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ORIGINAL ARTICLE

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Effect of *CYP2D6* genetic polymorphism on the population pharmacokinetics of tipifarnib

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Abstract *Objective:* Evaluate the effect of *CYP2D6* genotype on the pharmacokinetics of tipifarnib. *Methods:* A total of 268 subjects included in six clinical trials were treated orally with tablet formulation of tipifarnib, as a single dose or as multiple b.i.d. doses (range 50–600 mg), and/or intravenously following 1, 2, and 24 h infusions. A total of 2,575 tipifarnib concentrations were fitted to an open three-compartment linear disposition model with sequential zero-order input into the depot compartment, followed by a first-order absorption process, and lag time, using NONMEM V. The effect of *CYP2D6* genotype was explored as a covariate for tipifarnib systemic clearance and absolute bioavailability. Likelihood ratio test was used to compare these parameters in homozygous extensive metabolizers (EM) ($N=152$), heterozygous EM ($N=97$), or poor metabolizers (PM) ($N=19$). Computer simulations were undertaken to explore the *CYP2D6* genotype effect on the tipifarnib pharmacokinetics. *Results:* The ratio of tipifarnib systemic clearance for the heterozygous EM and the PM subjects, relative to the homozygous EM group, were 0.95 (95%CI 0.87–1.03) and 0.96 (95%CI 0.82–1.11), respectively ($\chi^2=2.376$, $df=2$, $P=0.305$). The ratio of tipifarnib absolute bioavailability for the heterozygous EM and the PM, relative to the homozygous EM, were 1.06 (95%CI 0.83–1.30) and 0.95 (95%CI 0.55–1.34), respectively ($\chi^2=1.398$, $df=2$, $P=0.497$). *Conclusions:* These results indicate that *CYP2D6* genetic polymorphism does not appreciably influence the pharmacokinetics of tipifarnib. Hence, concomitant administration of potent *CYP2D6* inhibitors is anticipated to have little or no significant impact on the systemic exposure to tipifarnib.

Keywords Genetic polymorphism · CYP2D6 · Pharmacokinetics · Tipifarnib

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Introduction

Tipifarnib (R115777, Zarnestra®) is a potent, selective and competitive inhibitor of the enzyme farnesyltransferase (FTase) [1, 2]. This enzyme is important in the processing and activation of signaling molecules linked to cell proliferation and malignant transformation, such as Ras, Rho-B, Rac, and lamin proteins [1]. Inhibition of FTase by tipifarnib induces antileukemic and antitumoral activity, which has been demonstrated in both in vitro and in vivo animal models [2]. Tipifarnib is believed to exert its antitumor activity, at least in part, by preventing the farnesylation of proteins involved in mechanisms that extend beyond Ras, including RhoB, G-proteins, centromere-binding proteins, and probably others [1].

In humans, tipifarnib is rapidly absorbed after oral intake. Peak plasma concentration generally reached within 4 h after administration, and then plasma concentrations decline in a bi-exponential manner and the half-life associated with the first disposition phase was 2–5 h [3]. The terminal half-life was 16–20 h and constituted only a small portion of the overall area under the plasma concentration versus time curve [4, 5]. As a consequence, minimal accumulation is seen upon twice-daily adminis-

tration, indicating that the first disposition phase is the most prominent phase of the tipifarnib plasma concentration–time profile. Little or no evidence of time-dependent drug metabolism is observed following repeated administration over an 8-week period [3].

In vitro and in vivo studies demonstrated that phase II metabolism, particularly, hepatic N-glucuronidation, followed by urinary excretion of the glucuronide, is a route for tipifarnib elimination as 14% of tipifarnib dose is excreted as the glucuronidated parent compound. In addition, oxidative N-deamination, oxidative N-demethylation, and loss of the methyl-imidazole moiety are the major phase I metabolic pathways involved in tipifarnib metabolism. In vitro experiments using cytochrome P450 (CYP) isozyme-selective chemical inhibitors and *E. coli* expressing individual human CYP isozymes indicated CYP3A4 was a predominant enzyme involved in the metabolism of tipifarnib and, also, suggested that CYP2D6 isozyme may contribute to tipifarnib phase I oxidative metabolism.

In particular, CYP2D6 appears to mediate the formation of the inactive N-demethylated metabolite (R130525) (unpublished observations, Johnson & Johnson Pharmaceutical Research and Development, J&JPRD). In fact, at a 6 μM tipifarnib concentration in microsomes prepared from a heterologous CYP expression systems in *E. coli*, the tipifarnib metabolism rate for CYP2D6 and CYP3A4 was 94.8 and 34.4 pmol/min pmol CYP, respectively. In studies with pooled human liver microsomes, the potent CYP2D6 inhibitor quinidine (10 μM) produced a 15% reduction in tipifarnib metabolism rate. Taken together, these preclinical observations collectively suggest that CYP2D6 may potentially contribute to tipifarnib oxidative metabolism in humans. Therefore, drug metabolism studies in humans are intended to confirm the preclinical data in a clinically relevant context and to obtain a quantitative estimate of the relative role of CYP2D6 for interindividual pharmacokinetic variability of tipifarnib. This paper reports the CYP2D6 isozyme-specific results of a comprehensive clinical pharmacogenomic screening program as part of a rational drug development strategy to decipher the risk for metabolism-based drug-drug interactions.

CYP2D6 is a prototypical example whereby the initial identification of a polymorphic phenotypic distribution of drug oxidation by characterization of extensive metabolizers (EM) and poor metabolizers (PM) of debrisoquine [6] and sparteine [7] was later shown to result from the presence of variant alleles in the gene encoding the enzyme [8]. In vivo, CYP2D6 activity varies more than 100-fold in the general population [9]. Due to the presence of more than 50 *CYP2D6* alleles that encode an enzyme with inactive, decreased, increased, or normal catalytic function [10, 11]. CYP2D6 isozyme is responsible for the oxidative biotransformation of many structurally diverse compounds and, in conjunction with other members of the CYP family, contributes to the metabolism of numerous compounds that include up to 20% of commonly prescribed drugs [8].

In Caucasians, the most common non-functional or null alleles associated with a PM phenotype are *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, and *CYP2D6*6* [10, 12]. Because many of the CYP2D6 substrates have a narrow therapeutic window, genotyping subjects for variant alleles might be of value in estimating individualized dose requirements in clinical practice. Moreover, antidepressants (e.g., fluoxetine) may be co-administered to many cancer patients and may inhibit CYP2D6 catalytic function in vivo. Hence, understanding the role of CYP2D6 isozyme in tipifarnib pharmacokinetics in humans may help to rationalize pharmacotherapy during concurrent treatment with CYP2D6 inhibitors in the clinic. Thus, to determine the influence of genotype in representative cancer patients without the need for a separate Phase I trial in cancer patients, use of sparse sampling with population pharmacokinetic modeling was utilized. The objective of the present study was to evaluate the effect of the *CYP2D6* genotype on clinical pharmacokinetics of tipifarnib utilizing a population pharmacokinetic analysis.

Subjects and methods

Subject description and clinical data

The population analysis of tipifarnib was based on data from 268 subjects who have participated in four Phase I studies and two Phase II/III studies during the clinical development of tipifarnib. Those studies were conducted in accordance with principles for human experimentation as defined in the *Declaration of Helsinki* (1983) and were approved by the Human Investigational Review Board of each study center. Written informed consent was obtained from each subject after being told the potential risks and benefits, as well as the investigational nature of the study. A separate written informed consent was obtained from all subjects included in the current analysis for the collection of blood samples used for the express purpose of genotyping the cytochrome P450 enzymes (as well as other genes) and relating these results to the disposition of tipifarnib.

The aims of those trials were to assess the safety and effectiveness of tipifarnib administered as monotherapy. Table 1 provides a summary of the type of subjects in each study, corresponding tipifarnib dosing schemes, and pharmacokinetic sampling schedules. Plasma concentration profiles from subjects included in these trials, who additionally were genotyped for determination of CYP2D6 status, were analyzed.

Drug assay

All venous blood samples were collected in heparinized tubes, centrifuged and separated plasma was stored at −20° and transported to J&JPRD (Beerse, Belgium) for analysis. Tipifarnib plasma concentrations were measured using either a high-performance liquid chromatography

Table 1 Characteristics of clinical studies, pharmacokinetic sampling, and subject genotype used in pooled analysis

Study (no. of subjects)	Study type and indication	Therapy duration	Dosage	Pharmacokinetic sampling (number of samples)	Genotype homo EM	Genotype hetero EM	Genotype Hetero PM
1 ($N=11$)	Phase I/healthy volunteers	Single dose	25 mg IV infusion (1 h) or 1×50 mg oral tablet	Frequent sampling to 48 h (257)	6	3	2
2 ($N=31$)	Phase I/advanced cancer	Three consecutive 4-day regimens	1) Continuous IV infusion (60–240 mg/day) 2) IV infusion BID (2 h) 3) 2×100 mg oral tablet administered BID	Frequent sampling to 10 h on fourth day of each regimen (700)	20	9	2
3 [25] ($N=9$)	Phase I/advanced cancer with normal liver function	5 days	2×100 mg oral tablet administered BID	Frequent sampling to 12 h on days 1 and 5 (135)	15	14	3
4 ($N=32$)	Phase I/healthy volunteers	Two single doses	1×50 mg oral tablets	Frequent sampling to 48 h (799)	57	39	4
5 [26] ($N=100$)	Phase III/advanced colorectal cancer	Chronic treatment	3×100 mg oral tablet administered BID	Sparse sampling: Cycle 1: prior to day 15 am dose and on day 22 Cycle 2: two samples on Day 1 (308)	50	30	5
6 [27] ($N=85$)	Phase II/refractory or relapsed acute myeloid leukemia	21 days per cycle	6×100 mg oral tablet administered BID	Sparse sampling: day 1: ≥1 h after am dose; day 8: two samples after am dose; prior to day 15 dose Frequent sampling to 12 h after first dose (20 patients) (368)			
Total	268			2,575	152	97	19
%					56	36	7

with ultraviolet detection (HPLC-UV) method (Studies 1, 2, 6) or a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method (Studies 3, 4, 5). A cross validation study between both techniques was performed and the results evidenced the interchangeability of both techniques (unpublished observations, J&JPRD). The lower limit of quantification for the HPLC-UV and LC-MS/MS methods was 1.00 and 0.50 ng/ml, respectively. The mean overall coefficient of variation was less than 7.1% across the validated range of concentration, which included up to 5,000 ng/ml. More detailed information about the HPLC-UV and LC-MS/MS method has been published elsewhere [3, 17].

Genotype determination

Pharmacogenomic blood samples were sent to J&JPRD for analysis. Genomic DNA was isolated from leukocytes in whole blood samples obtained at screening using the DNA Isolation kit for mammalian blood supplied by Boehringer-Mannheim (Cat. No. 1667327). Subsequently, the genotyping was carried out to detect the *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, and *CYP2D6*6* alleles. In brief, genotyping for the *CYP2D6*3*, *CYP2D6*4*, and *CYP2D6*6* alleles was performed using the commercially available kits from the Applied (formerly PE) Biosystems (Foster City, CA; Cat. No. 4312554, 4312555, and 4312556, respectively). The analysis of the *CYP2D6*5* allele was performed using a long-PCR technology, as described previously [13]. In some cases, subjects were also genotyped using other methodologies, e.g., PCR-RFLP [12] and TaqMan® gene dose PCR assay [14]. The latter assay is capable of characterizing the number of *CYP2D6* copies, but cannot inform on the variant type at each genetic loci.

The *CYP2D6* genotypes were classified into three groups according to the presence and number of any of the four evaluated non-functional alleles. Accordingly, subjects were assigned to groups with no active gene (PMs, $N=19$), one active gene (heterozygous EMs, $N=97$), or two active genes (homozygous EMs, $N=152$) [12, 15]. Since that TaqMan® gene dose PCR assay was only available at certain moment in time, only one clinical study could be analyzed using that technique. Therefore, ultrarapid metabolizers could not be detected in the other five studies conducted earlier. For consistency across the dataset, the metabolizer genotypes were assigned across all studies based on the presence or absence of gene deletions but not gene duplications. The frequency of the CYP2D6 EMs and PMs, the number of patients, and pharmacokinetic samples included in this population analysis are shown in Table 1.

Software

Nonlinear mixed effects modeling by extended least squares regression using the NONMEM® V level 1.1 software package (GloboMax, Hanover, MD, USA) [16] including NM-TRAN (version III level 1.0) and

PREDPP (version IV level 1.0), was used to model tipifarnib plasma concentration versus time profiles. Compilations were achieved using Microsoft FORTRAN 77 Powerstation compiler (version 4.00). First order estimation (FO) method was used to estimate model parameters. Graphical and all other statistical analyses, including evaluation of NONMEM® outputs, were performed using S-Plus® 2000 Professional package for Windows (Insightful Inc., Data Analysis Products Division, Seattle, WA).

Pharmacostatistical model

A population pharmacokinetic model previously developed [17] was used as a reference model to analyze the tipifarnib plasma concentration versus time data. Briefly, an open, three-compartment disposition model with linear elimination from the central compartment was used to describe the pharmacokinetics of tipifarnib in plasma after intravenous infusion. Systemically available tipifarnib is cleared from the body according to a linear process, which was influenced by total bilirubin. The volume of the central compartment was directly correlated with body weight. Oral absorption was modeled as a sequential zero-order input into the depot compartment, followed by a first-order absorption from depot compartment to the systemic circulation, and a lag time. The structural model parameters were defined in terms of clearance (CL); volume of distribution for central (V2), shallow (V3), and deep (V4) compartments; intercompartmental flow for shallow (Q3) and deep (Q4) compartments; lag time (LAG); duration of the zero-order release process (D); first-order absorption rate constant (KA); and absolute bioavailability (F). Previous population pharmacokinetic analysis [17] evidenced statistically significant difference between healthy volunteers and cancer patients with respect to CL, V2, Q4, V4 and KA. As a consequence, these parameters were allowed to differ between healthy volunteers and cancer patients.

Absorption of tipifarnib was highly variable, with observed lag-times ranging from negligible to greater than 2 h. The subject population was assumed to consist of two subpopulations with different typical values of lag time. This feature was implemented using the mixture model, where each subject is assigned to one of the subpopulations and the estimated probabilities associated with each subpopulation are estimated. Individual values of the lag time were constrained to less than 6 h using the logit transformation. The same transformation was also applied to bioavailability, with a constraint ranging from 0 to 1.

Except for oral bioavailability and lag time, the between (BSV) and within (WSV) subject variability in all other pharmacokinetic parameters were assumed to follow the lognormal distribution, and the magnitude was expressed as a coefficient of variation (CV)[24]. As previously described [17], correlations between the random effects Q3 versus V3, CL versus Q4 and V4, and KA versus LAG were fixed to 1. Residual variability was evaluated using an additive error model after natural logarithmic transformation of the measured plasma concentrations and model predictions. Two random effects were included to account for the residual variability for full pharmacokinetic profiles as well as isolated measurements of tipifarnib.

Analysis of genotype effect

The reference model did not include effects of CYP2D6 metabolic genotype on structural pharmacokinetic parameters. Reference model was fit to the data and the resulting minimum value of the objective function (MVOF) was considered as a starting value in evaluating the effect of the *CYP2D6* genotype on tipifarnib pharmacokinetics. Due to the limited total number of PM subjects, it was assumed that the magnitude of the *CYP2D6* genotype effect (if any) was the same for healthy volunteers and cancer patients.

For graphical exploration and preliminary statistical analysis of the influence of the *CYP2D6* genotype, empirical Bayes estimates of the individual pharmacokinetic parameters were computed using the POSTHOC option of the NONMEM® program. Plots were produced and an analysis of variance test (ANOVA) was performed between interindividual random effect and *CYP2D6* genotype.

In order to evaluate the effect *CYP2D6* genotype on the tipifarnib pharmacokinetic model, it was assumed that genotype might affect either CL, or F, or both parameters. No impact on distribution or absorption kinetic parameters was assumed. The implementation used compared the tipifarnib systemic clearance and absolute oral bioavailability in heterozygous EM and PM subjects to those parameters in the homozygous EM subjects, considered as the reference. Statistical significance of the effect of the homozygous EM, heterozygous EM, and PM was examined using the likelihood ratio test that is equivalent to testing the significance of the change in the log likelihood value obtained for various models. For each run, NONMEM® computes the MVOF, a statistic equal (up to a constant) to minus twice the log likelihood of the data. In the case of hierarchical models, the change in the minimum value of the objective function (ΔMVOF) produced by the inclusion of a parameter is asymptotically distributed as χ^2 with the number of degrees of freedom equal to the difference between the number of parameters in models under comparison. As the first-order method was employed, a ΔMVOF of at least 10.81 ($\alpha = 0.001$, 1 degree of freedom) was used to define statistical significance for the addition of a single parameter [18].

The goodness of fit was assessed by the examination of the following: scatter plots of observed versus population and individual predicted tipifarnib concentrations; scatterplots of population weighted residuals versus predicted concentrations and time; the precision of the parameter estimates as measured by the asymptotic standard errors and 95% confidence intervals based on normality

assumptions; and changes in the estimates of interindividual and residual variability for the specified models.

Simulations

Based on the fixed and random parameter estimates obtained from the model that implements the genotype effect on CL and F simultaneously, the steady-state tipifarnib pharmacokinetic profile after a 600-mg twice-daily dose regimen was simulated for the homozygous EM, heterozygous EM, and PM groups (1,000 subjects per group) in order to generate the population 80% prediction interval.

Results

The population pharmacokinetic analysis of tipifarnib was based on data from 262 Caucasian and 6 African-American. All non-Caucasian were homozygous EM, and three of them were healthy subjects. A summary of the other patient characteristics at baseline is presented in Table 2 by *CYP2D6* metabolic genotype. No statistical significance differences in patient characteristics at baseline were found between the *CYP2D6* genotypes.

Approximately 6% of collected pharmacokinetic samples were excluded because the measured concentration was below the limit of quantification or the sample was associated with an unclear dosing or sampling record. Tipifarnib plasma concentration of ultra-rapid metabolizers could have been preferentially excluded, however, a detailed evaluation evidenced that the vast majority of exclusions corresponded to plasma samples collected before the drug administration, or more than 30 h after the administration of a single low dose of tipifarnib to healthy volunteers. Therefore, it is unlikely that tipifarnib plasma concentration of ultra-rapid metabolizers could have been preferentially excluded.

The reference model, an open three-compartment disposition model with linear elimination and sequential zero-order release to the depot compartment and first-order absorption from the depot compartment, after lag time, was fitted successfully to the available pharmacokinetic data of tipifarnib (Fig. 1). Table 3 shows the population pharmacokinetic parameter estimates (and corresponding relative standard errors) for tipifarnib in healthy volunteers and cancer patients. Attempts to estimate the population pharmacokinetic parameters using the first-order conditional estimation method with or without interaction were unsuccessful. Overall, population and individual model predictions adequately described the data. Diagnostic plots showed random, uniform scatter around the line of identity and indicate an absence of bias (Fig. 2), while histograms of individual random effects on parameters showed approximately normal distribution (data not showed). These results clearly demonstrate the adequacy of the model to describe the time course of the tipifarnib plasma concentrations.

The highest correlation observed between random effects was the correlation between CL and F. However, it was relatively small ($r^2 = -0.21$) and when incorporated into the population pharmacokinetic model a limited reduction on the objective function was observed ($\Delta MVOF = 0.040$). Therefore, no further correlations were included in the reference model. In addition, attempts to employ a residual variability model incorporating the two tipifarnib assays did not significantly improve the fit.

The ANOVA of the empirical Bayesian estimates for the interindividual random effects (as obtained from the

Table 2 Summary of subject characteristics at baseline

Subject characteristics	Genotype homo EM[a] ($N=152$)	Genotype hetero EM[b] ($N=97$)	Genotype hetero PM[c] ($N=19$)	*P* value
Age (y)	56.7 (19.0–80.0)	59.9 (26–77.0)	56.8 (30.0–81.0)	0.123
Body weight (kg)	75.0 (43.0–120)	72.2 (41.0–95.0)	74.6 (48.0–114)	0.986
Body surface area (m^2)	1.88 (1.38–2.54)	1.85 (1.32–2.21)	1.92 (1.45–2.42)	0.698
Sex (%)				0.542
Male	60.8	53.7	56.2	
Female	39.2	46.3	43.8	
Subject status (%)				0.331
Healthy	13.8	17.5	26.3	
Cancer	86.2	82.5	73.7	
ALT (IU/l)	30.6 (3.0–194)	36.1 (4.0–185)	26.9 (9.0–92.0)	0.292
AST (IU/l)	27.9 (4.0–125)	33.6 (5.0–132)	24.4 (6.0–41.0)	0.059
Alkaline phosphatase (IU/l)	208 (35–1256)	221 (36–1392)	121 (37–409)	0.236
Lactate dehydrogenase (IU/l)	566 (94–7901)	662 (94–3122)	568 (205–1840)	0.533
Total bilirubin (μmol/l)	11.1 (2.0–41.0)	9.8 (2.0–37.0)	10.7 (6.0–21.0)	0.232
Serum albumin (g/l)	38.7 (24.0–53.0)	38.4 (23.0–49.0)	41.3 (27.0–48.0)	0.298
Total protein (g/l)	72.5 (48.0–102)	72.3 (53.0–90)	69.7 (24.0–80)	0.425
Creatinine clearance[d] (ml/min)	93.0 (36.0–150)	92.8 (41.0–150)	96.1 (30.0–150)	0.898

Continuous variables expressed as median (range). Categorical variables are expressed as percentage

[a] Range of observations: 1.2–2,825 ng/ml

[b] Range of observations: 1.0–1,665 ng/ml

[c] Range of observations: 1.4–1,142 ng/ml

[d] Creatinine Clearance was calculated using Cockroft–Gault formula. Values higher than 150 ml/min were truncated to 150 ml/min

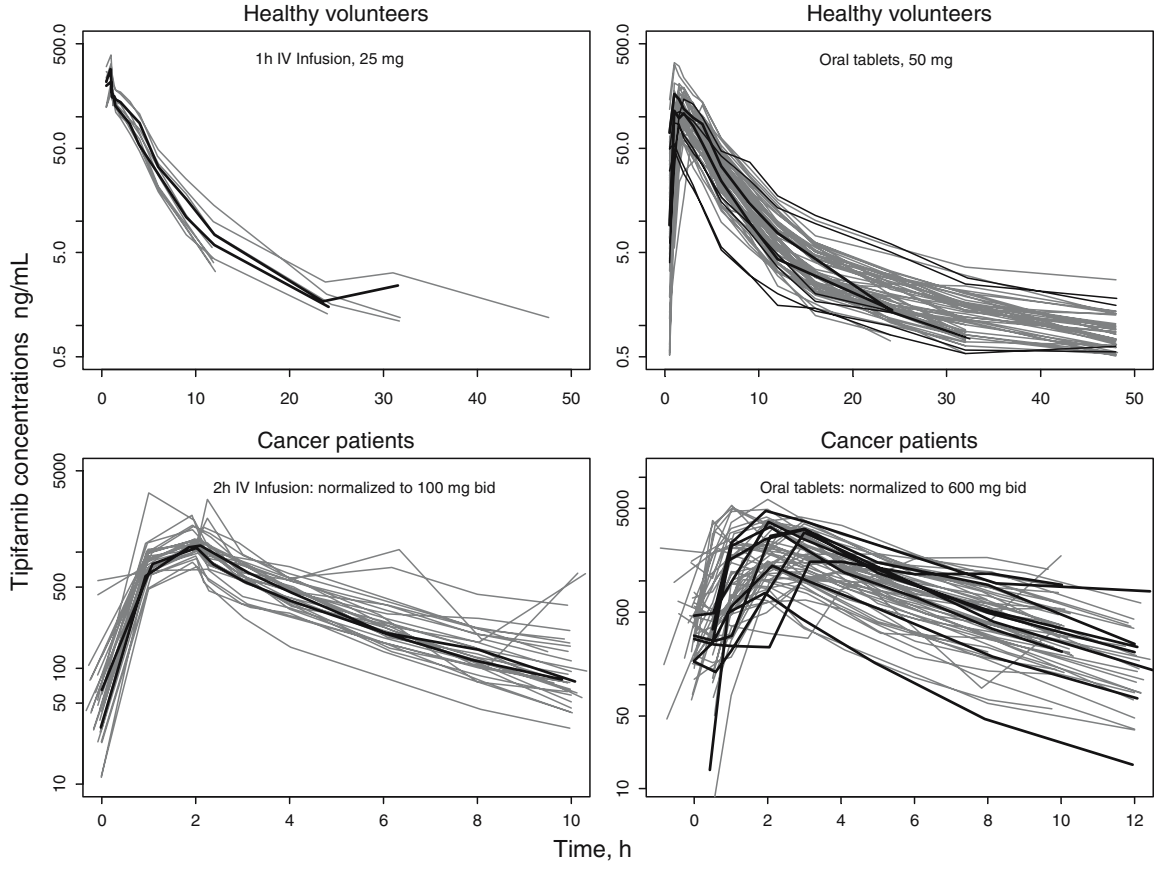

Fig. 1 Observed tipifarnib concentrations versus time since last dose for healthy volunteers (*upper*) and cancer patients (*lower*) after intravenous infusion (*left*) and oral (*right*) administration. *Bold lines* represent PM subjects

Table 3 Population pharmacokinetic parameters of tipifarnib in healthy volunteers and cancer patients

Pharmacokinetic Parameter	Typical value			Variability[h]	
	Cancer subjects*	Healthy subjects	Ratio healthy subjects:cancer subjects*	Between subjects (%)	Within subjects (%)
CL (L/h)[a]	22.6 (5.49)	28.7	1.27 (6.60)	26.1	12.3
V_2 (L/70 Kg)	39.0 (18.4)	25.0	0.64 (26.2)	32.3	–
Q_3 (L/h)	5.65 (11.1)	5.65	–	48.9	50.6
V_3 (L)	123 (15.0)	123	–	53.9[c]	–
Q_4 (L/h)	23.0 (27.6)	161	7.01 (29.1)	24.2[d]	–
V_4 (L)	26.0 (18.3)	66.6	2.56 (14.5)	25.7[e]	–
D_1 (h)	1.12 (3.02)	1.12	–	46.6	269
K_A (h^{-1})	0.60 (13.2)	1.80	3.00 (25.2)	53.2	36.5
F_{abs} (%)	30.2 (5.83)	30.2	–	0.72[g]	0.85[g]
T_{LAG} (h)[b,f]				0.76[f,g]	0.58[g]
Subpopulation 1	0.10 (22.0)	0.10	–		
Subpopulation 2	0.23 (18.0)	0.23	–		

*Results expressed as parameter (*RSE* relative standard error of parameter estimate, %)
[a] Clearance normalized for a bilirubin of 9 μmol/l. The normalization coefficient is equal to $(TBIL/9)^{\theta 1}$, where TBIL is bilirubin (expressed as μmol/l), and θ1 is −0.103 (RSE = 57.8%)
[b] Proportion of patients in subpopulation 1 is 60.3 (RSE = 35.7%)
[c] Correlation between IIV of Q_3 and V_3 set to 1. Expansion factor of V_3 is 1.50 (RSE = 14.2%)
[d] Correlation between IIV of CL and Q_4 set to 1. Expansion factor of Q_4 is 0.876 (RSE = 70.3%)
[e] Correlation between IIV of CL and V_4 set to 1. Expansion factor of V_4 is 0.972 (RSE = 20.8%)
[f] Correlation between IIV of K_A and T_{LAG} set to 1. Expansion factor of T_{LAG} is 2.18 (RSE = 63.3%)
[g] Expressed as standard deviation of the logit domain
[h] Residual variability, expressed as percentage: full PK profiles, 21.2 (RSE = 20.5%); isolated measurements, 15.8 (RSE = 45.6%)

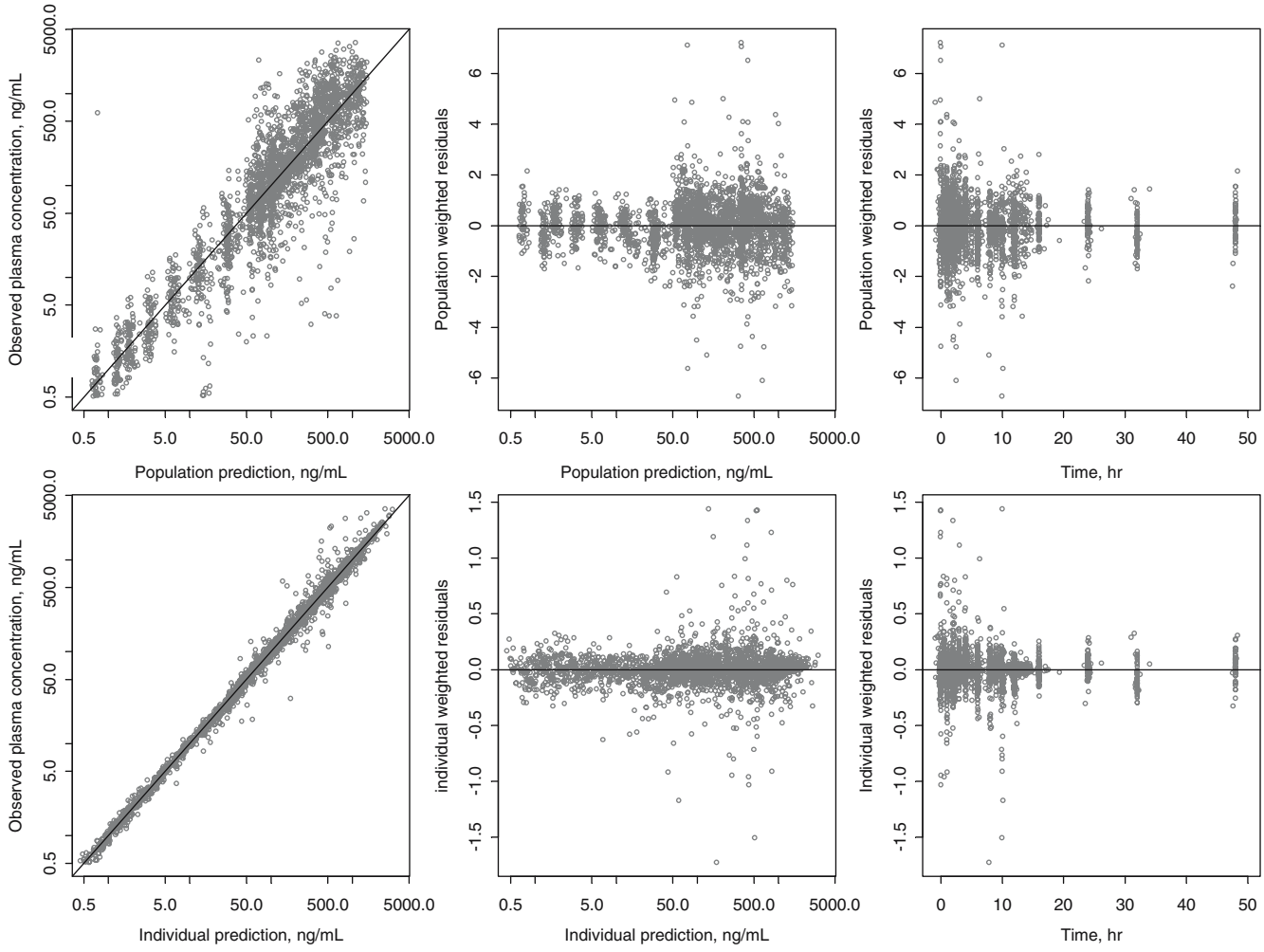

Fig. 2 Observed tipifarnib concentration versus population (*upper left panel*) and individual (*lower left panel*) predicted tipifarnib concentrations, population weighted residuals versus the population predicted concentrations (*upper middle panel*) and time (*upper right panel*), and individual weighted residuals versus the individual predicted concentrations (*lower middle panel*) and time (*lower right panel*)

reference model) versus the *CYP2D6* metabolic genotype did not demonstrate a statistical significance. However, boxplots exhibited a slight trend for the PM subjects to have a different distribution of BSV than the EM subjects, most probably because the difference in the sample size and the fact that the Bayesian estimates for the inter-individual random effect are shrunk to the mean.

Testing the effect of *CYP2D6* metabolic genotype on CL ($\chi^2=2.376$, $df=2$, $P=0.305$), bioavailability ($\chi^2=1.398$, $df=2$, $P=0.497$), and both ($\chi^2=3.088$, $df=4$, $P=0.543$) by incorporating the covariate into the NONMEM model also demonstrated that CYP2D6 status was not a significant predictor of these pharmacokinetic parameters. In fact, the ratio of tipifarnib systemic clearance for the heterozygous EM and the PM subjects, relative to the homozygous EM group, were 0.95 (95%CI 0.87–1.03) and 0.96 (95%CI 0.82–1.11), respectively. The ratio of tipifarnib absolute bioavailability for the heterozygous EM and the PM, relative to the homozygous EM, were 1.06 (95%CI 0.83–1.30) and 0.95 (95%CI 0.55–1.34), respectively. Figure 3 presents the histogram of the Bayesian estimates of tipifarnib systemic clearance and absolute bioavailability from the reference model for healthy volunteers and cancer patients, by *CYP2D6* metabolic genotype.

The simulated steady-state concentration–time profile after a 600-mg twice-daily dose regimen is provided in Fig. 4. In that Fig. 4, substantial overlap in the tipifarnib concentration-time profiles between the homozygous EM, heterozygous EM, and PM groups was observed. Overall, the magnitude of the *CYP2D6* effect on tipifarnib pharmacokinetics was small in comparison with the unexplained variabilities (residual, WSV and BSV).

Discussion

The CYP2D6 enzyme plays an important role in the metabolism of many clinically important drugs. Owing to

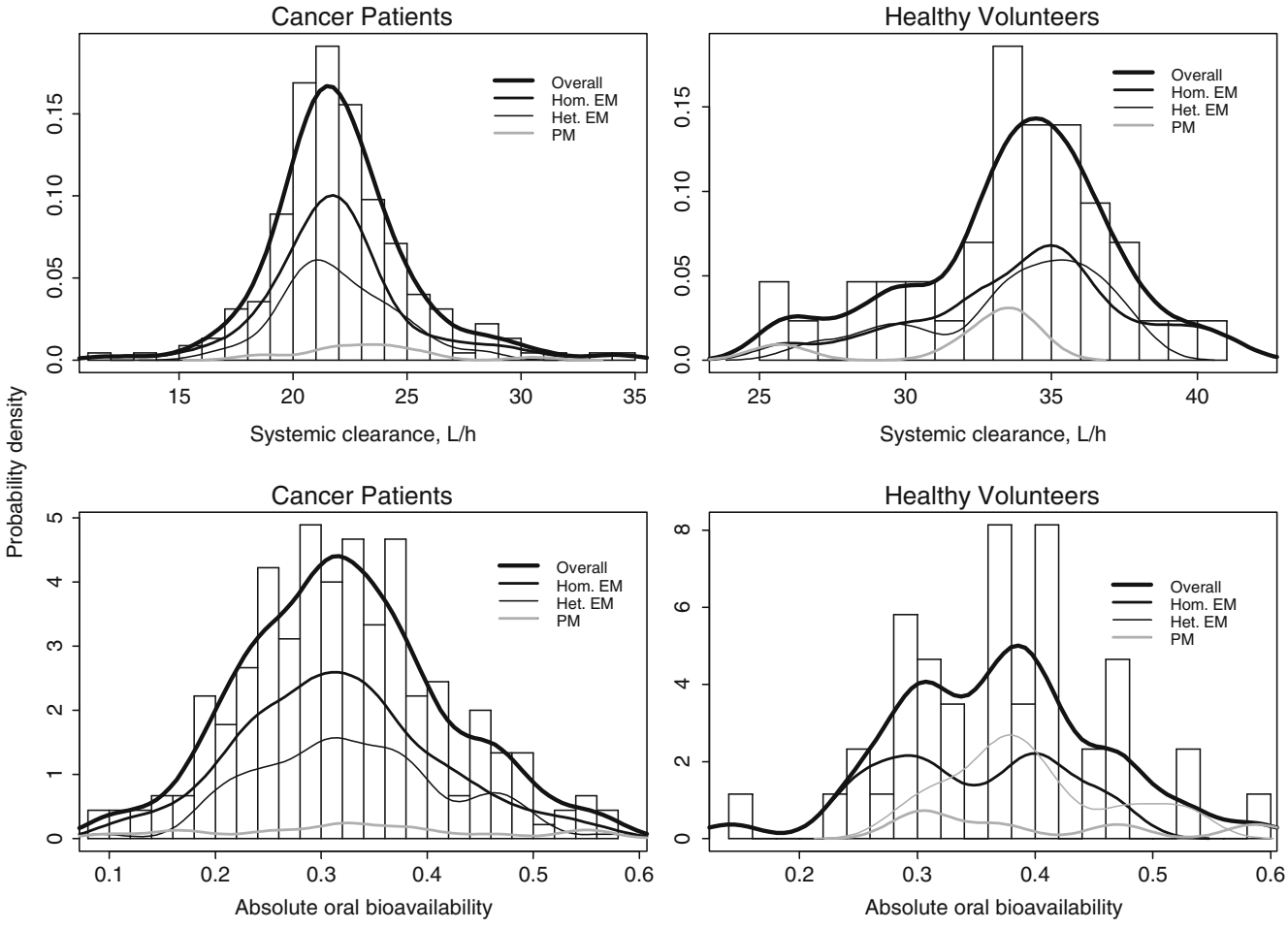

Fig. 3 Systemic clearance (*upper*) and absolute bioavailability (*lower*) estimates for tipifarnib from Model 1 and probability densities for homozygous extensive metabolizers (Hom. EM), heterozygous extensive metabolizers (Het. EM), and poor metabolizers (PM) for cancer patients (*left*) and healthy volunteers (*right*)

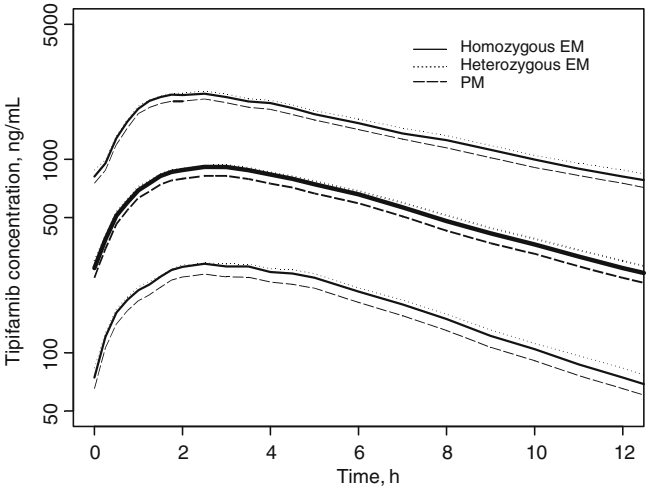

Fig. 4 Simulated steady-state concentration–time profiles (percentiles 10, 50 and 90) after a 600-mg twice-daily dosage regimen for cancer patients as a function of the *CYP2D6* genotype

the extremely polymorphic nature of the *CYP2D6* gene locus and the vast number of CYP2D6 inhibitor compounds used in the clinic, large variations of the CYP2D6 enzyme activity can be anticipated within any given population. Genotyping is often employed in clinical trials in order to investigate the CYP2D6-dependent metabolism and to predict subjects' phenotype in a relatively noninvasive manner [19, 20]. Moreover, heterozygous carriers of variant alleles can be identified and may provide an explanation for potential differences in the pharmacokinetics of CYP2D6 substrates within the EMs. For selected drugs such as tricyclic antidepressants [21], genotype-based dose adjustments have been recommended to minimize the risk of serious adverse events in PMs [21].

In the present study, subjects who previously received tipifarnib monotherapy were classified according to the presence of the non-functional alleles *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*6*, and *CYP2D6*5* into homozygous EM, heterozygous EM, and PM groups. The frequencies for each of these genotypic groups (Table 1)

were consistent with the distributions described previously in Caucasian populations [12, 19, 22]. In addition, the genotype frequencies were in equilibrium with the Hardy–Weinberg equation. According to this equation, the predicted incidence of PM and heterozygous EM was 6.1 and 37.2%, which is similar to the observed incidence of PM and heterozygous EM, 7,1% (95%CI 4.0–10.2%) and 36.2% (IC95% 30.4–41.9%), respectively.

The primary objective of the present analysis was to evaluate the effect of the *CYP2D6* genotype on the pharmacokinetics of tipifarnib using a population pharmacokinetic model previously developed over 1,083 cancer subjects. In that analysis, body weight and total bilirubin were identified as statistically significant covariates, with limited clinical relevance. As no other covariates (except healthy status) did significantly affect the pharmacokinetics of tipifarnib and similar distributions of patient characteristics at baseline were found between the *CYP2D6* genotypes, only body weight and total bilirubin were used as a covariates in the pharmacokinetic model in order to avoid that the absence of covariate effects in the model could mask the effect of *CYP2D6* genotype. Nevertheless, similar results were obtained when no covariates were included in the model (data not shown).

The parameter estimates obtained using the reference model were consistent with a recent report describing a meta-analysis of tipifarnib pharmacokinetics, including the differences between healthy volunteers and cancer patients, and the effect of patient characteristics [17]. In cancer patients, the absolute bioavailability of tipifarnib is about 30% and the total plasma clearance is 23 l/h (383 ml/min). Thus, assuming the drug is moderately to lowly extracted by the liver, subjects with low activity of the liver drug metabolizing enzymes may have a decreased clearance and/or increased bioavailability.

Multiple approaches were used to compare tipifarnib systemic clearance and absolute oral bioavailability in homozygous EM, heterozygous EM, and PM subjects. Methods included graphical analyses, ANOVA applied to empirical Bayes estimates of individual pharmacokinetic parameters, likelihood ratio testing, and calculation of the asymptotic 95% confidence intervals. No statistically significant differences were observed between homozygous EM, heterozygous EM, and PM groups using these methods. In all these analyses the magnitude of the *CYP2D6* genotype effect (if any) was assumed to be the same for healthy volunteers and cancer patients. However, in some pharmacokinetic parameters, such as Q4 and KA, important differences between healthy volunteers and cancer patients were evidenced. Therefore, an exploratory analysis on the effect of *CYP2D6* genotype on tipifarnib pharmacokinetic parameters was performed separately for healthy volunteers and cancer patients (data not shown) and similar results were found. Therefore, the differences between healthy volunteers and cancer patients in Q4 and KA have minimal impact on the results of the current analysis.

In order to provide additional evidence about the sensitivity of the population pharmacokinetic approach to detect changes in tipifarnib clearance that could be detected using the current dataset, an estimation of the magnitude of the difference between heterozygous EM and PM groups, relative to homozygous EM group, was calculated. With the current distribution of subjects between the *CYP2D6* genotypes, it was estimated that a 7.8 and 14.2% reduction in tipifarnib clearance in heterozygous EM and PM groups, relative to homozygous EM, could have been detected.

The potential interference of the comedication was ruled out before the start of the analyses. In the two studies conducted in healthy volunteers, the administration of concomitant medication, including CYP2D6 inhibitors, was not allowed in the clinical study protocol. In cancer patients, the effect of steroids, antiemetics ($5HT_3$-inhibitors, metoclopramide and domperidone), azole antifungals, benzodiazepines, ciprofloxacin, gemcitabine and amphotericin B was evaluated in a population pharmacokinetic analysis [17]. Notably, none of these top 10 concomitant medications were CYP2D6 inhibitors and no co-medication effect on tipifarnib oral clearance was identified. In addition, the limited number of the subjects (less than 0.5%) received concomitant treatment with fluoxetine or paroxetine, precluded any formal statistical analysis of the potential relationship between the presence or absence of these comedication and the tipifarnib pharmacokinetics. Therefore, it is unlikely that the interference of comedication on these studies might change the conclusion derived from the current analysis.

Taken together, these results indicate that *CYP2D6* genetic polymorphism does not appreciably influence the pharmacokinetics of tipifarnib and the relative role of CYP2D6 enzyme for variability in tipifarnib exposure is small. Apparently, other metabolic pathways are more important in man, and the CYP2D6 deficiency has little impact on tipifarnib pharmacokinetics. These results are also consistent with preliminary observations in a limited number of healthy subjects with detailed pharmacokinetic data (Fig. 1). Arguably, a prospective genetically-stratified panel study in extensive and poor metabolizers might have helped to address the role of *CYP2D6* genotype in tipifarnib metabolism. However, since a number of drug metabolism pathways such as UGTs and CYP3A4, CYP3A5, CYP2C9 and CYP2C19 may potentially play a role in tipifarnib metabolism, an alternative strategy to optimize the clinical trial resources and expedite the drug development timeframes was selected and it consisted with use of the sparse pharmacokinetic data, population pharmacokinetic analysis and genotyping for functional variants in drug metabolizing enzymes in Phase II and Phase III trials. However, the pharmacokinetic/pharmacogenomic analyses using the population approach was limited to the *CYP2D6* genotype because it was the only gene that has been considered as a valid biomarker for decision-making process with respect to drug–drug interactions and the sample size allow us to

perform a formal statistical. The results of the current population pharmacokinetic analysis confirm the preliminary findings in healthy volunteers, and extend it to a larger and clinically more relevant sample of cancer patients.

The clinically relevant consequences are twofold. First, no dose adaptation based on *CYP2D6* genotype is needed since PM patients will not have an increased tipifarnib exposure as compared to EM. A further application of the findings is the consideration of the effect of coadministration of potent CYP2D6 enzyme inhibitors. As an example, administration of quinidine, which is a known inhibitor of CYP2D6, effectively renders EM individuals as phenotypically equivalent to PM of CYP2D6 substrates [23]. The results of the present analysis have important implications regarding the lack of potential impact of the concurrent use of CYP2D6 inhibitors on the clinical pharmacokinetics of tipifarnib.

In summary, an open pharmacokinetic model with three-compartment linear disposition and a sequential zero-order input to the depot compartment and first-order absorption, after a lag time, adequately describes the plasma concentration–time profiles of tipifarnib after intravenous and oral administration in healthy volunteers and cancer patients. Genetic polymorphism of the CYP2D6 enzyme has no statistically significant influence on the pharmacokinetics of tipifarnib. Therefore, no dose adaptation in patients having low or no CYP2D6 enzyme activity is needed. In addition, the concomitant administration of a potent CYP2D6 inhibitor is anticipated to have little or no impact on the clinical pharmacokinetics of tipifarnib. From a drug development science perspective, the present integrated population pharmacokinetic-pharmacogenetic investigation further demonstrates the value of population pharmacokinetic modeling and *CYP2D6* genotyping in clinical trials to evaluate the effects of patient-specific factors for the development of an effective dosing strategy as an integral part of rational drug evaluation and development.

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