.4%	66.4%	89.0%

13.3.3   Statistical Power Implications

The protocol was amended on September 16, 2012 (recommended by the COAG DSMB and approved by the NHLBI Director), to reduce the required sample size from 1,238 to 1,022. Extensive justification is provided in the Amendment Section of the Protocol.

Projections for Statistical Power: Power of at least 80% should be achieved for both the overall cohort analysis and the primary subgroup analysis. The power assessment for the full cohort ($\alpha_A = 0.04$) through June 2013 is given in the figure below, assuming: a detectable difference of 5.0%, the lower bound for a clinically relevant detectable absolute difference in PTTR (red line); and 5.5%, the difference in PTTR targeted in the original protocol (blue line). The standard deviation of PTTR is assumed to be 25%. Sample sizes are provided on the horizontal axis.

Statistical power for overall cohort analysis (left axis) through June 2013
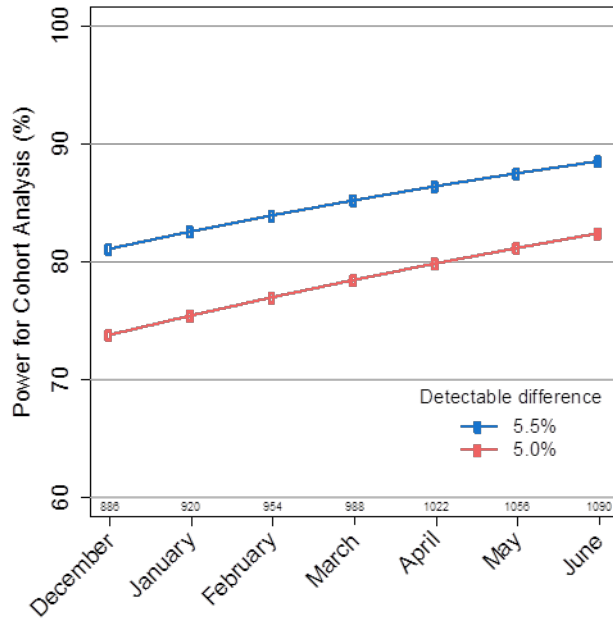
It is seen that by the end of April 2013, after a sample size of 1022 is achieved, there will be at least 80% power for the overall cohort analysis to detect an absolute difference of at least 5% in PTTR between groups.

We also examine power under assumptions of 'worst-case' recruitment through April 2013 (see figures below), assuming: detectable differences of 5.0% and 5.5% for the overall cohort analysis (left panel); and a detectable difference of 9.15% for the primary subgroup analyses (right panel) with different subgroup sizes (60%, 50%, and 40% for the proportion with ≥ ± 1.0mg/day predicted initial dose difference between the clinical and the genetic algorithms). The standard deviation of PTTR is assumed to be 25%. In each panel, the accrual rate is either assumed to be the 'best estimate' of 2.0 participants per center per month (represented in darker color) or that obtained under the 'worst-case' scenario of 1.5 participants per center per month (represented in lighter color). Corresponding sample sizes for the overall cohort analysis (left panel) are provided on the horizontal axis ('best estimate' on top; 'worst case' on bottom).

Statistical power for overall cohort analysis (left) and primary subgroup analysis (right) through April 2013 under different accrual rates

It is seen that by the end of April 2013, after a sample size of 1022 is achieved, there will be at least 80% power for the primary cohort analysis to detect an absolute difference of 5% in PTTR between groups and to detect an absolute difference of 9.15% between groups in the primary subgroup analysis. Even under the 'worst-case' scenario, we still will be able to detect an absolute difference of 5.5% in PTTR for the overall cohort analysis and 9.15% for the primary subgroup analysis if the size of the subgroup is > 40%.

13.4 Interim Analyses

A possible mid-course correction might be considered by the DSMB if the parameters postulated in the sample size computation prove to be incorrect (e.g., variability of the PTTR outcome, and distribution of the allelic variants). Because departures from these assumptions may lead to a total sample size that is underpowered for the specified detectable difference, or conversely, overpowered, the results of these evaluations may necessitate a modification in the sample size. In assessing the need for a sample size adjustment, data will neither be unblinded nor assessed for the primary outcome. In addition, a sample size adjustment should not impact the overall design of the study.

We will use the two-stage procedure outlined by Wittes and Brittain (1990) to design an "internal pilot study",[177] for assessing the need for a sample size adjustment based on an under or over estimate of the variance of PTTR:

1 Use a prior variance estimate $\gamma 2$ to calculate a pre-planned sample size, denoted by N. We assumed a standard deviation of 30 and calculated a sample size of 1238.
2 Use 25% of the pre-planned sample size as the size of the internal pilot study. Despite the name given to the process, no additional data are collected.

3 Use data from the patients in the internal pilot study to re-estimate the variance, denoted by s2.
We will calculate the final sample size N* from the two-stage procedure as:

176 N* = (tN-1, 1-α/2 + tN-1, 1-β)2s2/δ2,where δ denotes the specified detectible difference and tn, p denotes the upper 100(1 - p) percentage point of the t-distribution with n degrees of freedom.

We do not assume that the pre-planned sample size represents a minimum sample size (i.e., the final sample size based on the "internal pilot study" will not be less than the pre-planned sample size). In this case, the "internal pilot study" is known as unrestricted (e.g. the sample size re-estimation could involve either an increase or decrease from the original estimated sample size). There is a concern that an alteration of sample size based on an interim analysis of the variance could impact on the stipulated α level. Without considering the effect size the COAG study is powered to detect, the increase in alpha (bias) for ratios of prior variance estimate to the true variance greater than one (e.g. the prior variance estimate was too large), can be in the 2nd digit (0.05 vs. 0.06) when re-estimating at 25% of the pre-planned sample size. For ratios less than 1 (e.g. the prior variance estimate was too small), the differences in alpha are negligible.179 Overall, as the ratio of the prior variance estimate to the square of the effect size (γ2 /δ2) increases to 10, the bias in alpha goes to 0. In our case, this ratio 302/5.52 (Protocol line 2180 and Table 13.1) is approximately 30, considerably greater than 10. The re estimated SD would have to be less than 17.4 for theta to be less than 10, assuming the effect size of 5.5. It is therefore, unlikely that we will need to adjust hypothesis tests regarding the primary outcome.

The CTCC presented computations of sample size re-estimation to the DSMB, showing the point estimate of the estimation of the process as well as the confidence interval around the point estimate. Since the PTTR outcome is a continuous variable, presenting the current standard deviation estimate should not, in and of itself, divulge information on the magnitude of the outcome difference between the two arms, if any, although to be certain, we will still protect against unblinding. The DSMB may consider factors in addition to the sample size re-estimate, including distribution of the allelic variants, distribution of differences in predicted stable doses at time of randomization, drop-out rate, study maturity etc., at their discretion, in determining whether to recommend a modification for sample size to the Director, NHLBI. This process will ensure that the study is adequately powered to detect the specified clinically relevant difference and ensure patient safety.

13.5   Computation of PTTR

The main missing data problem will be a result of participants missing INR values within protocol specified windows needed to compute the primary outcome of PTTR. Various approaches will be used to address the missing INR problem. For the most commonly expected missing data, those INRs missing between other protocol scheduled windows, we will linearly interpolate the missing INRs using the method of Rosendaal.[78] As an example, if an outpatient misses the scheduled INR at the first week 2 visit but has INRs at study visits day 4/5 and the second week 2 visit, then our approach will proceed by connecting the known INRs between the known visits with a straight line and using the

reading on the line at the middle day as the imputed value of INR for the missed visit. The Rosendaal approach has been shown to be relatively robust to missing data, particularly for the primary measure of PTTR.[79] The approaches to dealing with the less commonly expected missing data for INR are outlined in Table 13.10 for the primary outcome PTTR. We believe that the approaches outlined are reasonable, relying on basic assumptions regarding the behavior of INR in the presence of missing data.

****Table 13.10. Approaches to dealing with missing INRs

Missing Data Status	PTTR Computation
No study protocol INRs	Missing, PTTR = "."
Only 1 INR on Day 4/5	If INR day 4 or 5: <2, PTTR = 0; 2-3, PTTR = 0.5 > 3, PTTR = 0
Missing INR on day 4/5, but INRs available thereafter	Compute PTTR with available data, with INR date 4/5 = 1
Temporary discontinuation to allow INR to become 1 (e.g., before surgical procedure) of warfarin after day 4/5 but before final 30 day INR	Compute PTTR with available data prior to interruption because INRs, if any, after restart are not meaningful
Permanent discontinuations after day 4/5	Compute PTTR with available, data prior to discontinuation

**See Below Clarification and Revised Table

Clarification of method for calculating the percent time in therapeutic range (PTTR) for patients with temporary discontinuation of warfarin.

The above Table 13.10 was modified to account for patients who temporarily discontinue warfarin. In order to avoid biased results that could result if patients' data were censored after temporary hold (for example, patients with high INRs who have their doses held for several days, as per protocol, would be systematically censored, and this could be related to the intervention), a refinement of the PTTR calculation was made. This decision was made prior to examining any study results or unblinding. Table 13.10 Revised, reflects the changes, and further details are provided below:

Table 13.10 - Revised.   Approaches to dealing with missing INRs

Missing Data Status	PTTR Computation
No study protocol INRs	Missing, PTTR = "."
Only 1 INR on Day 4/5	If INR day 4 or 5: <2, PTTR = 0; 2-3, PTTR = 0.5 > 3, PTTR = 0
Missing INR on day 4/5, but INRs available thereafter	Compute PTTR with available data, with INR date 4/5 = 1
Any temporary discontinuation after day 4/5	If 5 or fewer days: Compute PTTR with all available INRs If >5 days: Compute PTTR with all available INRs for 5 days after hold, then use all INRS after restart and concatenate
Permanent discontinuations after day 4/5	Compute PTTR with available INRs up through 5 days after discontinuation

A "restart" is defined as starting warfarin after the drug had been held for at least one day. For patients who have the drug held for 5 days or fewer, all available INRs will be

used in the calculation of the PTTR. For those who have the drug held for more than 5 days, any INRs measured in the 5 days after the drug was held will be used in the calculation of PTTR (in order to be consistent with the method of calculating PTTR for those with 5 or fewer days of warfarin holds). Once the drug is restarted, the first INR drawn will then be used to calculate PTTR from that point on. The overall PTTR will be concatenated between the courses of warfarin therapy; that is, for both short and longer term holds, a single PTTR will be calculated for a patient using all INRs available during the time on warfarin (i.e., on the drug and up to 5 days after the dose was held, and INRs after dosing was restarted). For patients who have their warfarin permanently discontinued, the PTTR will be calculated using all INRs through 5 days after discontinuation (consistent with the method of calculating PTTR for those with dose holds).

14   Study Organization

Figure 14.1 below depicts the study organization. Management of this project will be provided through the Clinical Trial Coordinating Center (CTCC), with Dr. Stephen Kimmel serving as the Principal Investigator and Dr. Jonas Ellenberg serving as Co-PI. Together they will assume primary responsibility for all issues related to the management of the project, including coordination of research activities and coordination of the clinical sites. The CTCC will be organized into three cores: Biostatistics; Informatics and Operations; and Scientific Administration. Together these units will perform or direct all tasks required for the successful completion of the trial.
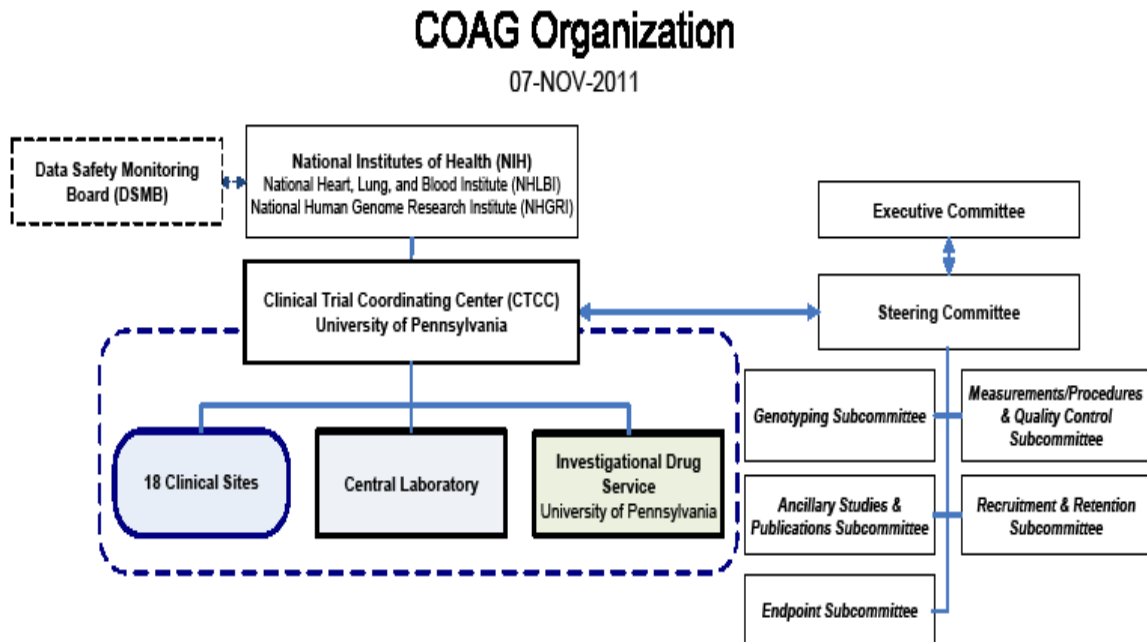
The success of the COAG trial depends on the coordination of efforts among the NIH, CTCC, clinical site investigators, Central Laboratory, and other participating organizations. Facilitating an atmosphere of scientific collaboration and cooperation among all participating individuals and institutions will be critical to the study's success.

The CTCC will collaborate with the NIH and participating investigators in exploring, evaluating, and developing strategies for the trial, and will work closely with the Steering Committee (SC) to enhance achievement of project goals and milestones. The project management strategy is to plan all operational aspects of the study such that the scope and objectives are well defined, evaluable, and achievable. The project management strategy will accomplish key shared objectives, including coordination of research activities, documentation of progress, compliance with regulatory agencies and guidelines, milestone-driven evaluation, frequent assessment of priorities and progress, active involvement of the clinical site investigators, and ongoing evaluation of progress by the independent DSMB. Proven tools and technology will be utilized to enhance the clinical research network infrastructure, provide optimal informatics technology, and facilitate communication.

14.1 *National Heart, Lung, and Blood Institute (NHLBI) and National Human Genome Research Institute (NHGRI)*

The Atherothrombosis and Coronary Artery Disease (ACAD) Branch, Division of Cardiovascular Diseases, NHLBI will be responsible for oversight and administration of the scientific conduct of the trial. Representatives from the Office of Acquisitions, Division of Research Activities, NHLBI, Office of Biostatistics Research, NHLBI, and Office of Population Genomics, National Human Genome Research Institute (NHGRI) will work with the CTCC to develop and implement the study.

14.2 *Clinical Trial Coordinating Center*

The Clinical Trial Coordinating Center (CTCC) at the University of Pennsylvania (Penn) will work collaboratively with appropriate project members to provide a broad range of services and collaboration regarding study design, data collection and quality assurance, logistics of meetings, statistical analysis, interpretation of results, and manuscript preparation. Specifically, the CTCC will be responsible for coordinating protocol writing activities (including statistical design); development of operational and analytical methodology; generation and distribution of reports; development and maintenance of the trial databases and related website; development and design of forms and a Manual of Operations; selection, training, and monitoring of the clinical sites and Central Laboratory; and data management and analysis. All of these activities will be performed under the direction of the NHLBI Program Office, the Steering Committee (SC) and the Executive Committee (EC). The CTCC will work with these investigators and the study subcommittees to ensure that all trial components are rigorously and successfully completed.

14.3 *Steering Committee*

The Steering Committee will provide scientific leadership for the trial. It will be composed of clinical site investigators, the CTCC principal investigator, NHLBI and NHGRI representatives and other experts. The list below includes the current members of the SC:

Voting Members (8)	Robert Califf, MD (Chair) Jeffrey L. Anderson, MD, FACC, FAHA, MACP Michael D. Caldwell, MD, PhD, FACS Charles S. Eby, MD Brian F. Gage, MD, MSc Samuel Z. Goldhaber, MD Robert G. Hart, MD Julie A. Johnson, PharmD *Other members from Clinical Sites, Central Lab on a rotating basis
NHGRI (1 vote)	Teri A Manolio, MD, PhD Lucia A. Hindorff, PhD, MPH Ebony Bookman, PhD
NHLBI (1 vote)	Nancy L. Geller, PhD Dihua Xu, PhD Yves D Rosenberg, MD, MPH Suzanne Goldberg, MSN, RN
CTCC (1 vote)	Stephen E. Kimmel, MD, MSCE Jonas H. Ellenberg, PhD Benjamin French, PhD
Ad hoc (non-voting) Members	Maryellen de Mars, PhD Ann M. Staten, RD

Subcommittees will be composed of experts appointed by the SC, and will make recommendations to the SC. The following subcommittees are planned, with others to be established by the SC as needed:

- Endpoint Classification Subcommittee
- Publications Subcommittee
- Ancillary/Substudy Proposals Subcommittee
- Genotyping Subcommittee
- Quality Control Subcommittee

14.4 Executive Committee

The Executive Committee (EC) will include the SC Chair (or designee), the CTCC Principal Investigator, and NHLBI Project Officer. The EC will run the day-to-day operations of the trial as an extension of the SC. Protocol changes and other major issues will be discussed by the EC for presentation, review and approval by the SC.

14.5 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be established to monitor data and oversee participant safety. Members will be appointed by the NHLBI. The Board will include experts in the areas of thromboembolic disease, anticoagulation treatment, pharmacogenetics, clinical trials, biostatistics and bioethics. The SC chair, and CTCC PIs will attend DSMB meetings (open sessions only) as well as representatives of the NHLBI.

Interim trial results will be submitted to the DSMB, which will meet approximately twice a year to monitor patient safety and advise the NHLBI about the trial progress. The CTCC will provide data to the DSMB Chair to ensure early identification of major adverse outcomes of the trial. The DSMB has the responsibility to recommend whether the trial

should continue, whether the protocol should be modified, or whether there should be early termination. The DSMB may request a futility analysis of the study. A charter will describe the specific responsibilities and operating procedures of the DSMB.

15 Ancillary Studies

To enhance the value of the COAG trial, the Steering Committee welcomes proposals from individual investigators to carry out ancillary studies. Nevertheless, to protect the integrity of the COAG trial, such ancillary studies must be reviewed and approved by the Ancillary/Substudy Proposal Subcommittee and the Steering Committee before their inception or submission of a proposal for external funding consideration.

Ancillary submissions will be reviewed by the Ancillary Subcommittee and comments will be collated and returned to the Investigator. A detailed Ancillary Study Policy that describes the submission, review and approval process will be developed by the Ancillary/Substudy Proposal Subcommittee for the COAG trial.

15.1 Ancillary Studies

An ancillary study is one based on information from COAG trial participants in an investigation or analysis that is relevant to, yet not described in, the COAG trial protocol, and derives support from non-COAG trial funds. It is anticipated that a typical ancillary study will propose the collection of additional data not collected or analyzed as part of the routine COAG trial dataset. However, ancillary studies that use existing COAG trial data, without the need for additional data collection, may be performed as well; these studies will also require separate funding for the effort required to complete the analyses. Ancillary studies may be submitted by the investigators within the COAG trial or by investigators without a prior relationship to the COAG trial. Ancillary studies require external (non-COAG trial) funding. Examples include studies funded by investigator-initiated NIH research awards (e.g., R01s), grants from academic institutions, or private sources (e.g., private foundations, pharmaceutical companies).

COAG study investigators are encouraged to consider ancillary studies and to involve other investigators, within and outside of COAG study personnel. Participation in an ancillary study is subject to the approval of the COAG Ancillary/Substudy Proposal Subcommittee and the National Heart, Lung, and Blood Institute (NHLBI). The following features will be significant in determining approval:

   a. Participant burden.
   b. The proposed study must require the unique characteristics of the COAG trial.
   c. The proposed study must meet requirements of the highest scientific merit.
   d. The investigators must have adequate resources to effectively complete the project.
   e. The proposed study procedures must be consistent with all COAG study policies.

15.2 Data Access

The Steering Committee will authorize access to study data and biospecimens. Investigators must submit a proposal requesting approval to access COAG trial data/specimens. The COAG trial will participate in the NHLBI Central Repository for study data and specimens.

All data access will follow guidelines described in the NHLBI Limited Access Data Policy (www.nhlbi.nih.gov/resources/deca/policy_new.htm), the NIH Data Sharing Policy (http://grants.nih.gov/grants/gwas/index.htm), and the Policy for Sharing of Data Obtained in NIH Supported or Conducted Genome-Wide Association Studies (GWAS) http://grants.nih.gov/grants/gwas/index.htm) with regard to documentation, content, storage and timing.

16   Publication Policy

The success of the COAG trial will depend largely on the number and quality of its scientific publications and presentations. The purpose of the policy established is to encourage and facilitate the presentation of COAG trial analyses while providing guidelines that ensure appropriate use of the COAG data, timely completion of manuscripts and presentations, equitable access to authorship, and adherence to established principles of authorship.

A limited number of COAG manuscripts, such as the major design paper and the main paper describing treatment effects on the major endpoints, will be authored by the COAG Study Group with reference to all investigators in an appendix. For some major papers, named authors may be suggested by the Publications Subcommittee with final determination by voting members of the Steering Committee. Named authorship for other papers can be suggested by the proposal's originator and will include a limited number of named authors who comprise the Writing Group. As allowed by the editorial policy of individual journals, an appendix describing the structure of the COAG organization and containing the names of all the COAG investigators will be included with publications.

The COAG trial Publications Subcommittee will establish a comprehensive policy for dissemination of COAG trial information. The policy will describe very specific processes for authorship, submission and review of manuscripts and will be based on the Uniform Requirements for Manuscripts Submitted to Biomedical Journals as developed by the International Committee of Medical Journal Editors (ICMJE).

17   Closeout Procedures

The CTCC will be responsible for oversight and management of all aspects of the study close-out phase, including indirect oversight of clinical sites, the Investigational Drug Service (IDS), and the Central Laboratory. During this phase, the CTCC will resolve all outstanding data problems and prepare for database closure and final analyses.

17.1   Study Closure

The CTCC will conduct the following activities during the close-out phase:

   a. Over the course of the trial, the CTCC will monitor the drug accountability documentation at clinical sites and the IDS. A final report of this drug accounting will be prepared during the close-out phase.
   b. The CTCC will notify the IRB of the study status.
   c. The CTCC will establish procedures with the Central Laboratory for depositing samples in the NHLBI Repository.
   d. The CTCC will meet all contract deliverable requirements, including the Final Financial Status Report and the Final Progress Report.

e. The CTCC will prepare a data dissemination plan that is in alignment with the [NIH Public Access Policy](#) document.

f. The CTCC will instruct the clinical sites in a process for storage of research records.

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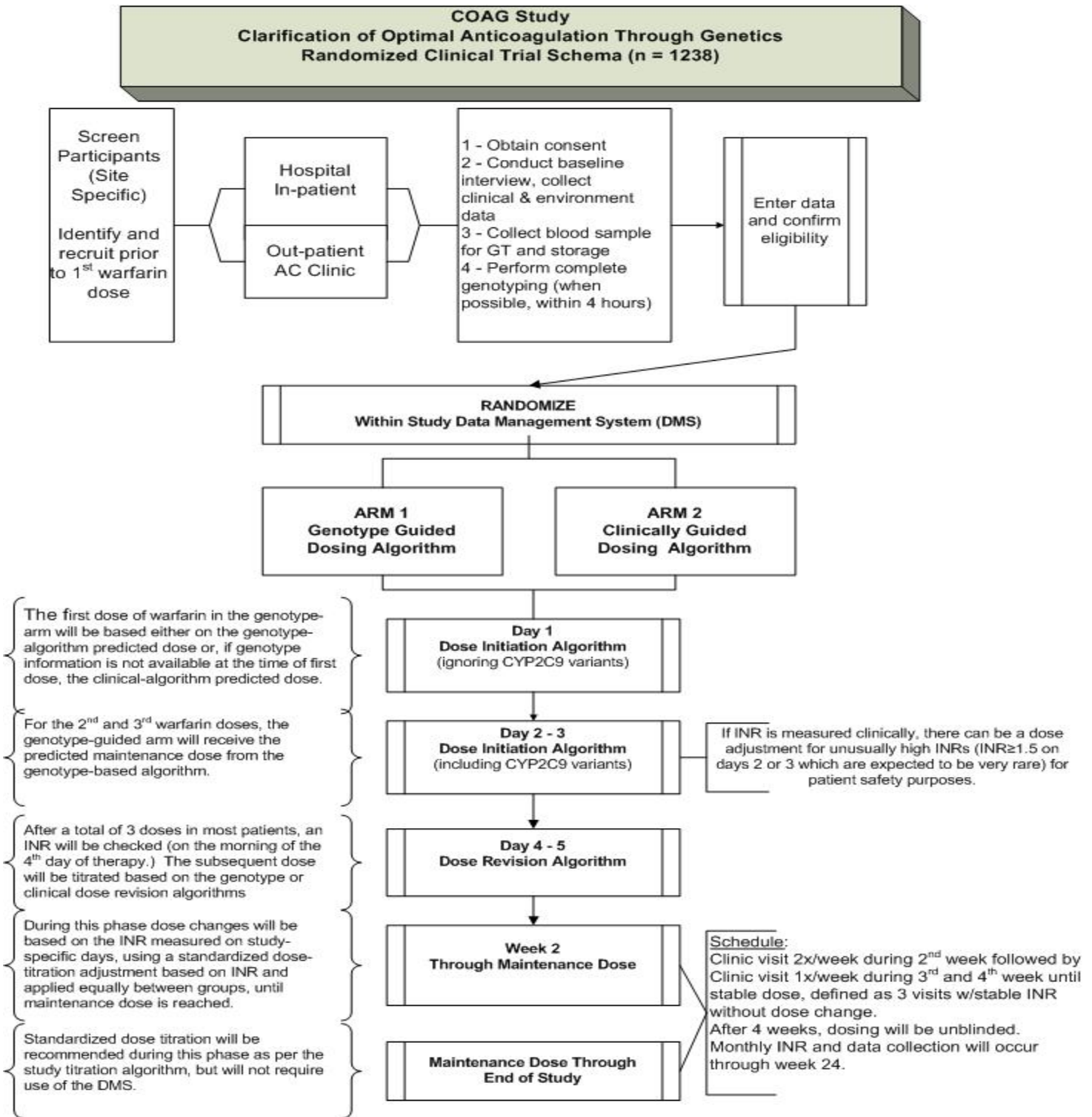
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APPENDIX A: Informed Consent Template (attached separately)

APPENDIX B: Patient Flow Diagram

COAG Study
Clarification of Optimal Anticoagulation Through Genetics
Randomized Clinical Trial Schema (n = 1238)

Screen Participants (Site Specific)

Identify and recruit prior to 1st warfarin dose

Hospital In-patient

Out-patient AC Clinic

1 - Obtain consent
2 - Conduct baseline interview, collect clinical & environment data
3 - Collect blood sample for GT and storage
4 - Perform complete genotyping (when possible, within 4 hours)

Enter data and confirm eligibility

RANDOMIZE
Within Study Data Management System (DMS)

ARM 1
Genotype Guided Dosing Algorithm

ARM 2
Clinically Guided Dosing Algorithm

The first dose of warfarin in the genotype-arm will be based either on the genotype-algorithm predicted dose or, if genotype information is not available at the time of first dose, the clinical-algorithm predicted dose.

For the 2nd and 3rd warfarin doses, the genotype-guided arm will receive the predicted maintenance dose from the genotype-based algorithm.

After a total of 3 doses in most patients, an INR will be checked (on the morning of the 4th day of therapy.) The subsequent dose will be titrated based on the genotype or clinical dose revision algorithms

During this phase dose changes will be based on the INR measured on study-specific days, using a standardized dose-titration adjustment based on INR and applied equally between groups, until maintenance dose is reached.

Standardized dose titration will be recommended during this phase as per the study titration algorithm, but will not require use of the DMS.

Day 1
Dose Initiation Algorithm
(ignoring CYP2C9 variants)

Day 2 - 3
Dose Initiation Algorithm
(including CYP2C9 variants)

If INR is measured clinically, there can be a dose adjustment for unusually high INRs (INR≥1.5 on days 2 or 3 which are expected to be very rare) for patient safety purposes.

Day 4 - 5
Dose Revision Algorithm

Week 2
Through Maintenance Dose

Schedule:
Clinic visit 2x/week during 2nd week followed by Clinic visit 1x/week during 3rd and 4th week until stable dose, defined as 3 visits w/stable INR without dose change.
After 4 weeks, dosing will be unblinded. Monthly INR and data collection will occur through week 24.

Maintenance Dose Through End of Study

APPENDIX C: Blinding Diagram

Pill Bottles to Replicate Usual Care (may be able to use fewer dose sizes)

1 mg   2 mg   2.5mg   3 mg   4 mg   5 mg   6 mg   7.5 mg   10 mg

For QD same dosing: Pull appropriate dose bottle and take 1 per day

Bottle 1: Take 1 tablet every day.

For 2 dose dosing: Pull 2 bottles and label one bottle for appropriate days (e.g., M/W/F) and the other for the other days (e.g., T/Th/S/Su):

Bottle 1: Take 1 tablet every Mon, Wed, & Fri

Bottle 2: Take 1 tablet every Tues, Thurs, Sat, & Sunday

Could load 7-day pill boxes too, although more complicated.

Appendix D: Data Management Plan

Appendix E: Conflict of Interest Policy

Guidelines for: AVOIDING CONFLICTS OF INTEREST IN MULTICENTER CLINICAL TRIALS

National Heart, Lung, and Blood Institute
National Institutes of Health

Updated: September 6, 2000

In 1995, the Department released its final rule on "Objectivity in Research" (Federal Register, July 11, 1995). Under this rule, an investigator must disclose to an official in his or her institution "any Significant Financial Interests (and those of his/her spouse and dependent children) that would reasonably appear to be affected by the research proposed for funding by the PHS. The institutional official(s) will review those disclosures and determine whether any of the reported financial interests could directly and significantly affect the design, conduct, or reporting of the research and, if so, the institution must, prior to any expenditure of awarded funds, report the existence of such conflicting interests to the PHS Awarding Component and act to protect PHS-funded research from bias due to the conflict of interest."

These are minimum requirements. Individual institutions may interpret them and implement them somewhat differently, and investigators may decide to go beyond them. Also, in certain circumstances, these rules may need to be adapted to the specific research program. For example, it would be reasonable for investigators in multicenter clinical trials to come up with a study-wide policy on conflict of interest, as different interpretations of the guidelines by different investigators and their institutions may be inappropriate. Certainly, the credibility of the study might depend on all of the investigators having stronger policies for conflict than are mandated by the PHS. Even if one or two of the investigators have financial interests in a drug or device being evaluated in the trial, or in a competitor of the drug or device, questions may arise as to the validity and interpretation of the trial results. An example of this is TIMI-1, where some of the investigators had considerable financial interest in tPA, leading to Congressional investigation.

Most, but not all, clinical trial investigator groups have since developed conflict of interest guidelines. These have ranged from disclosure of interests to prohibition against buying or selling stock in a company manufacturing one of the interventions, to having any equity. In some cases, consulting or giving paid talks for the manufacturers has been discouraged.

Any clinical trial assessing an intervention for which there is or might be an IND (for drugs) or an IDE (for devices) has additional requirements. Details about these requirements may be obtained from www.fda.gov/oc/guidance/financialdis.html.

In summary, the integrity of the study must not be compromised by financial interests. To assure that, investigators cannot have financial arrangements that reward a particular study outcome, proprietary interest in the intervention being tested, significant equity in the manufacturer of the intervention, or significant payments of other sorts. Specified financial arrangements must be disclosed. The sponsor of the IND or IDE is responsible

for collecting information about financial interests. The NHLBI, even if it is not the sponsor of the IND or IDE, will take an active role in ensuring that these requirements are followed.

It is in the Institute's interest to strongly advise the investigative group to develop guidelines that avoid any perception that the study design, conduct, and data analysis and interpretation might have been biased by investigator conflict. To help investigators comply with the PHS regulations, the Institute Project Officer or Program Scientist and the Principal Investigator(s) must discuss the conflict of interest policy for the study at an early stage in the protocol development process. Although the Institute does not specify exactly what policy a given study should develop, it needs to remind the investigative group that study credibility depends on reasonably strict guidelines. These policies should be clearly spelled out in the protocol. In addition, the Project Officer or Program Scientist should be apprised of conflicts that arise and the corrective actions by the investigator's local institution. As noted in regulations, the Institute "may at any time inquire into the Institutional procedures and actions regarding conflicting financial interests in PHS-funded research, including a requirement for submission of, or review on site, all records pertinent to compliance with this subpart."

INFORMED CONSENT TEMPLATE
CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY

Study Title:	**A Randomized Controlled Clinical Trial of Clarification of Optimal Anticoagulation Through Genetics**
Study Key Name:	**COAG Study**
Principal Investigator:	**[Insert Name, Address, and Phone Number of the Principal Investigator]**
Emergency Contact:	**[Insert Emergency Contact Insert Phone Number/Pager, etc]**

Introduction

We invite you to take part in a clinical research study called Clarification of Optimal Anticoagulation through Genetics (COAG) at the (NAME OF INSTITUTION). This form is called a consent form. The purpose of this consent form is to give you information to help you decide if you want to be in this study. Please read this form carefully before deciding whether you want to take part. This information will also be discussed with you by the research team. If there is anything you do not understand about this study, please ask the study doctor and/or research team any questions you have before you make your decision. If you decide to take part in this study, you will be asked to sign this form and you will be given a signed copy of this form.

Being in this research study is voluntary. You do not have to take part in this study if you do not want to. If you do take part in this study, you can leave the study at any time, and for any reason. You do not have to participate in this research study in order to receive treatment.

This study is sponsored by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH). This study will be conducted at about 18 clinical centers throughout the United States of America. It will include a total of about 1,022 participants and the study will end in July 2013.

Why you are being asked to take part in this research study?

You are invited to take part in this study because your doctor recommends that you begin taking the medication warfarin for at least one month. Warfarin is also known by the "trade name" *Coumadin®* and is in a class of medications called anticoagulants or "blood thinners." Warfarin works by reducing the clotting ability of the blood. It is used to stop blood clots from forming or growing larger in your blood and blood vessels.

Warfarin is prescribed for many conditions, including for people with certain types of irregular heartbeat, people with replacement or mechanical heart valves, people who have suffered a heart attack, people who have had orthopedic surgery, or who have a history of having blood clots. Warfarin is used to prevent or treat deep vein thrombosis (swelling and blood clot in a vein), pulmonary embolism (a blood clot in the lung), and strokes (a blood clot in the brain).

What is the purpose of this research study?

The purpose of this research study is to find out the best way to start warfarin treatment.

Individuals vary in the dose of warfarin they need to have safe and effective levels of blood thinning. Individuals can vary in how their bodies use (break down) and react to warfarin. Therefore, you may need a different dose of warfarin than another person. Some of these differences are due to differences in age, race, sex, weight, and medical conditions.

Along with these differences, the use of genetic information may also help doctors find the dose of warfarin that you need. Genes are like a set of instructions that tell your body's cells what to do. Genes carry the messages that tell cells when and how to make certain chemicals necessary for the growth and health of the body. Researchers have found that certain genes may affect how a person's body will break down or react to warfarin. If genetic information can help doctors better determine the best dose of warfarin before it is first given, this may help the doctors to get you to the correct levels of blood thinning and thereby reduce the risk of bleeding or the risk of developing a blood clot. This study will test whether doctors can improve the control of blood thinning by using genetic information. This genetic testing is done by a blood test that will be conducted on everyone in this study.

There are likely to be many genetic differences (also called "variations") that contribute to a person's response to warfarin, but not all of them have been discovered yet. Recent scientific advances allow researchers the ability to search for all possible genetic changes that might be related to warfarin response. These are often called "genome-wide association studies" or "sequencing studies." These

studies will also be conducted on everyone in the COAG study to determine even better ways of giving warfarin treatment in the future.

Throughout your treatment with warfarin, you will have regular blood tests, called INR (International Normalized Ratio), to check how the warfarin in your blood is working. This test is done frequently as part of regular medical care for all patients on warfarin in order to help your doctor provide you with the dose of warfarin that it is right for you. It is sometimes necessary to change the dose of warfarin to avoid too much thinning of the blood which can lead to serious bleeding, or too little thinning of the blood which can allow clots to form in the blood vessels. Frequent INR blood tests will be done on everyone in this study. This is common in patients using warfarin and would be done whether or not you decide to participate in the study.

Several things such as food, alcohol, other medications, or medical conditions, can change how your body breaks down or responds to warfarin. This can change your INR and the dose of warfarin that you need. This study will also look at how these factors (such as other medications) can affect warfarin therapy and how genetics might change your response to these other factors.

How long will I be in this research study?

Your participation in research study visits will be for six (6) months. All study visits will end in July 2013. If you are enrolled in the study after February 2013, your participation will end in July 2013, and your study visits will be for less than six months. Future studies that use information from this research study will continue after your participation in the research study ends.

How many people will take part in this study?

The total number of people in the study will be about 1,022, from 18 medical centers in the United States. Approximately (*xxx number)* people will be enrolled in this study at the [name of institution].

What is involved in this research study?

If you agree to be in this research study, information will be collected to see if you are eligible for the study. This is called a screening visit which will occur before you receive your warfarin treatment in the hospital or at your first out-patient visit to the anticoagulation clinic.

Screening Visit
At your screening visit you will be interviewed by the research coordinator and asked questions about your medical health and health habits related to smoking and alcohol use. You will be asked to answer questions about your medical

condition, medical history, food and diet information, the quality of your life, and medications you are taking.

A blood sample (approximately 3 teaspoons) will be taken from you. The laboratory at [INSTITUTION NAME] will use part of this sample to determine the genetic differences (variations) that may be used in the study to help determine your warfarin dose. The rest of this blood sample will be sent to a central laboratory. This laboratory will perform quality checks on the results from the laboratory at [INSTITUTION NAME]. The rest of your samples will be stored and used to study genes and other factors in the blood that may affect how people respond to warfarin.

Study Participation
If the research doctor determines that you are eligible to participate in this study, you will be randomly assigned by chance (like flipping a coin) to one of two treatment groups. One group will receive their warfarin dose based on a formula that uses clinical information only (such as age and weight) to calculate a person's warfarin dose. This is called the "clinical-guided arm" of the study. The other group will receive warfarin based on a formula that uses clinical and genetic information to calculate a person's warfarin dose. This is called the "genotype-guided arm" of the study.

The treatment assignment to either the clinical-guided group or the genotype-guided group is a blinded assignment. This means that the study doctor, the study staff, and you will <u>not</u> know which treatment group you are in. The reason for a blinded assignment is to make sure that the study meets strict scientific standards and that the results are therefore accurate. For the first 4 weeks you are in the study, the dose of warfarin you get will not be known to the study doctor, the study staff, or to you. However, this information is available to your study doctor in case of an emergency.

Taking the Study Medication
After you are enrolled in the study you will receive a daily dose of the warfarin study medication based on the study group that you are in. You will not know the dose of the warfarin. After you receive the first few doses, your dose will again be changed based on formulas designed for your study group. After this, the frequency of INR blood tests and dose of your warfarin will be adjusted using a standard method of medical care for warfarin therapy. You will be given instructions about how and when to take the study medication. If at any time your health care provider feels that your dose should not be determined by the study methods, your dose can be changed.

Clinic Visits While Taking the Study Medication
You will be scheduled for your clinic visits for the first month. While receiving warfarin medication, you will be asked to return to the clinic for all scheduled visits so that the safety and effectiveness of the study treatment can be checked.

These visits are part of the regular care that all patients on warfarin get. During the first and second week, you will come to the clinic twice each week. During the third and fourth week, you will come to the clinic once each week. In addition to the clinic visits, a phone call between you and the research coordinator may be needed to find out how you are adjusting to the study medication and to report any medication side effects you may be experiencing. After the fourth week, you will come to the clinic once a month for the rest of the study (up to month 6). During these visits, you will also be asked to complete questionnaires to assess your quality of life, preferences and use of inpatient and outpatient medical services. Depending on your INR results, you may need to come to the clinic more frequently. ==Your final study visit will be at month six (6),or for a shorter period if you are enrolled after February 2013.==
`
Clinic Visits When Not Taking Study Medication
Should you or the study doctor decide to stop your warfarin medication before the final study visit at month six, you will be asked to continue your participation in the study even if you are not taking study medication. Your continued participation in the study will involve your completing study questionnaires and providing follow-up information until the final study visit at month six (6).

What are the risks of taking part in this research study?

There are risks in taking warfarin and there are risks from being in a research study. These are described below. Also, there may be risks or side effects we do not know about yet.

Warfarin Risks (These are risks that can occur to all patients on warfarin, whether they are in this study or not)

Side effects of warfarin therapy: Most side effects relate to how warfarin works. To minimize the risk of bleeding, health care providers try to keep your blood thinning in the correct range. However, even when your blood thinning is in the proper range, you might have side effects. Some people may experience hair loss or skin rashes, but this is rare. If you notice something wrong that you feel may be caused by your medication, call your doctor.

Common Side Effects: Warfarin can cause slight bleeding—you may notice gum bleeding while brushing your teeth, an occasional nosebleed, easy bruising, bleeding after a minor cut that stops within a few minutes, or menstrual bleeding that is a little heavier than usual.

Serious Side Effects: Warfarin can cause serious and even life-threatening bleeding problems. The following are signs of more serious bleeding that mean you should contact your doctor or go to the hospital emergency room: red, dark, coffee or cola colored urine; bowel movements that are red or look like tar;

bleeding from the gums or nose that does not stop quickly; vomit that is coffee colored or bright red; anything red in color that you cough up; severe pain, such as a headache or stomach ache; sudden appearance of bruises for no reason; menstrual bleeding that is much heavier than normal; a cut that will not stop bleeding within 10 minutes; dizziness or weakness.

Use of Other Medications: When warfarin is taken with other medicines it can change the way the warfarin work. It can also change the way those other medicines work. It is important to talk with your health care provider and study staff about all of the other medicines that you are taking, including prescription medicines, over-the-counter medicines, antibiotics, vitamins, or herbal products. You also should talk with your health care provider before starting any new medicines or stopping any of your current medicines.

Diet and Alcohol: The foods you eat can affect how well warfarin works for you. Before starting a weight loss plan while taking warfarin, you should first discuss it with your doctor. Alcohol can affect your warfarin dosage but it does not mean you must avoid all alcohol. Serious problems can occur with alcohol and warfarin when you drink more than two (2) drinks a day or when you change your usual diet or alcohol consumption.

Pregnancy Risks: Because warfarin might be harmful to a pregnant woman and/or the unborn child, women of childbearing potential must have a negative pregnancy test at the time of screening if they wish to participate in this trial. Women of childbearing potential also must agree to use a reliable form of contraception (birth control) during this study. Please note that the rhythm method is not a medically accepted form of birth control.

Medically acceptable birth control methods for this study include:
- hormonal methods (birth control pills, or injected or implanted contraceptive),
- intrauterine device (IUD) with spermicide,
- condom with spermicide or
- diaphragm with spermicide.

Even if you use a medically acceptable birth control method, you could still become pregnant. If you suspect that you are pregnant, it is important to the safety of your unborn child that you tell your health care provider immediately. They will determine if warfarin should be stopped. If you must continue on anticoagulation therapy, it will be supervised by the doctor/health care provider you have chosen to care for you during your pregnancy. You must also notify the study doctor/staff immediately.

Research Risks (These are risks that can occur from participating in this study.)

Risks associated with drawing blood: Some possible risks and discomforts you could experience during this study include physical discomfort such as a sharp sting from the needle used to collect blood from your arm. There is a small chance that you will develop a bruise or an infection at the needle site, or you may feel lightheaded or faint.

Risks of the Study Dosing: The goal of the study is to try to keep your blood thinning in the correct range. If the use of one of the study dosing methods leads to a higher amount of blood thinning than desired, this may increase your risk of side effects from warfarin (as detailed in the "**Warfarin Risks**" section above). If the use of one of the study dosing methods leads to a lower amount of blood thinning than desired, this may increase your risk of developing clots in your blood vessels.   It is important that you keep your scheduled visits so that the doctor can detect important changes in your levels.

Loss of Confidentiality: One possible risk is the loss of confidentiality about your medical information. A related possible risk is disclosure of your genetic information that could lead to discrimination against you in insurance or employment. There are some state laws that protect against genetic discrimination by employers or insurance companies, and a federal law protecting against such discrimination will take effect late in 2009. There is the unlikely risk that if people other than the researchers got your genetic information they could misuse it. The chance of this happening to you is very small.

Today, there are a limited number of possible ways of linking genetic information back to you. As research advances, there may be new ways of linking genetic information back to you that we cannot foresee now. Also, since we do not yet know the results of research, new risks may become known in the future that we cannot predict now. These new risks may include genetic associations with disorders other than warfarin dosing. Every attempt will be made to keep all information collected in this study strictly private.

Are there any benefits to taking part in this research study?

A direct benefit cannot be guaranteed. It is possible that, by being in this study, your levels of blood thinning will be improved. This could reduce your chances of having complications from warfarin therapy, reduce your chances of developing blood clots in your blood vessels, or improve the ability to better adjust your warfarin dose, reducing the need for repeat clinic visits.

You will be contributing to scientific knowledge and possibly helping other patients with this condition by what is learned from the study results. By participating in this study, you will be increasing knowledge about how genes work in individuals and how that relates to health and disease.

All of the warfarin that you need for the first 30 days of treatment will be provided by the research study, free of charge. After this time, the study will not be using blinded assignment of warfarin, and you will therefore fill your warfarin prescription like you do any other medications that you are taking.

What happens if you decide not to take part in this research study?

Participation in this research study is voluntary. You do not have to take part in the research project to continue to receive care at [insert name of institution]. If you decide not to take part in this research study, your current and future medical care at [*Insert name of Institution]* will not be affected in any way and you will receive the same standard of heath care given for warfarin therapy.

What if you want to leave the research study after it begins?

Once you start in this research study, you are free to stop at any time. If at any time during the study you choose to withdraw from the study, you will still receive the same health care you would have otherwise received. However, if you stop in the first 4 weeks of the study, the study will not provide your warfarin free of charge. This study is expected to end after all participants have completed the study, and all information has been collected. Your participation in this study may also be stopped at any time by the study doctor or the study Sponsor (NHLBI, NIH), without your consent because:

- The study doctor feels it is necessary for your health or safety. Such an action would not require your consent, but you will be informed if this decision is made, and the reason for this decision.
- New information suggests that taking part in the research study may not be in your best interests.
- You have not followed study instructions.
- The Sponsor or the study doctor has decided to stop the study for any other reason.

Will confidential health information be collected as part of this study?

Yes. We need to collect your health information to conduct this study and we will keep it confidential as required by law.

Authority to Collect Information: The authority to collect this information is under 42 USC [National Heart, Lung, and Blood Institute (NHLBI) – 42 USC 285b]. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. We will ask you to provide information about your medical conditions, treatments, health habits, and the quality of your life. We will collect medical record information related to any hospitalizations and out-patient treatments you receive while participating in this study. We will also collect information from your billing records on the costs related to any hospitalizations and treatment services.

Every attempt will be made to keep your health information private. [Insert text of site specific protected health information (PHI)]

Personal identifying information such as your name, address, and phone number will be collected. It will be used by the study staff to contact you for study related purposes such as scheduling, or to give you health information. This information will only be available to the local study staff members. Your study information will be

given a unique code number. The key to this unique code will be kept in a locked file or a password-protected computer file at [*insert name of institution*].

The University of Pennsylvania serves as the Clinical Trial Coordinating Center for this research study. All of the study information from the research centers, without your identifying information, will be stored in secure computer files at the University of Pennsylvania by unique code. All study information will be sent to the Coordinating Center by secured internet connection.

To help us protect your privacy, a Certificate of Confidentiality from the National Institutes of Health has been obtained. This Certificate makes it much more difficult to force the researchers to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below. The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects.

A Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information. No voluntary disclosure of information that would identify you as a participant in this research study will be made, without your written consent.

Some members of the research project will have permission to see your identifying information in order to ensure that the study is being performed properly. They will be required to keep this information confidential. Authorized representatives of the Sponsor, the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH), and the Institutional Review Board at *(insert name of institution)*, may have access to and may copy medical or research records that identify you by name. This step is necessary to ensure the accuracy of the research findings and your safety and welfare.

During the study, blood samples and your genetic sample will be stored at a Central Laboratory at Washington University in St. Louis, MO under a code number that will not have any personal identifying information. Information about your samples will be kept in a secure computer file that can be used only by authorized staff members.

The results of this study may be shown at meetings or published in journals so other doctors and health professionals know about the study. You will not be identified by name or other personal information in any written publication or presentations about the study. Other researchers who are approved through standard, approved agreements may be permitted to analyze the data without your personal identifying information. This information may include other identifiers such

as dates of medical tests and services, but <u>will not</u> include your name or any other primary identifiers such as address, Social Security number, or Medicare number.

At the end of the study, all forms with your name or other identifying information will be stored in a locked facility at [insert name of institution] for a period of at least XXX [fill in site-specific requirements] years. Only the study doctor or study staff members assisting the doctor will have access to these forms. After XXX five years [to be filled in relevant to institution/State], the forms will be destroyed.

Also at the end of the study, the Coordinating Center will provide to the National Heart, Lung, and Blood Institute (NHLBI) the information collected from the study, without personal identifying information such as your name, address, Social Security number, or Medicare number.

Blood samples taken from you during the study will be considered donated by you to medical research. After the main study has ended, the blood samples will be transferred to a National Heart, Lung, and Blood Institute (NHLBI) laboratory storage center, which saves samples from many research projects around the country to conduct large research studies. Your name or other information that could identify you will not appear on the blood samples.

The study information and/or blood samples may be shared with other scientists who meet NHLBI requirements. These requirements include treating the study information and/or blood samples as medically confidential, obtaining approval from their Human Subjects review boards, and agreeing not to share the information or blood samples with other researchers. NHLBI policies regarding data availability, especially genetic data availability, are subject to change. The investigators will continue to ensure that current NHLBI guidelines are followed.

Study information and blood sample data will be stored in a secure computer file. under a code number that will not have any personal identifying information. The information in this computer file will be available on an Internet database that is available only to researchers who have been approved by the NHLBI and under security standards that are reviewed by researchers and public advocates. Researchers who plan to access coded medical information or other information from the databases will not know who you are nor have access to the code linking genetic data to you.

Only certain study investigators who are working directly with the genetic data will have the master code that links your name with the code number. This master code will be kept in a secure location at [Insert name of institution].

Contacting you about the results of the study

Once the entire study is completed, in <mark>about 1 year</mark>, you will be informed of the results. At that time, we will contact you by phone to obtain your current mailing address so that we may provide you with a written summary of the study results.

Contacting you about the results of genetic testing

Results of your genetic findings from this research study <u>will not </u>be reported to you unless that information would change your medical care. If we find that you have a genetic condition that may have potentially important meaning for your health and treatment, we will contact you if you have given us consent to do so. Results from genetic testing will not be placed in your medical record, or shared in any way with your relatives, personal physician, or insurance companies, unless you request the research staff, in writing, to do so.

What happens to my health information if I leave the study?

You can leave the study at any time or ask that your health information not be used. If you ask that we no longer collect your health information, then you will have to leave the study.

If you choose to leave the study, but will let the researchers use or share your personal health information, you will be asked to fill out a form, called the "Withdrawal from Study" form.

If you do not want us to collect, use or share your health information anymore, you must send a letter to the study doctor. In the letter, you must say you changed your mind and that you will not allow us to use and share your health information anymore. We will then ask you to fill out a form, called a "Withdrawal of Study Participation and Consent/Authorization" form.

Even if you take back your permission for us to use your information, we may still use the information about you that we collected before you left the study. We do this because we need to know what happens to everyone who starts a research study for the study to be valid.

If you leave the study, you can ask that your blood samples be destroyed. You may also ask that your coded medical and genetic information not be released in the future. However, information that has already been distributed will not be able to be recalled.

Financial Costs and Compensation

You will not have to pay to be in this study. All procedures and tests specifically required by the study (for example the genetic tests) will be covered by the study. However, all other procedures and tests that are part of routine medical care (like the INR tests) will need to be covered by you or your medical insurance.

You will receive payment for your participation in this study. You will receive $50 for completing the initial survey and the first 5 days of the study; $50 for completing first 30 days of the study (from days 6 to 30), $50 for completing the next 2 months of the study (up to the 3 month visit), and $50 for completing the

final 3 months of the study (up to the 6 month visit if you are on warfarin for this period of time). The total will be $200 if you complete your study visits and are in the study for 6 months.

Warfarin, the study medication, will be provided free of charge during the first 4 weeks of your participation in the study. After one month, you or your medical insurance will have to pay for it. [*This section should be customized per site. Parking/transportation reimbursement, if provided, should be itemized.*]

What if I get hurt or ill from my participation?

While it is not likely that you will suffer major health problems as a result of your participation in this study, the medical treatment that is a part of this study carries a small risk of serious health problems. Of course, should a problem occur, or should you need emergency medical help, necessary emergency care would be provided and the investigator working with you would help you find a doctor to continue your care if needed. Any cost of medical care that results from such a health problem will be your responsibility and will not be paid for by the National Heart, Lung, and Blood Institute, the study investigators, or the hospital or clinic conducting this study.

New Information

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in the study. We will notify you as soon as possible if such information becomes known.

Contact Persons for Study

If at any time you have questions, concerns, or comments about this study or experience a research-related injury, you should contact [*Insert Principal Investigator's name*] at [*Insert telephone number*].

Institutional Review Boards/Subject Rights

The _____[*Insert your Institution's name*] has a committee called the Institutional Review Board (IRB). It is their responsibility to make sure that the possible benefits of participating in the study are greater than the possible risks and that people in the study are informed about risks and benefits. If you would like more information or have questions about your rights as a research subject, you can contact the Office of Regulatory Affairs at the _____ by phoning _____. [*Insert appropriate information for your clinical site's IRB*]

Statement of Voluntary Participation

I have read the above information about this research study. I have been given an opportunity to ask questions about it and to discuss it with [*Insert Principal Investigator's name or authorized personnel*]. All of my questions/concerns have been answered to my satisfaction. I understand that I need to contact the [*Insert your Institution's name and telephone number*], if I move or change my telephone number. My signature below indicates my voluntary participation in this research study. It also indicates that no procedures associated with this study have been performed on me prior to my signing this consent.

Alternatives to Participation

Genetic testing to study response to warfarin dosing is a requirement of participation in this study**.** Your alternative is not to participate in this research study. Your warfarin treatment will then be determined according to standard medical care.

Refusal or Withdrawal of Participation

I understand that I may refuse to participate or withdraw from the study at any time without consequence to my present or future care at the [*Insert your Institution's name*].

Documentation of Consent

The original and one copy of this consent form will be kept in a research folder and a copy of this Consent Form will be given to you to keep.

Supplement to the COAG Study Informed Consent

Permission for Future Use of Your COAG Blood Sample and Information Collected in the COAG Study

Introduction

Once your participation in the COAG study has ended, we would like to use your genetic blood specimen (sample) and medical information for future research and we request your permission to do this. Allowing your genetic material and medical information to be used in these future studies is voluntary and you can refuse to participate. You do not have to provide this permission in order to be part of the COAG Study.

What will happen if I agree to future use of my specimen and information?

By signing this form, you will allow the National Institutes of Health (NIH) to store and save your blood sample and data in a "sample bank" on a long-term basis and to make decisions about how your samples and data will be used in the future.

The genome-wide association studies and sequencing studies that will allow COAG researchers to search for all possible genetic changes related to your body's response to warfarin, also provide information that can be used to study many other conditions. This is different from studying genetic changes that might relate to your response to warfarin, which is part of the COAG study.

Your information may be useful for genome-wide association studies or other genetic studies of other conditions. This research may include genetic or biology studies that are not about warfarin therapy. Your blood sample also may be used for genetic testing to study genes or other materials in the blood related to other diseases, such as heart disease. Your blood samples may be shared with scientists from private research companies. Another example for future use of your blood sample would be that one or more laboratories selected by the NIH might study your genetic data to identify possible genetic changes that might be related to a particular condition. More tests could be performed on the sample to find out which of those changes are actually associated with disease. Laboratories participating in these future studies will NOT receive personally identifying information on you; they will only receive coded specimens.

How will my identity be protected?

If you agree to participate in this kind of study, your samples and medical information will be coded (assigned a unique study number) to allow the researchers to link your blood sample to the other information that you provide through the COAG study, such as age, gender, race, diagnosis, disease history, medical treatments. Information that might identify you personally will NOT be provided to the researchers. This information will be saved in a computer file along with information from the other research participants. The information in this computer file will be available on an Internet database that is available only

to NIH-approved researchers and under security standards that are reviewed by researchers and public advocates. Researchers who plan to access coded medical information or other information from the databases will not know who you are nor have access to the code linking genetic data to you.

Researchers who plan to use your genetic material for future scientific study will have to request and receive all of the necessary approvals from the National Institute of Health, National Heart, Lung, and Blood Institute before using your sample. Samples will only be released to scientists who are qualified and prepared to conduct a research study and who will follow the confidentiality policy. NIH policies regarding data availability, especially genetic data availability, are subject to change. The investigators will continue to ensure that current NIH guidelines are followed.

Information obtained from the <u>analysis of future research studies</u> will be anonymous and cannot be used to identify you. Information related to these types of research studies may be put in an open Internet database, which means that it will be available to anyone on the Internet.

What are the risks involved in allowing future use of my genetic specimen and information?

There are no physical risks to you. The main risk is that someone could get access to the data we have stored about you. If that data suggested something serious about your health, it could be misused. For example, it could be used to make it harder for you to get or keep a job or insurance. There are laws against this kind of misuse, but they may not give full protection. We believe that the chance of these things ever happening is extremely small. However, we cannot make guarantees. Your privacy and the confidentiality of your data are very important to us and we will make every effort ot protect them. Your name or any other personally identifying information will NOT be used in any published reports from future research performed on your specimen.

What are the benefits of allowing future use of my genetic specimen and information?

Information gained from research on your blood samples may be used for the development of diagnostic procedures or new treatments for major diseases. Your blood samples will not be sold to any person, institution, or company for financial gain or commercial profit. However, neither you nor your heirs will gain financially from discoveries made using the information and/or specimens that you provide. There will be no direct benefit to you from allowing your specimen to be kept and used for future research.

Contacting you in the future

We can only use your information again if special committees called the Institutional Review Boards, let us. These committees may want us to talk to you again before we do another study using your information, or the committees may also let us do research without talking to you again if we keep your health information private. If

these committees do require us to talk to you again, we will attempt to re-contact you to give you an opportunity to participate in future studies that are approved by these committees. You may tell us that you do not wish to be re-contacted. You may also tell us that you do not want us to use your specimen (sample) and information in future studies.

Agreement to Participate in the COAG Study

Instructions: For each permission, please **CIRCLE *"YES"*** or ***"NO"*** and write your initials and today's date in each row where indicated.

1.   I agree to participate in the COAG study, which includes the use of genetic data and measurements of other factors in the blood to study my response to warfarin dosing.

YES Initials: _____ Date: _____   *NO* Initials: _____ Date: _____

2.   I understand that the genetic data collected from me are considered research results. If the research results suggest that I have a genetic condition that may have potentially important meaning for my health and treatment, I agree to allow the COAG study to notify me and with my permission to notify my physician.

YES Initials: _____ Date: _____   *NO* Initials: _____ Date: _____

Agreement for Future Use of My COAG Blood Sample and Information Collected in the COAG Study

Instructions: For each permission, please **CIRCLE *"YES"*** or ***"NO"*** and write your initials and today's date in each row where indicated.

1. I give permission to study my genetics and other biological factors for other health conditions besides response to warfarin therapy.

YES Initials: _____ Date: _____   *NO* Initials: _____ Date: _____

2. I agree to allow future studies to make my genetic and other information available on a controlled access website to approved researchers. Such information cannot be used to identify me. I give permission to have my coded genetic information and coded medical information placed in this special database for use only by approved researchers.

YES Initials: _____ Date: _____   *NO* Initials: _____ Date: _____

3. I agree to allow researchers from private companies to have access to my DNA and genetic data which may be used to develop laboratory tests or pharmaceutical therapies that could benefit other people.

YES Initials: _____ Date: _____   *NO* Initials: _____ Date: _____

6. *I give permission to be contacted in the future to see if I am willing to provide additional biological samples or follow-up information about my health or medical care.*

YES Initials: _____ Date: _____ *NO* Initials: _____ Date: _____

SIGNATURES

I have read and received a copy of this consent form. I understand that my signature below means that I voluntarily agree to participate in this study.

Printed Name	Signature of Participant	Date

Complete ONLY if patient is unable to sign:

Printed Name of Legally	Signature of Legally Authorized	Date
Authorized Representative	Representative	

_____

(Note relationship with participant)

Completed ONLY if patient or their legal representative is unable to read this consent form and an impartial witness is present for the entire discussion:

Printed Name	Signature of Witness	Date

I certify that I have discussed the study purpose, potential benefits, and risks with the below named participant and/or his/her authorized representative, using language that is understandable and appropriate. I have answered any questions that have been raised and have witnessed the signature of this subject. I have explained the information contained in this document to the subject on the date stated on this consent form.

Printed Name	Signature of Person Obtaining Consent	Date