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Impact of \textit{CYP2C8*3} on paclitaxel clearance: a population pharmacokinetic and pharmacogenomic study in 93 patients with ovarian cancer

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Abstract

The primary purpose of this study was to evaluate the impact of CYP2C8*3 and three genetic ABCB1 variants on the elimination of paclitaxel. We studied 93 Caucasian women with ovarian cancer treated with paclitaxel and carboplatin. Using sparse sampling and nonlinear mixed effects modeling, the individual clearance of unbound paclitaxel was estimated from total plasma paclitaxel and Cremophor EL. The geometric mean of clearance was 385 l/h (range 176-726 l/h). Carriers of CYP2C8*3 had 11% lower clearance than non carriers, P=0.03. This has not been demonstrated before in similar studies; the explanation is probably the advantage of using both unbound paclitaxel clearance and a population of patients of same gender. No significant association was found for the ABCB1 variants C1236T, G2677T/A and C3435T. Secondarily other candidate SNPs were explored with possible associations found for CYP2C8*4 (P=0.04) and ABCC1 g.7356253C>G (P=0.04).
Introduction

Paclitaxel is used to treat cancer of the breast, lung, ovary and other neoplasms. In ovarian cancer the standard of care in most clinics is post-surgery combination chemotherapy with carboplatin and paclitaxel. Although the initial response rate is as high as 70-85%(1), most patients with advanced stages eventually relapse and die of the disease. Paclitaxel is dosed normalized to body surface area (BSA) and in most regimes infused over 1, 3 or 24 hours. The dose limiting toxicities are neutropenia and neuropathy. There is a large inter-individual variability in toxicity and therapeutic effect of paclitaxel in ovarian cancer treatment which remains a clinically relevant problem with implications on survival and quality of life of the patients and practical with regard to the handling of dose delay, dose reduction or cessation of the treatment(2). Several possible causes of this variability have been suggested; especially the notion that single nucleotide polymorphisms (SNPs) in certain genes could cause significant changes in the affinity and/or expression of key transporters or metabolizing enzymes. Paclitaxel is metabolized to inactive compounds by CYP2C8 and CYP3A4 in the liver(3-5), and is also a substrate for the ATP driven efflux pump P-glycoprotein encoded by the ABCB1 gene(6). A host of other candidate genes linked to paclitaxel were picked for exploratory analysis: they include CYP3A5, ABCC1, ABCC2, ABCG2, ABCC10, CYP1B1 and SLCO1B3(7-13). These are all drug transporters except CYP3A5 which has a substrate range similar to CYP3A4, and CYP1B1 which has been implied to have a role in taxane metabolism and therapeutic effects(7). Transporters can facilitate elimination by actively transporting the drug into a metabolizing cell or by actively exporting drug to the bile.

Total plasma paclitaxel elimination has previously been shown to be nonlinear(14). This has been attributed to saturable transport(15) and saturable binding(16) but also to paclitaxel being trapped in micelles formed by adjuvant Cremophor EL(17-19). Considering the unbound clearance of paclitaxel instead of total plasma clearance can therefore be expected to give a clearer estimate of the impact of genetic variants on the elimination processes.
The aim of the present study was to evaluate the elimination capacity of patients with the
CYP2C8*3, ABCB1 C1236T, G2677T/A and C3435T variants in a confirmatory type analysis and other
variants in CYP2C8 and ABCB1 as well as variants in CYP1B1, CYP3A4/5, SLCO1B3, ABCC1, ABCC2,
ABCG2 and ABCC10 in an exploratory analysis. The study was initiated based on reports on
CYP2C8*3 as having decreased paclitaxel metabolism(20;21) and reports of a functional significance
of ABCB1 SNPs with particular interest in C1236T, G2677T/A and C3435T(22-24); the other candidate
SNPs entered the study after it was initiated but the a priori distinction between confirmative and
exploratory candidates was kept in order to meet the issue of multiple testing.
We present a prospective pharmacogenetic study on the population pharmacokinetics of paclitaxel
in 93 Scandinavian Caucasian female patients with epithelial ovarian/peritoneal cancer.

Patients and methods

Patients

Eligibility criteria included: candidates for paclitaxel/carboplatin chemotherapy, clinical or
histopathological diagnosis of epithelial ovarian cancer, fallopian tube cancer or peritoneal cancer,
age ≥18 years, Caucasian, WHO / ECOG performance status ≤2, no concurrent malignant diseases
and adequate contraception for women of child-bearing potential. All patients provided written and
verbal consent before entering the study as per Helsinki declaration. All patients were scheduled for
six to nine courses of paclitaxel (175 mg/m²), 3 hour iv-infusion followed by carboplatin (AUC 5-6
mg*min/ml), every third week. Paclitaxel was administered in formulation with Cremophor EL (CrEL;
polyoxyethylene glycerol triricinoleate 35) at 5 ml per 30 mg paclitaxel. All patients were pretreated
with steroid and histamine receptors H1 and H2 antagonists as recommended by national
guidelines. Concurrently used drugs were recorded and compared to known inhibitors of CYP3A4/5
and CYP2C8. The study was approved by regional ethics committees of Southern Denmark and Lund,
Sweden. Patients were recruited at oncology departments in Denmark and Sweden.
Blood sampling, paclitaxel and cremophor EL analysis

Three consecutive samples were collected from one cycle from each patient. Plasma was sampled at noted time points; approximately at 3 hours (just prior to end of infusion), at 5-8 hours and at 18-24 hours after start of paclitaxel infusion. Aliquots of whole blood for DNA extraction was kept at -20 °C or less. The total concentration of paclitaxel in plasma was estimated using a liquid-liquid extraction method and High-Performance Liquid Chromatography (HPLC) with UV detection. Paclitaxel was purchased from Sigma, St. Louis, MI, and docetaxel from Fluka, Buchs, CH. The extraction method was performed as described by Alexander et al.(25). The sample was transferred to a conical 300 μl polypropylene HPLC micro vial. An aliquot of 40 μl was injected into a LaChrom HPLC system (Merck-Hitachi, Darmstadt, Germany) and monitored at λ= 227 nm on the UV-detector. The separation was performed on a 50 x 2.1 mm Gold C18 column (2.1 μm particle size) (Thermo, San Jose, CA) at 30°C using isocratic elution. The mobile phase consisted of acetonitrile: Milli-Q water: glacial acetic acid (35:65:0.1 v/v %). The flow rate was 0.2 ml/min. Calibration curves ranging up to 10 000 nmol/l was produced for each day of analysis and showed a good linearity (r=0.997). The intraday variability was < 8.5 % for paclitaxel. The interday variability (n=5 days) was investigated for six concentration levels (75, 300, 750, 1 500, 3 500 and 8 500 nmol/l) and was < 6 % for paclitaxel. The accuracy for paclitaxel ranged from 89.2 to 101.8 %. The lower limit of detection was 20 nmol/l and the lower limit of quantification was 25 nmol/l for paclitaxel.

Cremophor EL concentrations were determined by an earlier described method with minor changes(26). The samples were analysed in duplicate with a standard curve ranging from 0.1–1.0 % and a spiked quality control sample. The sample concentration was interpolated from the non-linear standard curve by second-order polynominal regression using GraphPad Prism version 5.0a (GraphPad software, USA). The intra- and interday variation (C.V.) of the quality control sample was found to be 7.0 % and 7.4 %, respectively (n=10) and the intra- and interday accuracy (%-difference from the spiked “true value”) was +1 % and -3 %, respectively. The limits of quantification were set by the standard curve which covered all plasma samples analysed in the study.
Pharmacokinetic analysis

The clearance of unbound paclitaxel was estimated from total paclitaxel concentrations and CrEL concentrations in a nonlinear mixed effects analysis. This approach is described by Henningsson et al(27); in brief, we used a two compartment structure with total paclitaxel concentrations \( C_p \) and unbound concentration \( C_u \) described with the following equation:

\[
C_p = C_u + 3.31 \times C_u + 4.46 \times [\text{CrEL}] \times C_u + \frac{C_u \times B_{\text{max}}}{K_m + C_u}
\]

Where \([\text{CrEL}]\) is the measured Cremophor EL concentration, \( B_{\text{max}} = 0.2311 \times [\text{AAGP}] - 0.017 \), \([\text{AAGP}]\) is the measured \( \alpha \)-acid glycoprotein concentration and \( K_m = B_{\text{max}}/9.41 \). Constants are the fixed estimates reported by Henningsson et al. The covariate model proposed by Henningsson et al(27) with BSA explaining IIV (inter-individual variability) of clearance and volumes of distribution and bilirubin explaining IIV of unbound clearance was used without any changes as the basic model. Additionally age, performance status, smoking, alcohol, albumin, alkaline phosphatase and alanine aminotransferase were tested as covariates in a trial and error manner using likelihood ratio test in NONMEM. Age and performance status further explained IIV of clearance, lowering the objective function value (OFV) 13.5 and 18.5 points respectively. Assuming that OFV approximately is chi-squared distributed, a true effect of the covariates seemed very likely (P-value < 0.001) and both were added in the final model. No other covariates significantly contributed to IIV. Empirical Bayes estimates of unbound clearance of paclitaxel were obtained and are herein referred to as clearance.

All analyses were performed with the first-order conditional estimation method with interaction in NONMEM VI (Beal & Sheiner, University of California, San Francisco, CA) and graphical diagnostics were obtained using the software Xpose(28) and PsN(29).

Genotyping procedures

Genomic DNA was extracted from whole blood using standard methods. SNPs in \textit{CYP2C8}, \textit{CYP3A4}, \textit{SLCO1B3} (except G767C) and \textit{ABCB1} (except A-1G) were determined using Pyrosequencing as previously described(30-32). The variants in \textit{CYP1B1}, \textit{CYP3A5}, \textit{ABCC1}, \textit{ABCC10} and G767C in
SLCO1B3, A-1G in ABCB1 and rs2273697 in ABCC2 were genotyped using TaqMan® pre-designed SNP genotyping assays by Applied Biosystems (Foster city, CA). SNPs in ABCG2 and rs17222723 and rs8187710 in ABCC2 were genotyped using pairs of allele specific primers (ASP) and a locus specific primer (LSP) in SYBRgreen real time PCR assays. The used primers were: rs8187710 (ABCC2) ASP: 5’-CCTAGA CAACGGGAAG ATTATAGAGTG-3’/5’-TCCTAGA CAACGGGAAG ATTATAGAGTA-3, LSP: 5’-GCTAG AATTTTGTGC TGTTACATT C-3’; rs17222723 (ABCC2) ASP: 5’-CCCACCGCTAA TATCAAACAT ATAGAAC-3; rs2231142 (ABCG2) ASP: 5’-CCTCTGACGGTAGAAAAACTMAM-3’, LSP: 5’-GCCACTTTAT CCAGACCTAA CTCTTG-3; rs2231137(ABCG2) LSP: 5’-CACCTAGTGTGTTGCA ATCTGATTATCT-3’, ASP: 5’-CCATTGGTGTTTCCTTGTGACAY-3’. All real time assays were performed on ABI PRISM 7700 or StepOnePlus from Applied Biosystems(Foster city, CA). Genotype calling using SYBRgreen was carried out by visual assessment by two persons separately.

**ABCB1 haplotype inference**

Haplotypes of the six ABCB1 SNPs were inferred using the software package PHASE, version 2.1 by Stephens et al(33;34). The program infers haplotype frequencies and the most likely individual haplotype using a Bayesian statistical method. We ran PHASE, with default settings 10 times. All runs returned 100% consistent calls. The inferred population frequencies agreed well with Kroetz et al(35) who investigated the haplotype structure of the ABCB1 gene in 100 Caucasian from a human variation panel and the frequencies estimated by the EM algorithm in STATA 10 (StataCorp, Texas, USA). In order to test an allele dose effect the resulting 13 haplotypes (with phase information) were grouped into diplotype 1 to 4 based on the 1236C>T, 2677G>TA and 3435C>T SNPs in a fashion similar to Sissung et al(36). The “full wild-type” is thus CGC/CGC (diplotype 1) and the “full variant type” TTT/TTT (diplotype 3) and the heterotype CGC/TTT (diplotype 2)(Table 1). This approach can possibly reveal effects of interplay between cis variants and are proposed by some to add valuable information that is impossible to achieve by assessing the SNPs separately(37-39).
Statistical methods

Logtransformed clearance was assessed visually to be approximately normal distributed and was used throughout the study. Geometric means are antilog of means of the log transformed data. All tests are two sided. For the confirmatory type analysis, P-values of 0.05 or less are considered statistical significant. For association testing multiple regression (trend) test was used; thus assuming an allele dose effect. Both raw P-values and values for the test controlled for BSA, age and performance status are reported; all computations were carried out using STATA 10 (StataCorp, Texas, USA). Inferred haplotypes in \textit{ABCB1} were tested for association to clearance using the software package BIMBAM version 0.99 by Servin and Stephens(40), assuming normal distribution of clearance; the program was run with default settings.
Results

Patients

Total plasma paclitaxel and Cremophor EL concentrations were available from 93 patients. They were recruited from four hospital centers in Denmark: Odense 66 (71 %), Herlev 12 (13 %), Herning 7 (8 %), Vejle 4 (4 %) and one in Sweden: Lund 4 (4 %). Median age was 60.1 years (range, 32-79.4 years) (Table 2). One additional patient was omitted from analysis because the 18-24 hour sample gave consistent results of 3172 ng/l (45-fold higher than mean) and a vial mix-up could be excluded. Two patients received bevacizumab from the 2nd cycle (subsequent to the plasma sampling cycle). Three patients used a CYP3A4/5 inhibitor prior to or during the course where PK samples were taken. One patient used ketoconazole, the two others used fluconazole. The individual clearance estimates of unbound paclitaxel for these three patients were 383 l/h, 456 l/h and 282 l/h respectively (Figure 1). No significant correlation between genotypes and BSA, age, bilirubin and performance status was found (data not shown).

Paclitaxel pharmacokinetics

The nonlinear mixed effects model fitted the data well as indicated by goodness-of-fit plots (not shown) and previous data using this model (Table 3). Overall geometric mean of clearance of unbound paclitaxel was 385 l/h (range 176-726 l/h). Typical estimates of compartment volumes and inter compartmental clearances and relative standard errors were similar to those earlier reported (27;41)(Table 3). The model predicts that: an increase in BSA of 0.1m² increases the typical value of clearance by 6.2 %, an increase in age of 10 years decrease the typical value of clearance by 6 % and the typical value of clearance for patients with WHO/ECOG performance status of 0-1 was 396 l/h compared to 304 l/h for performance status 2. The average measured Cremophor EL concentration and range were 4.10 vol/vol % [2.63-6.67 %], 3.32 vol/vol % [2.25-5.81 %] and 2.07 vol/vol % [1.10-3.39 %] for the first, second and third sampling respectively.
Results of genotyping and haplotype inference

Twenty-two single nucleotide polymorphisms (SNPs) were analyzed in 10 genes. Candidate SNPs were split, a priori, into two groups; one for confirmative type analysis and one for exploratory analysis (Table 4). Allele frequencies were all similar to frequencies reported in the NCBI SNP database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp). The genotype distributions were all in Hardy-Weinberg equilibrium (numbers not shown) except CYP2C8*1B, ABCG2 (c.421C>A) rs2231142 and CYP1B1*3 where the P-values of Pearson Chi2 statistic were 0.03, 0.05 and 0.01 respectively. Inferring haplotypes in ABCB1 resulted in 13 haplotypes (data not shown) with frequencies from 0.5% to 27.4%. Frequencies were comparable with a previous report by Kroetz et al (35).

Genotype vs. paclitaxel clearance

The confirmative analysis: The CYP2C8*3 genotype was associated with an 11% lower clearance of unbound paclitaxel, which was statistically significant with a P-value of 0.03 (Figure 1). No significant correlation was found between clearance and ABCB1 C1236T, G2677T/A and C3435T genotypes (Table 4). Analysis of ABCB1 “haplotypes” performed using the BIMBAM software showed no association with clearance for any single SNP or any two or three SNP combination (data not shown). No significant association was found between diplotype group 1-3 and clearance (P-value of 0.12) using linear regression trend-test controlled for BSA, age and performance status (Table 1).

Explorative analysis: seven patients with the CYP2C8*4 (c.792C>G) variant had 18% lower mean clearance (geometric) of unbound paclitaxel than wild-type individuals (Table 4), P-value 0.04. The one individual carrying both the CYP2C8*3 and *4 variant had a clearance of 270 l/h. For the intronic SNP in ABCC1 (rs504348) carrying one or two variant alleles were associated with 7% lower clearance of unbound paclitaxel, P-value 0.04. P-values for the exploratory analysis are reported without any correction for multiple testing and should be interpreted accordingly.
Discussion

Our study tests the hypothesis that genetic variants in \textit{CYP2C8} and \textit{ABCB1} contribute to the observed inter-individual variability in paclitaxel clearance. We found evidence supporting a reduced clearance among patients carrying the \textit{CYP2C8}*3 and possibly also the *4 variant. These results confirm the finding in a pilot study on 33 ovarian cancer patients treated in Sweden\cite{42}. To our knowledge this is the largest prospective study reporting this association. The results, however, are in somewhat contrast to Henningsson et al\cite{43} who found no correlation between clearance and \textit{CYP2C8}*3, \textit{CYP2C8}*4 and \textit{ABCB1} C3435T in 97 patients (male and female) treated with paclitaxel (80-225\,mg/m$^2$) and Marsh et al\cite{44} who found no correlation between clearance and \textit{CYP2C8}*3, \textit{CYP2C8}*4, \textit{ABCB1} C1236T and \textit{ABCB1} G2677T in 93 patients with breast cancer treated with 24-hour paclitaxel infusion (575-775\,mg/m$^2$). This discrepancy can be explained by chance or: in case of the study by Henningsson et al, by gender in so far that Joerger et al\cite{45} demonstrated a 20\% higher elimination of paclitaxel in males than in females, and in the case of the study by Marsh et al by the nonlinear elimination of paclitaxel reported in the studied population\cite{46}. In the pilot study of 33 patients\cite{42}, the rare genotype 2677GA was associated with increased clearance of paclitaxel compared to 2677TT and 2677GG. In the present study five patients carried the 2677A allele but although the patient with the highest clearance among all patients (726 l/h) carried this allele no significant association was found in this study (data not shown). Baker et al\cite{47} studied docetaxel in 92 patients and found no correlation between docetaxel clearance and \textit{ABCB1}, \textit{ABCC2} and \textit{SLCO1B3} variants, but they did report a 64\% increase in clearance for the simultaneous presence of \textit{CYP3A4}*1\text{B} and \textit{CYP3A5}*1\text{A}. This finding was not replicated in our study (data not shown).

In the exploratory analysis a possible association between an intronic SNP in \textit{ABCC1} (rs504348) and clearance was found. To our knowledge this has not been reported in the literature before. However, replication in future studies are warranted before any conclusions can be made regarding this association because the multiple testing makes a spurious finding more likely. The only support
in the literature is contradicting evidence supporting a role of ABCC1 in taxane metabolism in vitro\cite{48,49} and a single report of a possible relationship to a clinical (in vivo) outcome\cite{50}. The geometric mean of paclitaxel clearances across diplotype 1-3 is 399, 381 and 355 l/h which suggest a trend; however it did not reach the level of statistical significance (P-value 0.12).

While not the aim of the study, our results support the finding by Joerger et al\cite{45} that age is negatively correlated and BSA positively correlated to paclitaxel clearance; the 11 % decrease in clearance of unbound paclitaxel explained by CYP2C8 variant is comparable in size to the effects of gender (20 %, male > female), age (-11 % per 20 years) and BSA (11 % per 0.2m$^2$) reported by Joerger et al. Somewhat surprisingly the patients’ performance status seem to have strong negative impact on the clearance; mean clearance and 10$^{th}$ and 90$^{th}$ percentiles observed were 397 l/h [300;490 l/h] and 291 l/h [176;468 l/h] for performance status 0-1 and 2 respectively. This impact on clearance of a “non-fixed” variable provides a competing and dynamic biological explanation for clearance that certainly should be controlled for in the analysis. Omitting patients with performance status 2 from analyses like this might provide a clearer signal, but on the other hand information about an important subgroup would be lost. Although we did find an association between CYP2C8*3 and clearance of unbound paclitaxel, and causality of the association is scientifically plausible - the magnitude of the effect (i.e. approximately 11%) is somewhat disappointing. Even if we assume that the clearance is tightly correlated to the efficacy or toxicity of paclitaxel – genotyping for CYP2C8*3 in clinical practice is not rational at this point in time because the effect is too small. The variability is likely explained by multiple small effect factors both genetic and environmental. It can be speculated that if individualized treatment with paclitaxel is to become a reality in the future and CYP2C8*3 genotyping is to play a role therein it must be in combination with multiple other factors.

In conclusion the study provides evidence supporting a contribution from the CYP2C8*3 variant and possibly also the CYP2C8*4 and ABCC1 (rs504348) variants to the variability of paclitaxel clearance. In future studies (of paclitaxel) it might be worthwhile to consider pooling patients with the CYP2C8*3 and *4 variant into one group. Our finding is important in general because it adds to our
understanding of inter-individual variability in pharmacokinetics and in particular because it contributes to the growing field of taxane pharmacogenomics.
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Conflicts of interest/Disclosure

None declared.
References


correlation of one allele with P-glycoprotein expression and activity in vivo.


(46) Somlo G, Doroshow JH, Synold T, Longmate J, Reardon D, Chow W et al. High-dose paclitaxel in combination with doxorubicin, cyclophosphamide and peripheral blood progenitor cell rescue in patients with high-risk primary and responding metastatic breast carcinoma:
toxicity profile, relationship to paclitaxel pharmacokinetics and short-term outcome.

*Br. J. Cancer* 2001; **84**: 1591-1598.


Table 1 - *ABCB1* diplotype constructs from the three loci: C1236T, G2677T/A and C3435T vs. Clearance of unbound paclitaxel. P-value 0.12

<table>
<thead>
<tr>
<th>#</th>
<th>Diplotype</th>
<th>N=93</th>
<th>Nucleotide by phase</th>
<th>Mean CL (l/h)</th>
<th>10th²</th>
<th>90th²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wt/Wt</td>
<td>9</td>
<td>CGC/CGC</td>
<td>399</td>
<td>264</td>
<td>641</td>
</tr>
<tr>
<td>2</td>
<td>Wt/full var</td>
<td>27</td>
<td>CGC/TTT</td>
<td>381</td>
<td>296</td>
<td>478</td>
</tr>
<tr>
<td>3</td>
<td>full var/full var</td>
<td>18</td>
<td>TTT/TTT</td>
<td>355</td>
<td>291</td>
<td>437</td>
</tr>
<tr>
<td>4</td>
<td>Other</td>
<td>39</td>
<td><em><strong>/</strong></em></td>
<td>399</td>
<td>282</td>
<td>552</td>
</tr>
</tbody>
</table>

Observed number of patients assigned to the different diplotypes based on the inferred (phased) haplotype. Type 4 is any other combination of nucleotides (denoted *****). A linear regression analysis (trend-test) across diplotype 1 to 3, controlled for body surface area, age and performance status showed no statistical significant correlation with clearance, P-value 0.12 (uncontrolled P-value 0.17).

1) Geometric mean clearance of unbound paclitaxel

2) 10th and 90th percentile observation of clearance
Table 2 - Patient summary

<table>
<thead>
<tr>
<th>Characteristics for n=93 evaluable patients</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.1 (32-79.4)</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.71 (1.27-2.37)</td>
</tr>
<tr>
<td><strong>Baseline biochemistry</strong></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (U/l)</td>
<td>22 (6-160)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>42 (27 – 51)</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>87.5 (45 – 322)</td>
</tr>
<tr>
<td>Bilirubin (μmol/l)</td>
<td>5 (2-28)</td>
</tr>
<tr>
<td>α-acid glycoprotein (g/l)</td>
<td>1.08 (0.5 – 2.75)</td>
</tr>
<tr>
<td><strong>WHO/ECOG Performance status</strong></td>
<td>N (%)</td>
</tr>
<tr>
<td>0</td>
<td>53 (57 %)</td>
</tr>
<tr>
<td>1</td>
<td>31 (33 %)</td>
</tr>
<tr>
<td>2</td>
<td>9 (10 %)</td>
</tr>
<tr>
<td><strong>Tumor type</strong></td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>59 (63 %)</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>20 (22 %)</td>
</tr>
<tr>
<td>Fallopian tube</td>
<td>4 (4 %)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>10 (11 %)</td>
</tr>
<tr>
<td><strong>FIGO stage</strong></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>25 (27 %)</td>
</tr>
<tr>
<td>II</td>
<td>10 (11 %)</td>
</tr>
<tr>
<td>III</td>
<td>39 (42 %)</td>
</tr>
<tr>
<td>IV</td>
<td>13 (14 %)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>6 (7 %)</td>
</tr>
<tr>
<td><strong>Tumor grade</strong></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>15 (16 %)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>23 (25 %)</td>
</tr>
<tr>
<td>Poorly or undifferentiated</td>
<td>36 (39 %)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Undetermined</td>
<td>19 (20 %)</td>
</tr>
</tbody>
</table>

1) Eastern Cooperative Oncology Group

2) International Federation of Gynecology and Obstetrics
Table 3 – Population pharmacokinetic parameter estimates for unbound paclitaxel with RSE(%)  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate¹ (RSE%)</th>
<th>Henningsson et al (27) Estimate² (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL² (l/h) for PS =0-1</td>
<td>396 (3.2)</td>
<td>343 (3.5) PS0-2</td>
</tr>
<tr>
<td>CL (l/h) for PS=2</td>
<td>304 (7.8)</td>
<td></td>
</tr>
<tr>
<td>V1 (l)</td>
<td>296 (17.8)</td>
<td>418 (7.1)</td>
</tr>
<tr>
<td>Q (l/h)</td>
<td>156 (13.7)</td>
<td>188 (13)</td>
</tr>
<tr>
<td>V2 (l)</td>
<td>793 (4.6)</td>
<td>1010 (4.2)</td>
</tr>
<tr>
<td>BSA on V1</td>
<td>1.11 (49.2)</td>
<td>1.45 (14)</td>
</tr>
<tr>
<td>BSA on CL</td>
<td>0.62 (22.6)</td>
<td>0.65 (22)</td>
</tr>
<tr>
<td>BSA on V2</td>
<td>0.48 (28)</td>
<td>0.87 (18)</td>
</tr>
<tr>
<td>Age on CL</td>
<td>-0.006⁵ (31.2)</td>
<td>n/a</td>
</tr>
<tr>
<td>Bilirubin on CL</td>
<td>fixed</td>
<td>-0.012 (21)</td>
</tr>
<tr>
<td>IIV on CL (CV%)³</td>
<td>17 (32)</td>
<td>20 (24)</td>
</tr>
<tr>
<td>IIV on V2 (CV%)³</td>
<td>16 (84)</td>
<td>16 (37)</td>
</tr>
<tr>
<td>IIV CrEL Binding (CV%)⁴</td>
<td>9.6 (130)</td>
<td>14 (31)</td>
</tr>
</tbody>
</table>

CL, clearance of unbound paclitaxel; PS, performance status; V, compartment volume; Q, intercompartmental clearance; BSA, body surface area; IIV, inter-individual variability; CV%, coefficient of variation in %; CrEL, cremophor EL; RSE, relative standard error.

1) Estimates for the typical individual with BSA 1.71m², bilirubin 5 μM and age of 60.7 years  
2) Estimates for the typical individual with BSA 1.76m² and bilirubin 5 μM  
3) \( \text{CL} = 396 \times (1 + 0.62 \times (\text{BSA} - 1.71)) \times (1 - 0.012 \times (\text{Bilirubin-5})) \times (1 - 0.006 \times (\text{Age-60.7})) \)  
4) RSE% is related to the corresponding variance term, \( \omega^2 \)  
5) Bootstrap (resampling 2 000 times): median 0.00498, 97.5% c.i.[-0.00911 : -0.00059:], 99.55% c.i.[-0.01093 : 0.00048]
<table>
<thead>
<tr>
<th>Gene / allele</th>
<th>Effect</th>
<th>Reference homozygote</th>
<th>Heterozygote</th>
<th>Variant homozygote</th>
<th>P-value</th>
<th>SNP ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C8</td>
<td></td>
<td>N²</td>
<td>CL² (10th-90th)</td>
<td>N²</td>
<td>CL² (10th-90th)</td>
<td>N²</td>
</tr>
<tr>
<td>1196A&gt;G (*3)</td>
<td>K399R</td>
<td>74</td>
<td>395 (297-490)</td>
<td>19</td>
<td>350 (238-458)</td>
<td>0.03*(0.04)</td>
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<tr>
<td>ABCB1</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1236C&gt;T</td>
<td>G412G</td>
<td>29</td>
<td>391 (270-569)</td>
<td>45</td>
<td>393 (299-490)</td>
<td>19</td>
</tr>
<tr>
<td>2677G&gt;T/A⁷</td>
<td>A893S/T</td>
<td>26</td>
<td>387 (270-490)</td>
<td>42(GT)</td>
<td>396 (299-490)</td>
<td>20(TT)</td>
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<tr>
<td>3435C&gt;T</td>
<td>I1145I</td>
<td>11</td>
<td>403 (326-548)</td>
<td>44</td>
<td>387 (282-490)</td>
<td>38</td>
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<tr>
<td>CYP3A4</td>
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<tr>
<td>219-237G&gt;A</td>
<td>frameshift</td>
<td>84</td>
<td>388 (297-490)</td>
<td>9</td>
<td>360 (176-726)</td>
<td>0.30(0.36)</td>
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<tr>
<td>SLCO1B3</td>
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<tr>
<td>699G&gt;A</td>
<td>M233I</td>
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<td>326</td>
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<td>377 (299-481)</td>
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<tr>
<td>767G&gt;C</td>
<td>G256A</td>
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<td>386 (298-481)</td>
<td>26</td>
<td>383 (291-490)</td>
<td>0.63(0.89)</td>
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<td>1294C&gt;G (*3)</td>
<td>V432L</td>
<td>30</td>
<td>389 (270-530)</td>
<td>36</td>
<td>401 (298-490)</td>
<td>27</td>
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<td>7356253C&gt;G</td>
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<td>1249G&gt;A</td>
<td>V417I</td>
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<td>396 (297-552)</td>
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<tr>
<td>3563T&gt;A</td>
<td>V1188E</td>
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<td>386 (296-490)</td>
<td>5</td>
<td>370 (176-569)</td>
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<tr>
<td>4544G&gt;A</td>
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<td>389 (296-490)</td>
<td>3</td>
<td>355 (176-569)</td>
<td>0.72(0.52)</td>
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<td>ABCG2</td>
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<tr>
<td>421C&gt;A</td>
<td>Q141K</td>
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<td>374 (291-478)</td>
<td>32</td>
<td>408 (315-548)</td>
<td>0.4(0.09)</td>
</tr>
<tr>
<td>34G&gt;A</td>
<td>V12M</td>
<td>87</td>
<td>385 (291-490)</td>
<td>4</td>
<td>395 (296-726)</td>
<td>0.68(0.83)</td>
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<td>2759T&gt;C</td>
<td>I920T</td>
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<td>386 (297-478)</td>
<td>43</td>
<td>386 (291-548)</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4 - Clearance of unbound paclitaxel as function of observed genotypes
Legend Table 4:

1) Type and sequence position of nucleotide substitutions with star notation where applicable
2) Single letter nomenclature amino acid substitution where applicable
3) N, denotes the number of patients in the group. For some patients genotypes were unavailable
4) Geometric mean clearance of unbound paclitaxel with 10th and 90th percentiles observed in parentheses
5) P-values for the linear regression analysis (trend) of clearance and genotype controlled for body surface area, age and performance status with uncontrolled P-values shown in parentheses
7) The 5 patients carrying the A allele were omitted from the analysis
Figure 1 - Estimates of clearance of unbound paclitaxel as function of CYP2C8*3 genotype for 93 patients with ovarian cancer.

The clearance of the three patients concomitantly using a CYP3A4/5 inhibitor are marked with black diamonds (none carried the *3 variant). Means are indicated by the horizontal line.

The linear regression analysis of clearance and genotype controlled for body surface area, age and performance status showed that patients carrying the CYP2C8*3 variant had a statistically significant lower mean clearance with P-value of 0.03 (uncontrolled P-value 0.04). Wt, wild-type.