

## Lack of Association of Single-Nucleotide Polymorphisms in *Pregnane X Receptor*, *Hepatic Nuclear Factor 4 $\alpha$* , and *Constitutive Androstane Receptor* with Docetaxel Pharmacokinetics

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**Abstract Purpose:** This study aims to describe a population pharmacokinetic model for docetaxel in Asian breast cancer patients and to evaluate the effects of single-nucleotide polymorphisms (SNP) in the *cytochrome P450 3A (CYP3A)* gene expression regulators, *constitutive androstane receptor (CAR)*, *pregnane X receptor (PXR)*, and hepatic nuclear factor 4 $\alpha$  (*HNF4 $\alpha$* ), on the pharmacokinetics of docetaxel.

**Experimental Design:** Docetaxel was given as an i.v. infusion of 75 mg/m<sup>2</sup> over 1 h to 101 female breast cancer patients. *CAR*, *PXR*, and *HNF4 $\alpha$*  were comprehensively sequenced. Docetaxel concentrations were measured using a liquid chromatography/tandem mass spectrometry method and its population pharmacokinetic variables, and the covariate effects of clearance predictors were estimated using a nonlinear mixed effects model.

**Results:** Final estimates for docetaxel clearance was 47.1 L/h/70 kg/1.75 m. Between subject variability in docetaxel clearance was 22.5%. Covariates that showed significant association with docetaxel clearance included body size,  $\alpha$ 1 acid glycoprotein and liver function. SNPs identified in the coding regions of *CAR* and *HNF4 $\alpha$*  and 5' untranslated region of *PXR* in this Asian breast cancer cohort did not seem to improve predictability of docetaxel clearance.

**Conclusions:** SNPs identified in *CYP3A* gene expression regulators *CAR*, *HNF4 $\alpha$* , and *PXR* in the Asian female breast cancer population do not seem to have any significant effect on the clearance of docetaxel, a *CYP3A* substrate.

Docetaxel (Taxotere, Sanofi-Aventis), a member of the taxane class of antineoplastic agents, exerts its antitumor effect by binding to and promoting stabilization of the microtubular network. Stabilization of the microtubule bundle causes cell cycle arrest and apoptosis (1–3). Large variability in docetaxel pharmacokinetics between patients has been reported with important implications for its pharmacodynamic responses (4–10). This unpredictable pharmacokinetic behavior of

docetaxel has been identified as the main factor, limiting its use, and has been postulated to be attributable to its dependence on cytochrome P450 3A4 (*CYP3A4*)–mediated metabolism for inactivation.

Phenotyping strategies with probes targeted at the *CYP3A4* pathway, docetaxel's main route of metabolism, have been tested in multiple studies as a possible tool for individualizing docetaxel dosing (11–13). Yet, even with the identification of genetic polymorphisms in *CYP3A*, the large variation in *CYP3A* expression and activity has not been explained by these polymorphisms (14, 15).

From a molecular perspective, recent evidence has shown that *CYP* expression is partly controlled by target genes regulated at the transcriptional level by gene regulators. These include the constitutive androstane receptor (*CAR*), *pregnane X receptor (PXR)*, and *retinoid X receptor* from the steroid family of nuclear receptors, as well as transcriptional factors, such as hepatic nuclear factor 4 $\alpha$  (*HNF4 $\alpha$* ; ref. 16) and *HNF3 $\gamma$*  (17). *In vitro* studies have shown that *CAR*, *PXR*, and *HNF4 $\alpha$*  interact and affect *CYP2C9* expression (18). Whereas *PXR* and *CAR* together are known to modify *CYP3A4* gene expression, *PXR* has been identified as the dominant regulator (19, 20). In the current study, four single-nucleotide polymorphisms (SNP) were identified by sequencing *CAR*, *PXR*, and *HNF4 $\alpha$*  in a group of Asian women with breast cancer. One SNP was identified each for *CAR* and *PXR*, whereas two 2 SNPs were

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**Table 1.** Patient characteristics ( $n = 95$ )

Characteristic	Value (mean $\pm$ SD)
<b>Demographics</b>	
Docetaxel dose (mg)	114.5 $\pm$ 12.3
Age, y	49.3 $\pm$ 9.9
<b>Karnofsky performance score</b>	
80%/90%/100%	4:12:79
<b>Race</b>	
Chinese/Malay/Indian/others	59:26:8:2
<b>Breast cancer grades</b>	
2:3:4	6:42:39
Height (cm)	155 $\pm$ 5.8
Weight (kg)	58.6 $\pm$ 14
Body surface area (m <sup>2</sup> )	1.55 $\pm$ 0.16
<b>Baseline biochemistry measurements</b>	
Bilirubin ( $\mu$ mol/L)	6.1 $\pm$ 4.3
ALT (units/L)	29.1 $\pm$ 17.3
AST (units/L)	28.2 $\pm$ 16.5
Alkaline phosphatase (units/L)	83.6 $\pm$ 32.9
Lactate dehydrogenase (units/L)	623 $\pm$ 336.3
$\alpha$ 1 acid glycoprotein (g/L)	0.86 $\pm$ 0.39
Serum creatinine ( $\mu$ mol/L)	65.2 $\pm$ 13.8
Creatinine clearance, calculated based on a 70-kg person (mL/min)	106.4 $\pm$ 26.4

identified for HNF4 $\alpha$ . The hypothesis of this study was that these SNPs, in the coding regions of exon 5 in *CAR* and exons 1C and 4 of *HNF4 $\alpha$*  and the 5' untranslated region in exon 1 of *PXR*, may have a role in regulating CYP3A expression, thus displaying an effect on the clearance of a CYP3A substrate, such as docetaxel. The aims of this study are to establish a population pharmacokinetic model for docetaxel in Asian breast cancer patients and to determine if SNPs in *CAR*, *PXR*, and *HNF4 $\alpha$*  can explain between subject variability in docetaxel clearance.

## Materials and Methods

**Study design.** This is a single-centered, open-labeled, randomized phase II study of two different schedules of sequential docetaxel and Adriamycin chemotherapy given once every 3 weeks in stages II to IV breast cancer patients. To be eligible for this study, all patients must be female; above the age of 18; with histologically or cytologically proved stages II to IV breast cancers that have measurable primary breast tumor(s) of diameter 2.0 cm or larger; have a minimum Karnofsky performance score of 70; estimated life expectancy of at least 12 weeks; adequate hematopoietic, hepatic, and renal functions [defined, respectively, as follows: absolute neutrophil count  $\geq 1.5 \times 10^9/L$ , WBC count  $\geq 3.5 \times 10^9/L$ , platelet count  $\geq 100 \times 10^9/L$ , hemoglobin  $\geq 9$  g/dL, total bilirubin  $\leq 1.5 \times$  upper limit of normal, alanine aminotransferase (ALT) or aspartate aminotransferase (AST)  $\leq 2.5 \times$  upper limit of normal (or  $\geq 5 \times$  in those with liver metastases), alkaline phosphatase  $\leq 2.5 \times$  upper limit of normal, serum creatinine concentration  $\leq 1.5 \times$  upper limit of normal]; and adequate contraception for women of child-bearing potential.

Patients who were pregnant; received concurrent treatment with other anticancer therapy within 30 days at accrual; received medications known to be CYP3A substrates, inhibitors, or inducers within 1-month study entry were excluded from this study. The study protocol was approved by the institution's review board, and all patients gave written informed consent.

**Genotyping procedures.** Whole blood was collected from patients, and DNA from peripheral mononuclear cells was extracted for comprehensive sequencing of *PXR*, *CAR*, and *HNF4 $\alpha$*  as previously described (21).

**Pharmacokinetic analysis.** Docetaxel was given as a 75 mg/m<sup>2</sup> i.v. infusion over 1 h, and blood samples were taken to determine docetaxel pharmacokinetics during the first dose of docetaxel at baseline, 1, 2, 4, 7, and 24 h after docetaxel infusion. Determination of docetaxel concentrations was done by isocratic liquid chromatography/tandem mass spectrometry method described previously (22). Analytic grade docetaxel reference standard was a gift from Aventis Pharmaceuticals SA.

**Table 2.** Population pharmacokinetic variables and covariate effect estimates from final pharmacokinetic model

Model no.	Model description	$\Delta$ OBJ	Degrees of freedom	P
1	Base model (one compartment; no covariates)	—	—	—
2	Base model (two compartments; no covariates)	-758.53	2	<0.001
3	#2 + SNFWT	-8.994	3	<0.05
4	#4 + A1AG	-12.09	1	<0.001
5	#5 + Hep	-0.353	1	0.55
6	#5 + Hep1	-5.555	1	<0.05
7	#7 + albumin	0	1	—
8	#7 + age	0	1	—
9	#7 + race	-1.247	1	0.26
10	#7 + KPS	-1.99	1	0.16
11	#7 + tumor grade	-1.061	1	0.3
12	#7 + HNF4 $\alpha$ exon 4	-0.085	2	0.96
13	#7 + PXR	-0.721	2	0.7
14	#7 + HNF4 $\alpha$ exon 1C	-3.762	2	0.15
15	#7 + CAR	-3.82	2	0.15
16	#7 + HNF4 $\alpha$ exon 1C + PXR	-9.934	12	0.62
17	#7 + CAR + PXR	-10.038	12	0.61
18	#7 + HNF4 $\alpha$ exon 1C + CAR	-12.574	12	0.4
19	#7 + HNF4 $\alpha$ exon 1C + PXR + CAR	-19.704	35	>0.9
20	#20 + covariance block for CL and Q	-63.558	1	<0.0001

Abbreviations: CL, clearance; A1AG,  $\alpha$ 1 acid glycoprotein; Hep, AST and/or ALT above upper limit of normal; Hep1, AST and/or ALT and/or lactate dehydrogenase above upper limit of normal; KPS, Karnofsky performance score.

**Table 3.** Final estimates of docetaxel population pharmacokinetic variables in female Asian breast cancer patients based on model #20 in Table 2

Variable	Median population estimate (between subject variability)	Bootstrap (95% CI)
<b>Fixed effects</b>		
V1 (L/h/70 kg/1.75 m)	17.0 (20.9%)	(15.0 to 19.0)
Q (L/h/70 kg/1.75 m)	11.7 (31.9%)	(10.0 to 13.7)
V2 (L/h/70 kg/1.75 m)	173.0 (0.6%)	(150.0 to 200.03)
CL (L/h/70 kg/1.75 m)	47.1 (22.5%)	(39.1 to 58.4)
MRT (h)	4.04	(3.27 to 5.0)
A1AG	-0.18	(-0.24 to -0.08)
FFAT	0	—
FHEP1	-0.14	(-0.31 to 0.01)
<b>Covariate effects of SNPs</b>		
<i>CAR 180 C&gt;T</i>		
Wild type (n = 24)	0	—
Heterozygous (n = 39)	-0.7	(-0.88 to -0.32)
Homozygous (n = 32)	-0.44	(-0.78 to 0.16)
<i>PXR -24381 A&gt;C</i>		
Wild type (n = 55)	0	—
Heterozygous (n = 31)	0.13	(-0.62 to 2.13)
Homozygous (n = 9)	-0.6	(-0.91 to 0.79)
<i>HNF4<math>\alpha</math> 49 A&gt;G (Exon 1C)</i>		
Wild type (n = 23)	0	—
Heterozygous (n = 40)	-0.07	(-0.56 to 1.07)
Homozygous (n = 32)	-0.024	(-0.69 to 0.68)
<i>CAR-PXR interaction terms</i>		
Wild type – wild type (n = 10)	0	—
Wild type – heterozygous (n = 10)	-0.54	(-0.8 to 0.15)
Wild type – homozygous (n = 4)	0.81	(-0.44 to 4.03)
Heterozygous – wild type (n = 24)	1.81	(0.4 to 4.1)
Heterozygous-heterozygous (n = 12)	0.46	(-0.46 to 3.15)
Heterozygous-homozygous (n = 3)	5.77	(1.04 to 16.7)
Homozygous – wild type (n = 21)	0.2	(-0.41 to 1.56)
Homozygous-heterozygous (n = 9)	-0.33	(-0.73 to 1.23)
Homozygous-homozygous (n = 2)	1.38	(-0.07 to 5.51)
<i>HNF4<math>\alpha</math> exon 1-PXR interaction terms</i>		
Wild type – wild type (n = 18)	0	—
Wild type – heterozygous (n = 5)	0.65	(-0.33 to 2.72)
Wild type – homozygous (n = 0)	—	—
Heterozygous – wild type (n = 21)	0.04	(-0.53 to 1.33)
Heterozygous-heterozygous (n = 14)	0.74	(-0.27 to 3.28)
Heterozygous-homozygous (n = 5)	0.29	(-0.67 to 4.7)
Homozygous – wild type (n = 16)	0.27	(-0.36 to 1.75)
Homozygous-heterozygous (n = 12)	0.98	(-0.21 to 4.16)
Homozygous-homozygous (n = 4)	0.54	(-0.56 to 4.64)
<i>HNF4<math>\alpha</math> exon 1-CAR interaction terms</i>		
Wild type – wild type (n = 5)	0	—
Wild type – heterozygous (n = 8)	0.02	(-0.57 to 1.55)
Wild type – homozygous (n = 10)	0.29	(-0.45 to 2.09)
Heterozygous – wild type (n = 11)	0.004	(-0.61 to 1.28)
Heterozygous-heterozygous (n = 20)	-0.01	(-0.61 to 1.38)
Heterozygous-homozygous (n = 9)	0.39	(-0.41 to 2.74)
Homozygous – wild type (n = 8)	-0.06	(-0.58 to 1.16)
Homozygous-heterozygous (n = 11)	0.23	(-0.53 to 2.35)
Homozygous-homozygous (n = 13)	0.54	(-0.34 to 2.82)
<i>Residual error</i>		
Proportional error (%)	30.0	—
Additive error (mg/L)	0	—

Abbreviations: V1, volume of distribution in the central compartment; Q, intercompartmental clearance between central and peripheral compartments; V2, volume of distribution in peripheral compartment; FHEP1, liver function index when AST and/or ALT were above upper limits of normal.

Pharmacokinetic variables and their variability were estimated using nonlinear mixed effect modeling (NONMEM version V release 1.1, GloboMax LLC). The first-order conditional estimation method with the interaction option was used with a convergence criterion of six significant digits. Relations between docetaxel clearance and that of

covariates (age, sex, race, weight, height, body surface area, tumor grade, creatinine clearance,  $\alpha$ 1 acid glycoprotein, albumin, and liver function) were tested for statistical significance. Creatinine clearance was calculated based on the Cockcroft and Gault formula, but with body weight fixed at 70 kg. Effect of patient's weight and height on docetaxel

clearance was modeled as a separate size descriptor, called the normal fat weight (NFWT), which was centered on 1 and standardized at the weight of a 70-kg person with height of 175 cm (NFWT<sub>STD</sub>). The NFWT descriptor contained estimates of maximum fat free mass in kilograms, 50% of the maximum actual weight (WT<sub>50</sub>) in kilograms, and a fat fraction (FFAT).

**Derivation of normal weight for a standard subject with height of 1.75 m and weight of 70 kg.** Lean body mass for standard subject in kilograms,

$$LBM_{STD} = LBW_{MAX} \times 1.75^2 \times \frac{70}{(WT_{50} + 70 \times 1.75^2)}$$

Normal fat weight for standard subject with height of 1.75 m and weight of 70 kg,

$$NFWT_{STD} = LBM_{STD} + FFAT \times (70 - LBM_{STD})$$

Likewise, the NFWT for any subject can be calculated based on the subject's measured height and weight.

**Derivation of the size descriptor, standard normal fat weight, which is centered on 1.** Standard normal fat weight (SNFWT),

$$SNFWT = \frac{NFWT}{NFWT_{STD}}$$

Liver function was categorized as the covariates HEP or HEP1. HEP was defined as having AST and/or ALT above the institutional upper limits of normal at 50 units/L and 70 units/L, respectively, whereas HEP1 was defined having alkaline phosphatase above the institutional upper limit of normal at 130 units/L.

The covariate effects of each of the SNPs from *CAR*, *PXR*, and *HNF4α* were applied to docetaxel clearance and investigated via NONMEM.

$$CL = CL_{POP} \cdot (1 + \beta_{SNP})$$

for homozygous or heterozygous SNPs, wherein  $\beta_{SNP} = 0$  for the wild type and is the estimated covariate effect for heterozygous and homozygous SNPs of *CAR*, *PXR*, or *HNF4α*, where applicable. This covariate modeling method was similar to that reported by Henningson et al. (23). The influence of body size was introduced using allometric scaling,

$$CL = (CL_{POP}) \cdot \left( \frac{NFWT}{NFWT_{STD}} \right)^{3/4}$$

Finally, based on reported overlapping functions between *HNF4α* and *CAR* or *PXR* in the induction of *CYP3A* (24) gene expression, a full model with the covariate effects of individual SNPs and combinations of all interaction permutations between *HNF4α* exon 1C and *CAR* or *PXR* SNPs on the nonrenal component of docetaxel clearance were introduced as shown:

$$CL = CL_{POP} \cdot (1 + \beta_{CAR}) \cdot (1 + \beta_{PXR}) \cdot (1 + \beta_{HNF4\alpha Exon1C}) \cdot (1 + \beta_{CAR/PXR}) \cdot (1 + \beta_{HNF4\alpha Exon1C/PXR}) \cdot (1 + \beta_{HNF4\alpha Exon1C/CAR})$$

where  $CL_{POP}$  is the population value for the nonrenal component of population clearance;  $\beta_{CAR}$ ,  $\beta_{PXR}$ ,  $\beta_{HNF4\alpha Exon1C}$ ,  $\beta_{CAR-PXR}$ ,  $\beta_{HNF4\alpha Exon1C-PXR}$ , and  $\beta_{HNF4\alpha Exon1C-CAR}$  are the covariate effects of *CAR*, *PXR*, *HNF4α* Exon1C variants, and their interaction terms. The covariate effects for wild type(s) were set to 0 as the reference for variants.

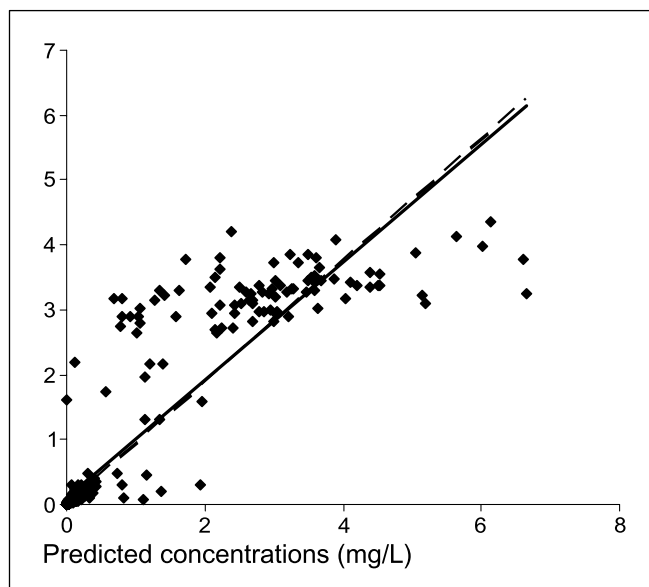
The random effects for between subject variability of the pharmacokinetic variables in the model were described by an exponential model for random effects. Model discrimination was based on changes

of the NONMEM's objective function value (OBJ). A decrease in OBJ ( $\Delta OBJ$ ) of  $>3.84$  ( $P < 0.05$ ; degree of freedom, 1) was considered statistically significant. Models were compared based on visual inspection of diagnostic plots. A bootstrap sampling method with replacement was conducted on the full covariate model using 1,000 bootstrap replications. This was used to construct the 95% confidence intervals (95% CI) of the variables. If the 95% CI of any SNP effect overlapped 0, it was interpreted as not having any significant influence on the clearance of docetaxel.

## Results

A total of 95 of the 101 patients accrued for this study had both docetaxel concentrations and genotyping data that could be used for this study. In total, 466 docetaxel concentration measurements were available for pharmacokinetic modeling. Although four variants were identified from the three genes studied, *Met49Val* and *Thr130Ile* in exons 1C and 4, respectively, of *HNF4α*, *PXR* 5' untranslated region -24381A>C, and *CAR* exon 5 *Pro180Pro*, this SNP and that of its interaction terms with SNPs in the other three genes were excluded in the covariate modeling step of this study, because variants in *HNF4α* exon 4 were very rare, with only two patients (2.1%) being heterozygous for the variant and none with homozygous mutation.

Table 1 summarizes baseline demographic and biochemistry characteristics of the patients. A summary of all the models tested is listed in Table 2 for comparison. In constructing the basic structural model, a two-compartment model showed significant improvement over a one-compartment model, both in terms of objective function improvement and visual inspection of individual patient fit plots. The different size descriptors, including weight, height, body surface area, and SNFWT, sex, race, tumor staging, Karnofsky performance score, and creatinine clearance were tested on the model as a covariate



**Fig. 1.** Goodness-of-fit diagnostic plots for model-predicted docetaxel concentrations (mg/L) against actual measured concentration (mg/L). Line of identity (perforated line) and line of best-fit (solid line) are overlapping. Pearson's correlation,  $R^2 = 0.833$ .

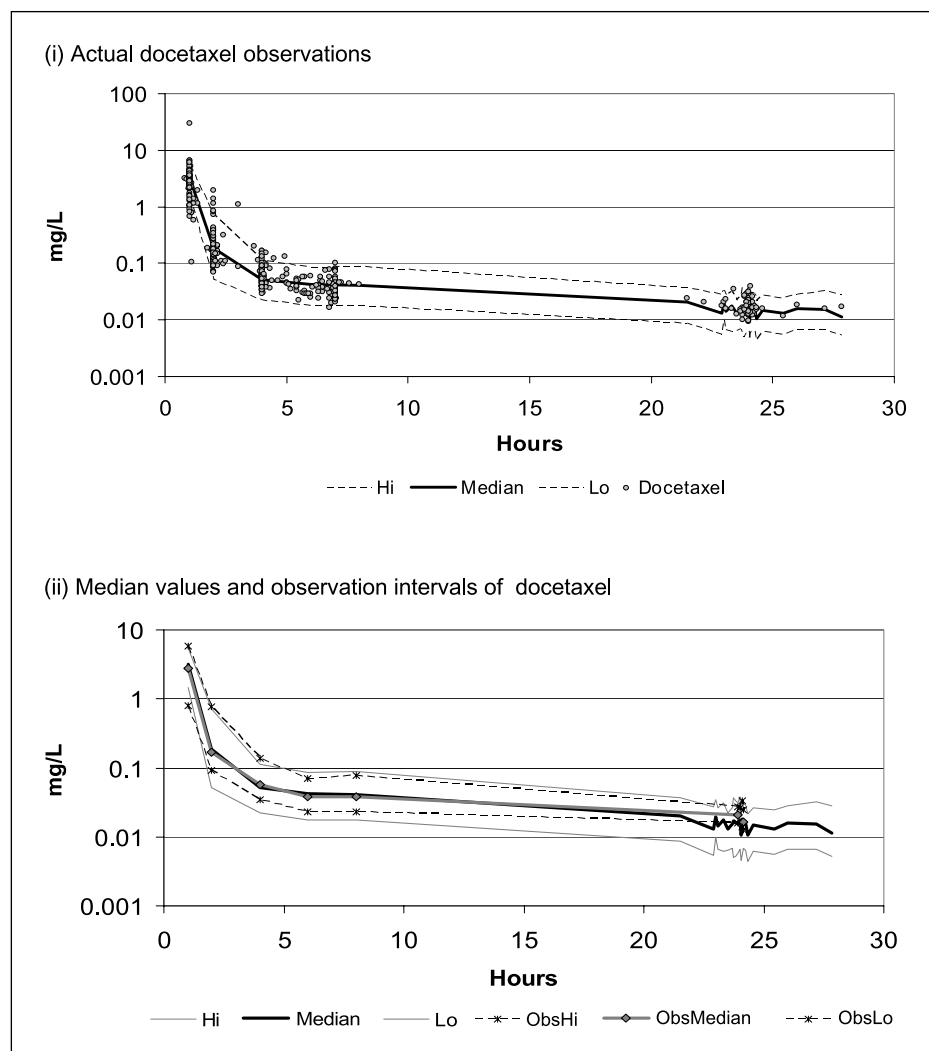
**Table 4.** Mean docetaxel clearance, their SDs for *CAR* and *PXR* variants derived from the individual estimates based on model #20 in Table 2, and the *P* values compared with wild type

<i>CAR-PXR</i> variants	Docetaxel clearance (L/h/70 kg/1.75 m)		
	Mean	SD	<i>P</i>
Wild type – wild type ( <i>n</i> = 10)	27.44	6.62	1.0
Wild type – heterozygous ( <i>n</i> = 10)	23.89	4.95	0.719
Wild type – homozygous ( <i>n</i> = 4)	25.72	4.03	0.999
Heterozygous – wild type ( <i>n</i> = 24)	25.48	7.14	0.954
Heterozygous-heterozygous ( <i>n</i> = 12)	24.02	7.12	0.714
Heterozygous-homozygous ( <i>n</i> = 3)	31.3	9.64	0.918
Homozygous – wild type ( <i>n</i> = 21)	27.08	4.37	1.0
Homozygous-heterozygous ( <i>n</i> = 9)	30.1	5.74	0.922
Homozygous-homozygous ( <i>n</i> = 2)	31.24	3.33	0.967

of clearance (CL). The covariates that resulted in a significant change in OBJ between two nested models were SNFWT,  $\alpha$ 1 acid glycoprotein, and HEP1.

None of the genes alone or in dual combination managed to exert a large enough effect to show a statistically significant  $\Delta$ OBJ between two nested models. Information on the individual covariate effects of each gene and the interaction terms between any two genes present in this cohort, when compared

against the wild types, were available through model 20. The final estimates of the population pharmacokinetic variables, together with their covariate effects, are listed in Table 3. There was a suggestion that the *CAR* 180 C>T heterozygous mutation has a negative effect on docetaxel clearance, whereas *CAR* 180 C>T heterozygous mutation – *PXR* -24381 A>C wild-type mutation and *CAR* 180 C>T heterozygous mutation – *PXR* -24381 A>C homozygous mutation had an increased effect on



**Fig. 2.** Validation of the final pharmacokinetic model through visual predictive check with a simulated dataset of 100 × 95 patients. A, a plot of actual observations of docetaxel concentrations (gray dots) versus the median values (thick line) and 90% prediction intervals (perforated lines) of the simulated dataset over ~ 24 h. B, a plot of the median values of observed docetaxel concentrations (thick black line), its 90% observation intervals (perforated black lines) versus the median values of the simulated dataset (thick gray line) and its 90% prediction intervals (thin gray lines).



docetaxel clearance when compared with wild type(s). Figures 1 and 2 show the respective goodness-of-fit diagnostic plot and visual predictive check with 90% CI of the final docetaxel model.

## Discussion

We have developed a population pharmacokinetic model for docetaxel in Asian breast cancer patients. The covariates that showed statistically significant improvement to model fitting included renal function, a weight descriptor SNFWT (derived from height, weight, sex, and a predicted fat content), which did better than body surface area,  $\alpha$ 1 acid glycoprotein concentrations, and an index for abnormal liver chemistries based on AST and ALT. Because the final estimate for the fat fraction, FFAT, was 0, the size descriptor was essentially similar to lean body mass. These covariates have all been previously identified to be associated with docetaxel clearance (6, 7, 12, 25). Because docetaxel is known to undergo extensive hepatic metabolism with the CYP3A pathway, it is expected that differences in liver function tests will have profound effect on its clearance. Conversely, because <10% of docetaxel is eliminated by urinary excretion (26) and all patients in this study have renal function indices with the reference range, it was not unexpected that creatinine clearance did not exhibit statistical significance on docetaxel clearance.

Covariates that were tested but did not exert an effect significant enough to be retained in our final model, as determined by the magnitude of  $\Delta$ OBJ, but had previously been reported to have significant independent effect on docetaxel clearance elsewhere were age (5, 25, 27), body surface area (5, 25, 27), and albumin (7, 25). In this study, we also tested sex, tumor grade, and performance status against docetaxel clearance, but these covariates did not improve model fitting significantly.

The genotype model (model 19 versus model 7) was unable to show any statistically significant improvement in model fitting, thereby rejecting the genetic variants as predictors for docetaxel clearance. A covariance block added to capture correlations between docetaxel clearance (CL) and intercompartmental clearance (Q) had significant effect in improving model fit.

Based on the covariate effects and their 95% CIs listed in Table 3, interactions between *CAR* 180 C>T heterozygous variant and *PXR* -24381 A>C homozygous variant, *CAR* 180

C>T homozygous variant, and *PXR* -24381 A>C homozygous variant seemed to show much larger covariate effects than their respective wild types. However, the sample sizes of patients with combinations of these two genotype variants were very small, that is, three and two, respectively. In addition, the 95% CI of the latter straddled 0. To be able to detect a 20% (7 L/h/70 kg/1.75 m) difference in clearance from the wild type, a sample size of 26 per subgroup will be required, assuming a power of 90% and an  $\alpha$  of 0.05. Hence, the sample sizes for these two subgroups were probably too small to detect if a difference in clearance from wild type truly existed.

Further confirmation that the large covariate effects were probably a result of insufficient numbers in those subgroups were done via a one-way ANOVA analysis with Dunnett's *t* test, using the wild type(s) as control on SPSS for Windows, release 13.0. The *P* values for *CAR* heterozygous-*PXR* homozygous and *CAR* homozygous-*PXR* homozygous variants were 0.918 and 0.967, respectively. Closer examination of the mean docetaxel clearances in these two groups also showed that they were not greater than those of other *CAR*-*PXR* variants (Table 4).

Allelic frequencies in the coding regions of *PXR* were found to be relatively low in the Dutch population, and only three linkages were found between *PXR* gene and the *CYP3A* gene (28). Hence, it is possible that *PXR* SNPs in the exonic regions may not play a role important enough in explaining *CYP3A* expression variability. In an *in vitro* study by Chen et al., evidences seem to point to possible cross-talk between *CAR*-*PXR* sites and *HNF4* $\alpha$  binding sites in *CYP2C9* promoter region (18).

## Conclusions

The results of the genotype covariate model in this study showed that the SNPs in *CAR*, *PXR*, and *HNF4* $\alpha$  did not have significant pharmacokinetic implications on the clearance of docetaxel, a *CYP3A* substrate. It is likely that for these regulator genes to have an effect on *CYP3A* expression, which in turn has to be large enough to show as having functional implications on the clearance of its substrates, a host of other factors and a more complex regulating mechanism are involved.

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## References

- Ringel I, Horwitz SB. Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. *J Natl Cancer Inst* 1991;83:288-91.
- Cortes JE, Pazdur R. Docetaxel. *J Clin Oncol* 1995; 13:2643-55.
- Clarke SJ, Rivory LP. Clinical pharmacokinetics of docetaxel. *Clin Pharmacokinet* 1999;36:99-114.
- Bruno R, Hille D, Riva A, et al. Population pharmacokinetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer. *J Clin Oncol* 1998;16: 187-96.
- Bruno R, Vivier N, Veyrat-Follet C, Montay G, Rhodes GR. Population pharmacokinetics and pharmacokinetic-pharmacodynamic relationships for docetaxel. *Invest New Drugs* 2001;19:163-9.
- Hirth J, Watkins PB, Strawderman M, Schott A, Bruno R, Baker LH. The effect of an individual's cytochrome CYP3A4 activity on docetaxel clearance. *Clin Cancer Res* 2000;6:1255-8.
- Goh BC, Lee SC, Wang LZ, et al. Explaining interindividual variability of docetaxel pharmacokinetics and pharmacodynamics in Asians through phenotyping and genotyping strategies. *J Clin Oncol* 2002;20: 3683-90.
- Rudek MA, Sparreboom A, Garrett-Mayer ES, et al. Factors affecting pharmacokinetic variability following doxorubicin and docetaxel-based therapy. *Eur J Cancer* 2004;40:1170-8.
- Baker SD, Li J, ten Tije AJ, et al. Relationship of systemic exposure to unbound docetaxel and neutropenia. *Clin Pharmacol Ther* 2005;77:43-53.
- ten Tije AJ, Verweij J, Carducci MA, et al. Prospective evaluation of the pharmacokinetics and toxicity profile of docetaxel in the elderly. *J Clin Oncol* 2005; 23:1070-7.
- Rivory LP, Slaviero K, Seale JP, et al. Optimizing the erythromycin breath test for use in cancer patients. *Clin Cancer Res* 2000;6:3480-5.
- Puisset F, Chatelut E, Dalenc F, et al. Dexamethasone as a probe for docetaxel clearance. *Cancer Chemother Pharmacol* 2004;54:265-72.
- Yamamoto N, Tamura T, Murakami H, et al. Randomized pharmacokinetic and pharmacodynamic study of docetaxel: dosing based on body-surface area compared with individualized dosing based on cytochrome P450 activity estimated using a urinary metabolite of exogenous cortisol. *J Clin Oncol* 2005; 23:1061-9.

14. Baker SD, van Schaik RH, Rivory LP, et al. Factors affecting cytochrome P-450 3A activity in cancer patients. *Clin Cancer Res* 2004;10:8341–50.
15. Lepper ER, Baker SD, Permenter M, et al. Effect of common CYP3A4 and CYP3A5 variants on the pharmacokinetics of the cytochrome P450 3A phenotyping probe midazolam in cancer patients. *Clin Cancer Res* 2005;11:7398–404.
16. Jover R, Bort R, Gomez-Lechon MJ, Castell JV. Cytochrome P450 regulation by hepatocyte nuclear factor 4 in human hepatocytes: a study using adenovirus-mediated antisense targeting. *Hepatology* 2001;33:668–75.
17. Bort R, Gomez-Lechon MJ, Castell JV, Jover R. Role of hepatocyte nuclear factor 3 $\gamma$  in the expression of human CYP2C genes. *Arch Biochem Biophys* 2004;426:63–72.
18. Chen Y, Kissling G, Negishi M, Goldstein JA. The nuclear receptors constitutive androstane receptor and pregnane X receptor cross-talk with hepatic nuclear factor 4 $\alpha$  to synergistically activate the human CYP2C9 promoter. *J Pharmacol Exp Ther* 2005;314:1125–33.
19. Bertilsson G, Heidrich J, Svensson K, et al. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci U S A* 1998;95:12208–13.
20. Quattrochi LC, Guzelian PS. Cyp3A regulation: from pharmacology to nuclear receptors. *Drug Metab Dispos* 2001;29:615–22.
21. Hor SY, Lee SC, Wong CI, et al. PXR, CAR, and HNF-4 $\alpha$  genotype and their association with pharmacokinetics and pharmacodynamics of docetaxel and doxorubicin in Asian Patients. *Pharmacogenom J*. Epub 2007 Sep 18.
22. Wang LZ, Goh BC, Grigg ME, et al. A rapid and sensitive liquid chromatography/tandem mass spectrometry method for determination of docetaxel in human plasma. *Rapid Commun Mass Spectrom* 2003;17:1548–52.
23. Henningson A, Marsh S, Loos WJ, et al. Association of CYP2C8, CYP3A4, CYP3A5, and ABCB1 polymorphisms with the pharmacokinetics of paclitaxel. *Clin Cancer Res* 2005;11:8097–104.
24. Wei P, Zhang J, Dowhan DH, Han Y, Moore DD. Specific and overlapping functions of the nuclear hormone receptors CAR and PXR in xenobiotic response. *Pharmacogenom J* 2002;2:117–26.
25. Bruno R, Vivier N, Vergniol JC, De Phillips SL, Montay G, Sheiner LB. A population pharmacokinetic model for docetaxel (Taxotere): model building and validation. *J Pharmacokinetic Biopharm* 1996;24:153–72.
26. de Valeriola D, Brassine C, Gaillard C, et al. Study of excretion balance, metabolism and protein binding of C<sup>14</sup> radiolabelled taxotere (TXT) (RP56976, NSC628503) in cancer patients. *Proc Am Assoc Cancer Res* 1993;34:2221.
27. Slaviero KA, Clarke SJ, McLachlan AJ, Blair EY, Rivory LP. Population pharmacokinetics of weekly docetaxel in patients with advanced cancer. *Br J Clin Pharmacol* 2004;57:44–53.
28. Bosch TM, Deenen M, Pruntel R, et al. Screening for polymorphisms in the PXR gene in a Dutch population. *Eur J Clin Pharmacol* 2006;62:395–9.

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## Lack of Association of Single-Nucleotide Polymorphisms in *Pregnane X Receptor, Hepatic Nuclear Factor 4 $\alpha$* , and *Constitutive Androstane Receptor* with Docetaxel Pharmacokinetics

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