Genetics informatics trial (GIFT) of warfarin to prevent deep vein thrombosis (DVT): rationale and study design

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The risk of venous thromboembolism (VTE) is higher after the total hip or knee replacement surgery than after almost any other surgical procedure; warfarin sodium is commonly prescribed to reduce this peri-operative risk. Warfarin has a narrow therapeutic window with high inter-individual dose variability and can cause hemorrhage. The genetics-informatics trial (GIFT) of warfarin to prevent deep vein thrombosis (DVT) is a 2² factorial-design, randomized controlled trial designed to compare the safety and effectiveness of warfarin-dosing strategies. GIFT will answer two questions: (1) does pharmacogenetic (PGx) dosing reduce the rate of adverse events in orthopedic patients; and (2) is a lower target international normalized ratio (INR) non-inferior to a higher target INR in orthopedic participants? The composite primary endpoint of the trial is symptomatic and asymptomatic VTE (identified on screening ultrasonography), major hemorrhage, INR ≥ 4, and death.

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Introduction

Warfarin sodium and other vitamin K antagonists adjusted to achieve a target INR of 2–3, can be used to prevent and treat venous thromboembolism (VTE, for example, pulmonary embolism or deep venous thrombosis), but are associated with the doubling of hemorrhagic risk.¹ This risk is greatest during the first weeks–months of warfarin therapy,²–⁶ when the therapeutic dose is generally determined by trial and error. To reduce hemorrhagic risk, experts recommend prescribing a predicted therapeutic dose to patients who are beginning warfarin, rather than use a standard loading dose.⁷–⁹ The genetics-informatics trial (GIFT) of warfarin to prevent deep vein thrombosis (DVT) compares two algorithms of predicting the optimal warfarin dose—one using both genetic and clinical information, and the other using clinical factors alone. These algorithms are available through a non-profit web-based application (http://www.WarfarinDosing.org) created by some of the authors. This application tailors warfarin doses to individual participants’ clinical vs clinical plus pharmacogenetic profiles.

Although there is overwhelming evidence that single-nucleotide polymorphisms in the cytochrome P-450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1) genes influence warfarin dose requirements, it is unclear whether prospective pharmacogenetic dosing reduces adverse events.¹⁰,¹¹ CYP2C9 is associated with warfarin inactivation and VKORC1 is
associated with the mechanism of action of warfarin. Two multi-centered, randomized trials in progress (EU-PACT;\textsuperscript{12} COAG NCT00839657) are investigating pharmacogenetic-based warfarin dosing using \textit{VKORC1} and \textit{CYP2C9}. A third gene, cytochrome P-450 4F2 (\textit{CYP4F2}), has also been associated with warfarin dose requirements,\textsuperscript{13} but its effect on warfarin safety has yet to be evaluated. Small, single-centered trials\textsuperscript{14–18} have not detected any reduction in major adverse events from genotyping, but have been underpowered to detect a putative difference.

The first aim of GIFT is to evaluate how pharmacogenetic management affects incidence of adverse outcomes. \textit{GIFT} will recruit elderly, hip and knee arthroplasty participants receiving warfarin prophylaxis. This population is at high risk for both thrombotic and hemorrhagic events. The rate of symptomatic VTE after the hip or knee replacement surgery is much higher than after almost any other procedure,\textsuperscript{19} and these rates are highest in the elderly.\textsuperscript{20} Studying this high-risk population will therefore power \textit{GIFT} to test for a difference in outcomes between pharmacogenetic and clinical warfarin dosing.

The second aim of \textit{GIFT} is to test the safety and effectiveness of two target INR ranges in arthroplasty participants. The American College of Chest Physicians (ACCP) recommends a target INR of 2.5 while the American Academy of Orthopaedic Surgeons (AAOS) recommends a target INR \textless 2.0. The AAOS argues that a lower target INR is safer from the standpoint of hemorrhagic risk, and is non-inferior in preventing pulmonary embolism.\textsuperscript{21} These incongruous therapeutic targets have not been directly compared in arthroplasty participants. Prior studies that compared different INR goals in participants with a history of deep vein thrombosis, (DVT) provided conflicting results. The randomized trial by Ridker \textit{et al}.\textsuperscript{22} showed that an INR target of 1.5–2.0 could prevent VTE recurrence without increased risk of major bleeding compared with placebo. In contrast, Kearon \textit{et al}.\textsuperscript{23} found that an INR of 2–3 was more effective than an INR of 1.5–1.9 in preventing VTE recurrence and equally safe. The Ridker trial, in conjunction with studies showing that target INR values \textless 2.5 are safe and effective in orthopedic participants,\textsuperscript{24,25} has led many orthopedic surgeons to target lower INR values.\textsuperscript{26} The best way to clarify this question is to do a multi-centered trial, as proposed in this study.

**Materials and methods**

**Study population**
\textit{GIFT} plans to enroll 1600 Medicare beneficiaries undergoing elective total hip or knee replacement surgery at Barnes-Jewish Hospital, Intermountain Healthcare, the Hospital for Special Surgery, and University of Utah Health Care. Research coordinators will recruit and obtain written consent from participants before surgery in accordance with the Declaration of Helsinki. Inclusion and exclusion criteria are listed in Table 1. This high-risk population will be screened 4–6 weeks after surgery for asymptomatic DVT by Doppler ultrasound. Assuming an average rate of DVT of 15%, the trial is powered to answer two novel questions: are pharmacogenetic-based dosing and targeting lower INR safer and more effective approaches to the prevention of VTE than clinically based warfarin dosing and traditional INR target range?

**Blinding and randomization**
Using a $2 \times 2$ factorial design, we will randomize participants to each of the following:

1. Pharmacogenetic vs clinical dosing of warfarin.
2. Higher target INR of 2.5 vs a lower target INR of 1.8.

**Table 1 Summary of inclusion and exclusion criteria**

| Inclusion criteria                                      | Exclusion criteria                                                                 |
|---------------------------------------------------------|-----------------------------------------------------------------------------------|
| Elective hip or knee arthroplasty (not hip fracture)    | Currently taking warfarin                                                        |
| > 65 years of age                                       | Incarcerated or institutionalized                                                  |
| Has Medicare Part B                                     | Thrombocytopenia (platelets < 75 K)                                              |
| Warfarin prophylaxis anticipated for at least 4 weeks   | History of venous thromboembolism                                                 |
| Reliable telephone access                               | History of thrombophilia                                                          |
| Willing to give consent (english only)                  | History of major bleeding or bleeding disorder (for example, hemophilia, von Willebrand disease and so on) |
|                                                          | Planned administration of any anti-coagulant other than warfarin (except heparin flushes) |
| Willing/able to have Doppler ultrasound at 4–6 week     | Warfarin genotypes or prior therapeutic warfarin dose known                       |
| follow-up visit                                         | Warfarin allergy                                                                  |
| Baseline INR < 1.35                                     | Unlikely to be compliant (for example, history of non-compliance, substance abuse and so on) |
| Life expectancy > 6 months                              | Planned administration of interacting medications, except those taken into account by http://WarfarinDosing.org* |
|                                                          | Alcoholism not in remission for past 6 months                                     |

Abbreviations: INR, international normalized ratio; VTE, venous thromboembolism.

*Carbamazepine, rifampin, barbiturates, phenytoin.
Randomization will be stratified by site, race and whether participants undergo knee or hip arthroplasty. Lists for block randomization will be prepared in advance by the trial statistician and monitored prospectively. Participants will be randomized after they have been genotyped. Patients and study clinicians will be blinded to patient genotype and study arm, but not to daily warfarin dose. The protocol is to initiate warfarin with similar doses in patients who do or do not have the $\text{CYP2C9}^*$2 or *3 variants. After two warfarin doses, subsequent doses are reduced to accommodate the decreased metabolism of S-warfarin conferred by these alleles for affected patients. These initial doses are based on pharmacokinetic principles\(^{27}\) that we have prospectively validated.\(^{28,29}\) This strategy of prescribing the initial two doses as though all participants were $\text{CYP2C9}^*1^*1$, in combination with the substantial contributions of clinical factors on warfarin dose requirements, should prevent inadvertent unblinding of genotype.

In contrast to genotype, randomization to standard vs lower target INR value will not be double-blinded. Technicians performing screening Doppler ultrasounds and physicians adjudicating outcomes will be blinded to trial arm, genotype and target INR.

**Warfarin dosing**

The research team will be responsible for warfarin dosing for all participants from the time of surgery until the completion of treatment, 4–6 weeks post-operatively. Dosing will be guided by http://www.WarfarinDosing.org for a minimum of the first 11 days of treatment (Figure 1).

Patients whose INR values are therapeutic after day 11 will remain on their predicted maintenance dose; others will have their warfarin dose adjusted empirically. Patients with a target INR of 1.8 will have an INR range of 1.5–2.1, whereas those assigned to a target INR of 2.5 will have an INR range of 2.0–3.0. Beginning with the INR measured after the first 11 days of warfarin therapy, we will dose patients per standard of care, titrated to their target INR.

**Use of http://WarfarinDosing.org**

Although prior studies have used pharmacogenetic or clinical dosing protocols for warfarin initiation, GIFT extends the length of the dosing protocol to the first 11 days of therapy. The GIFT algorithms predict stable warfarin doses over days 1–11 of therapy and incorporate clinical, laboratory and genetic data gathered from sites across North America (including Missouri, New York and Utah), Europe and Asia.\(^{28,30–33}\) The pharmacogenetic models explained 53–73% of the variability in warfarin dose with median absolute dosing errors of \(\leq 7\) mg/wk.

To facilitate dosing strategies for GIFT and for the public at large, we have made the non-profit, decision-support web application, http://www.WarfarinDosing.org, available to the public for investigational use. The website has more than 1000 visitors per week, providing continuous feedback. This extensive testing, feedback and improvements to the website have produced an accurate, reliable and user-friendly tool critical to support pharmacogenetic study and practice. In March 2011, the US Food and Drug

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**Figure 1** Dose estimates from the nonprofit, decision-support web application, www.WarfarinDosing.org
Administration issued an Investigational Device Exemption for use of http://WarfarinDosing.org and participant recruitment began on 24 March.

INR testing will be performed per standard practice—daily for inpatients, bi-weekly for non-therapeutic outpatients and weekly for therapeutic outpatients. Each day that an INR is available, the GIFT research coordinator will enter it into http://WarfarinDosing.org to obtain a daily estimated dose and an estimated maintenance dose to be used subsequently (until the next INR value). When an INR is not drawn, patients will continue to receive their estimated maintenance dose. The website also indicates whether the dose of warfarin given on the day of INR testing should differ from the estimated therapeutic dose—a feature that allows http://WarfarinDosing.org to compensate for missed doses, large doses or other dosing errors.

**Data collection**
In addition to key variables being entered into the study website for randomization and dosing purposes, all study data will be entered into the GIFT database (GIFT DB). Clinical data including the patient’s age, race, gender, height, weight, medications, and medical and surgical history will be obtained at the time of recruitment. Blood samples for research purposes will be collected to support genotyping and ancillary studies. DNA will be extracted from de-identified blood samples collected in EDTA tubes to determine subjects’ genotypes for CYP2C9*2 (430C>T),*3 (1075A>C), CYP4F2 V433M (rs2108622 G>A) and VKORC1–1639 G>A. Study laboratory technicians will input the genotype results into http://www.WarfarinDosing.org. Genotyping for CYP2C9 and VKORC1 will be performed using either the GenMarkDx eSensor genotyping platform or the Simple Probe Warfarin Genotyping Reagents (Idaho Technology, Salt Lake City, UT, USA), and LightCycler instrumentation (Roche, Indianapolis, IN, USA). Genotyping for CYP4F2 will be performed by using either the GenMark eSensor genotyping platform or a melt-curve analysis, with fluorescent resonance energy transfer (FRET) probes, and LightCycler instrumentation (Roche), which was developed by ARUP Laboratories. The Central GIFT Genotyping Laboratory, directed by Dr Eby, will use Pyrosequencing for verification of all genotypes. The Centers for Medicare & Medicare Services (CMS) will reimburse for the genotyping costs using the Coverage with Evidence Development mechanism, as implemented on 5 April 2010 (http://www.cms.gov/transmittals/downloads/R111NCD.pdf).

For brevity purposes, genotyping results will not be available to anyone besides the laboratory technicians. Daily warfarin doses, INR values and other results will be entered into the GIFT DB along with new medications, the results of Doppler ultrasound testing at post-operative week 4–6 and all adverse events. Adverse events will be consistently reported using an electronic case report form.

**Safety**
Following surgery, participants in the hospital will be monitored daily for adverse events, including VTE (DVT or pulmonary embolism), bleeding, stroke or myocardial infarction, by members of the clinical research team. If symptomatic VTE has not been objectively documented during the period of warfarin therapy, participants will have a Doppler ultrasound of their legs at the time of warfarin completion (4–6 weeks after surgery). The Biostatistics and Data Management Core will generate reports for the PI and the Data Safety and Monitoring Board (DSMB) regarding rates of adjudicated outcomes. The DSMB will safeguard study participants’ safety, assess effectiveness of study procedures and monitor the overall conduct of the study. The DSMB is comprised of national experts in clinical trials, cardiology, pharmacogenetics, warfarin research, orthopedics and statistics. The DSMB meets twice annually.

**Primary study outcomes**
The primary outcome for Aim 1 is the composite of VTE, major hemorrhage, INR≥4 or death, and for Aim 2 the composite of non-fatal VTE or death (Table 2). Major hemorrhage includes overt bleeding causing a fall in hemoglobin level of 20 g l

| Aim | Factor | Endpoints, primary | Endpoints, secondary | Hypothesis, primary | Statistical test |
|-----|--------|--------------------|---------------------|---------------------|------------------|
| 1   | Genetic vs. clinical dosing | VTE, major hemorrhage, death, or INR≥4 | INR control | Decreased event rate associated with genetic dosing in whole population and in subgroup whose clinical and genetic predicted doses differ by >1 mg per day. | $\chi^2$-test (partitioning alpha) |
| 2   | Target INR 2.5 vs. 1.8 | VTE or death | INR control, bleeding | The event rate is non-inferior in the lower target INR arm | $\chi^2$-test |

**Abbreviations**: INR, international normalized ratio; VTE, venous thromboembolism.
warfarin for pharmacogenetic vs. clinical dosing and time to supra-therapeutic INR.

Power and statistical analyses
Aim 1. Primary endpoint for clinical vs. pharmacogenetic warfarin dosing. For Aim 1, we will analyze the primary endpoint in the whole population and in the subgroup whose clinical and genetic predicted doses differ by \( \geq 1.0 \text{ mg per day} \) (~50% of the population) using a two-sided \( \chi^2 \)-test. To preserve the type I error rate of this co-primary endpoint, we will partition our alpha for the tests in the whole group and the subgroup, as described below.

If we recruit 1600 participants and have an 18% drop-out rate, we will have 1312 participants left for analysis. Using these figures and an overall type I error rate of 0.05, we have >95% power to detect a difference in the rate of the co-primary composite endpoint.

We estimate VTE rates (defined as clinically overt VTE or ultrasound detected VTE at 4–6 weeks) of 18% in participants randomized to clinical dosing and 15% in participants randomized to genetic dosing. Historically, DVT rates with warfarin therapy after joint arthroplasty are variable, often with rates around 25%. However, because seminal studies (for example,\(^{35,36}\)) screened for DVT using a more sensitive test, venography, we predict a lower DVT rate in GIFT where participants will be evaluated by Doppler ultrasound.

We suspect that the rate of VTE in the subgroup whose clinical and genetic predicted doses differ by \( \geq 1.0 \text{ mg per day} \) will be 1.6 times as high as that in the remaining population. This 1.6-fold increase is an estimate based on a threefold increased risk of adverse events in participants who carry at least one copy of CYP2C9*2 and/or CYP2C9*3, but no clear increase in patients homozygous for VKORC1-1639 AA.\(^{37}\) Major bleeding and death will be uncommon in the trial. In the clinical arm, we anticipate that the rate of major bleeding will be 2.4% and the rate of death will be 1.0%, for a total of 3.4%. In the pharmacogenetic arm, we anticipate the rate of major bleeding or death will be 2.6% (the estimated 32% relative risk reduction in major bleeding is based on a meta-analysis of clinical trials\(^{38}\) and a similar reduction in a large observational study).\(^{39}\) Based on prior research, we estimated the rate of INRs \( \geq 4.0 \) in clinical and pharmacogenetic arms to be 12.3 and 7.4%, respectively.\(^{31}\)

We anticipate that half of the bleeding events will be associated with INRs \( \geq 4.0 \), and account for this correlation in our power calculations.

Alpha partitioning. To preserve a type 1 error rate of 5% for Aim 1, we partitioned the alpha between the whole group and the subgroup analysis, as recommended.\(^{40}\) The subgroup consists of participants for whom pharmacogenetic and clinically predicted (per baseline algorithm\(^{28}\)) doses differ by \( \geq 1.0 \text{ mg per day} \). Due to correlation between outcomes in main study and in the subgroup, Bonferroni splitting would be overly conservative.

Partitioning the alpha in this manner maximizes power for Aim 1 while limiting the overall type I error rate to 0.05.

We elected to partition the alpha \( \text{a priori} \), as it maximizes the power for the test in the whole group, without jeopardizing the power in the subgroup.\(^{40}\) Because the two endpoints are collinear, we used simulation to determine possible pairs of alpha values that preserved the overall 0.05 type 1 error. We selected our alpha value of 0.044 for the tests in the whole group, 0.01 in the subgroup and 0.05 for the total alpha.

Aim 2. Primary endpoint for low vs. high target INR. We hypothesize that orthopedic participants randomized to a target INR of 1.8 will have a rate of VTE or death that is no higher (non-inferior) than those treated with a target INR of 2.5. Using the average of our estimates above, we expect the rate of VTE or death with standard warfarin therapy and Doppler US screening to be 16.5%. We will have 80% power to reject the null hypothesis of a difference greater than 5% (the non-inferiority margin) in the two arms. Patients with different target INRs will be combined when comparing clinically vs pharmacogenetically based dosing, but we will also conduct analyses stratified by target INR values and assess for an interaction.

Contingency plan for statistical analyses
If randomization were to result in an unbalanced distribution of any clinical variable associated with VTE (that is age, body mass index, hormonal replacement therapy or male gender\(^{20}\)), we will adjust for the imbalance using logistic regression.

Secondary study outcomes
Percentage of time in therapeutic range. We will compare the time spent in therapeutic range time in therapeutic range among the first 30 days of warfarin for pharmacogenetic vs clinical dosing in a regression model using linear interpolation of INR values between measurements.\(^{41}\)

Time to first event. We will compare time to first supra-therapeutic INR (the number of days until INR > target INR + 1.5) using the log-rank test or Cox-proportional hazard model, as appropriate. We will censor participants at the time of withdrawal, loss to follow-up or death. Likewise, we will compare time to the first major or minor bleeding event (safety endpoint).

Secondary statistical analysis of primary endpoint. As a secondary outcome, we will rank the components of the composite outcomes in ascending order of importance: INR \( \geq 4.0 \), asymptomatic DVT, symptomatic DVT, major bleed or pulmonary embolism, death and analyze with ordinal logistic regression.

Discussion
Recent studies emphasize the importance of certain genetic markers in explaining inter-individual variation in warfarin requirements (Table 3).\(^{42-44}\) Of particular importance, common single-nucleotide polymorphisms in the CYP2C9 gene (CYP2C9*2 and CYP2C9*3) are associated with...
impaired warfarin metabolism, decreased dose requirements and increased time necessary to achieve stable levels of anticoagulation. Further, single-nucleotide polymorphisms in vitamin K epoxide reductase complex 1 (VKORC1) correlate with warfarin sensitivity. In recognition of the clinical relevance of these genetic variants, the Food and Drug Administration in 2007, and again in 2010, approved increasingly detailed revisions to the labeling for Coumadin, a popular formulation of warfarin, to recommend lower dosing have not been sufficiently powered to determine whether pharmacogenetic testing prevents adverse events. The results from GIFT will illuminate how pharmacogenetic testing significantly reduced the rate of major hemorrhages. Given these conflicting results, clinicians and researchers are uncertain as to whether they should use genetic testing when initiating warfarin therapy.

GIFT will evaluate novel strategies to improve the safety and effectiveness of warfarin therapy and test the safety and effectiveness of different target INR ranges. Although there is evidence of genotypic influence on warfarin requirements, prior studies of pharmacogenetic vs. clinical warfarin dosing have not been sufficiently powered to determine whether pharmacogenetic testing prevents adverse events. The results from GIFT will illuminate how pharmacogenetic management affects INR control and clinical outcomes while testing the non-inferiority of a target INR of 1.8 vs a target INR of 2.5. GIFT is complementary to other studies (Table 3) and has several advantages: larger sample size, inclusion of high-risk participants, systematic screening of study participants for clinical outcomes, consistent availability of genotype before the first warfarin dose, use of CYP4F2 and dosing algorithms that guide warfarin dose for at least the initial 11 days of therapy.

**Conflict of interest**

The authors declare no conflicts of interest.

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