A Pharmacogenetic Predictive Model for Paclitaxel Clearance Based on the DMET Platform

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Abstract

Purpose: Paclitaxel is used in the treatment of solid tumors and displays high interindividual variation in exposure. Low paclitaxel clearance could lead to increased toxicity during treatment. We present a genetic prediction model identifying patients with low paclitaxel clearance, based on the drug-metabolizing enzyme and transporter (DMET)-platform, capable of detecting 1,936 genetic variants in 225 metabolizing enzyme and drug transporter genes.

Experimental Design: In 270 paclitaxel-treated patients, unbound plasma concentrations were determined and pharmacokinetic parameters were estimated from a previously developed population pharmacokinetic model (NONMEM). Patients were divided into a training- and validation set. Genetic variants determined by the DMET platform were selected from the training set to be included in the prediction model when they were associated with low paclitaxel clearance (1 SD below mean clearance) and subsequently tested in the validation set.

Results: A genetic prediction model including 14 single-nucleotide polymorphisms (SNP) was developed on the training set. In the validation set, this model yielded a sensitivity of 95%, identifying most patients with low paclitaxel clearance correctly. The positive predictive value of the model was only 22%. The model remained associated with low clearance after multivariate analysis, correcting for age, gender, and hemoglobin levels at baseline ($P = 0.02$).

Conclusions: In this first large-sized application of the DMET-platform for paclitaxel, we identified a 14 SNP model with high sensitivity to identify patients with low paclitaxel clearance. However, due to the low positive predictive value we conclude that genetic variability encoded in the DMET-chip alone does not sufficiently explain paclitaxel clearance. Clin Cancer Res; 19(18); 5210–7. ©2013 AACR.

Introduction

Paclitaxel is a highly active anticancer drug with a broad spectrum of activity. It is used in the treatment of several solid tumors, such as breast, ovarian, and non–small cell lung cancer (NSCLC). Paclitaxel stabilizes cellular microtubules and thereby blocking chromosomal segregation and mitosis, eventually inducing apoptosis (1).

Paclitaxel is metabolized in the liver by cytochrome P450 (CYP) CYP2C8 and 3A4. The anion organic transporting polypeptide OATP1B3 was identified as an important influx transporter (2), whereas the efflux of paclitaxel was shown to be mediated by ABCB1 (P-glycoprotein) and ABCC2 transporters (3, 4). The pharmacokinetics of this agent are known for its large interindividual variability, which could have important safety consequences and may also affect treatment outcome. The source of this large variability remains to be elucidated.

It has been suggested that genetic variation in the genes involved in the metabolism of paclitaxel could explain part of the mentioned variability. As a candidate gene approach, single-nucleotide polymorphisms (SNP) in CYP2C8, CYP2A5, and ABCB1 (3435C > T, 2677G > T and 1236C > T) have been tested, but these studies yielded contradictory results (5–10).

Another approach to study genetic variants that could potentially influence paclitaxel pharmacokinetics and toxicity is by using a more broad approach, such as the drug-metabolizing enzyme and transporter (DMET) genetic platform (Affymetrix). This platform includes a large
Translational Relevance

During the last 20 years, paclitaxel has become a standard cytotoxic drug in the treatment of several malignancies, such as breast, ovarian, and non–small cell lung cancer. However, due to its wide interpatient variability in plasma exposure, efficacy, and toxicity profiles of this compound are still quite unpredictable for the individual patient. Dose-limiting toxicities resulting from high systemic concentrations could lead to prematurely discontinuation of treatment or to treatment-related hospitalizations. It is therefore important to identify patients at risk for (extremely) high exposures to the drug, allowing these patients to be monitored more closely or to apply dose reductions. In this study, we explored the potential association of 1,936 genetic variants in 225 drug metabolizing enzyme and drug transporter genes (DMET-platform) with paclitaxel unbound clearance.

Materials and Methods

Patients

Patients treated with paclitaxel for different tumor types were included in a prospective trial studying pharmacokinetics, pharmacodynamics, and pharmacogenetics (registered at www.trialregister.nl as NTR2311, study number MEC 03.264).

The inclusion criteria in this study were (i) a histologic or cytologic confirmed diagnosis of cancer treated with paclitaxel, (ii) aged 18 years or older, (iii) World Health Organization performance score of 0 or 1, and (iv) adequate hematopoietic, hepatic, and renal functions according to the product information of paclitaxel (11). CYP3A4 and CYP2C8 inducers and inhibitors were not allowed during the course of paclitaxel treatment (12). All patients provided written informed consent before study participation. The trial was approved by the Medical Ethical Committee of the Erasmus University Medical Center (Rotterdam, NL).

Pharmacokinetic analysis

Paclitaxel pharmacokinetic limited sampling with 4 to 5 samples in up to approximately 24 hours after the start of infusion was conducted and was repeated once or twice in following cycles according to each patients’ individual consent. For all samples lithium heparin was used as anti-coagulant. A validated UV detection high-performance liquid chromatography method (13) or a validated liquid chromatography/mass spectrometry method. Based on a method used for docetaxel (14), was used to quantitate paclitaxel in plasma. Measured samples for total paclitaxel plasma concentrations and a previously developed population pharmacokinetic model (15–17) were used to calculate paclitaxel pharmacokinetic parameters in each individual patient. It is known that the total plasma concentrations of paclitaxel do not show linear pharmacokinetics in contrast with the “free” plasma concentrations. This is caused by the formulation vehicle of paclitaxel, Cremophor EL, which is responsible for disproportionate drug accumulation in the plasma fraction and therefore causes a decrease in free circulating drug (18, 19). For this reason, pharmacokinetic model parameters were parameterized to be on the basis of unbound concentrations in the modeling and ‘unbound’ instead of total paclitaxel clearance was used in the covariate analyses of this study. The individual pharmacokinetic parameters were estimated as Empirical Bayes estimates within the nonlinear mixed-effect modeling software NONMEM (version 7, Icon Development Solutions).

Genetic variant analysis using the DMET platform

DNA of 293 patients was extracted from whole blood using MagnaPure LC (Roche Diagnostics GmbH), according to manufacturer’s instructions. The Affymetrix DMET Plus Premier Pack (Affymetix) was used to genotype genomic DNA of all paclitaxel-treated patients as described by Dumaual and colleagues (20). Genotypes of all SNPs on the DMET assay were reported either as “call” or as “no call”. Markers on the DMET assay with call rates less than 90% were excluded from analysis. After removal of duplicate measurements, patients with missing clearance data and removal of low-call rate assays, data for 270 patients were eligible for data analysis.

Marker selection

As shown in the flowchart (Fig. 1), genetic variants were excluded from the analysis if the genotype was identical in all patients, leaving 1,048 variant reporting different genotypes for analysis. The remaining genotypes were tested for Hardy–Weinberg equilibrium and SNPs deviating from this equilibrium (P > 0.05) were excluded. This left 770 variants for the prediction analysis. The cohort of patients was split into training and a validation set. To guarantee a sufficient number of minimal observations for analysis, each genotype (wild-type, heterozygote, or homozygote variant) of a particular SNP should occur in at least 5% of patients, which equals 7 patients in the training set. Identification of variants, which were significantly associated with low clearance, was conducted using a Naive Bayes formula in the training set. The conditional probability was estimated for each SNP:

\[ P(A|B) = \frac{P(A \& B)}{P(B)} \]

Here, A is clearance, whereas B is the genotype. This expresses the chance of a patient having low clearance (coded as 1), whereas having a homozygote wild-type, a heterozygote, or a homozygote variant genotype. Each of
the possible genotypes has a probability of occurring, which is used as a weight in the prediction analysis. To select variants associated with paclitaxel clearance, a wild-type or variant genotype should be present in at least half of the patients with low clearance in the training set, and to have a $P(A|B) > 0.2$.

This resulted in the selection of 20 SNPs. Four SNPs (rs2359612, rs8050894, rs9934438, and rs9923231) were in complete linkage disequilibrium and belonged to the same gene (VKORC1). Of these SNPs, the SNP with the highest allelic frequency, rs9923231, was selected. Similarly, 2 other SNPs (rs7793861 and rs7797834) were in complete linkage disequilibrium in the CYP51A1 gene. Here, rs7797834 was selected because this SNP had the highest allelic frequency. Next, the remaining 16 SNPs were included in a multivariate logistic regression model. Each of these SNPs was added to the model one by one and SNPs were included in the signature if they increased the likelihood ratio of the fitted model, leaving 14 SNPs for final analysis.

**SNP signature**

In developing, the predictive model patients were divided in having either low clearance, thus assumed to have an increased risk of toxicity during paclitaxel therapy or normal (or high) clearance. Low clearance was defined as a clearance 1 SD below the mean clearance of the entire cohort. Low clearance was coded as 1, the other clearances as 0. The 14 selected SNPs (Table 3) were used to build the SNP signature predicting low clearance in paclitaxel-treated patients. For each of these SNPs, the conditional probabilities estimated in the training samples were used. For each individual sample a total probability weighted score was calculated on the basis of the sum of all 14 probabilities of having the selected SNPs. The scores of the samples in the training-cohort were associated with clearance in a ROC curve. From this ROC-curve, we selected the threshold where all patients with truly low clearance were identified correctly (i.e., 100% sensitivity). Finally, the probability-weighted scores of the samples from the validation-cohort were calculated and compared with the selected threshold. If the score was above the threshold the sample was predicted as "low clearance", otherwise as "rest". The predicted calls were then compared with the actual clearance data.

**Statistical analysis**

Data are presented as median with ranges, unless stated otherwise. Differences between validation and training set were tested with the $\chi^2$ test for binary covariables and differences between validation and training set in continuous variables were tested with the Mann–Whitney test. Logistic regression was used to study the influence of covariables on the association between the SNP signature and a patient having low clearance. Variables tested in this model were age, gender, and hemoglobin levels before start of therapy because these variables have previously been shown to influence paclitaxel treatment. For example, male patients have higher paclitaxel metabolism than female patients (21). Paclitaxel elimination is also negatively correlated with age (21). Furthermore, hemoglobin levels have been shown to be a prognostic factor in cancer treatment (22–24). $P$ values were all two-sided and $P$ values < 0.05 were considered statistically significant. Analysis was conducted with STATA version 11 (StataCorp LP) and SPSS version 20.0 (SPSS Inc).
Results

Patients

In the analysis, 270 Caucasian patients treated with paclitaxel were included. This group was divided in a training \((n = 140)\) and a validation set \((n = 130)\), so that the numbers of patients in the low clearance category are equally distributed between the training and validation set. The training and validation set displayed the same patient characteristics (Table 1). The median age of the whole cohort was 61 years (range: 18–82 years) and esophageal cancer was the main diagnosis (49%). Patients were treated with a median dose of 170 mg (range: 50–560 mg). Patients received paclitaxel weekly or every 3 weeks in different combination regimens. Patients receiving a weekly dose of 50 mg/m² paclitaxel in combination with radiotherapy, as a preoperative regimen for resectable esophageal cancer, were also included (25). There was no statistical significant difference between the training and validation set with respect to clearance \((P = 0.66)\), gender \((P = 0.64)\), tumor type \((P = 0.98)\), smoking status \((P = 0.56)\), hemoglobin levels \((P = 0.31)\), or platelets counts \((P = 0.72)\) at start of therapy, excluding potential selection bias between the training and validation set.

Influence of variables on paclitaxel pharmacokinetics

In the total cohort, we tested the influence of the variables age, gender, and hemoglobin levels before start of therapy on unbound paclitaxel clearance because these variables have previously been shown to influence paclitaxel treatment. Age was not significantly correlated with paclitaxel pharmacokinetics in our dataset \((R = 0.09; P = 0.16)\). Gender had a significant effect on paclitaxel unbound clearance. In our cohort males had higher median clearance than females \((mean 541 L/h vs. 432 L/h; P < 0.00001)\). We found a significant but weak correlation between hemoglobin levels at start of paclitaxel therapy and paclitaxel unbound clearance \((R = 0.2; P = 0.001)\).

Genetic signature predicting low metabolism

The mean clearance of all patients was 488 ± 149 L/h (Table 2). Therefore, the threshold for having low clearance was 339 L/h, which was 1 SD below the mean of the total cohort. In total 14 SNPs located on 11 different chromosomes were selected for the prediction model (Table 3). Included in the model were SNPs in the genes: SLC22A11 \((rs1783811)\), GSTZ1 \((rs7975)\), SLC28A2 \((rs1060896)\), VKORC1 \((rs9923231)\), PGP3 \((rs2952151)\), CDA \((rs1048977)\), EPHX1 \((rs1051740)\), CYP20A1 \((rs1048013)\), SLC6A6 \((rs2341970)\), CRIP3 \((rs2242416)\), GSTA4 \((rs13197674)\), AKAP9 \((rs7785971)\), CYP51A1 \((rs7797834)\), and CYP2D7P1 \((rs28360521)\). The probabilities of having a homozygote wild-type, heterozygote, or homozygote variant for each SNP are listed in Table 3. The sum of these probabilities gives each patient a probability score and these scores were used to generate a ROC-curve. From this curve a threshold was selected (2.12) at which point, a 100% sensitivity was achieved to identify low clearance patients in the training set. All patients with a probability score more than 2.12, were scored as having low clearance and all patients with a probability score less than

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
</tr>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Median age, years (range)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Median dose (mg; range)</td>
</tr>
<tr>
<td>Primary tumor site, n (%)</td>
</tr>
<tr>
<td>Esophagus</td>
</tr>
<tr>
<td>Ovary</td>
</tr>
<tr>
<td>Cervix</td>
</tr>
<tr>
<td>Endometriat</td>
</tr>
<tr>
<td>Breast</td>
</tr>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>Head/Neck</td>
</tr>
<tr>
<td>A (CUP)</td>
</tr>
<tr>
<td>Testis</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

Abbreviations: A (CUP), adenoma (carcinoma of unknown origin).
*Continuous data are given as median with range in parentheses and categoric data are given as number of patients with percentage of the total population in parentheses.
2.12, were scored as not having low clearance. Subsequently, we validated this predictive model in the validation cohort. Figure 2 shows paclitaxel clearance levels according to the predicted group; the median paclitaxel clearance in the group predicted as low clearance \((n = 93)\) was significantly less than the group predicted as "rest" \((n = 37; 461 \text{ vs. } 525 \text{ L/h}; P = 0.01)\). With the cut off score of 2.12, 20 of 21 patients with truly low clearance were predicted by the signature as having low clearance, yielding the model a sensitivity of 95% and a positive-predictive value of 22% (Table 4, Fig. 2). Patients with a positive-SNP prediction model had an OR of 9.9 (95% confidence interval (CI), 1.3–76.4; \(P = 0.028\)) of having low clearance. When tested in a multivariate logistic regression model to correct for the influence of age, gender, and hemoglobin levels at start of therapy, the SNP prediction model was independently associated with low clearance (OR, 10.9; 95% CI, 1.4–86.3; \(P = 0.024\)). None of the other tested variables significantly improved the model.

### Discussion
During the early years of the 21st century, emphasis has been on somatic tumor mutations that can predict disease course and treatment outcome and could therefore aid in selecting the appropriate therapy for an individual.

### Table 2. Paclitaxel clearance in both sets

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>n</th>
<th>Training set</th>
<th>n</th>
<th>Validation set</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel clearance (L/h)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>480 (138–1,037)</td>
<td>270</td>
<td>474 (138–1,037)</td>
<td>140</td>
<td>494 (239–618)</td>
<td>130</td>
</tr>
<tr>
<td>Low clearance-group</td>
<td>286 (138–339)</td>
<td>44</td>
<td>258 (138–328)</td>
<td>23</td>
<td>302 (239–339)</td>
<td>21</td>
</tr>
<tr>
<td>No low clearance-group</td>
<td>512 (340–1,037)</td>
<td>226</td>
<td>505 (340–1,037)</td>
<td>117</td>
<td>518 (345–858)</td>
<td>109</td>
</tr>
</tbody>
</table>

aData are represented as median with ranges.
bPaclitaxel clearance in based on unbound concentrations.

d\(P\) value < 0.05.

e\(P\) value < 0.01.

### Table 3. Selected SNPs for the conditional probability gene signature and probability score

<table>
<thead>
<tr>
<th>Genetic variation</th>
<th>Gene (Chr)</th>
<th>SNP Value</th>
<th>SNP Value</th>
<th>SNP Value</th>
<th>(P) (SNP = 0)*</th>
<th>(P) (SNP = 1)*</th>
<th>(P) (SNP = 2)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1783811</td>
<td>SLC22A11 (Chr11)</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>0.154</td>
<td>0.106</td>
<td>0.230</td>
</tr>
<tr>
<td>rs9757</td>
<td>GSTZ1 (Chr 14)</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>0.205</td>
<td>0.16</td>
<td>0</td>
</tr>
<tr>
<td>rs1060896</td>
<td>SLC28A2 (Chr 15)</td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
<td>0.213</td>
<td>0.133</td>
<td>0.105</td>
</tr>
<tr>
<td>rs9923231</td>
<td>VKORC1 (Chr16)</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>0.235</td>
<td>0.119</td>
<td>0.136</td>
</tr>
<tr>
<td>rs2952151</td>
<td>PGAP3 (Chr17)</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>0.203</td>
<td>0.136</td>
<td>0.111</td>
</tr>
<tr>
<td>rs1048977</td>
<td>CDA (Chr1)</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>0.210</td>
<td>0.127</td>
<td>0.071</td>
</tr>
<tr>
<td>rs1051740</td>
<td>EPHX1 (Chr1)</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>0.091</td>
<td>0.119</td>
<td>0.214</td>
</tr>
<tr>
<td>rs1048013</td>
<td>CYP20A1 (Chr2)</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>0.226</td>
<td>0.106</td>
<td>0.2</td>
</tr>
<tr>
<td>rs2341970</td>
<td>SLC6A6 (Chr3)</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>0.208</td>
<td>0.115</td>
<td>0.111</td>
</tr>
<tr>
<td>rs2242416</td>
<td>CRIP3 (Chr6)</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>0.174</td>
<td>0.090</td>
<td>0.26</td>
</tr>
<tr>
<td>rs13197674</td>
<td>GSTA4 (Chr6)</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>0.111</td>
<td>0.103</td>
<td>0.234</td>
</tr>
<tr>
<td>rs7789971</td>
<td>AKAP9 (Chr7)</td>
<td>AA</td>
<td>AT</td>
<td>TT</td>
<td>0.111</td>
<td>0.109</td>
<td>0.241</td>
</tr>
<tr>
<td>rs7797834</td>
<td>CYP51A1 (Chr7)</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>0.241</td>
<td>0.095</td>
<td>0.167</td>
</tr>
<tr>
<td>rs28630521</td>
<td>CYP2D7P1 (Chr22)</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>0.207</td>
<td>0.098</td>
<td>0.143</td>
</tr>
</tbody>
</table>

Abbreviations: SLC22A1; solute carrier family 22 (organic anion/urate transporter) member 11, GSTZ1; glutathione transferase zeta 1, SLC28A2; solute carrier family 28, (sodium-coupled nucleoside transporter) member 2, VKORC1; Vitamin K epoxide reductase complex subunit 1, PGAP3; post-GPI attachment to proteins 3, CDA; cytidine deaminase, EPHX1; epoxide hydrolase 1 microsomal (xenobiotic), CYP20A1; cytochrome P450 family 20 subfamily A polypeptide 1, SLC6A6; solute carrier family 6 (neurotransmitter transporter) taurine member 6, CRIP3; cysteine-rich protein 3, AKAP9; A kinase anchor protein 9, CYP51A1; cytochrome P450 family 51 subfamily A polypeptide 1, CYP2D7P1; cytochrome P450 family 2 subfamily D polypeptide 7 pseudogene 1.

*SNP values as coded on the DMET platform.

*Probability that a patient is homozygote wild-type for each SNP, given that this patient has a clearance < 339.6 L/h.

*Probability that a patient is heterozygote for each SNP, given that this patient has a clearance < 339.6 L/h.

*Probability that a patient is homozygote for the variant allele for each SNP, given that this patient has a clearance < 339.6 L/h.
patient. However, germline-genetic variation, as present in normal tissue can influence the pharmacokinetics and pharmacodynamics of an anticancer drug regardless of tumor-type and therefore also affects treatment outcome and toxicity (26). Knowledge of this germline variation could therefore significantly contribute to a truly individualized pharmacotherapy of anticancer drugs.

In this study, we present the findings of a pharmacokinetic–pharmacogenetic study that relates multiple genetic variants in metabolic enzymes and transporters to the unbound clearance of paclitaxel, to identify patients with low clearance who are potentially at risk for increased toxicity. This analysis resulted in the development of a genetic signature, predictive for low paclitaxel clearance, containing 14 SNPs, which yielded a high-sensitivity, but a low-positive predictive value, when tested in a validation cohort.

To the best of our knowledge, this is the first large-scale application of the DMET platform to explain the pharmacokinetic variability of a commonly used anticancer drug. The traditional candidate gene approach is most often used in pharmacogenetic cancer research (27–29). However, this method is only able to identify a limited number of genetic variants, which are plausible candidates within the current knowledge of the field. The DMET platform enables us to study genetic variants in all currently known drug metabolizing enzymes and transporters simultaneously, making this a tool with high potential for pharmacogenetic research (26).

Our genetic prediction model yielded a sensitivity of 95% when tested in the validation cohort. However, the model had a positive-predicting value of only 22%. This means that this model identifies almost all patients with truly low clearance, at the cost of a high percentage of false positives. Also, 36 of 37 patients (97%) have truly no low clearance after testing negative for the genetic prediction model, meaning that they do not have an increased toxicity risk. To be clinically applicable, we reasoned that a prediction model should have besides a high-sensitivity also a high-positive predictive value. The latter is not reached in our study.

In previous studies several SNPs have been associated with paclitaxel clearance or toxicity, but contradictory findings have been reported. For example, ABCB1 3435 C>T was associated with paclitaxel clearance (30) and ABCB1 2677 G>T/A was associated with response to paclitaxel (31). The combination of ABCB1 3435 C>T and ABCB1 2677 G>T/A has been linked to neutropenia and patients with at least one ABCB1 3435 C>T showed a trend toward more development of neurotoxicity during paclitaxel therapy (6). Also, SNPs in CYP2C8 have been related to paclitaxel therapy response. For example, CYP2C8*3 carriers had higher rates of complete response than noncarriers (32). On the same note, CYP2C8*3 carriers were found to have lower paclitaxel clearance than noncarriers (8) and a higher risk of neurotoxicity (9, 10). However, none of these previous identified associations were found in other studies (5, 33).

Table 4. Results of validation of SNP prediction model in validation seta

<table>
<thead>
<tr>
<th>SNP prediction model</th>
<th>Truly low clearance</th>
<th>Truly no low clearance</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted low clearance</td>
<td>20 (15)</td>
<td>73 (56)</td>
<td>93 (72)</td>
</tr>
<tr>
<td>Predicted no low clearance</td>
<td>1 (1)</td>
<td>36 (28)</td>
<td>37 (28)</td>
</tr>
<tr>
<td>N</td>
<td>21 (16)</td>
<td>109 (84)</td>
<td>130 (100)</td>
</tr>
</tbody>
</table>

aCells represent the absolute number of patients with percentage of total patients in parentheses.
Interestingly, in the currently developed model, none of the SNPs that were previously associated with paclitaxel clearance or toxicity were included. However, this may partly be due to the fact that even this DMET-chip is not fully covering all existing drug metabolizing enzyme and transporter genes known today. For instance, CYP3A4*22, that we recently correlated with paclitaxel-induced neurotoxicity (34) is not (yet) available on the chip. In addition, epoxide hydrase 1 (EPHX1) and glutathione S-transferase alpha 4 (GSTA4) have previously been associated with docetaxel clearance in a small study which related selected genes in metabolism and signaling of reactive oxygen species (35, 36). Also, it was found that EPHX1 was upregulated in gemcitabine resistant NSCLC cells (37). None of the other 12 SNPs that were selected for the prediction model have previously been associated with clearance or toxicity of taxanes. Because of the discrepancy between the previously associated candidate SNPs with paclitaxel clearance and the outcome of this DMET analysis, we presume it is unlikely that common inherited genetic variability in drug metabolizing enzymes and transporters will contribute enough to explain the (large) interpatient variability in paclitaxel clearance. Several noninherited factors may mask the pharmacogenetic effects (38). However, Peters and colleagues described the response to paclitaxel treatment as having a high-heritability when assessing heritable drug-induced cell-killing on 125 lymphoblastoid cell lines derived from 14 families (39). If paclitaxel-induced toxicity and treatment outcome are more heritable than pharmacokinetics, remains to be elucidated. Our group is currently working on a study associating paclitaxel-induced toxicity to genetic variability as encoded on the DMET platform. To conclude, we developed a validated genetic prediction model in a large cohort of paclitaxel-treated patients to identify patients at risk of low clearance. Although this validated prediction model for paclitaxel clearance had a high-sensitivity, its positive-predictive value was too low to be of direct clinical use. Strikingly, the genes that are reported to influence paclitaxel pharmacokinetics were not identified in this analysis; we therefore conclude that genetic variability in DMET genes does not substantially contribute in explaining a large part of the interpatient variability in paclitaxel clearance. If these genes can explain the interpatient variability in the pharmacokinetics of other drugs should be explored in further studies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: A.-J.M. de Graan, L. Elens, J. Verweij, R.H.N. van Schaik, R.H.J. Mathijssen
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