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# The Impact of CYP3A5\*3 and CYP2C8-HapC on

# Paclitaxel/Carboplatin-Induced Myelosuppression in Patients with

# **Ovarian Cancer**

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## **Conflicts of interest**

The authors declare no conflicts of interest.

Running title: CYP3A5\*3, CYP2C8-HapC and Paclitaxel

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**Abstract:** The influence of genetic variants on paclitaxel-induced toxicity is of considerable interest for reducing adverse drug reactions. Recently the genetic variants CYP2C8\*3, CYP2C8-HapC, and CYP3A5\*3 were associated with paclitaxel-induced neurotoxicity. We therefore investigated the impact of CYP2C8-HapC and CYP3A5\*3 on paclitaxel/carboplatin-induced myelosuppression and neurotoxicity. Thirty-three patients from a prospective pharmacokinetics study were genotyped using pyrosequencing. Patients with variant alleles of CYP2C8-HapC were found to have significantly lower nadir values of both leukocytes and neutrophils (p < 0.05) than wild type patients. CYP3A5\*3/\*1 patients were shown to have borderline significantly lower nadir values of leukocytes (p = 0.07) than \*3/\*3 patients. Combining the two genotypes resulted in a significant correlation with both leukopenia and neutropenia (p = 0.01). No effect of these genetic variants on the neurotoxicity could be shown in this rather small study, but their importance for paclitaxel-induced toxicity could be confirmed.

### **INTRODUCTION**

Paclitaxel in combination with carboplatin is the standard chemotherapy regimen in ovarian cancer, but it is associated with significant myelosuppression and neurotoxicity. The predictability of the adverse effects therefore has considerable significance. Paclitaxel is metabolized by CYP2C8 clinical to  $6-\alpha$ p-3'-hydroxypaclitaxel bv CYP3A4/3A5<sup>1-5</sup>. hydroxypaclitaxel and to Polymorphisms in these genes may influence the clinical effects and pharmacokinetics of paclitaxel and several single nucleotide polymorphisms (SNPs) have been investigated. It has been demonstrated that CYP2C8\*3 is associated with reduced clearance of total<sup>6</sup> and unbound<sup>7</sup> paclitaxel and that the same allele has been associated with sensory neurotoxicity.<sup>6,8</sup> However, other groups have not been able to demonstrate the same association.<sup>9-10</sup> Leskelä *et al.* recently found an association between paclitaxel-induced neurotoxicity and the genetic variants CYP2C8\*3, CYP2C8-HapC, and CYP3A5\*3.<sup>8</sup> To validate these results, we investigated the impact of CYP2C8-HapC and CYP3A5\*3 on paclitaxel/carboplatin-induced myelosuppression and neurotoxicity in a previously published pharmacokinetics study of 33 patients with ovarian cancer.<sup>6</sup>

#### **MATERIAL AND METHODS**

The patients included in this study were treated with 175 mg/m<sup>2</sup> (or 135 mg/m<sup>2</sup>, n = 3) of paclitaxel plus carboplatin (AUC= 5 or 6) every three weeks for at least six cycles, except for one patient who only received four cycles due to severe neurotoxicity.<sup>6</sup> Myelosuppression was evaluated as the nadir leukocyte and neutrophil counts during the whole treatment period and according to CTC (Common Terminology Criteria) scale version 2. In addition to CTC scale assessment, 23 patients underwent a more detailed evaluation of sensory neuropathy using the method of Cassidy et al., <sup>11</sup> which generated N-scores of 0 to 17 on a neurotoxicity severity scale. The genotypes were investigated by Pyrosequencing<sup>®</sup> as previously described,<sup>6</sup> but with new primers. The forward bio-CTTAGATTTGCATTTTGAGACTAT, primer the primer reverse CAGCAGAAGAAGAATTAGTGAG, and the sequencing primer GAAAGAATTAGTGAGCTTTAAC were used for CYP2C8-HapC (rs1113129, G>C). To determine the CYP2C8-HapC genotype, the nucleotides were added in the following dispensation order in the Pyrosequencer<sup>®</sup>: GACAGCGTA. The bio-ATGTACCACCCAGCTTAA forward primer and ACACACAGCAAGAGTCTCA

reverse primer were used to amplify the region around CYP3A5\*3 (rs776746, A>G). The sequencing primer GGTCCAAACAGGGAA was annealed to the template and the dispensation order CGAGATACTCG was used to determine the CYP3A5\*3 genotype of the patients. The association of the CYP3A5\*3 and CYP2C8-HapC genotypes with toxicity was tested using Fisher's exact test when evaluating the toxicity according to the CTC scale. The Mann-Whitney U test was used to compare the N-score and nadir values of leukocytes and neutrophils between genotypes.

#### RESULTS

Five of the 33 patient were heterozygous for CYP3A5\*3 and 28 were \*3/\*3. As regards CYP2C8-HapC, two patients were homozygous for the genetic variant, 11 were heterozygous and the rest were wild-type. For CYP2C8-HapC, the toxicity found in the wild-type patients was compared with that found in the other patients. A significant association was found between myelosuppression according to the CTC scale and both CYP3A5\*3 and CYP2C8-HapC genotypes. CYP3A5\*3 homozygously variant patients showed a significantly lower risk of developing leukopenia (p = 0.01, Fisher's exact test) than heterozygous \*3/\*1 patients. Five out of 28 patients in the \*3/\*3 group developed severe (CTC grade 3–4) leukopenia, compared to four out of five patients in the \*3/\*1 group (Table 1). A similar trend was found for \*3/\*3 genotypes regarding neutropenia, but this was not statistically significant. CYP2C8-HapC variants \*1/C + C/C were associated with a significant (p = 0.01, Fisher's exact test) increased risk of developing neutropenia compared to wild-type patients (\*1/\*1). Ten out of 13 patients in the \*1/C + C/C group suffered from severe life-threatening neutropenia (CTC grade 3–4), compared to six out of

20 patients in the \*1/\*1 group (Table 1). A trend towards an increased risk of leukopenia was also associated with CYP2C8-HapC, although this was not statistically significant. We could not find an association between neuropathy according to the CTC scale and either CYP2C8-HapC or CYP3A5\*3 in this population.

The nadir values of leukocytes and neutrophils during the whole treatment were compared between the different genotypes of CYP3A5\*3 and CYP2C8-HapC (Figure 1). Patients with CYP2C8-HapC \*1/\*1 had significantly higher median leukocyte and neutrophil nadir counts than patients with \*1/C + C/C (p = 0.03 and p = 0.02, respectively). A similar trend difference in median nadir values for leukocytes and neutrophils was also observed between the different genotypes of CYP3A5\*3. Patients with the CYP3A5\*3/\*3 genotype tended to have borderline significantly higher median nadir leukocyte and neutrophil values than \*3/\*1 patients (p = 0.07 and p = 0.14, respectively). We also combined the genotypes so that patients with the CYP3A5\*3/\*3 and CYP2C8\*1/\*1 genotypes were compared with the CYP3A5\*3/\*3 and CYP2C8\*1/\*1 genotypes, patients with the CYP3A5\*3/\*3 and CYP2C8\*1/\*1 genotypes showed significantly higher nadir values (p = 0.01) and neutrophils (p = 0.01) as compared to the rest of the patients.

#### DISCUSSION

Our data are consistent with the data published by Leskelä *et al.*<sup>8</sup> in the sense that both CYP3A5\*3 and CYP2C8-HapC are associated with adverse reactions to paclitaxel therapy. Like Leskelä et al., we found less toxicity in patients with the CYP3A5\*3/\*3 genotype than in the wild-type patients. However, we found that carriers of the CYP2C8-HapC variant had a higher risk of neutropenia, in contrast to Leskelä *et al.* who found a lower risk of neuropathy in this group of patients.<sup>8</sup> This may either be due to the different types of toxicity being studied or to dose changes made in response to the acquired toxicity, which could have prevented the development of the other form of toxicity in that particular patient. Paclitaxel is a neurotoxic drug in a dose-dependent manner and this adverse event is also the doselimiting toxicity. However. both paclitaxel and carboplatin cause myelosuppression, so the leukopenia and neutropenia seen in this study could have been caused by either of these drugs. We have previously found that CYP2C8\*3 is associated with increased neuropathy<sup>6</sup> in accordance with the findings of Leskelä et al.<sup>8</sup> Both CYP2C8\*3<sup>6-7</sup> and CYP2C8-HapC<sup>12</sup> have previously been shown to be associated with a lower clearance of paclitaxel, which may explain the higher degree of toxicity in these patients. Leskelä et al. also put forward a hypothesis that metabolites may affect the tissues and be associated with the adverse drug reactions, without themselves being cytotoxic.<sup>13</sup> This could explain why CYP3A5\*3, which is associated with a splice variant resulting in a low CYP3A5 activity,14 is also associated with low toxicity. Our toxicity data are, in fact, consistent with this hypothesis, as we found that the genotypes associated with low CYP2C8 and high CYP3A5 activity are associated with myelosuppression. We have also previously shown that a change in metabolic activity may be associated with a shift in the metabolic patterns in patients treated with paclitaxel.<sup>6</sup> Leskelä *et al.* found an association between CYP2C8\*3 and neuropathy,<sup>8</sup> in accordance with our previous data.<sup>6</sup> However, we could not confirm an association between neuropathy and either CYP2C8-HapC or CYP3A5\*3 in our small series. A final genetic model was presented by Leskelä *et al.* combining the CYP3A5\*3, CYP2C8-HapC, and CYP2C8\*3 genotypes. When using this model in our series (CYP2C8\*3 data from our previous study<sup>6</sup>), we could not find any significant association with toxicity (data not shown); however, combining CYP3A5\*3 and CYP2C8-HapC resulted in a significant association with myelosuppression.

Previous studies have suggested that there is an association between genotype and paclitaxel-induced toxicities. As mentioned above, both Gréen *et al.*<sup>6</sup> and Leskelä *et al.*<sup>8</sup> found associations between the genotypes of the metabolizing enzymes and toxicity. However, studies by Henningsson *et al.*<sup>10</sup> and Marsh *et al.*<sup>9</sup> did not find an association between toxicity and the ABCB1, CYP2C8, or CYP3A5 genotypes. These discrepancies may be due to the use of a wide range of infusion times and dosages, the use of a combination of docetaxel and paclitaxel, lack of adjustment for the dose-dependent nature of paclitaxel-induced neuropathy, and non-inclusion of the functional CYP2C8-HapC genotype.

In conclusion, these results show that genotyping may be a feasible approach for the individualization of paclitaxel chemotherapy and that the CYP2C8-HapC and CYP3A5\*3 alleles are SNPs of interest. However, a large part of the clinical response to paclitaxel cannot currently be explained by these genotypes alone. New candidate genes and larger studies are necessary to elucidate the true impact of patient genotype on the individual response to paclitaxel therapy.

Toxicity	CYP3A5	CTC Grading		p value	CYP2C8-HapC CTC Grading			<i>p</i> value
		0–2, n	3–4, n			0–2, n	3–4, n	
Leukopenia	*3/*3	23	5		*1/*1	17	3	
	*3/*1	1	4	0.01	*1/C + C/C	7	6	0.11
Neutropenia	*3/*3	16	12		*1/*1	14	6	
·	*3/*1	1	4	0.17	*1/C + C/C	3	10	0.01
		0, n	1–2, n			0, n	1–2, n	
Neurosensory	*3/*3	15	12		*1/*1	11	9	
-	*3/*1	5	0	0.13	*1/C + C/C	9	3	0.27

Table 1. Comparison between CYP3A5\*3 and CYP2C8-HapC genotypes and toxicity associated with paclitaxel treatment in ovarian cancer

CTC = Common Terminology Criteria for adverse events, \*3/\*3 = homozygously variant patients, \*3/\*1 = heterozygous patients, \*1/\*1 = wild type, \*1/C + C/C = heterozygous + homozygously variant patients, n = No. of patients.



**Figure 1.** Comparison of the nadir values of leukocytes and neutrophils during paclitaxel treatment in ovarian cancer patients depending on the CYP3A5\*3 and CYP2C8-HapC genotypes. Patients carrying the CYP3A5\*3/\*3 genotype were compared with the patients carrying the \*3/\*1 genotype and patients carrying one or two variants of the CYP2C8-HapC were compared with the rest of the patients. The CYP3A5\*3 and CYP2C8-HapC genotypes were also combined so that patients carrying both CYP3A5\*3/\*3 and CYP2C8\*1/\*1 were compared with the rest of the patients, i.e. those carrying at least one of the CYP3A5\*1 or CYP2C8-HapC alleles. The two patients with that were homozygously variant for CYP2C8-HapC had leukocyte nadir values of 3.5 and 4.2, and neutrophil nadir value of 2.2 and 2.2 cells/mm<sup>3</sup>.

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