Special Report

A Systems Approach to Designing Effective Clinical Trials Using Simulations

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Background**—**Pharmacogenetics in warfarin clinical trials have failed to show a significant benefit in comparison with standard clinical therapy. This study demonstrates a computational framework to systematically evaluate preclinical trial design of target population, pharmacogenetic algorithms, and dosing protocols to optimize primary outcomes.

Methods and Results**—**We programmatically created an end-to-end framework that systematically evaluates warfarin clinical trial designs. The framework includes options to create a patient population, multiple dosing strategies including genetic-based and nongenetic clinical-based, multiple-dose adjustment protocols, pharmacokinetic/pharmacodynamics modeling and international normalization ratio prediction, and various types of outcome measures. We validated the framework by conducting 1000 simulations of the Applying Pharmacogenetic Algorithms to Individualize Dosing of Warfarin (CoumaGen) clinical trial primary end points. The simulation predicted a mean time in therapeutic range of 70.6% and 72.2% (P=0.47) in the standard and pharmacogenetic arms, respectively. Then, we evaluated another dosing protocol under the same original conditions and found a significant difference in the time in therapeutic range between the pharmacogenetic and standard arm (78.8% versus 73.8%; P=0.0065), respectively.

Conclusions**—**We demonstrate that this simulation framework is useful in the preclinical assessment phase to study and evaluate design options and provide evidence to optimize the clinical trial for patient efficacy and reduced risk. **(*Circulation*. 2013;127:517-526.)**

Key Words: bioinformatics ■ clinical trials ■ individualized medicine ■ modeling ■ pharmacogenetics ■ simulations ■ warfarin

Pharmacogenetic-based warfarin dosing has been shown to most accurately predict individual dose requirements.[1] Surprisingly, clinical trials testing the efficacy of pharmacogenetics-guided dosing in warfarin therapy have yet to demonstrate a decrease in out-of-range international normalization ratios (INRs), improvement of outcome (time in therapeutic range, TTR), or a significant decrease in thromboembolic or hemorrhaging events in comparison with nonpharmacogenetic dosing approaches.[2] The variability in warfarin dosing (>20-fold difference) is strongly influenced by interindividual pharmacokinetics/pharmacodynamics (PK/PD)[3] and pharmacogenetics coupled with the drug's narrow therapeutic index. Nevertheless, warfarin remains the most widely prescribed oral anticoagulant for the prevention and treatment of thromboembolic events,[1] and scientific studies continually suggest that warfarin dosing is the most promising example of personalized medicine. The challenge of warfarin dosing and promise of pharmacogenetics has resulted in >50 algorithmic articles about warfarin therapy ranging from clinical nongenetic maintenance dose algorithms[1,4] to genetic-based maintenance dose algorithms[1,4–7] to dosing nomograms[8–12] to PK/PD models to predict INR[3,13,14] – many evaluated in several races and ethnicities.[15–20]

Three prospective randomized clinical trials compared TTR between pharmacogenetic-based dosing of warfarin and standard clinical dosing.[5,21,22] Although providing compelling evidence that pharmacogenetic-based dosing is beneficial, these 3 trials were underpowered to conclusively demonstrate a benefit of pharmacogenetic-guided therapy.[2,5] To date, the Applying Pharmacogenetic Algorithms to Individualize Dosing of Warfarin (CoumaGen) trial is the largest randomized control trial (n=200) that tested pharmacogenetics-guided warfarin initiation by the use of both CYP2C9 and VKORC1 variants in comparison with standard clinical initiation. The CoumaGen trial failed to demonstrate a significant improvement in the percentage of INRs outside of therapeutic range (1.8–3.2). However, post hoc

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analysis determined a benefit for 2 genotypic subsets of the population.[5]

We hypothesize that the primary reason that warfarin clinical trials have failed to adequately demonstrate the value of pharmacogenetic dosing is the lack of a systematic preclinical method to evaluate the optimal combination of initial dosing (both standard clinical and pharmacogenetic), INR monitoring frequency, and initiation and maintenance protocols with the goal to maximize TTR in a broad assortment of study populations with different individual and population characteristics. Currently, clinical trials are not designed from a systems perspective and fail to take into consideration the entirety of potential treatment options.[23] Salinger et al[24] provided the first pharmacogenetic trial design with the use of existing warfarin data and models to simulate differences in effect sizes and dose adjustments for CYP2C9 poor metabolizers. Our study is distinct in that we validate the framework in the context of an actual clinical trial and demonstrate a systematic approach to predict clinical trial outcomes.

Here, we present the use of a comprehensive warfarin clinical trial simulation framework by using a systems approach to clinical trial design for personalized warfarin treatment. Our approach includes 5 adjustable modeling components to produce a robust in silico clinical trial simulation platform: (i) simulate a patient population, (ii) calculate an initial dose (clinical and pharmacogenetic), (iii) adjust dose by using a protocol, (iv) predict INR via PK/PD modeling, and (v) calculate outcome measurements. We demonstrate the accuracy of this approach by reproducing the CoumaGen clinical trial outcomes, CoumaGen Simulation 1, and then reanalyze the simulation evaluating a new dosing protocol, CoumaGen Simulation 2, to determine whether this new study design is significantly more beneficial for the same population. We demonstrate that this simulation framework is useful in the preclinical assessment phase to study and evaluate design options and provide evidence to optimize the clinical trial for patient efficacy and reduced risk.

Methods

Simulated Patient Population

We simulated a warfarin patient population, referred to as clinical avatars, with the use of a Bayesian model. The prior probabilities for the Bayesian model required a statistical characterization of the patient population including age, sex, weight, height, race, body surface area (calculated from height and weight), smoking status, deep vein thrombosis status, amiodarone use status, and genotypes for

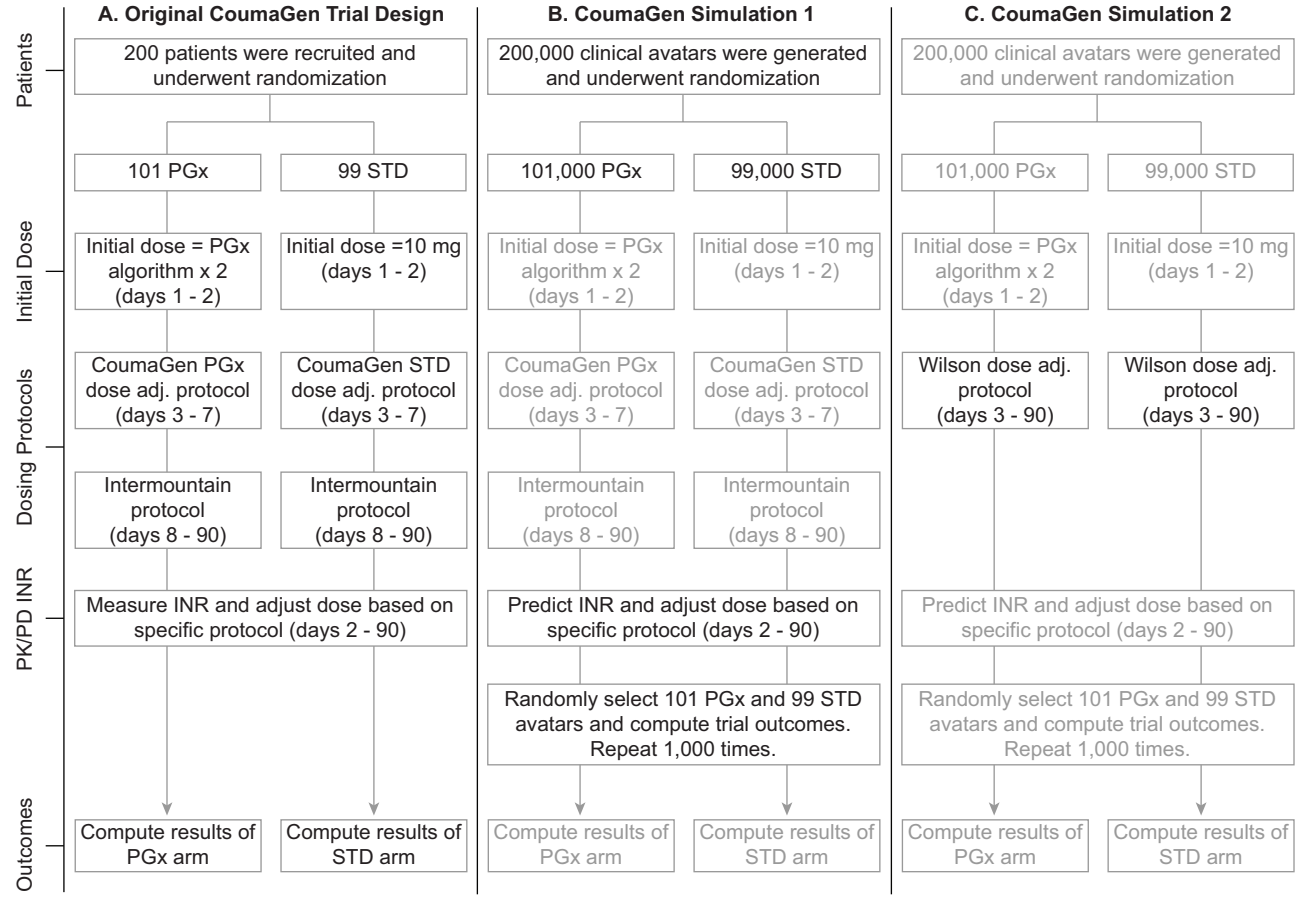

Figure 1. Overview of the simulation framework illustrating the systems approach to clinical trial modeling and prediction. **A**, Study design for the original CoumaGen trial highlighting the 5 major components: patients, initial dose, dosing protocols, PK/PD INR, and outcomes. **B**, Our computational framework to validate the simulations by using the CoumaGen study design. Black text represents new features, whereas gray text represents those features in common with **A**. **C**, Application of the framework by fixing all features (gray text) with the exception of the dosing protocol as in **B**. adj. indicates adjusted; INR, international normalization ratio; PGx, pharmacogenetic arm; PK/PD, pharmacokinetic/pharmacodynamics; and STD, standard clinical arm.

Table 1. Baseline Patient and Clinical Avatar Characteristics and Allelic Variant Frequencies

	CoumaGen Actual		Clinical Avatars	
Characteristics	PGx	STD	PGx	STD
n	101	99	101	99
Age, y (mean)	63.2	58.9	62.5	58.3
Male, %	49.5	56.6	51.3	55.1
Weight, kg, mean±SD	92.1±24.6	94.7±24.2	89.8±24.3	91.9±24.3
DVT, %	18.8	28.3	18.6	28.6
White, %	94.1	94.9	94	95
CYP2C9*2, %				
Wild-type (CC)	82	76.5	82.2	76.7
Het (CT)	18	23.5	17.8	23.3
Hom (TT)	0	0	0	0
CYP2C9*3, %				
Wild-type (AA)	89	87.6	88.9	87.9
Het (AC)	10	11.3	10.2	11.1
Hom (CC)	1	1	1	1
VKORC1 1173, %				
Wild-type (CC)	50.5	34.7	50.5	34.6
Het (CT)	35.4	50	35.6	50.2
Hom (TT)	14.1	15.3	13.9	15.2

Clinical avatars characteristics are based on results from 1000 simulations. DVT indicates deep vein thrombosis; Het, heterozygous; Hom, homozygous; PGx, pharmacogenetic arm; SD, standard deviation; and STD, standard clinical arm.

CYP2C9*2, CYP2C*3, VKORC1-1173, and VKORC1-1639. The CoumaGen trial data used age, sex, weight, height, and genotypes as their variables. We estimated the smoking, deep vein thrombosis, and amiodarone status for white Americans by using data from the Centers for Disease Control and Prevention and the 2000 US Census, because the CoumaGen trial participants were predominately white (95%). To produce physiologically realistic clinical avatars, we used the US Census 2007 to 2008 Table 209 (http://www.census.gov/compendia/statab/2012/tables/12s0210.pdf), which details height and weight distributions as functions of age and sex. Data in this dependency format was not available for the CoumaGen trial, so we transformed the existing normal distributions to match the actual population in the CoumaGen trial. We *z*-transformed the distributions for persons 40 to 49, 50 to 59, and 60 to 69 years of age and scaled them according to the mean and standard deviation for the CoumaGen trial pharmacogenetic and standard arms, respectively. We then produced a dependency table by sampling from these distributions and calculating the percentages for each age/sex category for use in the Bayesian model. The Bayesian model was implemented in TETRAD IV[25] to produce the clinical avatar trial populations and is accessible through our web site (http://clinicalavatars.org).

CoumaGen Warfarin Dosing (CoumaGen Simulation 1)

CoumaGen Simulation 1 followed the dosing schedule as specified in the CoumaGen clinical trial.[5] The standard arm dosing followed the 10-mg warfarin nomogram from Kovacs et al[10] shown to achieve rapid therapeutic INR without an increase in major bleeding or number of INR measurements. Specifically, 10-mg doses were administered on days 1 and 2 followed by dose adjustment based on INR according to the Kovacs protocol for days 3 to 7 (online-only Data Supplement Table I). For days 8 to 90, CoumaGen used the Intermountain Healthcare warfarin dosing protocol (online-only Data Supplement Table II).

The CoumaGen pharmacogenetic arm dosing followed a previously developed regression equation:

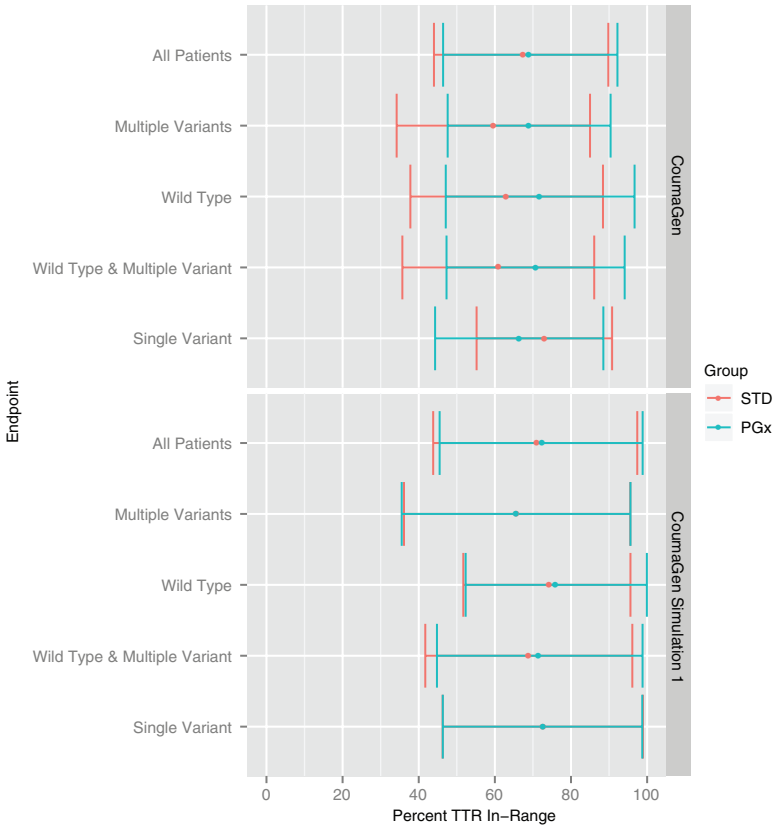

Figure 2. Primary outcomes (TTR) stratified by genotype subsets for the CoumaGen and CoumaGen Simulation 1 studies. Mean (dot) and standard deviation (bars) are plotted for the standard clinical (STD) and pharmacogenetic (PGx) arms. Genotype subsets are grouped according to the original clinical trial. TTR indicates time in therapeutic range.

Table 2. Statistical Characterization of Time in Therapeutic Range for 1000 Clinical Trial Simulations

	CoumGen Simulation 1		CoumaGen Simulation 2	
Characteristics	PGx	STD	PGx	STD
All patients				
Mean±SD*	72.0±26.6	70.5±26.8	78.8±11.9	73.7±13.6
Median	72.0	70.6	78.8	73.7
Range†	63.2–80.2	62.8–78.1	75.1–82.0	70.0–78.1
Wild type				
Mean±SD*	76.0±23.6	73.6±21.8	85.6±10.2	85.6±9.9
Median	76.0	73.6	85.6	85.6
Range†	61.5–88.0	57.6–88.4	80.2–90.9	79.0–91.7
Wild type and multiple variant				
Mean±SD*	71.8±26.9	69.1±27.0	79.9±12.6	72.4±15.8
Median	71.8	69.1	79.9	72.4
Range†	60.7–82.2	58.0–83.0	74.1–85.3	64.5–78.8
Single variant				
Mean±SD*	72.4±26.0	72.4±26.2	76.9±10.4	75.4±10
Median†	72.4	72.4	76.9	75.4
Range†	55.9–85.5	59.8–88.9	71.2–83.2	70.7–80.5
Multiple variant				
Mean±SD*	65.6±29.9	65.9±29.6	71.7±11.2	63.2±12.1
Median	65.6	65.9	71.8	63.2
Range†	47.0–84.2	48.9–83.0	63.6–78.8	55.1–69.9

Results tabulated based on 1000 simulations for each clinical trial. SD indicates standard deviation; PGx, pharmacogenetic arm; STD, standard clinical arm; and TTR, time in therapeutic range.

*Standard deviation reported is the mean standard deviation from 1000 simulations and is representative of the actual observed variation.

†Reported range is for the mean TTR from 1000 simulations.

$$\begin{aligned}&\text{Predicted maintenance dose (mg/d)}\\&= (1.64 + exp\,[3.984 + "*1*1"\,(0)\\&\quad + "*1*2"\,(-0.197) + "*1*3"\,(-0.360)\\&\quad + "*2*2"\,(-0.265) + "*2*3"\,(-0.947)\\&\quad + "*3*3"\,(-1.892) + Vk_{CT(-0.304)}\\&\quad + Vk_{TT(-0.569)} + Vk_{CC(0)}\\&\quad + age(-0.009) + male\_sex(0.094)\\&\quad + female\_sex(0) + weight\_kg(0.0003)])/7 days\_per\_week\end{aligned}$$

Where *1, *2, and *3 refer to CYP2C9 wild type (*1) or variant (*2 or *3) genotypes, respectively, and Vk refers to VKORC1 (C1173T) wild type (CC) or variant (CT or TT). For the initial dose (days 1 and 2), twice the pharmacogenetic dose was administered followed by a dose adjustment based on INR by multiplying standard-arm changes by a pharmacogenetic algorithm coefficient for days 3 to 7 (online-only Data Supplement Table III). The pharmacogenetic algorithm coefficient was defined as the ratio of the estimated individual weekly dose determined by the pharmacogenetic algorithm to the standard weekly dose of 35 mg. For days 8 to 90, CoumaGen used the Intermountain Healthcare warfarin dosing protocol.

Wilson Warfarin Dosing (CoumaGen Simulation 2)

The Wilson warfarin-dosing simulation was executed using a third dosing protocol based on the nomogram by Wilson et al[12] (online-only Data Supplement Table IV). The protocol used the same starting doses for days 1 to 2 as in the CoumaGen trial, 10-mg/d for the standard arm and 2 times the pharmacogenetic dose for the pharmacogenetic arm. For days 3 to 90, the protocol increased or decreased the dose proportionally based on low or high INR values, respectively.

Dose to Pill Conversion

All predicted doses were converted to the closest pill combination by taking the minimum distance between the dose value and the nearest pill combination. We assumed up to 3 pills selected from any of the following standard doses: 0.5, 1, 2, 2.5, 3, 4, 5, 6, and 7.5 mg/d. The maximum dose was set to 15 mg/d. Possible dose combinations ranged from 0 to 15 mg in 0.5-mg increments.

The Predictive INR Model

We extended a previously developed INR model based on PK/PD principles to model daily warfarin dosing for each clinical avatar.[14] In brief, a PK/PD model was estimated from a clinical trial of 150 Italian patients. We only considered the PK/PD effects of S-warfarin because it is 3 to 5 times more potent than R-warfarin.[3,24] We modeled the pharmacokinetic effects with the use of a 2-compartment model with first-order input and first-order elimination and modeled the pharmacodynamics effects by the use of a 2-chain transit compartment model. We implemented the INR model in R with the use of the published parameters. We used a random log normal distribution to estimate the variability of the S-warfarin clearance rate, the volume of the central compartment, EC_{50} value for VKORC1 genotype, and the volume of the peripheral compartment, because the covariance data were unavailable. To represent reasonable physiological ranges, we restricted the values to be within the 25th and 75th percentiles. We also corrected errors in the published INR model (personal correspondence with Hamberg AK, 2010). Specifically, we set the age effect on S-warfarin clearance to −0.91% change per year and the short transit chain rate constant to K_{tr2} = 6/mean transit time$_2$. The predicted daily concentration over time was solved directly by using a PK/PD equation for a 2-compartment model.[26] We set the bioavailability of warfarin to 0.9 and reduced the dose by half because we only considered S-warfarin. The PK/PD equation included a time component in hours, which we calculated for the entire duration of the trial (90 days × 24 hours/d = 2160 hours). The remaining parameters were set according to the original model. We use linear superposition[27] to reflect the fact that the metabolic half-life of warfarin is ≈60 hours and included the residual warfarin concentration plus the current daily dose when modeling dose concentration over time. Superpositioning assumes that each dose of the drug acts independently and that the rate and extent of absorption and the average systemic clearance are the same for each dosing interval, and that linear pharmacokinetic applies.[27] We recorded hourly warfarin concentrations (rows) by day (columns) and summed across the rows at 24-hour intervals to estimate the daily residual warfarin concentration. We used the S-warfarin concentration in combination with the estimated parameters to solve a system of coupled nonlinear ordinary differential equations, using the deSolve package[28] in R, to calculate the daily INR value for each avatar.

Measuring End Points

The primary end point we considered was TTR. We defined the TTR as the percentage of time an individual avatar had an INR between 1.8 and 3.2, as specified in the CoumaGen trial, during the 90-day simulation. Although we calculated daily INR values, we only considered those days in which the INR would have been checked in the clinic according to the specific protocol. We stratified the clinical avatars based on genotypes and performed the same calculation.

Clinical Trial Simulations

CoumaGen was a randomized controlled trial between pharmacogenetic and clinical-based (empirical without genetics) warfarin dosing in patients initiated on oral anticoagulation. The study recruited 101 patients into the pharmacogenetic (PGx) arm and 99 patients into the standard clinical (STD) arm. We created a sufficient number

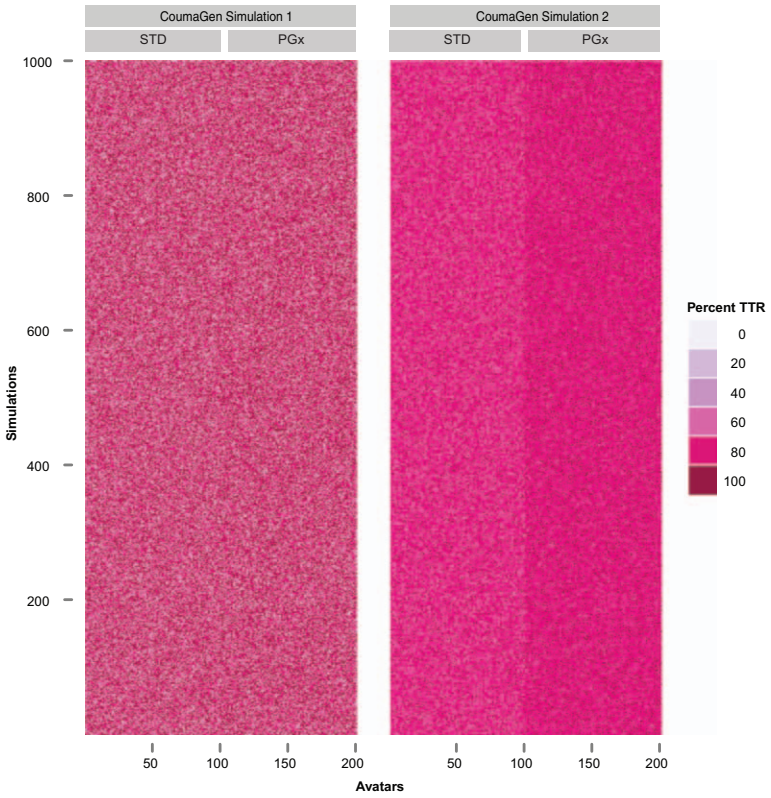

Figure 3. Heat maps representing the TTR for each of the 200 avatars across all 1000 clinical trial simulations for both CoumaGen Simulation 1 and 2 stratified by study arm, standard clinical (STD) and pharmacogenetic (PGx). The percentage of TTR was scaled between 0 to 1 for plotting purposes, where higher values indicate higher percent TTR (darker color). TTR indicates time in therapeutic range.

of clinical avatars (n=200 000) to conduct 1000 simulations of the CoumaGen clinical trial. From this large population we randomly recruited 101 avatars for the PGx arm and 99 for the STD arm. We then simulated daily dose and INR for each avatar following a specific protocol for that arm for 90 days. We repeated this process 1000 times in parallel. We recorded doses, INR, INR-monitoring frequency, and population statistics for each clinical trial simulation and across all 1000 simulations to calculate and produce the mean, standard deviation, and probability value (unpaired *t* test) for TTR for each study arm. We also calculated the mean of the means to produce robust aggregated population results from the 1000 clinical trial simulations (Table 1). Importantly, the model was not fit to the actual CoumaGen outcome data or predicted end points. The simulations produced predictions, which were then directly compared with the CoumaGen results. We implemented all simulations and calculations in R[29] by using our cloud computing environment.[30]

Statistical Analysis

Where appropriate, we computed means, standard deviations, and quantiles to statistically characterize CoumaGen actual, clinical avatar, and simulation results. A log-linear model fit by iterative proportional fitting was used to estimate expected cell frequencies to verify that the structure of the data representing the simulated population of avatars matched the expected structure from the Bayesian model.[31] We compared the percent TTR between the CoumaGen Simulation 1 and CoumaGen Simulation 2 results with the use of an unpaired *t* test. Median, 5th, and 95th percentiles were plotted in the examination of the distribution of INRs and dose over time, as well.

Genotype and Metabolizer Subsets

Allele types for CYP2C9 *2 are defined as: wild type, C/C; single variant, C/T or T/C; and multiple variant, T/T. Allele types for CYP2C9 *3 are: wild type, A/A; single variant, A/C or C/A; and multiple variant, C/C. Allele types for VKORC1 1173 are: wild type, C/C; single variant, C/T or T/C; and multiple variant, T/T. CYP2C9 metabolizer status was defined as extensive or normal for wild-type homozygous alleles, intermediate for heterozygous alleles, and poor for homozygous variant alleles.

Results

Simulation Framework Overview

We used a systems approach to develop a warfarin clinical trial simulation framework to predict trial outcomes for a specific patient population following a specific treatment protocol. The framework is modular and incorporates options for creating a patient population, multiple dosing strategies including genetic-based and nongenetic clinical-based, multiple-dose adjustment protocols, PK/PD modeling, and INR prediction, and various types of outcome measures, as well. We programmatically combined these components, which were derived from previously published studies, to create an end-to-end framework that systematically evaluates warfarin clinical trial designs. We then used the simulation framework to conduct 2 clinical trial simulations: CoumaGen Simulation 1 and CoumaGen Simulation 2 depicted in Figure 1.

Clinical Avatar Population Validation

We used iterative proportional fitting to show that the clinical avatar population produced by the Bayesian model was statistically similar to the CoumaGen population. Iterative proportional fitting estimates the contingency table expected values (in each table cell) based on the marginal totals of the table. We used this method to compare estimated and actual contingency tables generated by permutations of the variables in the avatar population. Specifically, we directly tested that a variable dependency prescribed in the Bayesian model of the population was also present in the simulated clinical avatar population. To confirm that the dependencies we prescribed during clinical avatar generation persisted in the resulting data, we fit a log-linear model to all relevant associations and

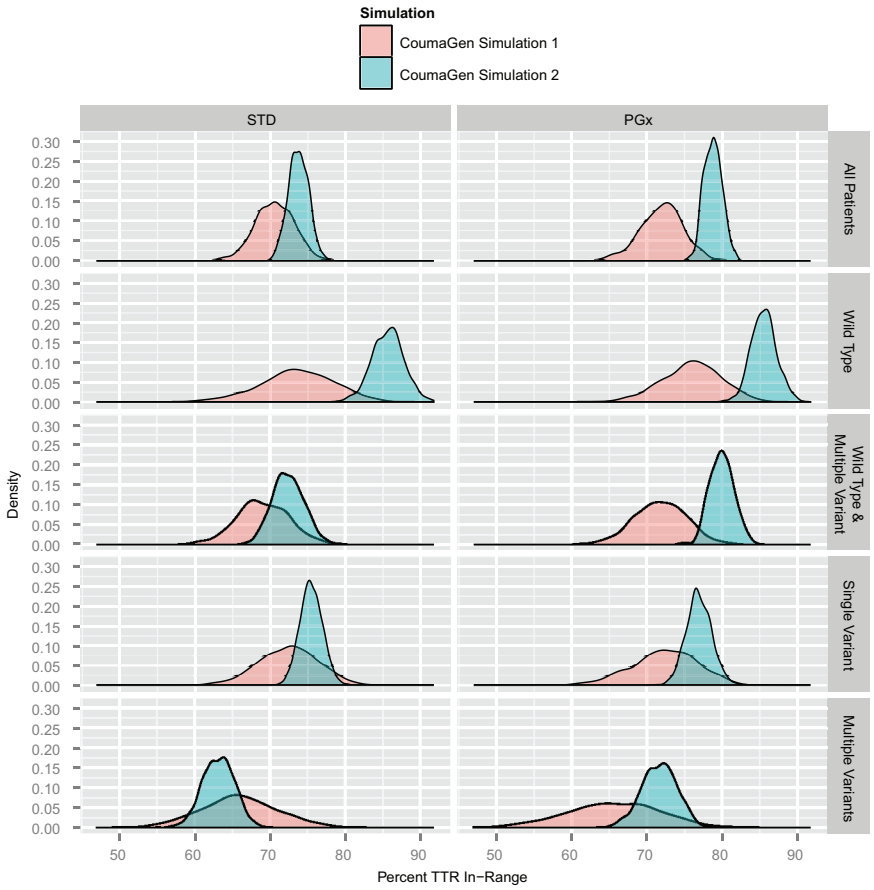

Figure 4. Density plots of the primary outcomes (TTR) for each study arm, standard clinical (STD) and pharmacogenetic (PGx), stratified by genotype subsets for the CoumaGen Simulation 1 and 2 studies. There is less variance in the CoumaGen Simulation 2 than in the Simulation 1 because of differences in protocol designs. Genotype subsets are ordered from extensive metabolizer (wild type) to poor metabolizer (multiple variants) status and illustrate the sensitivity of the model to predict changes in TTR corresponding to known genetic factors. TTR indicates time in therapeutic range.

calculated probability values by using the Pearson χ^2 statistic. The test indicated that there were no significant differences ($P \leq 0.05$) between the dependencies in the clinical avatar population and the actual CoumaGen population (Table 1). In addition, we confirmed that nonprescribed dependencies were not present in the clinical avatar data.

Warfarin-INR PK/PD Model Validation

We validated the extended PK/PD model by reproducing the S-warfarin concentration and genotype with age graphs from the original article.[14] For the S-warfarin concentration, we created 1000 clinical avatars, all 71 years of age with wild-type genotypes (CYP2C9, *1/*1; VKORC1, G/G) and a unique set of PK/PD parameters. We administered a single 10-mg dose and simulated the predicted concentration over 72 hours. We visually compared our simulated S-warfarin concentration with the original plot of actual patient data (online-only Data Supplement Figure I). At 12 hours, our simulation captures the majority of actual patient data with an S-warfarin concentration between 0.2 and 0.4 mg/L and again at 36 hours (0.1–0.25 mg/L) and 60 hours (0.025–0.15 mg/L). The model also captures the variation observed in the actual patient population, indicating the validity of the PK/PD parameters. Next, we created avatars to match the genotype and age properties (online-only Data Supplement Figure II). A comparison between the resulting plots and the original published plots indicated qualitatively consistent INR values over time for each age/genotype combination. The results also indicate that *1/*1 individuals behave as expected with INR values between 2 and 3, whereas *3/*3 individuals are more sensitive to warfarin dose and have higher INRs, as expected.

Clinical Trial Simulation Validation

We validated the entire framework by simulating the CoumaGen clinical trial, CoumaGen Simulation 1 (Figure 1A and 1B) and performed a quantitative and qualitative comparison. The CoumaGen Simulation 1 accurately reproduced the primary TTR outcome of the CoumaGen trial (Figure 2), predicting a mean TTR of 70.6% and 72.0% in the STD and PGx arms, respectively. As in the CoumaGen trial, the mean difference was not statistically significant ($P_{\text{simulation}}$=0.47 versus P_{CoumaGen}=0.47). Analysis of the predicted results by genotype subsets (see Methods) demonstrated similar agreement with the CoumaGen results, with no statistical difference between predicted and actual TTRs for multiple variants, wild type, and single variant subsets (P>0.05). CoumaGen Simulation 1 indicated a nonsignificant 2.7% reduction in out-of-range INRs ($P_{\text{simulation}}$=0.44) for the wild type and multiple variants subgroup in the PGx arm in comparison with CoumaGen, which reported a significant 9.8% reduction in out-of-range INRs (P_{CoumaGen}=0.03) in the same groups. The number of INR measurements for the CoumaGen Simulation 1 was accurately predicted to be 10±1.4 (mean±SD) days for both arms, a difference that was not significant ($P_{\text{simulation}}$=0.38). The simulation predicted ≈1 additional INR measurement in comparison with the CoumaGen trial, which reported 7.2±2.3 and 8.1±3.5 days for the PGx and STD arms, respectively.

Redesign of CoumaGen Using a Systems Approach

We sought to test if a rationally motivated modification of the CoumaGen dosing protocol would result in a significant difference in outcome between the PGx and STD arms (Figure 1C). Consequently, we fixed all model and simulation components of the CoumaGen Simulation 1 – avatars, initial dosing, PK/PD parameters, and TTR outcome calculations, with the exception of the replacement of part of the CoumaGen dosing protocol with the Wilson protocol for days 3 to 90. We reran the entire simulation, called CoumaGen Simulation 2, and calculated the outcomes exactly as in the first simulation (Table 2). The mean TTR for the PGx arm was significantly higher than the STD arm in the CoumaGen Simulation 2 (78.8% versus 73.7%; P=0.0065, respectively), demonstrating that the Wilson protocol, which adjusts dose based on percentage change, predicted better management of the clinical avatars and was able to achieve a stable TTR for a longer period of time. The population-wide difference between the 2 study designs is clearly illustrated with a heat map of the percentage of TTR across all 1000 simulations for the CoumaGen Simulation 1 and 2 (Figure 3).

The CoumaGen Simulation *2* PGx protocol resulted in a higher mean TTR across all the genotype subsets than the corresponding CoumaGen Simulation 1 PGx protocol (Figure 4).

For all patients, the difference between the STD arms in the CoumaGen Simulation 1 and 2 was 3.1%, indicating similar TTR results despite different protocols. Conversely, the difference in TTR for the PGx arm was 6.8% higher, indicating that the Wilson protocol was more accurate at maintaining a therapeutic dose within the 90-day clinical trial time window. The CoumaGen Simulation 2 also exhibited a smaller TTR standard deviation for each genotype subset than the CoumaGen Simulation 1, indicating that the INR range was better controlled by the use of the Wilson protocol.

We further explored the differences between the 2 simulations by plotting the predicted INR for 90 days stratified by CYP2C9 genotypes (Figure 5). The predicted INR for extensive metabolizers (wild type *1/*1) and poor metabolizers (*3/*3) exhibit known behavior. That is, for *1/*1 patients, INR was well controlled within the therapeutic range (1.8 < INR < 3.2) for both STD and PGx arms. For the *3/*3 patients, we observed that the INR response rose to dangerously high levels (INR > 4), owing to decreased metabolism resulting in high circulating warfarin. There was little difference in the INR response between the STD and PGx arm for the CoumaGen Simulation 1. We also observed that the CoumaGen dosing protocol tended to optimize INR closer to 3, which may explain the lower TTR, because more avatars were closer to the cutoff of 3.2. The CoumaGen Simulation 2, using the Wilson protocol, tended to optimize INR closer to 2.5, resulting in a higher overall TTR. The avatars on the CoumaGen Simulation 2 reach therapeutic range faster, but their INRs overshoot before quickly adjusting to therapeutic range.

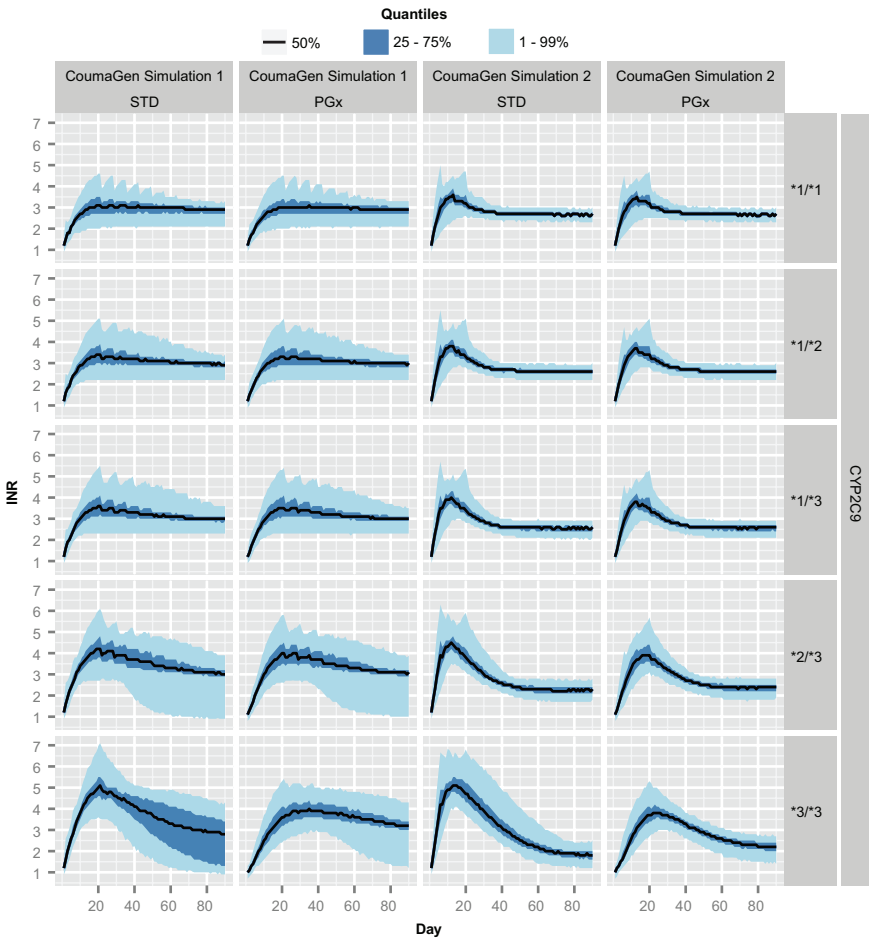

Figure 5. Quantile plot of predicted INR for each study arm, standard clinical (STD) and pharmacogenetic (PGx), for both CoumaGen Simulation 1 and 2 stratified by CYP2C9 genotypes. The INR increases and decreases directly correspond to changes in the dosing as specified in the dose-adjustment protocols. CYP2C9 genotypes are ordered from extensive (*1/*1) to poor (*3/*3) metabolizer status. INR indicates international normalization ratio.

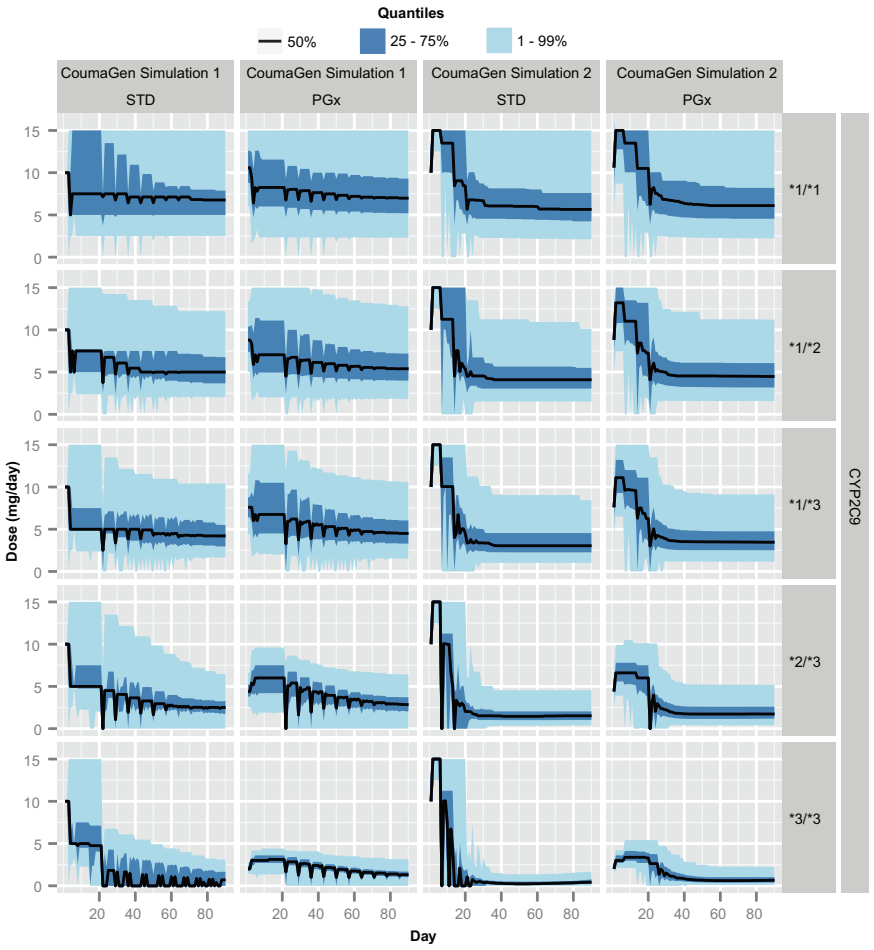

Figure 6. Quantile plot of predicted dose (mg/d) for each study arm, standard clinical (STD) and pharmacogenetic (PGx), for both CoumaGen Simulation 1 and 2 stratified by CYP2C9 genotypes. The dosing increases and decreases directly correspond with dosing changes specified in the dose-adjustment protocols. CYP2C9 genotypes are ordered from extensive (*1/*1) to poor (*3/*3) metabolizer status.

Next, we examined differences in dosing between the 2 simulations stratified by CYP2C9 genotypes (Figure 6). The dosing plots for the CoumaGen Simulation 1 demonstrate the conservative nature of the CoumaGen protocol, resulting in many relatively small dose changes and lengthening the time to reach therapeutic range. Conversely, the Wilson protocol, used in CoumaGen Simulation 2, was more aggressive in response to out-of-range INRs and was able to quickly achieve therapeutic range.

Finally, to gain further insight into the simulation framework, we conducted a broad sensitivity analysis (online-only Data Supplement Figures III and IV). Overall, the simulation framework is sensitive to genotypic changes in CYP2C9 and VKORC1 and different drug-dosing protocols. The framework is relatively insensitive to changes in age. These results are consistent with our expectations based on the underlying simulation models. In addition, Figures 4 through 6 illustrate the sensitivity of TTR, INR, and dose, respectively, to simulation type by CYP2C9 genotype. In general, the TTR for CoumaGen Simulation 1 exhibits higher variance and lower TTR across all genotype subsets than CoumaGen Simulation 2. Figures 5 and 6 allow comparisons between INR and dosing response both within and across groupings. For example, it is possible to observe a decrease in dose owing to high INR. Based on this analysis, we conclude that the modeling and simulation framework is robust and the predictions are qualitatively consistent with current physiological and genetic knowledge.

Discussion

We present a new systems approach to model, simulate, and predict outcomes of clinical trials. We demonstrate the utility and accuracy of the system, first by simulating the CoumaGen clinical trial and then by comparing the predicted results to the actual CoumaGen outcomes. Subsequently, we used the modular nature of the simulation framework to replace the more conservative CoumaGen protocol with the relatively more aggressive Wilson protocol and resimulated the CoumaGen clinical trial design with the new dosing protocol. Our initial simulation results validated the framework and are consistent with the original CoumaGen trial, which failed to show a significant improvement in TTR for pharmacogenetic-guided dosing. When we replaced the original 3- to 90-day CoumaGen dosing protocol with the Wilson protocol, we predicted significantly higher TTR (P=0.0065) in the PGx arm than in the STD arm.

Our simulation framework can compare standard therapy with testable alternative therapies and provide trialists or regulators with a tool to examine a trial's TTR under a variety of different assumptions. For example, when conducting a noninferiority trial comparing novel oral anticoagulants to warfarin, regulatory authorities scrutinize TTR to assess whether the constancy assumption was satisfied. This was evident in the ROCKET-AF (Rivaroxaban once daily, oral, direct factor Xa inhibition Compared with vitamin K antagonism for prevention of stroke and Embolism Trial in Atrial

Fibrillation) trial (NCT00403767) comparing rivaroxaban with warfarin.[32] There was considerable controversy regarding the low TTR (55%) for the warfarin control arm as not satisfying the constancy assumption in comparison with the 62% to 73% TTR observed in other recent clinical trials.[33] This prompted the Food and Drug Administration Advisory Committee to not recommend rivaroxaban for approval. The ROCKET-AF study argued that the primary reason for the lower TTR, in comparison with previous warfarin clinical trials, was due to differences in the study population. The Food and Drug Administration disagreed with the Advisory Committee's recommendation and approved rivaroxaban for stroke reduction in people with atrial fibrillation in November 2011. Using our framework, we could adjust the population parameters to compare previous trial populations with the ROCKET-AF population, conduct side-by-side simulations, estimate expected TTR in both cases, and provide statistical evidence to address the controversy. We note that, in our simulations of the CoumaGen trial, the observed TTR fell within the expected ranges of warfarin clinical trials (70% to 78%).

Despite its clinical efficacy and low cost, warfarin has many well-known limitations, including numerous interactions with other drugs, regular INR monitoring, and the need for multiple dose adjustments. Thus, noninferior, easier to administer, oral anticoagulants are an attractive option for clinicians and patients.[34] However, it has been observed that, when warfarin is used skillfully, the advantage of newer agents diminishes.[33] Our results suggest that the limitations of warfarin may be ameliorated if a more systematic approach was used to evaluate all possible dosing protocols and select optimal INR monitoring.

There are several areas to improve in this modeling and simulation system. First, the PK/PD model for warfarin metabolism was validated by the use of published data from an Italian population of white origin. Therefore, corresponding clearance rates used in this study may not accurately reflect US or other clinical trial populations. In addition, the clearance rates used for this simulation for the *2/*2 (n=5), *2/*3 (n=4), and *3/*3 (n=2) CYP2C9 genotypes are based on limited data, and the clearance rate for the *2/*2 genotype is lower than *2/*3 despite population-wide evidence to the contrary. In a recent update with more data, the values are corrected[3] but still may not generalize to other populations. Second, the 2 simulations assume perfect compliance by patients and physicians, which is not often accomplished in practice. Future versions of our framework will incorporate various rates of noncompliance. Third, the results of the simulations of the CoumGen Simulation 2 are predictions and have not been confirmed in an actual clinical trial.

In summary, we developed a systems-based clinical trial simulation framework for warfarin dosing and validated the framework against the CoumaGen trial. We demonstrate the utility of the framework by simulating the same clinical trial with the use of a relatively more aggressive dosing protocol and predict that the PGx arm is likely to perform significantly better than the STD arm. Our results suggest that the effect and value of personalized pharmacogenetic warfarin dosing requires careful preclinical trial design testing. The results indicate the importance of selecting the correct study population to fully realize the benefits of pharmacogenetic-guided dosing. Simulations are able to predict where and why a trial may fail to achieve its primary end point, and, through the iterative modeling process, we can assess alternative strategies such as different dosing protocols, study population, or outcome metrics. As individualized evidence-based drug and treatment plans continue to emerge, we envision an increasing need for pretrial modeling and simulations to predict outcomes and guide clinical trial development to achieve the best possible outcome.

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Disclosures

None.

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