Pharmacogenomic trial design: use of a PK/PD model to explore warfarin dosing interventions through clinical trial simulation

David H. Salinger[a], Danny D. Shen[b], Kenneth Thummel[c], Ann K. Wittkowsky[b], Paolo Vicini[a] and David L. Veenstra[b]

Objective **Variants of two genes, *CYP2C9* and *VKORC1*, explain approximately one third of variability in warfarin maintenance dose requirements. However, the clinical utility of using this information in addition to clinical and demographic data ('pharmacogenomic-guidance') is unclear, as few comparative clinical trials have been conducted to date. The objective of this study was to explore the incremental effect of pharmacogenomic-guided warfarin dosing under various conditions using clinical trial simulation.**

Methods **We used an existing pharmacokinetic/pharmacodynamic model to perform clinical trial simulations of pharmacogenomic-guided versus standard of care warfarin therapy. The primary outcome was the percentage of patient time spent in therapeutic range over the first month of therapy. We assessed the influence of the frequency of INR monitoring, and the use of a loading dose and dose increase delay in patients with *CYP2C9* variants.**

Results **Pharmacogenomic guidance resulted in a 3–4 percentage point absolute increase in time spent in therapeutic range over the first month of therapy compared with standard of care. The improvement in time in range was greater when the frequency of INR monitoring in both arms was assumed to be lower. The absolute difference increased to 6–8 percentage points with the use of a loading dose and dose increase delay in patients with a *CYP2C9* variant.**

Conclusion **Our initial results imply that pharmacogenomic-guided warfarin dosing may be more useful in settings with less intensive patient follow-up, and when adjustments are made for slower therapeutic response in patients with a *CYP2C9* variant. Further pharmacokinetic/pharmacodynamic model development may be useful for warfarin pharmacogenomic trial design.**

Pharmacogenetics and Genomics 2009, **19**:965–971

Keywords: pharmacodynamic, pharmacogenomic, pharmacokinetic, trial simulation, warfarin

Departments of [a]Bioengineering, [b]Pharmacy and [c]Pharmaceutics, University of Washington, Seattle, Washington, USA

Correspondence to David L. Veenstra, PharmD, Phd, University of Washington, Department of Pharmacy, Box 357630, Seattle, WA 98195, USA
Tel: +1 206 221 6936; fax: +1 206 299 9303; e-mail: veenstra@u.washington.edu

Current address: David H. Salinger, Amgen Inc., Seattle, Washington, USA; Paolo Vicini, Pfizer Global Research and Development, La Jolla, California, USA

Received 28 March 2009 **Accepted** 21 September 2009

Introduction

Warfarin is effective in reducing the risk of thromboembolic events, but is also one of the most common causes of serious adverse drug events [1]. Variants of *CYP2C9* and *VKORC1* have been shown to explain approximately one third of the variability in warfarin dose requirements, and *CYP2C9* variants have been associated with a higher risk of serious bleeding events [2,3]. Although there is strong evidence that genetics influence warfarin dose, there are limited data on the clinical impact of using this information in addition to clinical and demographic data to guide warfarin therapy. Anderson *et al.* reported no difference in time in therapeutic range (TTR) between pharmacogenomic-guided and standard dose initiation in an intensively monitored population, whereas Caraco *et al.* found a decrease in time to first therapeutic INR [4,5]. The optimal care setting and implementation strategies for pharmacogenomic (PGx)-guided dosing are not yet clear [6].

Pharmacokinetic–pharmacodynamic (PK/PD) models that incorporate the respective effects of *CYP2C9* and *VKORC1* variants on drug serum levels and INR have been developed for dosing of warfarin [7,8]. The implementation of such models within a dynamic dosing and monitoring framework affords a means of assessing the potential impact of PGx-guided dosing upon various measures of clinical outcome. We conducted a set of clinical trial simulations with the following objectives: (i) to estimate the magnitude of the effect size for PGx-guided versus standard care warfarin dose initiation, (ii) to explore the influence of INR monitoring frequency on the effect size, and (iii) to assess the potential benefits of dosing adjustments that account for the slower therapeutic response in patients with a *CYP2C9* variant.

Methods

A warfarin clinical trial simulation tool was developed as an R interface that calls upon the nonlinear mixed effects

1744-6872 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/FPC.0b013e3283333b80

modeling software NONMEM [9,10]. The simulator was designed with the capability of adjusting dosing and follow-up appointment scheduling based on a simulated participant's INR at each clinical appointment. We used a recently published population model of S-warfarin pharmacokinetics and pharmacodynamics developed by Hamberg and colleagues [8] to simulate warfarin concentration and effect, as well as variability, in a hypothetical clinical trial population. Participant demographics, including baseline INR, age, and *CYP2C9* and *VKORC1* genotype, were simulated based on the median and range for the continuous variables or fractions for the categorical distribution reported by Hamberg and colleagues. Each participant's dosing history, appointment schedule and INR were tracked during the simulated protocol. We did not assess the effects of parameter uncertainty in our simulations; in part because parameter uncertainty was low (generally $< 10\%$ standard error) compared with variability (26–99% coefficient of variation), so the expected effect on predictions was limited [11] and in part due to the lack of access to the full parameter estimate covariance matrix from the Hamberg analysis.

Simulation outcome

The primary outcome was the average percentage of time patients spent in the therapeutic range (INR 2–3) over the first month of therapy. The percentages of time spent above and below therapeutic range were also assessed. The amount of time patients spent above/in/below INR range was interpolated from INRs at individual clinic visits using the technique of Rosendaal *et al*. [12,13]. This method provides a more informative measure of patient exposure to over anticoagulation and under anticoagulation than the proportion of INR values above and below range, respectively, at specific clinic visits.

Warfarin initiation

We compared the following warfarin initiation protocols. In the 'standard' initiation, all hypothetical participants received 5 mg/day for days 1–4 if under age 75 years, and 2.5 mg/day otherwise. In 'pharmacogenomic' (PGx) initiation, day 1–4 doses were specified based on *CYP2C9* and *VKORC1* genotype as well as age of each hypothetical participant (Table 1) [14]. Although the initiation equation specified dosing based on patient weight, the Hamberg PK/PD model did not identify weight as an important covariate in warfarin PK–PD, and so we assumed a weight of 150 lb for all the participants [8].

Table 1 Pharmacogenomics-based dose initiation algorithm [14]

Factor	Dose adjustment
Base dose	5.67 mg/day
Age (years)	− 0.05*(age 61)
Weight (lbs)	+ 0.01*(WT 191)
CYP2C9 (each *2)	− 1.04*(number of *2)
CYP2C9 (each *3)	− 1.14*(number of *3)
VKORC1 (GG)	+ 1.85
VKORC1 (AA)	− 2.33

CYP2C9 adjustments as compared with *1/*1. *VKORC1* adjustments as compared with AG. Minimum dose 0.5 mg/day.

Warfarin maintenance

We adapted an algorithm developed by Wilson and colleagues (Table 2), which features moderately frequent INR monitoring and is reflective of a maintenance protocol for patients in an anticoagulation clinic setting [15]. We simulated dosing and INR over the first month of therapy. For comparison purpose, we implemented two parallel algorithms, which we refer to as 'more frequent' and 'less frequent' monitoring – corresponding to the lower and upper values of the follow-up range in the Wilson algorithm. All participants received follow-up appointments on days 4 and 8 to determine doses 4 to 7 and from 8 to 30, respectively. After day 8, all appointments were individually specified based on INR.

Trial simulation

For each protocol, Monte Carlo simulation was performed for 2000 participants and the percent of time participants were above/in/below INR range (INR 'in range' defined as 2–3) was assessed. Results were stratified based on patient's genotype: (a) patients with *CYP2C9* variants and any *VKORC1* genotype; (b) patients with *VKORC1* variants and *CYP2C9* wild type; (c) wild type for both *CYP2C9* and *VKORC1*; and (d) all patients. These groups are not mutually exclusive, and were defined to explore the relative effects of *CYP2C9* variants, which affect warfarin clearance and half-life, and *VKORC1* variants, which do not.

To explore the effect of the trial size, we performed multiple trial simulation of 200, 500, 1000, and 2000 participant trials. Owing to lengthy run times, we simulated 1000, 400, 200, and 100 trials of each size, respectively. The standard deviations (SDs) of percent above/in/below range time results (or standard error of

Table 2 Dose adjustment algorithm

		INR recheck (days) frequency	
INR	Suggested dose adjustment (for maintenance of INR in 2–3 range)	More	Less
≤ 1.3	Increase dose by 50%	5	7
1.4	Increase dose by 33%	5	7
1.5–1.8	Increase dose by 25%	5	7
1.9	Increase dose by 10%	7	14
2.0–2.8	No change	7[a]	14[a]
2.9–3.1	Reduce dose by 10%	7	14
3.2–3.5	Reduce dose by 25%	5	7
3.6–3.7	Reduce dose by 33%	5	7
3.8–4.4	Skip 1 day, reduce dose by 33%	3	7
4.5–5.0	Skip 2 days, reduce dose by 33%	3	7
5.1–6.0	Skip 3 days, reduce dose by 33%	3	7
≥ 6.1	Determined by physician[b]	–	–

Adapted from [15].
[a]Fourteen to twenty-eight days in the Wilson algorithm [15].
[b]Not implemented, treated as for INR 5.1–6.0.

the mean) were computed for each trial size. The SDs provide estimates of between-trial variability for trials of each sample size. Comparable results were independently obtained with a less computationally intensive approach based on bootstrap runs from a simulated pool of 2000 participants (results not shown).

Simulation strategy

The warfarin protocol simulation routine was written using a modular architecture as a function call in the R programming language with system calls to NONMEM for Monte Carlo pharmacokinetic/pharmacodynamic simulation and a system call to SED (*http://sed.sourceforge.net*) for changing the random seed in the NONMEM control file. After initial dosing and prescheduled appointments, each simulated participant receives individual appointment scheduling and dose adjustment, based on the specified protocol, for the duration of the simulation.

The simulation function allows the user to set simple inputs such as the number of participants to simulate, the number of days to simulate the protocol, random seeds for population simulation from both demographic and pharmacokinetic/dynamic variability, and prescheduled appointments that all study participants receive (e.g. days 4 and 8 to adjust doses 4–7 and 8 until the next individually scheduled appointment; scheduling is based on simulated INR before the appointment). More complex inputs to the simulation function include a list of dosing algorithms (functions) that collectively specify the current protocol (e.g. initiation, transition, and maintenance routines) and a function specifying when to switch to the next dosing algorithm (either for all participants based on protocol day or individually based on current INR or time in range).

The initial modules of the simulation create the NONMEM 'dataset,' which specifies for each participant the dose and observation schedule as well as demographic covariates (such as age, baseline INR, and *CYP2C9* and *VKORC1* variant) which are sampled from specified covariate distributions. The initial doses are specified based on the initiation algorithm and a default dose (to be changed as the simulation progresses) is specified for all later dose times. The dose amounts in this initial dataset are updated as the simulation progresses and doses are adjusted. Individual appointment schedules are specified and tracked through an array in R.

Once the initial dataset is constructed, the individual inputs to the pharmacokinetic and pharmacodynamic models are fully specified. NONMEM is then called to choose individual pharmacokinetic and pharmacodynamic model parameters (from within the specified distributions) and run the time-dependent simulation. The output includes daily INR values for each participant. For each day of the simulation, each participant with a scheduled appointment receives adjustment to future doses (through the appropriate dose selection algorithm) and a scheduled future appointment. The new doses are then updated in the dataset and the simulation is then run forward. Each participant with an appointment on the next simulation day then receives adjustment to future doses and a scheduled future appointment. For the multiple trial simulations, the trial simulation function is wrapped in a loop within which the appropriate random seeds are changed for each trial (thus simulating a new study population) and the running mean and SD of results (time above/in/below range) are computed.

Protocols simulated

The following protocols were simulated:

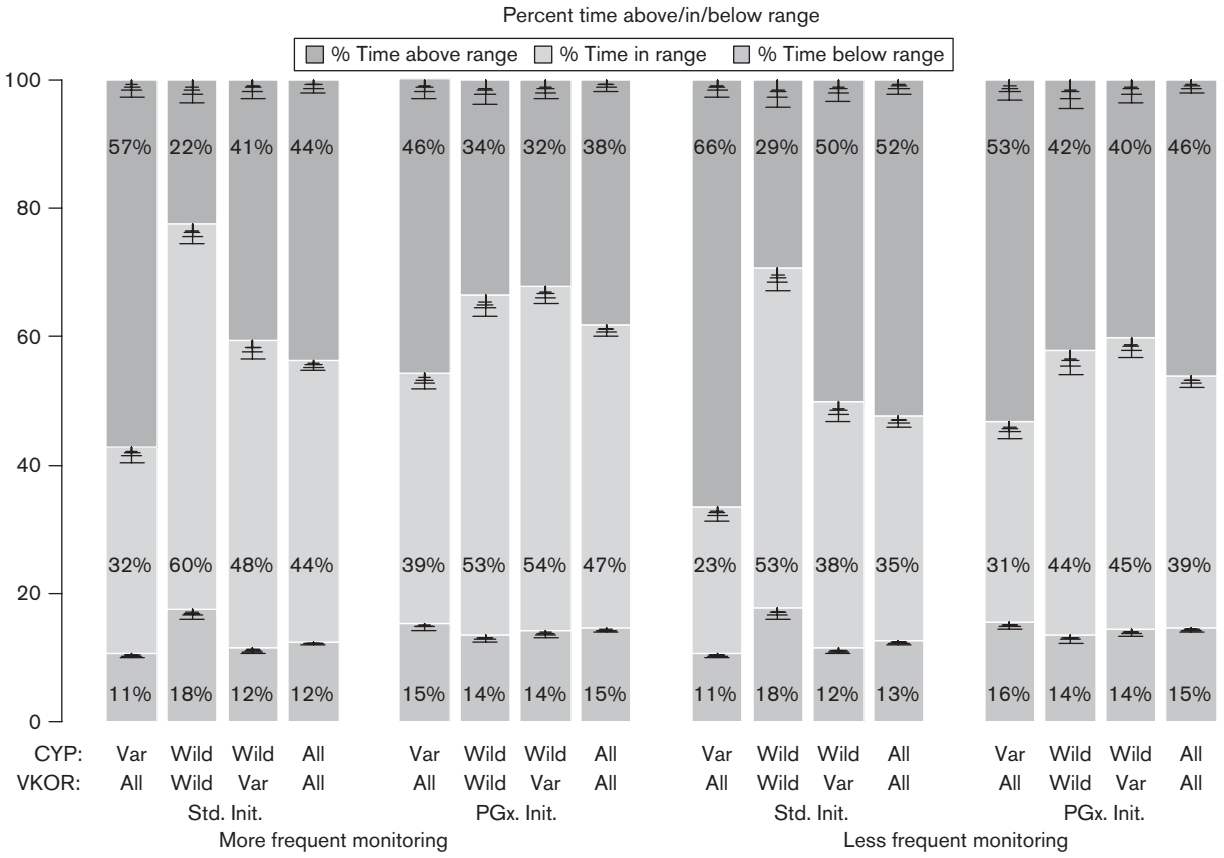
(1) Base-case protocol: standard dose initiation versus PGx-guided dose initiation, as described above, with more-frequent and less-frequent monitoring.
(2) A protocol similar to (1), except *CYP2C9* variants under the PGx initiation strategy received a loading dose $2\times$ the predicted maintenance dose on days 1–2 only, and no dose increase for the first 18 days (dose decrease was allowed).
(3) As a test of the effect of dose timing compliance, dosing times were allowed to vary randomly (normally distributed) with an SD of 4 h.
(4) As a test of the effect of missed doses, 10% of doses in the simulated population were skipped at random.
(5) To explore the impact of within-participant variability (drift) due, for example, to dietary variation (e.g. vitamin K intake), we allowed each participant's prescribed dose to wander in a random walk, starting at a dose multiplier of 1 (i.e. no change) with step chosen uniformly from the interval $(-0.025, 0.025)$ and with ultimate dose multiplier bounds of $(0.9, 1.1)$.

Results

The primary results are shown in Fig. 1: standard versus pharmacogenomic initiation (PGx), each with more frequent or less frequent monitoring. The results for each protocol are stratified by genotype. The vertical bars represent the percent time over the first month of therapy spent above, in, and below therapeutic range.

The key findings that can be extracted from this figure are as follows. First, under the more frequent monitoring scenario, the percentage of TTR for all patients was 44% for standard initiation versus 47% in the PGx initiation arm (fourth and eighth columns, respectively). In contrast, under the less frequent monitoring scenario, the TTR for both the standard and PGx arms were lower (35 and 39%, respectively). Thus the absolute increase in TTR due to PGx initiation was slightly larger in the less frequent monitoring scenario compared with the more frequent monitoring scenario (4Δ vs. $3\Delta\%$, $\Delta\%$ = absolute

Fig. 1



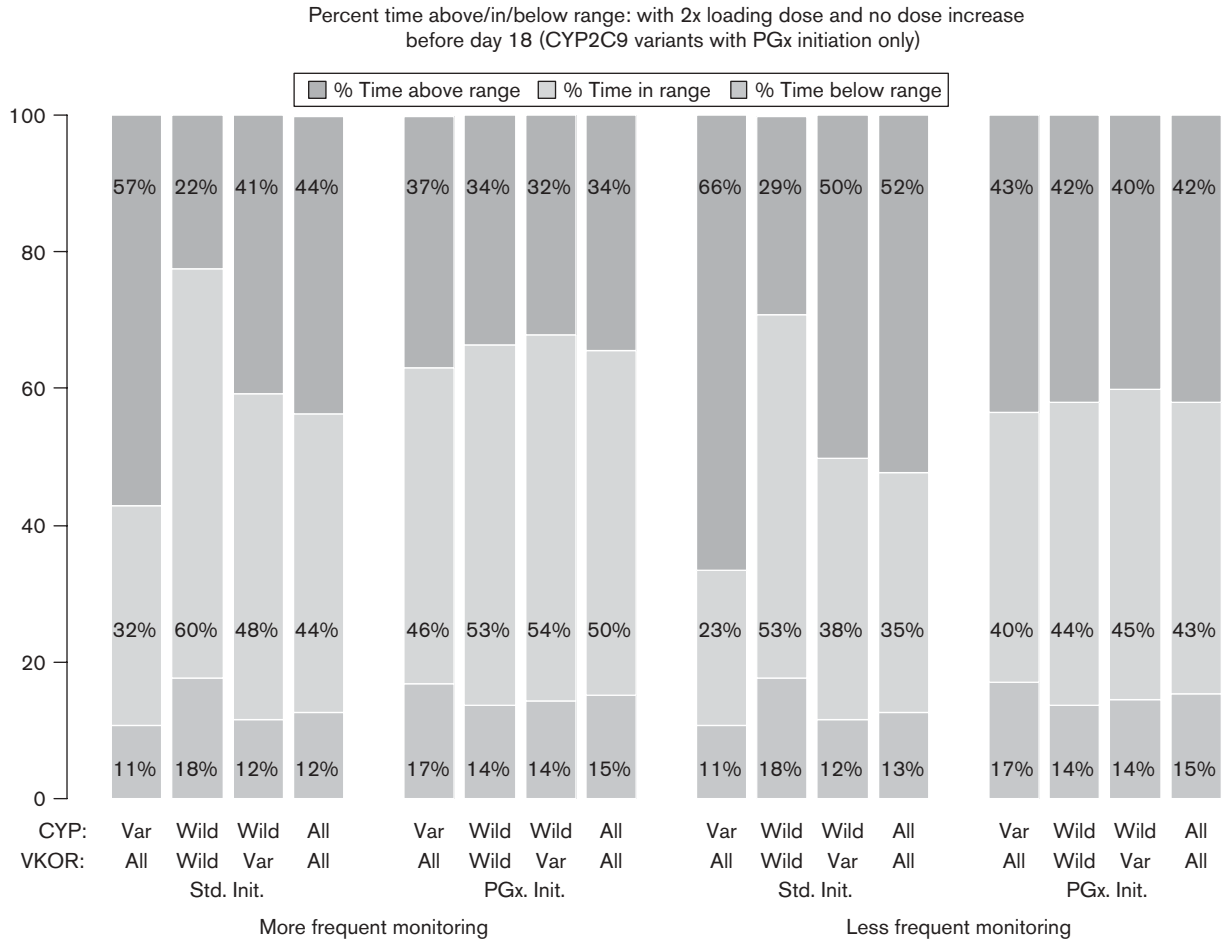
Trial simulation results from standard (Std.) versus pharmacogenomic-based (PGx-based) dose initiation (Init.) under two monitoring scenarios, over the first month of therapy. Each column shows time in, above, and below range for all 2000 simulated participants, as well as three subgroups, based on *CYP2C9* and *VKORC1* genetic polymorphisms. Each pair of clusters shows results for more and less frequent monitoring, respectively. Both more or less frequent monitoring trials are each initiated by either standard or PGx-based initiation of therapy. Error bars visualize standard deviation for population sizes of 200, 500, 1000, and 2000 (corresponding to decreasing error bars) (see text for further details).

change in %TTR). Second, the TTR improved for *VKORC1* variants by 6–7Δ% with PGx initiation. In contrast, TTR decreased for *CYP2C9*/*VKORC1* wild type/wild-type patients by 7–9Δ% with PGx initiation, despite higher starting doses of 6.0–8.4 mg. Third, the % time above range for *CYP2C9* variants was lower with PGx versus standard initiation by 11Δ% (46 vs. 57%) and 13Δ% (53 vs. 66%) in the more and less frequent monitoring protocols, respectively. Furthermore, in the standard versus PGx initiation protocols, 3.5 versus 1.0% of patient time was above an INR of 5 (more frequent monitoring) and 8.4 versus 3.8% (less frequent monitoring). Finally, the average number of clinic visits was 5.4 versus 5.2 (more frequent monitoring) and 3.8 versus 3.7 (less frequent monitoring) for standard and PGx initiation, respectively.

The results of protocol (2) in which patients with a *CYP2C9* variant were administered a 2X loading dose and did not have any dose increases for the first 18 days are shown in Fig. 2. The TTR for all patients in the PGx initiation simulations was 50 and 43% for more and less frequent monitoring, respectively, as compared with 47% ($\pm$0.6% for 2000-participant or $\pm$1.7% for 200-participant trials) and 39% ($\pm$0.7% for 2000-participant or $\pm$1.8% for 200-participant trials) for the base-case simulation. Compared with the base-case simulation (Fig. 1), this represents a doubling of the difference between PGx and standard initiation strategies (i.e. from 3–4Δ% to 6–8Δ%). These differences are within the simulation variability for the small ($N = 200$) trials, but not for the larger ($N = 2000$) trial. The improvement was primarily the result of a decrease in the % time above range for *CYP2C9* variants. However, the approximately 20% absolute decrease in time above range for patients with *CYP2C9* variants is offset to some degree by an approximately 6% absolute increase in the time below range – thus there is potentially a slight increase in thromboembolic risk.

In protocol (3) in which dose times were allowed to vary randomly, the results were nearly unchanged from the

Fig. 2



Trial simulation results similar to those in Fig. 1, except *CYP2C9* variants in the PGx-based initiation of therapy arm received double the predicted maintenance dose days 1–2 and no dose adjustment for the first 18 days (see text and Fig. 1 for further details). Init., initiation; Std., standard.

base-case simulation. When 10% of doses were skipped at random (protocol 4), the %TTR increased by 1–3% as a result of a small decrease in the % time above range for patients across genotype categories. Finally, in protocol (5) in which each participant's prescribed dose was allowed to wander in a random walk, the %TTR across all patients was slightly decreased (e.g. $< 1\%$).

Discussion

Comparison with observed data

The percent of TTR over the first month of therapy simulated in this study for standard initiation, 35–44%, is somewhat lower than what has been observed over the first month of therapy both in our outpatient academic medical center anticoagulation clinic (50%) and in a first-month analysis of the COUMAGEN trial (48%) [5,14]. The difference between the simulated and clinical data are primarily driven by a greater % time above range for *CYP2C9* variants in the simulations, 57–66%, versus 25–37% observed. These results imply that clinician specialists may be managing patients with *CYP2C9* variants more effectively than strict adherence to a simulated maintenance protocol. Overall, in the COUMAGEN randomized controlled trial, genotyping did not improve TTR, with 49.8% in the PGx group and 51.9% in the standard of care group, $P = 0.54$. These results again suggest that clinicians may be able to adapt to the impact of *CYP2C9* variants on warfarin dose and clearance in a way that is not reflected by the algorithm simulated in this study. Of note, however, 80% of patients in the COUMAGEN trial were inpatients and had INR monitored daily during initiation. Comparison with results of Caraco *et al.* is difficult because %TTR was reported between day 9 and 'stable' anticoagulation, rather than over a fixed time period [4].

Limitations

There are several important limitations of our study worth noting. There likely were systematic differences in the nature of the simulated individuals compared

with patients in the actual clinical studies. The simulated individuals were reflective of those in the Hamberg study, whereas observed data were from anticoagulation patients with potentially different clinical and demographic characteristics. The lack of reported correlation of the covariates could potentially cause systematic overestimation of demographic variability in our simulated participants.

The PK/PD model on which we based our analysis was developed using 150 patients, and likely did not have sufficient sampling of all patient subgroups [8]. For instance, the model suggests that *2/*2 patients have a greater pharmacodynamic response (INR) to warfarin than *2/*3 patients, which is in contrast to mechanistic and observed data [7,16]. In addition, weight was not found to be a predictive covariate, although dose prediction algorithms and PK/PD models have recognized it to be significant [2,17].

We found simulation of the dynamic, individualized doses and dose scheduling inherent with warfarin therapy to be challenging. In the early stage of our simulation work, several maintenance dosing and monitoring protocols were evaluated [5,18]. We found that implementation of protocols with less frequent (e.g. 7–10 days) INR monitoring for patients with an INR in or near therapeutic range during the first 1–2 weeks of therapy led to poor INR control over the first month of therapy. However, continual simulation of periods beyond 1 month led to extremely tight INR control, which also does not match clinical experience or literature data. A more realistic model, possibly incorporating variables such as intra-participant PK variability due to diet, etc., may be ultimately needed for realistic simulations beyond a 1-month time period. Given these limitations, the findings of this study should be considered exploratory in nature.

We allowed dose time to vary with an SD of 4 h to assess dose–time variation, but we did not account for decreased adherence in terms of proportion of dosing actually taken. Investigation of such effects is warranted, but beyond the scope of this study. Furthermore, although not evaluated in this study, an additional strategy to improve anticoagulation outcomes would be the use of a PK/PD model to individualize dosing, or algorithms that incorporate initial INR measurements with genotype information [8,19].

Implications

Our findings suggest that the effect of PGx initiation may be relatively small, but potentially clinically relevant in certain populations, such as those monitored less frequently than in an anticoagulation clinic. The clinical implications can be estimated based on epidemiologic studies of INR and the risk of clinical events. For example, in a recent study, van Walraven and colleagues [20] assessed 6400 patient years of anticoagulant exposure. Using their estimates for the risk of bleeding and thromboembolic events above, within, and below INR range, we estimate that pharmacogenomic-guided dosing in 1000 patients would avert approximately one bleed, with little difference in clotting events.

The results also imply that using information about the slower therapeutic response in patients with a *CYP2C9* variant may improve the effectiveness of the intervention. Furthermore, given the ability to identify patients that require a lower dose, and in addition, the subset of those that will be slow to respond to warfarin, the benefit-risk tradeoff of using a loading dose may be improved [18].

Our findings may have implications for trial design. For instance, using standard statistical power calculations (at 80% power), a trial with %TTR in the first month of therapy as the primary outcome, and assuming a %TTR of 39% in the standard care arm and 43% in the PGx arm based on the trial simulations in this study, would require 4800 patients. The simulation results using a $2\times$ loading dose and dose increase delay (39 and 47% %TTR) would imply a trial of 1200 patients.

Conclusion

The use of quantitative modeling approaches to explore trial design issues for pharmacogenomic interventions, involving dosage and monitoring refinements, offers significant promise. Further investment in PK/PD models and trial simulation approaches is warranted.

Acknowledgements

This study was supported in part by Grant# P41EB0019/5 (NIH National Institute or Biomedical Imaging and Bioengineering), Centers for Disease Control (CDC) Seed Funding for Public Health Genomics Research (DLV), Grant No. 1U18 GD000005-01 (CDC Office of Genomics and Disease Prevention), and the University of Washington Drug Metabolism, Transport, and Pharmacogenomic Research (DMTPR) Program (supported by unrestricted gifts from Abbott, Allergan, Amgen, Bend Research, Bristol-Myers Squibb, Eli Lilly, Hoffman La Roche, Johnson & Johnson, and Pfizer).

References

1 Gage BF, Lesko LJ. Pharmacogenetics of warfarin: regulatory, scientific, and clinical issues. *J Thromb Thrombolysis* 2008; **25**:45–51.
2 International Warfarin Pharmacogenetics Consortium. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med* 2009; **360**:753–764.
3 Limdi NA, Veenstra DL. Warfarin pharmacogenetics. *Pharmacotherapy* 2008; **28**:1084–1097.
4 Caraco Y, Blotnick S, Muszkat M. CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study. *Clin Pharmacol Ther* 2008; **83**:460–470.
5 Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, *et al*. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. *Circulation* 2007; **116**:2563–2570.

6 Eckman MH, Rosand J, Greenberg SM, Gage BF. Cost-effectiveness of using pharmacogenetic information in warfarin dosing for patients with nonvalvular atrial fibrillation. *Ann Intern Med* 2009; **150**:73–83.

7 Linder MW, Looney S, Adams JE III, Johnson N, Antonino-Green D, Lacefield N, *et al*. Warfarin dose adjustments based on CYP2C9 genetic polymorphisms. *J Thromb Thrombolysis* 2002; **14**:227–232.

8 Hamberg AK, Dahl ML, Barban M, Scordo MG, Wadelius M, Pengo V, *et al*. A PK–PD model for predicting the impact of age, CYP2C9, and VKORC1 genotype on individualization of warfarin therapy. *Clin Pharmacol Ther* 2007; **81**:529–538.

9 Team RDC. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing; 2007.

10 Beal SL, Sheiner LB, Boeckmann AJ. *NONMEM users guides 1989–2006*. Ellicott City, Maryland: Icon Development Solutions; 2006.

11 Samtani MN, Perez-Ruixo JJ, Brown KH, Cerneus D, Molloy CJ. Pharmacokinetic and pharmacodynamic modeling of pegylated thrombopoietin mimetic peptide (PEG-TPOm) after single intravenous dose administration in healthy subjects. *J Clin Pharmacol* 2009; **49**:336–350.

12 Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. *Thromb Haemost* 1993; **69**:236–239.

13 Torn M, Cannegieter SC, Bollen WL, van der Meer FJ, van der Wall EE, Rosendaal FR, *et al*. Optimal level of oral anticoagulant therapy for the prevention of arterial thrombosis in patients with mechanical heart valve prostheses, atrial fibrillation, or myocardial infarction: a prospective study of 4202 patients. *Arch Intern Med* 2009; **169**:1203–1209.

14 Meckley LM, Wittkowsky AK, Rieder MJ, Rettie AE, Veenstra DL. An analysis of the relative effects of VKORC1 and CYP2C9 variants on anticoagulation related outcomes in warfarin-treated patients. *Thromb Haemost* 2008; **100**:229–239.

15 Wilson SE, Costantini L, Crowther MA. Paper-based dosing algorithms for maintenance of warfarin anticoagulation. *J Thromb Thrombolysis* 2007; **23**:195–198.

16 Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, Rettie AE. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 2002; **287**:1690–1698.

17 Tham LS, Goh BC, Nafziger A, Guo JY, Wang LZ, Soong R, Lee SC. A warfarin-dosing model in Asians that uses single-nucleotide polymorphisms in vitamin K epoxide reductase complex and cytochrome P450 2C9. *Clin Pharmacol Ther* 2006; **80**:346–355.

18 Kovacs MJ, Rodger M, Anderson DR, Morrow B, Kells G, Kovacs J, *et al*. Comparison of 10-mg and 5-mg warfarin initiation nomograms together with low-molecular-weight heparin for outpatient treatment of acute venous thromboembolism. A randomized, double-blind, controlled trial. *Ann Intern Med* 2003; **138**:714–719.

19 Gage BF, Eby C, Johnson JA, Deych E, Rieder MJ, Ridker PM, *et al*. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. *Clin Pharmacol Ther* 2008; **84**:326–331.

20 Van Walraven C, Oake N, Wells PS, Forster AJ. Burden of potentially avoidable anticoagulant-associated hemorrhagic and thromboembolic events in the elderly. *Chest* 2007; **131**:1508–1515.