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# ABCB1 G1199A Polymorphism and Ovarian Cancer Response to Paclitaxel

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**Running title:** ABCB1 SNP G1199T/A and Response to Paclitaxel

**Key words:** ABCB1, paclitaxel, ovarian cancer, G1199T/A

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## **Abstract**

P-glycoprotein, encoded by the ABCB1 gene, confers multi-drug resistance to a variety of antineoplastic agents, e.g. paclitaxel. Recently, the G1199T/A polymorphism in the ABCB1 gene was shown to be important for the function of P-glycoprotein as well as for the resistance to several chemotherapeutic agents *in vitro*. We analyzed the allelic distribution of the G1199T/A and other polymorphisms in exons 11 and 12 of the ABCB1 gene in ovarian cancer patients treated with paclitaxel and carboplatin in order to evaluate their predictive value *in vivo*. The SNPs C1236T, G1199T/A and A1308G were determined using Pyrosequencing in 51 patients with advanced ovarian cancer and correlated to the progression free survival. The G1199T/A SNP was found to affect the progression free survival. Although only two heterozygous (G/A) patients were found their mean progression free survival was only 2 months as compared to 19 months for the wild-type patients. This is in accordance with the higher resistance for the 1199A genetic variant found *in vitro*. Genotyping of the ABCB1 gene may be important for determining the tumor resistance to paclitaxel and provide useful information for individualized therapy.

## **Introduction**

Crouthamel *et al.*<sup>1</sup> reported that the single nucleotide polymorphism (SNP) G1199T/A in ABCB1 is important for the *in vitro* resistance to several chemotherapeutic agents including paclitaxel. The transport activity of P-glycoprotein (P-gp), encoded by the ABCB1 gene, affects the pharmacokinetics of paclitaxel in several ways. Overexpression of P-gp on tumor cells resulting in an enhanced efflux of the drug is a known *in vitro* resistance mechanism for paclitaxel<sup>2,3</sup>. Clinical resistance and poor response have also been correlated with a high expression of P-gp in tumours<sup>4-6</sup>. Studies in knock-out mice and co-administrations of P-gp inhibitors have shown that P-gp limits the oral uptake of paclitaxel and mediates the direct excretion of the drug from the systemic circulation as well as the absorption (or re-absorption) of paclitaxel in the intestine<sup>7-9</sup>. The penetration of paclitaxel to the cerebrospinal fluid has also been shown to be dependent on the P-gp<sup>10,11</sup>.

Recently we showed that the SNP G2677T/A correlated with the response to paclitaxel treatment in ovarian cancer<sup>12</sup>, although other studies have given different results<sup>13,14</sup>. The importance of P-gp transport for the effects of paclitaxel treatment, our previous findings and the shown impact of the G1199T/A SNP on the *in vitro* resistance led us to investigate the clinical importance of this SNP during paclitaxel treatment of ovarian cancer.

## **Material and Methods**

To evaluate the relationship between the response to paclitaxel treatment and the G1199T/A and others SNPs in exon 11 and 12 of the ABCB1 gene (G1199T/A, C1236T and A1308G), we retrospectively identified the SNPs in 51 epithelial ovarian tumors (FIGO stage IIB-IV) included in a previous study<sup>12</sup>. All three SNPs were investigated due to their close proximity to each other. After primary surgery all patients had been treated with paclitaxel at a dose of

175 mg/m<sup>2</sup> or 135 mg/m<sup>2</sup> (n=5) in combination with carboplatin for at least four cycles. The patient and tumor characteristics are presented in the table.

Eleven tumours were collected from paraffin embedded tissues stored at the Division of Molecular and Immunological Pathology, Linköping University, and 40 tumors were fresh-frozen and obtained from a bio-bank at the Department of Oncology, Sahlgrenska Academy at Göteborg University. This study was approved by the regional ethics committees in Linköping, Sweden.

#### *DNA Isolation, PCR and Pyrosequencing*

Genomic DNA was isolated using QIAamp<sup>®</sup> DNA mini kits (VWR International, Stockholm, Sweden) according to the manufacturer's protocol. A 392 bp fragment of exons 11 and 12 in the ABCB1 gene was amplified using the forward primer biotin-GAGTGGGCACAAACCAGATA and reverse primer GTCATCTCACCATCCCCTCT. The reaction was based on the HotStarTaq master mixture (VWR International) and carried out on a Mastercycler gradient (Eppendorf) in a total volume of 25 µl, as previously described<sup>12</sup>. For the real-time sequencing of the PCR products and SNP analysis a Pyrosequencing PSQ96MA (Pyrosequencing AB, Uppsala, Sweden) was used. In short, single-stranded DNA was isolated from the PCR reactions using the Pyrosequencing Vacuum Prep Workstation (Pyrosequencing AB) and transferred into a 96-well plate. Three sequencing primers, CTTTTTCGAGATGGGTAA for G1199T/A, TGCACCTTCAGGTTCA for C1236T and ATAGAGCCTCTGCATCA for A1308G, were annealed to the single stranded DNA by heating the sample to 80°C for 2 min and then allowing it to cool to room temperature. The plate was then transferred to the PSQ96MA where the real time sequencing took place by

dispensing the nucleotides in the following order ACGATGCTGCACTGTATCATCTATCA.

All primers were obtained from Invitrogen (Paisley, U.K).

### *Statistical Analysis*

The statistical analysis was performed with the SPSS software package version 14.0 (SPSS Inc. Chicago, IL, USA). Kaplan-Meier plots were used to visualize and study differences in fractions of patients with progression-free survival (PFS) between different genotypes.

### **Results**

The PCR amplification of exon 11 and exon 12 of the ABCB1 gene resulted in single products of expected sizes (as judged by agarose gel electrophoresis). The pyrograms during real-time sequencing showed peaks corresponding to the theoretical outcome. The C1236T SNP was found at an allele frequency of 42% in Hardy-Weinberg distribution. Only the genetic variant 1308A was found in the material and two patients were found to be G/A heterozygous for the G1199T/A SNP. Both these patients had stage III serous tumors that were poorly differentiated. Due to the small number of patients, no significant correlations could be shown between the tumors' stage/grade and the progression free survival.

Patients G/A heterozygously mutated for the SNP G1199T/A were found to have a shorter progression free survival (mean PFS 2 months) as compared to the wild-type patients (mean PFS 19 months), see Figure. The SNP C1236T did not correlate to the treatment response.

### **Discussion**

As a follow-up of the findings by Crouthamel *et al.* that the genetic variant G1199T/A in ABCB1 is important for the resistance to several chemotherapeutic drugs *in vitro*, we

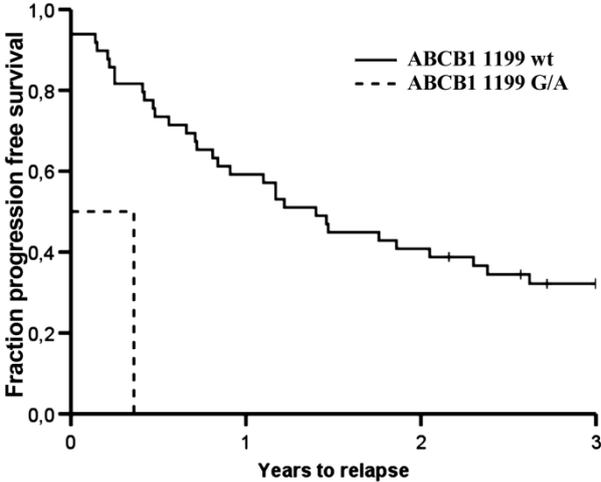
investigated the impact of this SNP on the response to paclitaxel-carboplatin treatment in ovarian cancer. Although we only found two heterozygous patients (1199G/A), they showed significant clinical resistance to paclitaxel-carboplatin treatment and a shorter progression free survival. This is in accordance with the 2.4 higher IC<sub>50</sub> value for paclitaxel for this genetic variant and the lower intracellular concentration of doxorubicin found in HEK-cells expressing the ABCB1 1199A variant as compared to the wild type transporter<sup>1</sup>. The increased drug resistance of cells expressing the 1199A variant has also been shown for other chemotherapeutic agents such as vincristine and vinblastine<sup>1,15</sup>.

The ABCB1 genotype especially the SNP G2677T/A has previously been shown to be important for the clinical effect of paclitaxel. In a study by Gréen *et al.* patients homozygously mutated (T/T or T/A) at position 2677 had a higher chance of responding to treatment. The number of mutated alleles were also shown to influence the response which improved with increasing number of variant alleles<sup>12</sup>. Two groups later published results from similar studies, but with different outcome<sup>13,14</sup>. However, the differences might be explained by variations in the composition and/or subdivision of tumor material and/or patients<sup>16</sup>. *In vitro* a slightly higher efflux of bodipy-FL-paclitaxel has also been shown in HeLa cells expressing the wild type P-gp than cells carrying a plasmid containing the 2677T variant<sup>17</sup>. In this system no effect of the G1199A genetic variant was seen on the efflux of paclitaxel. However, the fluorescent bodipy modification of the P-gp substrates and the usage of only one substrate concentration may have influenced the ability to distinguish differences between the genetic variants of the ABCB1 gene.

In conclusion, this study indicates that the SNP G1199T/A in ABCB1 influences the response to paclitaxel-carboplatin treatment in ovarian cancer. However, the potential importance of

the ABCB1 genotype as a predictive marker for paclitaxel treatment is still elusive and future studies will hopefully clarify the significance of these SNPs.

**Figure legend.** The effect of the ABCB1 SNP G1199T/A on the progression free survival in patients with advanced ovarian cancer treated with paclitaxel in combination with carboplatin.



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