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This is the authors’ version of the following article:

Henrik Green, Peter Söderkvist, Per Rosenberg, Rajaa A Mirghani, Per Rymark, Elisabeth Avall Lundqvist and Curt Peterson, Pharmacogenetic Studies of Paclitaxel in the Treatment of Ovarian Cancer, 2009, Basic and clinical pharmacology and toxicology, (104), 2, 130-137. which has been published in final form at: http://dx.doi.org/10.1111/j.1742-7843.2008.00351.x
Postprint available at: Linköping University Electronic Press http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-16525
Pharmacogenetic Studies of Paclitaxel in the Treatment of Ovarian Cancer

Henrik Gréen1, Peter Söderkvist2, Per Rosenberg3, Rajaa A. Mirghani4,§, Per Rymark5, Elisabeth Åvall Lundqvist6, and Curt Peterson1

1 Division of Drug Research, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden
2 Division of Cell Biology, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden
3 Department of Oncology, Linköping University Hospital, SE-581 85 Linköping, Sweden
4 Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska University Hospital, Huddinge, Karolinska Institutet, SE-141 86 Stockholm, Sweden
5 Department of Obstetrics and Gynecology, Central Hospital, SE-721 89 Västerås, Sweden
6 Department of Gynecologic Oncology, Radiumhemmet, Karolinska University Hospital, Solna, SE-171 76 Stockholm, Sweden
§ Present affiliation: Department of Clinical Toxicology, Central Laboratories & Blood Bank, King Fahad Medical City, Riyadh 11525, Kingdom of Saudi Arabia

Grant support: This study was supported by grants from the Swedish Cancer Society, Swedish Research Council - Medicine (3902), The Cancer Society in Stockholm, Gunnar Nilsson’s Cancer Foundation and the County Council in Östergötland.

Number of tables and figures: 4 tables and 3 figures

Running title: Paclitaxel pharmacogenetics in ovarian cancer

Keywords: paclitaxel, ovarian cancer, pharmacokinetics, CYP2C8, ABCB1

Corresponding author/Requests for reprints:
Henrik Gréen, Ph.D. M. Sc. Engineering Biology
Division of Drug Research, Clinical Pharmacology
Faculty of Health Sciences
Linköping University
SE-581 85 Linköping
Sweden

E-mail: henrik.green@imv.liu.se
Phone: +46 13 22 12 29
Fax: +46 13 10 41 95
Abstracts

The purpose of this study was to evaluate the role of sequence variants in the CYP2C8, ABCB1 and CYP3A4 genes and the CYP3A4 phenotype for the pharmacokinetics and toxicity of paclitaxel in ovarian cancer patients. Thirty-eight patients were treated with paclitaxel and carboplatin. The genotypes of CYP2C8*1B,*1C, *2, *3, *4, *5, *6, *7, *8 and P404A, ABCB1 G2677T/A and C3435T, as well as CYP3A4*1B, were determined by pyrosequencing. Phenotyping of CYP3A4 was performed in vivo with quinine as a probe. The patients were monitored for toxicity and twenty-three patients underwent a more extensive neurotoxicity evaluation. Patients heterozygous for G/A in position 2677 in ABCB1 had a significantly higher clearance of paclitaxel than most other ABCB1 variants. A lower clearance of paclitaxel was found for patients heterozygous for CYP2C8*3 when stratified according to the ABCB1 G2677T/A genotype. In addition, the CYP3A4 enzyme activity in vivo affected which metabolic pathway was dominant in each patient, but not the total clearance of paclitaxel. The exposure to paclitaxel correlated to the degree of neurotoxicity. Our findings suggest that interindividual variability in paclitaxel pharmacokinetics might be predicted by ABCB1 and CYP2C8 genotypes and provide useful information for individualized chemotherapy.
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Introduction

Paclitaxel in combination with carboplatin is the standard chemotherapy for ovarian cancer. Carboplatin doses are adjusted according to the renal function, whereas paclitaxel is used in standardized doses according to body surface area. The pharmacokinetics and the response to paclitaxel treatment vary greatly among individuals and one factor of importance for these differences might be the genetic variability. Our belief is that it would be important to be able to predict the highest yet safe starting dose for each individual to avoid undertreatment. Understanding the mechanisms behind the interindividual differences in the pharmacokinetics of paclitaxel should be the foundation for establishing individual dosages.

It has been suggested that the pharmacokinetics of paclitaxel are affected by several proteins, such as metabolic enzymes and drug transporters [1]. Systemic elimination of paclitaxel occurs by hepatic metabolism involving the cytochrome P450 (CYP) enzymes, CYP3A4 and CYP2C8 [2]. Paclitaxel is converted to \( p\)-3'-hydroxypaclitaxel by CYP3A4 [3] and CYP2C8 catalyzes the formation of 6\( \alpha \)-hydroxypaclitaxel [4]. These metabolites can be further oxidized to 6\( \alpha \)-, \( p\)-3'-dihydroxypaclitaxel [4, 5]. All three metabolites are less potent than the parent compound in inhibiting cell growth \textit{in vitro} [6, 7]. Several single nucleotide polymorphisms (SNPs) have been reported in the CYP2C8 gene and some alleles (*2, *3, *7, *8 and P404A) have been associated with decreased 6\( \alpha \)-hydroxypaclitaxel production \textit{in vitro} [8-11]. The CYP2C8*5 allele, a premature stop-codon, is also expected to encode an inactive protein [12]. However, the effects of the polymorphisms on paclitaxel pharmacokinetics \textit{in vivo} are still unclear. The large interindidual variation in CYP3A4 activity is more difficult to explain on a genetic basis [13], although the CYP3A4*1B seems to affect enzyme activity [14]. Therefore several groups have developed and validated probes for determination of the CYP3A4 activity \textit{in vivo} [14-16].
Paclitaxel is also a substrate for P-glycoprotein, a 170 kDa plasma membrane protein encoded by the ABCB1 gene that functions as an ATP-driven drug export pump. P-glycoprotein is believed to be an important factor in the resistance to [17, 18] and biliary elimination of many drugs, including paclitaxel [19, 20]. Different polymorphisms in the ABCB1 gene have been identified and of these SNPs, the linked G2677T/A (Ala893Ser/Thr) and C3435T (Ile1145Ile, wobble) have been associated with altered P-glycoprotein expression and phenotype [21-23]. Recently we showed that SNPs in the ABCB1 gene affect the response to paclitaxel treatment in ovarian cancer[24], although another study did not find the same correlation [25].

We initiated this pilot study to investigate the feasibility of genotyping ovarian cancer for CYP3A4, CYP2C8 and ABCB1 sequence variants and CYP3A4 phenotyping in vivo and its correlation to the pharmacokinetics and toxicity of paclitaxel as a basis for individualized chemotherapy.

**Material and Methods**

*Patient selection and characteristics:* A total of 38 Caucasian women to be treated with paclitaxel at 175 mg/m$^2$ in combination with carboplatin (AUC 5 or 6 according to Calvert’s formula) were included in the study. Paclitaxel was administered intravenously during a 3-h infusion at a dose of 175 mg/m$^2$ (n = 35) or 135 mg/m$^2$ (n = 3, dose reduction due to poor general condition) and at least six cycles of paclitaxel-containing chemotherapy were given (except for two patients, one received only one cycle due to septicemia and one patient was withdrawn from further paclitaxel treatment after four cycles due to severe neurotoxicity). The pharmacokinetic sampling was done during one cycle for each patient. Twenty-four
patients were chemotherapy naive and nine were treated after relapse. In 30 patients the diagnosis and histology were consistent with epithelial ovarian cancer and in 5 patients with peritoneal cancer. Remaining patients suffered from carcinoma in corpus uteri (n = 1), in cervix uteri (n = 1) and cancer of uncertain origin (ovarian or peritoneal, n = 1). No patient was on medication with digoxin, quinidine, ketoconazole or had previously shown any hypersensitivity against quinine or quinidine. The patients and tumour characteristics for those patients assessed for pharmacokinetics are presented in table 1.

This study was approved by the regional ethics committees and written informed consent was obtained from each patient.

Sampling and pharmacokinetic studies: Prior to chemotherapy (24—48 h) a 250 mg quinine tablet was given to the patient and a blood sample was drawn 16 h later in a heparinized tube to assess the in vivo CYP3A4 activity, as previously described [26, 27]. For pharmacokinetic analysis, blood samples were collected in EDTA tubes at the following time points: immediately before infusion of paclitaxel, 30 min and 1 h after start of infusion, immediately before stop of infusion, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h and 24 h after stop of infusion. Five patients were excluded from pharmacokinetic assessment due to incomplete sampling. After centrifugation, plasma samples were stored at -80°C until analysis. The rest of the blood samples were stored for DNA-extraction. We determined the concentrations of paclitaxel, 6α-hydroxypaclitaxel and p-3’-hydroxypaclitaxel using solid phase extraction, reverse-phase high-performance liquid chromatography and an ion trap mass spectrometer with a sonic spray ionization interphase, as described by Green et al. [28]. The areas under the plasma concentration-time curve (AUC_{0-24h}) were calculated using the trapezoid method (AUC_{24h-∞} < 5%).
Toxicity assessments: Toxic effects were documented according to National Cancer Institute Common Toxicity Criteria (NCI-CTC version 2.0) after the first chemotherapy cycle, at the first response evaluation (cycle 3 or 4) and after the final chemotherapy cycle containing paclitaxel. Hematological toxicity (leukocytes, neutrophils, platelets and hemoglobin) was recorded as the lowest value at any sampling occasion from the first cycle of chemotherapy to one month after the last cycle, and rated according to the CTC scale. Twenty-three patients also undertook a more extensive neurotoxicity assessment at cycle 3 or 4 and at the final cycle of chemotherapy. The evaluation consisted of 12 questions and five neurological tests according to Cassidy et al. and the severity of the toxicity resulted in a neurotoxicity score, Nscore [29]. The patients were also asked to rate their inconveniences due to neurological adverse effects on a scale from 0 = no notice of neurological adverse effects to 5 = unbearable. Both the patients’ rating and Nscore at first response evaluation and at the final cycle were used for evaluation of the patient’s individual neurotoxicity.

DNA isolation, PCR and pyrosequencing: Genomic DNA was isolated using QIAamp® DNA mini-kits (VWR International, Stockholm, Sweden) according to the manufacturer’s protocol. The quantity of DNA extracted was determined using absorbance spectroscopy (260 and 280 nm) and the DNA was diluted to 10 ng/µl for working solutions and stored at -20°C.

The PCR primers (table 2) for amplification of the genes were designed using the website Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and checked for specificity using the NCBI BLAST server (http://www.ncbi.nlm.nih.gov/blast/). One primer for each PCR product was biotinylated in its 5’-end for purification of single-stranded DNA. The sequencing primers were designed using the Pyrosequencing SNP primer Design Version
1.01 software (http://www.pyrosequencing.com/). All primers were obtained from Invitrogen (Paisley, UK).

HotStarTaq master mixture (VWR International) was used for PCR amplification and all reactions were carried out on a Mastercycler gradient (Eppendorf) in a total volume of 25 μl. Each reaction was optimized for annealing temperature (58°C) and MgCl₂ concentration (1.5 or 2.5 mM). The PCR primers were used at a concentration of 0.4 μM and each amplification used 25 ng of human genomic DNA as template. The following temperature cycles were used during the PCR: 1 cycle at 95°C for 15 min; 50 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s; followed by 1 cycle at 72°C for 10 min.

The sequences of all PCR products were verified using both forward and reverse primers on a MegaBACE 1000 (Amersham Biosciences, Uppsala, Sweden) and the sequences were consistent with the GenBank sequences AC005068 for ABCB1, AF136830-43 for CYP2C8 and AF280107 for CYP3A4.

The SNPs were analyzed by a Pyrosequencing PSQ96MA (Biotage, Uppsala, Sweden) according to the manufacturer’s protocol and as previously described.[24] In short, for each genotype, single-stranded DNA was isolated from the PCR reactions using the Pyrosequencing Vacuum Prep Workstation (Biotage) and Streptavidin Sepharose™ High Performance beads (Amersham Biosciences) that bind to the biotinylated primers. After washing in ethanol (70%, Kemetyl AB, Stockholm, Sweden), denaturation in 0.2 M NaOH (Sigma) and flushing with washing buffer (10 mM Tris-acetate, 5 mM magnesium acetate, pH 7.6, Sigma), the beads were then released into a 96-well plate containing annealing buffer (10 mM Tris-acetate, 5 mM magnesium acetate, pH 7.6, Sigma) and the specific sequencing
primer (table 2). Annealing was performed by heating the sample at 80°C for 2 min and cooling to room temperature. The plate was then transferred to the PSQ96MA and the real-time sequencing was performed according to the dispensation order presented in table 2.

The allele frequencies of the SNPs were also investigated in a Swedish reference population. DNA samples (n=195) were obtained from a regional DNA bank consisting of genomic DNA isolated from epidemiographically selected individuals in the southeastern part of Sweden, after obtaining their informed consent.

Statistical Analysis: The statistical analysis was performed with the SPSS software package version 14.0 (SPSS Inc., Chicago, USA). The Mann-Whitney U-test was used when comparing different pharmacokinetics parameters to the genotypes found in the material. For comparison of the tables of genotype and found toxicity, the generalized Fisher’s exact test was used. The P values for the two-sided exact significance are presented. Linear regression was used when comparing two continuous variables. No corrections were done for multiple statistical testing when analysing the toxicity data, which should be considered when interpreting the results.
Results

The pharmacokinetics of paclitaxel showed high interindividual variability as shown in table 3. Genotypes both in the patients and in a Swedish reference population were found to be in Hardy-Weinberg equilibrium (table 4). Some polymorphisms described in the literature could not be detected in our population.

**Paclitaxel pharmacokinetics, genotypes and CYP3A4 in vivo activity:** Patients carrying the G/A alleles in position 2677 of the ABCB1 gene showed a significantly higher clearance of paclitaxel (median 26.0 L/h, 95% CI 20.3-34.8) compared to wild-type patients (median 18.9 L/h, 95% CI 15.2-21.1) or patients with the T/T genetic variant (median 17.4 L/h, 95% CI 13.2-21.4); however, no significant difference could be shown compared to the G/T heterozygous patients (median 21.1 L/h, 95% CI 17.6-23.9) (fig. 1A). The clearance of paclitaxel also seemed to correlate with CYP2C8*3. However, due to the influence of ABCB1 G2677T/A the data had to be stratified and the only combination where the number of patients was high enough for statistical analysis (n>2) was the combination 2677G/T and CYP2C8*1/*1 versus 2677G/T and CYP2C8*1/*3. Patients carrying the 2677G/T and CYP2C8*1/*3 had a significantly lower clearance of paclitaxel (median 14.7 L/h, 95% CI 8.4-17.8) than patients carrying the 2677G/T and CYP2C8*1/*1 alleles (median 22.8 L/h, 95% CI 19.3-25.5) (fig. 1B). Multivariable analysis of the effect of CYP2C8*3 and ABCB1 G2677T/A on the clearance of paclitaxel resulted in a P-value of 0.076 for both factors (main effects only). None of the genotypes ABCB1 C1236T & C3435T, CYP2C8*1B, CYP2C8*1C or CYP2C8*4 could be shown to influence the clearance of paclitaxel, nor did the CYP3A4 enzyme activity *in vivo* correlate with the clearance of paclitaxel. However, a low CYP3A4 enzyme activity *in vivo* correlated with a high AUC_{0-24h} of 6a-hydroxypaclitaxel (R = 0.671, P < 0.001, data not shown), which is formed by CYP2C8.
Neurotoxicity, other adverse effects, genotype and paclitaxel exposure: The severity of the neurotoxicity (Nscore, n = 23) at the final cycle of chemotherapy correlated with the exposure of paclitaxel (AUC_{0-24h} paclitaxel) as shown in fig. 2 (R = 0.513, P = 0.012). The patients’ own rating of the neurological effects at first response evaluation correlated to the exposure of paclitaxel (R = 0.497, P = 0.016, fig. 3). Although not significant, patients with ABCB1 G2677T/A wild type seemed to have less sensory neuropathy compared to patients with heterozygous or homozygous genetic variants (P = 0.186, data not shown). The mean Nscore for each genotype was compared without finding any significant difference. Patients heterozygous for CYP2C8*3 had a higher risk of motor neuropathy (P = 0.034, data not shown). The CYP2C8*3 genotype also seemed to affect the hematological toxicity, especially the leukocytes (P = 0.067) and platelets (P = 0.02, data not shown). Half of the CYP2C8*3 heterozygous patients (n = 3) suffered extremely high hematological toxicity (leukocytes: grade 4 and platelets: grade 2 or 3) while the other half (n = 3) had a minor effect on their blood counts (leukocytes: grade 0 and platelets: grade 0 or 1). For other adverse effects registered using the CTC scale no linear correlations could be found between genotype and toxicity.

Discussion

In this pilot study, we found that the clearance of paclitaxel was influenced by the SNPs G2677T/A in ABCB1 and CYP2C8*3. The neurotoxicity correlated with the exposure to paclitaxel. In addition, the CYP3A4 enzyme activity in vivo affected the pathway of paclitaxel metabolism but not the total clearance. The genotypes found had similar allele frequencies in the patient and the reference populations and were in accordance with previous studies [8, 30, 31].
In this study patients heterozygous for CYP2C8*3 had a lower paclitaxel clearance compared to wild-type patients, which is in accordance with the lower $V_{\text{max}}$[11] and enzyme activity [9] found for recombinant CYP2C8*3 as compared to the wild type. Liver microsomes heterozygous for CYP2C8*3 (n=19) also had a lower paclitaxel 6-hydroxylase activity as compared to the wild type [8], although the activity did overlap and the results could not be reproduced in a smaller study (n=4) [32].

The CYP3A4 activity affected the metabolite pattern, but not the clearance of paclitaxel. A low activity correlated to a high AUC$_{0-24h}$ of 6α-hydroxypaclitaxel indicating that in patients with low CYP3A4 activity a higher proportion of paclitaxel is converted by CYP2C8.

We found that the G2677T/A SNP affect the clearance of paclitaxel. Patients carrying the G/A had a significantly higher clearance whereas patients homozygous for T/T had the lowest clearance of paclitaxel. We have previously shown that patients with two non wild type alleles in position 2677 (T/T or T/A) have a better response to paclitaxel treatment [24], although others have presented different results [25]. This effect can also be explained by an altered transport activity at the tumour site in combination with a change in clearance. The functional consequences of these SNPs on the transport of P-glycoprotein has not been studied extensively in vitro [23, 33-36]. Schaefer et al. showed that the T and A genetic variants in position 2677 had significantly different transport capacity, the maximum transport velocities of vincristine were increased by 1.5 and three-fold for the Ser893 (2677T) and the Thr893 (2677A) variants, respectively [36]. This is in accordance with our findings of a higher paclitaxel clearance for patients with the 2677G/A genetic variant, although only 3 patients with this variant were found. In another study the wild type showed a slightly higher efflux of
paclitaxel than the Ser893 variant [33], in agreement with our results but in contrast to Schaefer et al. For other substrates such as verapamil, vinblastine, calcein-AM, prazosin, bisantrene, forskolin, digoxin and cyclosporin A, the transport was not affected by known variants of P-glycoprotein, however, for each of these substrates only one concentration was tested [33-35].

Previously Yamaguchi et al. had studied 13 Japanese ovarian cancer patients receiving 175 mg/m² of paclitaxel and found that the ABCB1 genotypes T-129C, C1236T and G2677T/A affected the AUC of paclitaxel [37]. The patients having the lowest AUC and the highest clearance had the following ABCB1 genotype -129T/C, 1236C/C and 2677A/A which is in accordance with our results showing that the A-allele in position 2677 correlates to a high clearance of paclitaxel. This was not reproduced by Nakajima et al., who investigated the effect of the ABCB1 genotype (T-129C, C1236T, G2677T/A and C3435T) on the clearance of paclitaxel in 23 ovarian cancer patients (180 mg/m²) without finding a correlation [38]. However, they did find an effect of the ABCB1 genotype on the metabolites in that patients with a genetic variant in position 3435 had a higher AUC for p-3'-hydroxy paclitaxel as compared to the wild type. Sissung et al. did not find a correlation between the ABCB1 genotype and the pharmacokinetics of paclitaxel either, although the study is small (n = 26) and the difference in AUC of paclitaxel approaches significance (P = 0.18) for G2677T/A and C3435T [39]. In a study of dose-intense paclitaxel, doxorubicin and cyclophosphamide treatment of breast cancer no correlations were found between the genotype of several genes including CYP3A4, ABCB1 and CYP2C8 and paclitaxel clearance [40]. However, the higher dose (575-775 mg/m²) of paclitaxel and longer infusion time (24h) might explain the discrepancy compared to our result. A study in a Caucasian population investigated the clearance of unbound paclitaxel in cancer patients receiving paclitaxel as an i.v. infusion for
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1, 3 or 24 h at a dose of 80-225 mg/m².[41] The patients were genotyped for CYP2C8*2, CYP2C8*3, CYP2C8*4, CYP3A4*3, CYP3A5*3C and ABCB1 C3435T. Although they found a high interindividual variation in clearance of unbound paclitaxel (10-fold), no statistical significant association was observed between any variant genotype and the pharmacokinetics of paclitaxel [41]. This is in contradiction to our findings concerning a reduced elimination of paclitaxel in patients heterozygous for CYP2C8*3. Most in vitro studies as well as findings for repaglinide [42] and ibuprofen [43] suggest that CYP2C8*3 can affect the pharmacokinetics of its substrates. The discrepancy in the findings by Henningsson et al. and our results might be due to the use of a wide range of dosage and infusion times, since the SNPs might have different impacts at different substrate concentrations. We also found a impact of the ABCB1 G2677T/A SNP, which Henningsson et al. did not genotype for, and we had to stratify the data accordingly to evaluate the effect of CYP2C8*3.

The exposure of paclitaxel was significantly correlated to the neurotoxicity at the final chemotherapy cycle. The patient’s own grading of her neurological inconvenience at cycle 3 or 4 was associated with the exposure to paclitaxel, but not at the final cycle of chemotherapy, which might be due to dose reductions at later cycles. We also found a correlation between CYP2C8*3 and neuropathy, which is in accordance with the lower clearance of paclitaxel associated with this genotype. Previous studies have also shown that the paclitaxel exposure is associated with the degree of neurotoxicity [38, 44] as well as overall survival [45] and the response at end of chemotherapy [46]. Neuropathy has also been shown to correlate to the genotype of ABCB1 [39]. Patients with wild type for C3435T did not develop neuropathy as fast as other patients.
In conclusion these results show that genotyping might be a feasible approach for individualised chemotherapy of paclitaxel. It has been shown that a higher plasma concentration and especially the duration of paclitaxel concentrations above a threshold correlates with the response to chemotherapy [45-47] as well as to the toxicity [44, 47, 48]. In this study, we found that the clearance of paclitaxel is influenced by the SNPs G2677T/A in ABCB1 and CYP2C8*3, and that the neurotoxicity correlates with the exposure to paclitaxel. However, results from larger studies are necessary before paclitaxel dosages can be individualized according to the patient’s pharmacogenetic profile.

Acknowledgments

This study was supported by grants from the Swedish Cancer Society, Swedish Research Council - Medicine (3902), The Cancer Society in Stockholm, Gunnar Nilsson’s Cancer Foundation and the County Council in Östergötland. The authors wish to acknowledge the invaluable help of all the research nurses: Dagmar Gutemark and Britt-Lena Staberg in Linköping, Susanne Skarps in Västerås, Ninni Petersson and Anne Brandt in Stockholm, who monitored the patients, cared for the study and took care of all the blood samples and a lot of paperwork. We also thank Mats Fredriksson, Linköping University for his help with the statistics and Ingela Delby & co-workers for linguistic revision of the text.

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Table 1. Patient and tumor characteristics for patients assessed for pharmacokinetics

<table>
<thead>
<tr>
<th>Table 1. Patient and tumor characteristics for patients assessed for pharmacokinetics</th>
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<tbody>
<tr>
<td><strong>Median age (range)</strong></td>
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<tr>
<td><strong>FIGO stage</strong></td>
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<tr>
<td>I</td>
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<tr>
<td>III</td>
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<tr>
<td>IV</td>
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<tr>
<td><strong>Histology</strong></td>
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<td><strong>Tumor grade (FIGO)</strong></td>
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<td>Moderately differentiated</td>
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<tr>
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Table 2. PCR primers, sequencing primers and dispensation order for detecting the SNPs in ABCB1, CYP2C8 and CYP3A4

<table>
<thead>
<tr>
<th>Gene/Exon</th>
<th>Forward primer, 5'-3'</th>
<th>Reverse primer, 5'-3'</th>
<th>Allele/SNP</th>
<th>Sequencing primer</th>
<th>Dispensation order</th>
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</thead>
<tbody>
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<td>ABCB1</td>
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<td></td>
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<tr>
<td>Exon 12</td>
<td><code>biolGAGTGGGACAAACCAGATA</code></td>
<td><code>GTCACTCACCACATCCCCTCT</code></td>
<td>C1236T</td>
<td><code>TGCACCTTCAGGGTCA</code></td>
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<td><code>biolTAGCAATTGTACCATCATGTC</code></td>
<td><code>AAAAGATGTCTTTGAGAGATGG</code></td>
<td>G2677T/A</td>
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<td><code>GTGGTGTCACAGGAAGA</code></td>
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<td>CYP2C8</td>
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<tr>
<td>5'-region</td>
<td><code>GGGCTAAGTCTCCTATTTTTG</code></td>
<td><code>biolTTCTTTCAGTGCAATCTA</code></td>
<td>*1C</td>
<td><code>TTCCCTCAAGGTCA</code></td>
<td>GCAGTCAGAT</td>
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<td><code>biolCAGAGCTTAGCCTATCTGCA</code></td>
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<td>*3</td>
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<td>*6</td>
<td><code>TGCAGGAGGACACAG</code></td>
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<td>*2 &amp; *4</td>
<td><code>ATCTTACGTGCTCTATTTTG</code></td>
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<td><code>TACTTCTCTCCTACTCTGAGCTT</code></td>
<td><code>biolCCAAAAGTTTCTCCTTCTTCTTCTTCTT</code></td>
<td>*3 &amp; P404A</td>
<td><code>CGTGCTACATGATGACA</code></td>
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<td>CYP3A4</td>
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<tr>
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<td><code>biolCTTTGAGTTATTTATCTGAGG</code></td>
<td>*1B</td>
<td><code>GAGGACAGCATAAGACAGAAG</code></td>
<td>TGCAGAGAGAG</td>
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NOTE: `biol` – biotinylated nucleotide
Table 3. Pharmacokinetic parameters of paclitaxel and CYP3A4 in vivo activity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg)</td>
<td>295 (210-350)</td>
</tr>
<tr>
<td>Clearance (L/h)</td>
<td>18.9 (8.4-34.6)</td>
</tr>
<tr>
<td>$C_{\text{max}}$, Paclitaxel (mg/L)</td>
<td>3.53 (1.99-10.90)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-24\text{h}}$, Paclitaxel (mg*h/L)</td>
<td>13.4 (7.8-39.4)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-24\text{h}}$, 6α-OH-Pac (mg*h/L)</td>
<td>0.72 (0.21-2.77)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-24\text{h}}$, p-3'-OH-Pac (mg*h/L)</td>
<td>0.30 (0.10-0.98)</td>
</tr>
<tr>
<td>CYP3A4 enzyme activity (MR)</td>
<td>9.9 (2.2-41.3)</td>
</tr>
</tbody>
</table>
Table 4. The SNP frequencies in a Swedish population (n=195) and the allele distribution of the different SNPs in the 33 patients treated with paclitaxel

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Nucleotide Change</th>
<th>Swedish reference Allele freq. +/- 95% CI</th>
<th>Patients treated with paclitaxel</th>
<th>Wild type</th>
<th>Heterozygous genetic variants</th>
<th>Homozygous genetic variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1C</td>
<td>T-370G</td>
<td>10% +/- 3.0%</td>
<td>21</td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>*1B</td>
<td>C-271A</td>
<td>29% +/- 4.5%</td>
<td>22</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>*2</td>
<td>A805T</td>
<td>0%</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>*3</td>
<td>G416A, A1196G</td>
<td>11% +/- 3.1%</td>
<td>27</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>*4</td>
<td>C792G</td>
<td>6% +/- 2.4%</td>
<td>29</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>*5</td>
<td>475 Del A</td>
<td>0%</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>*6</td>
<td>G511A</td>
<td>0%</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>*7</td>
<td>C556T</td>
<td>0%</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>*8</td>
<td>C556G</td>
<td>0%</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>P404A</td>
<td>C1210G</td>
<td>0%</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1B</td>
<td>A-392G</td>
<td>4.40% +/- 2.0%</td>
<td>30</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ABCB1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex12</td>
<td>C1236T</td>
<td>T 46% +/- 4.9%</td>
<td>8</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Ex21</td>
<td>G2677T/A</td>
<td>G 56% +/- 4.9%</td>
<td>5</td>
<td>17 G/T 3 G/A</td>
<td>8 T/T</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T 42% +/- 4.9%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 2% +/- 1.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex26</td>
<td>C3435T</td>
<td>T 55% +/- 4.9%</td>
<td>4</td>
<td>19</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Note: * No significant difference could be found between male and females in the reference population. The 95% confidence intervals for the allele frequencies are given as +/- values. † CYP2C8*1C and *4 were present in a linkage disequilibrium and CYP2C8*1B was present mutually exclusive of *1C, *3 and *4.
Figure Legends

**Fig. 1.** The influence of different genotypes on the clearance of paclitaxel. A) The clearance of paclitaxel due to the ABCB1 genotype in position 2677. B) The effect of CYP2C8*3 on the clearance of paclitaxel is shown for patients with the ABCB1 genotype 2677G/T.

**Fig. 2.** Correlation between the AUC$_{0-24h}$ of paclitaxel and the severity of the neurotoxicity (Nscore) at A) first response evaluation (cycle 3 or 4) and at B) the final cycle of chemotherapy.

**Fig. 3.** Correlation of paclitaxel exposure (AUC$_{0-24h}$) to the patients’ own rating of their inconveniences due to neurological adverse effects at A) the first response evaluation (cycle 3 or 4) and at B) the final cycle of chemotherapy.
Paclitaxel pharmacogenetics in ovarian cancer

Fig. 1
Paclitaxel pharmacogenetics in ovarian cancer

Fig. 2

A

R = 0.214
P = 0.327

B

R = 0.513
P = 0.012

Neurological adverse effects
at course 3 or 4 - Nscore

AUC$_{0-24}$ paclitaxel (ng*h/ml)

Neurological adverse effects
at final course - Nscore

AUC$_{0-24}$ paclitaxel (ng*h/ml)
Fig. 3

A

B

R = 0.497
P = 0.016

R = 0.249
P = 0.252

AUC<sub>0-24</sub> paclitaxel (ng·h/ml)

Neurological adverse effects
at course 3 or 4 - patients' grading

Neurological adverse effects
at final course - patients' grading