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**Running title: Impact of *CYP2C8\*3* on paclitaxel clearance**

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

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**Keywords:** CYP2C8, ABCB1, P-glycoprotein, paclitaxel, cremophor, ovarian cancer

## **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*. Secondly 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  $\geq 18$  years, Caucasian, WHO / ECOG performance status  $\leq 2$ , 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 ( $175 \text{ mg/m}^2$ ), 3 hour iv-infusion followed by carboplatin (AUC 5-6  $\text{mg} \cdot \text{min/ml}$ ), every third week. Paclitaxel was administered in formulation with Cremophor EL (CrEL; polyoxyethyleneglycerol 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  $\lambda = 227$  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 polynomial 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.

## Pharmakokinetic 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:

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

Where [CrEL] is the measured Cremophor EL concentration,  $B_{\max} = 0.2311 \times [AAGP] - 0.017$ , [AAGP] is the measured  $\alpha$ -acid glycoprotein concentration and  $K_m = B_{\max}/9.41$ . 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 *CYP2C8*, *CYP3A4*, *SLCO1B3* (except G767C) and *ABCB1* (except A-1G) were determined using Pyrosequencing as previously described(30-32). The variants in *CYP1B1*, *CYP3A5*, *ABCC1*, *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'-GCG ATTTCTGAAA CACAATGAGGW-3', LSP: 5'-CCCACCGCTAA TATCAAACAT ATAGAAC-3; rs2231142 (*ABCG2*) ASP: 5'-CACTCTGACGGTGAGAGAAAATTAM-3', LSP: 5'-GCCACTTAT CCAGACCTAA CTCTTG-3'; rs2231137(*ABCG2*) LSP: 5'-CACCTAGTGTGGCA ATCTCATTATCT-3', ASP: 5'-CCATTGGTGTTCCTTGTGACAY-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 *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 2<sup>nd</sup> 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 0.1m<sup>2</sup> 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 Chi<sup>2</sup> 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 *CYP2C8* and *ABCB1* contribute to the observed inter-individual variability in paclitaxel clearance. We found evidence supporting a reduced clearance among patients carrying the *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(42). To our knowledge this is the largest prospective study reporting this association. The results, however, are in somewhat contrast to Henningsson et al(43) who found no correlation between clearance and *CYP2C8*\*3, *CYP2C8*\*4 and *ABCB1* C3435T in 97 patients (male and female) treated with paclitaxel (80-225mg/m<sup>2</sup>) and Marsh et al(44) who found no correlation between clearance and *CYP2C8*\*3, *CYP2C8*\*4, *ABCB1* C1236T and *ABCB1* G2677T in 93 patients with breast cancer treated with 24-hour paclitaxel infusion (575-775mg/m<sup>2</sup>). 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(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(46). In the pilot study of 33 patients(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(47) studied docetaxel in 92 patients and found no correlation between docetaxel clearance and *ABCB1*, *ABCC2* and *SLCO1B3* variants, but they did report a 64% increase in clearance for the simultaneous presence of *CYP3A4*\*1B and *CYP3A5*\*1A. This finding was not replicated in our study (data not shown).

In the exploratory analysis a possible association between an intronic SNP in *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(48;49) and a single report of a possible relationship to a clinical (in vivo) outcome(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(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<sup>2</sup>) reported by Joerger et al. Somewhat surprisingly the patients' performance status seem to have strong negative impact on the clearance; mean clearance and 10<sup>th</sup> and 90<sup>th</sup> 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.

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

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

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**

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

Poorly or undifferentiated	36 (39 %)
Undetermined	19 (20 %)

1) Eastern Cooperative Oncology Group

2) International Federation of Gynecology and Obstetrics

**Table 3 – Population pharmacokinetic parameter estimates for unbound paclitaxel with RSE(%)**

Parameter	Estimate <sup>1</sup> (RSE%)	Henningsson et al(27) Estimate <sup>2</sup> (RSE%)
CL <sup>3</sup> (l/h) for PS =0-1	396 (3.2)	343 (3.5) PS0-2
CL (l/h) for PS=2	304 (7.8)	
V1 (l)	296 (17.8)	418 (7.1)
Q (l/h)	156 (13.7)	188 (13)
V2 (l)	793 (4.6)	1010 (4.2)
BSA on V1	1.11 (49.2)	1.45 (14)
BSA on CL	0.62 (22.6)	0.65 (22)
BSA on V2	0.48 (28)	0.87 (18)
Age on CL	-0.006 <sup>5</sup> (31.2)	n/a
Bilirubin on CL	fixed	-0.012 (21)
IIV on CL (CV%) <sup>4</sup>	17 (32)	20 (24)
IIV on V2 (CV%) <sup>4</sup>	16 (84)	16 (37)
IIV CrEL Binding (CV%) <sup>4</sup>	9.6 (130)	14 (31)

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 1.71m<sup>2</sup>, bilirubin 5 µM and age of 60.7 years
- 2) Estimates for the typical individual with BSA 1.76m<sup>2</sup> and bilirubin 5 µM
- 3)  $CL = 396 * (1 + 0.62 * (BSA - 1.71)) * (1 - 0.012 * (Bilirubin - 5)) * (1 - 0.006 * (Age - 60.7))$
- 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]

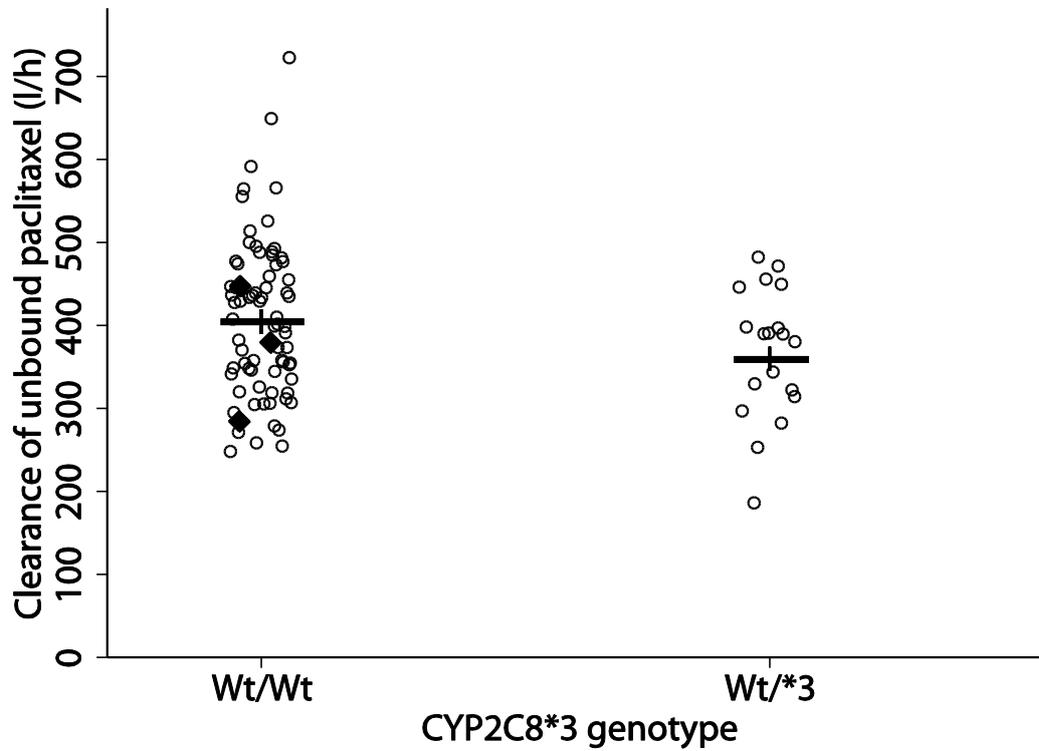
Gene / allele <sup>1</sup>	Effect <sup>2</sup>	Reference homozygote		Heterozygote		Variant homozygote		P-value <sup>5</sup>	SNP ID <sup>6</sup>
		N <sup>3</sup>	CL <sup>4</sup> (10th-90th)	N <sup>3</sup>	CL <sup>4</sup> (10th-90th)	N <sup>3</sup>	CL <sup>4</sup> (10th-90th)		
Candidate SNPs for confirmative analysis									
<b>CYP2C8</b>									
1196A>G(*3)	K399R	74	395 (297-490)	19	350 (238-458)			0.03*(0.04)	rs10509681
<b>ABCB1</b>									
1236C>T	G412G	29	391 (270-569)	45	393 (299-490)	19	359 (291-437)	0.25(0.25)	rs1128503
2677G>T/A <sup>7</sup>	A893S/T	26	387 (270-490)	42(GT)	396 (299-490)	20(TT)	356 (294-437)	0.20(0.26)	rs2032582
3435C>T	I1145I	11	403 (326-548)	44	387 (282-490)	38	378 (297-468)	0.83(0.43)	rs1045642
Candidate SNPs for exploratory analysis									
<b>CYP2C8</b>									
792C>G(*4)	I264M	86	391 (297-490)	7	321 (270-374)			0.04*(0.03)	rs1058930
15577956G>T (*1B)	-	49	395 (298-552)	43	373 (291-478)	1	461	0.75(0.36)	rs7909236
15578055A>C (*1C)	-	69	382 (291-478)	24	393 (300-552)			0.48(0.62)	rs17110453
<b>ABCB1</b>									
-1A>G	-	1	458	29	396 (270-592)	63	379 (297-477)	0.56(0.3)	rs2214102
61A>G	N21D	63	384 (282-490)	29	386 (298-478)	1	437	0.52(0.77)	rs9282564
1199G>A	S400N	83	385 (291-490)	10	386 (322-461)			0.74(0.99)	rs2229109
<b>CYP3A4</b>									
24616372T>C (*1B)	-	85	383 (296-490)	7	397 (270-641)			0.67(0.72)	rs2740574
<b>CYP3A5</b>									
219-237G>A	frameshift	84	388 (297-490)	9	360 (176-726)			0.30(0.36)	rs776746
<b>SLCO1B3</b>									
699G>A	M233I	1	326	19	377 (299-481)	73	388 (291-490)	0.99(0.46)	rs7311358
767G>C	G256A	67	386 (298-481)	26	383 (291-490)			0.63(0.89)	rs60140950
<b>CYP1B1</b>									
1294C>G (*3)	V432L	30	389 (270-530)	36	401 (298-490)	27	361 (300-470)	0.77(0.24)	rs1056836
<b>ABCC1</b>									
7356253C>G	-	65	394 (297-548)	27	368 (291-470)	1	332	0.04(0.15)	rs504348
<b>ABCC2</b>									
1249G>A	V417I	67	381 (291-490)	24	396 (297-552)	2	415 (368-468)	0.21(0.39)	rs2273697
3563T>A	V1188E	87	386 (296-490)	5	370 (176-569)			0.7(0.7)	rs17222723
4544G>A	C1515Y	75	389 (296-490)	3	355 (176-569)			0.72(0.52)	rs8187710
<b>ABCG2</b>									
421C>A	Q141K	61	374 (291-478)	32	408 (315-548)			0.4(0.09)	rs2231142
34G>A	V12M	87	385 (291-490)	4	395 (296-726)			0.68(0.83)	rs2231137
<b>ABCC10</b>									
2759T>C	I920T	46	386 (297-478)	43	386 (291-548)	4	373 (326-467)	0.88(0.89)	rs2125739

**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 10<sup>th</sup> and 90<sup>th</sup> 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
- 6) RS number referring to the single nucleotide polymorphism database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>)
- 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.