ABCB1 Genetic Variation Influences the Toxicity and Clinical Outcome of Patients with Androgen-Independent Prostate Cancer Treated with Docetaxel

Tristan M. Sissung, Caitlin E. Baum, John Deeken, Douglas K. Price, Jeanny Aragon-Ching, Seth M. Steinberg, William Dahut, Alex Sparreboom, and William D. Figg

Abstract

Purpose: Polymorphisms that are associated with ABCB1 expression and function may be linked to treatment efficacy and the development of neutropenia and neurotoxicity in patients with androgen-independent prostate cancer receiving docetaxel.

Experimental Design: Patients with androgen-independent prostate cancer treated with docetaxel alone (n = 23) or docetaxel and thalidomide (n = 50) were genotyped for the ABCB1 1236C>T, 2677 G>T/A, and 3435 C>T alleles by direct sequencing, and diplotypes were constructed using an EM algorithm. The data were then compared with duration to onset of peripheral neuropathy, neutropenia grade, and survival after docetaxel.

Results: For patients receiving docetaxel alone, individuals carrying a diplotype consisting of the 1236C-2677G-3435C linked alleles had improved overall survival after treatment (P = 0.0017). Additionally, patients treated with docetaxel and thalidomide carrying a diplotype consisting of the 2677T-3435T haplotype had a shorter median survival (P = 0.045). After adjusting for a particular set of polymorphisms and diplotype groupings, a hazard ratio of 10.87 was found for patients carrying the 2677GG genotype versus patients carrying other genotypes (P = 0.0048) in the docetaxel and thalidomide cohort. Among both treatment arms together, individuals carrying the 2677GG genotype also had a significantly longer time to neuropathy (P = 0.035). Finally, there was a strong trend toward patients carrying the 2677TT-3435TT diplotype having higher grades of neutropenia (P = 0.053).

Conclusion: The data suggest that docetaxel-induced neuropathy, neutropenia grade, and overall survival could be linked to ABCB1 allelic variants with ensuing negative implications for docetaxel treatment in patients carrying ABCB1 variant genotypes.

Taxanes are part of the larger family of anticancer drugs whose mechanism of action targets tubulin. Docetaxel binds to the β-tubulin subunit causing stabilization of microtubules leading to mitotic arrest and subsequent apoptosis (1, 2). Docetaxel is the standard of care for men diagnosed with androgen-independent prostate cancer (AIPC; ref. 2). Docetaxel has been given to men with AIPC in clinical trials where it has been found to prolong survival in men with this disease (3), although there is much interpatient variability in overall survival after docetaxel. The major dose-limiting toxicities for both drugs are the development of neutropenia and peripheral neuropathy (2).

ABCB1 (P-glycoprotein, multidrug resistance 1) is responsible for a large portion of the systemic efflux capacity toward docetaxel. Within the liver, ABCB1 is expressed in the canalicular domain of hepatocytes, where it transports docetaxel into the biliary duct, resulting in drug clearance (4, 5). ABCB1 is also responsible for the transport of many drugs, including docetaxel, between bodily compartments. For example, ABCB1 is expressed by endothelial cells of the blood-brain (6) and blood-nerve barriers (7, 8), and its expression at these sites can limit the exposure of the nervous tissue to substrate drugs by the active transport of these drugs from the nerves into the systemic circulation (9). ABCB1 is also expressed in hematopoietic precursors where it mitigates the exposure of these cells to potentially harmful substances (10, 11).

Tumor cells can also express ABCB1, resulting in the multidrug-resistant phenotype, wherein ABCB1-overexpressing tumors can have limited exposure to ABCB1 substrate drugs.
such as the taxanes, through efflux pathways (12). Indeed, non–drug-treated, high–Gleason grade prostate tumors have been found to have a more multidrug-resistant phenotype that is attributable to an up-regulation of ABCB1 (13). This multidrug resistance most likely results in poor prognosis after docetaxel treatment (14).

ABCB1 expression and protein folding are largely influenced by three single-nucleotide polymorphisms (SNP) in the ABCB1 gene. These are the ABCB1 1236C>T, 2677G>T/A (A893S/T), and 3435C>T transitions (15). It is also likely that diplotype combinations of these three alleles are more deterministic of the efflux capacity of individuals treated with these drugs, as protein folding, expression, and functional differences are more pronounced when certain ABCB1 variants are co-inherited—an increasing number of variant alleles (e.g., the 1236T-2677T-3435T haplotype) being associated typically with lower neutrophil counts after docetaxel treatment (14). This is attributable to an up-regulation of ABCB1 (13). This multidrug resistance most likely results in poor prognosis after docetaxel treatment (14).

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Materials and Methods

**Patients and treatment.** We evaluated ABCB1 genotypes using germline DNA obtained from 73 men with AIPC treated with either single-agent docetaxel (Aventis Pharmaceuticals) given i.v. over 1-h at a dose of 30 mg/m² (n = 23) or with a combination of docetaxel on the same schedule and oral thalidomide at a dose of 200 mg taken daily (n = 50; Celgene Corp). Inclusion and exclusion criteria and patient characteristics have been previously published (20). Toxicity was defined by the Cancer Therapy Evaluation Program/National Cancer Institute Common Toxicity Criteria (version 2.0). The National Cancer Institute Institutional Review Board approved the study and genotyping.

**Genotyping.** DNA was extracted from plasma using a QiaBlood DNA extraction kit (Qiagen) and stored at 4°C. Genotyping was conducted via direct sequencing at three ABCB1 loci using the following PCR primers: 1236C>T F1 5′-GTCTACTCTCATGTTACC-CATCTCG-3′ and R1 5′-TATCCGTGCACTACGTGAC-3′; 2677G>T/A F1 5′-AGGCTATAGGCTG-3′ and R1 5′-AGAAGA-CAGTGAGAACATTG-3′; and 3435C>T F1 5′-ATCTCACAG-TAATTTGGACGT-3′ and R1 5′-AACCCAAAACAGAAATGTG-3′. A 50-μL reaction was prepared for PCR amplification. The reaction consisted of 1 × PCR buffer, 2 mmol/L of each of the four deoxyribonucleotide triphosphates, 1.5 mmol/L magnesium chloride 20 mmol/L of the forward and reverse primers, and 1 unit of Platinum Taq DNA polymerase. PCR conditions were 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 64°C for 30 s, and 72°C for 30 s, with a final 7 min cycle at 72°C. Direct nucleotide sequencing PCR was conducted using the Big Dye Terminator Cycle Sequencing Ready Reaction kit V1.1 on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). The primer sequences for these reactions were as follows: 1236C>T F2 5′-GTCTACTCTCATGTTACC-CATCTCG-3′ and R2 5′-AGGCTATAGGCTG-3′; 2677G>T/A F2 5′-AGAAGAACAGTGAGAACATTG-3′; and 3435C>T F2 5′-ATCTCACAG-TAATTTGGACGT-3′ and R2 5′-AACCCAAAACAGAAATGTG-3′.

**Pharmacokinetics of docetaxel.** Blood specimens were obtained from patients receiving docetaxel (1-h infusion; 75 or 85 mg/m²), as described previously (21). Evaluation of docetaxel pharmacokinetics was done using samples obtained immediately before drug infusion and at 0.5, 1, 2, 4, 6, and 24 h after start of infusion. Concentrations of docetaxel in plasma were determined by a validated reverse-phase high-performance liquid chromatography method with UV detection (22). Pharmacokinetic variables of docetaxel were obtained by noncompartmental analysis using WinNonlin Professional Version 5.0 (Pharsight Corporation). Table 2 (plasma data, constant infusion).

Table 1. Genotype and allele frequencies of the studied variants

| Allelic variant* | Effect ‡ | n ³ | Genotype frequencies ⁴ | Allele frequencies ||
|------------------|----------|-----|------------------------|-----------------|||
|                  |          |     | Wt                     | Het             | Var             | p     | q     |
| ABCB1 1236C>T    |          | 23  | 13 (56.5)              | 8 (34.8)        | 2 (8.7)         | 0.739 | 0.261 |
| ABCB1 2677G>T/A  |          | 23  | 11 (47.8)              | 9 (39.1)        | 2 (8.7)         | 0.674 | 0.283 |
| ABCB1 3435C>T    |          | 23  | 7 (30.4)               | 10 (43.5)       | 6 (26.1)        | 0.522 | 0.478 |
| Docetaxel plus thalidomide (n = 50) |          |     |                        |                 |                |       |       |
| ABCB1 1236C>T    |          | 46  | 12 (26.1)              | 18 (39.1)       | 16 (34.8)       | 0.457 | 0.543 |
| ABCB1 2677G>T/A  |          | 50  | 15 (30.0)              | 20 (40.0)       | 15 (30.0)       | 0.500 | 0.500 |
| ABCB1 3435C>T    |          | 45  | 10 (22.2)              | 23 (51.1)       | 12 (26.6)       | 0.478 | 0.522 |

Abbreviations: p, frequency for wild-type allele; q, frequency for variant allele; Wt, homozygous wild-type allele patient; Het, heterozygous patient; Var, homozygous variant patient.

*Number represents position in nucleotide sequence.

†Number represents amino acid codon.

‡Genotype data were not available in all patients, as not all samples yielded sufficient DNA or PCR amplified.

§Number represent number of patients with percentage in parenthesis; the difference in the total number of patients is due to the fact that not all samples yielded sequencing data or showed PCR amplification.

|| Hardy-Weinberg notation for allele frequencies.

* A single Hispanic male was also included, and his genotype was 1236C>T, unknown; 2677G>T/A, wild-type; 3435C>T, wild-type.

**The 2677G>T/A polymorphism is trallelic, and two different SNPs are therefore presented.
Table 2. Diploptotype groupings in the initial exploratory analysis

<table>
<thead>
<tr>
<th>Diploptotype</th>
<th>Chromosome 1</th>
<th>Chromosome 2</th>
<th>Docetaxel population frequency (%)</th>
<th>Docetaxel and thalidomide population frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1236C&gt;T 2677G&gt;T/A 3435C&gt;T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploptotype 1</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>7 (32.3)</td>
</tr>
<tr>
<td>Diploptotype 2</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>*</td>
</tr>
<tr>
<td>Diploptotype 3</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>*</td>
</tr>
<tr>
<td>Diploptotype 4</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>*</td>
</tr>
<tr>
<td>Diploptotype 5</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Diploptotype 6</td>
<td>—</td>
<td>G</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Diploptotype 7</td>
<td>—</td>
<td>G</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Diploptotype 8</td>
<td>—</td>
<td>G</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Diploptotype 9</td>
<td>—</td>
<td>T</td>
<td>T</td>
<td>—</td>
</tr>
<tr>
<td>Diploptotype 10</td>
<td>—</td>
<td>T</td>
<td>T</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE: Diploptotypes were grouped based on whether or not the patient carried a fully wild-type or fully variant haplotype. This approach was based on a report by Kimchi-Sarfaty et al. where it was noted that protein affinity and folding was different between these haplotypes (15). * refers to any combination of alleles that is not mutually exclusive with another diplotype consisting of all three SNPs; †, any combination of alleles that is not mutually exclusive with another diplotype consisting of only the 2677 and 3435 SNPs. —, this SNP was excluded in the diplotypes 6 to 10 calculations.

Statistical considerations. The association between genotypes and overall survival was determined by the Kaplan-Meier method using a two-tailed log-rank test (23, 24). A Cox proportional hazards model analysis was done to determine whether any of the SNP or diplotype variables were jointly statistically significant (25, 26). P values for the survival analysis are presented without formal correction for multiple testing; however, in review of the exploratory nature of the analysis and the number of explorations done, univariate P values of <0.01 were considered statistically significant, whereas 0.01 < P < 0.05 would suggest trends.

The probability of development of a peripheral neuropathy during docetaxel therapy as a function of time, according to ABCB1 genotypes, was also analyzed using the Kaplan-Meier method and log-rank P values but using formal correction of P values to account for pooling of categories after examining results. The association between the clinical grade of neutropenia and genotypes was evaluated using a Kruskal-Wallis test for ordered columns; the association between the presence or absence of a double variant diplotype and grade of neutropenia was evaluated using an exact Cochran-Armitage test (27).

Results

Genotyping. The tested variants were all in Hardy Weinberg equilibrium (P < 0.05), with the following D' linkage values: 1236G>T-2677G>T/A D' = 0.81, 2677G>T/A-3435C>T D' = 0.77, and 1236G>T-3435C>T D' = 0.63 (P < 0.001). The genotype and allele frequencies for each SNP are reported in Table 1 and are similar to previously reported values. An EM algorithm was used to compute the most likely diplotype for each individual that consisted of two possible diplotypes (Helix Tree Software), and this information was used in subsequent analyses (see Table 2). Diplotypes were ordered to capture increasing genetic variation in the population, as it would be expected that each additional variant allele would alter protein folding and function to a greater degree based on relative synonymous codon usage, as reported by Kimchi-Sarfaty et al., where it was shown that there were transport affinity and tertiary structure differences between a protein encoded by the ABCB1 1236C-2677G-3435C haplotype versus the 1236T-2677T-3435T haplotype (ref. 15; see Table 2). To explore the association between diplotype and clinical outcome measures, individual diplotypes were initially compared with overall survival, development of neuropathy, and neutropenia grade. Based on the initial exploration, diplotypes were then grouped based on statistical similarity and the P value was corrected for multiple comparisons.

Genotype related to patient demographics. No association was found between any ABCB1 alleles or diplotype and age, Gleason score, ECOG, body surface area, hemoglobin (g/dL), lactate dehydrogenase (unit/L), AP (unit/L), or serum albumin (g/L; P > 0.05). However, a statistically significant association was observed between diplotypes 6 to 10 and the levels of prostate-specific antigen (PSA) observed before treatment (P = 0.0004; Table 3). There was no difference in on study PSA between the two treatment arms (P = 0.83; Wilcoxon rank-sum test), and thus on study PSA values of patients receiving docetaxel alone or in combination with thalidomide were combined between treatment arms. Men carrying diplotypes 9 and 10 had higher median PSA levels (median PSA, 135.6 ng/mL) compared with men carrying diplotypes 6 to 7 (median PSA, 61.65 ng/mL) and diplotype 8 (25.15 ng/mL). There was also an association between the ABCB1 3435C>T SNP and PSA (P = 0.0002), wherein patients carrying the ABCB1 CT genotype had a lower PSA than individuals carrying homozygous wild-type and variant alleles (median PSA, 34.5 ng/mL, 98.70 ng/mL, and 148.0 ng/mL, respectively). However, these data may be obscured by the fact that there were only 17 individuals carrying homozygous wild-type alleles, whereas two of them had a PSA of >900 ng/mL. Although 3435CC and 3435CT are statistically different from one another when individuals carrying the 3435CT genotype are compared with individuals carrying 3435CC and 3435CT, there remains a statistically significant difference (P = 0.0016) where PSA is greater in those men carrying the 3435CT genotype (median PSA, 148 ng/mL) than in pooled 3435CC and 3435CT genotypes (median PSA, 50.1 ng/mL). Thus, variant alleles in ABCB1 seem to be associated with a higher plasma level of PSA before receiving docetaxel-based treatment.

Genotype related to survival. Initial Kaplan-Meier analyses revealed that none of the SNPs alone was associated with a significant difference in overall survival after treatment with
single-agent docetaxel (all \( P > 0.05 \); Table 3). Within the cohort treated with both docetaxel and thalidomide, both the \( ABCB1 \) 1236C>T and the 2677G>T/A polymorphisms were associated with survival (\( P = 0.0028 \) and \( P = 0.018 \), respectively; Table 3). The \( ABCB1 \) 3435C>T polymorphism was not associated with survival in the docetaxel and thalidomide–treated cohort (\( P > 0.05 \); Table 3). Those patients carrying the 1236CC genotype survived significantly longer than individuals carrying the 1236CT and 1236TT genotype (45.1 months versus 20.1 months), whereas patients carrying the 2677GG genotype also had improved overall survival when compared with individuals carrying genotypes consisting of the 2677T or 2677A alleles (52.4 months versus 21.4 months; Table 3). However, a similar exploratory analysis was conducted in the docetaxel cohort with diplotype groupings (based on all three SNPs) that indicated a diplotype grouping consisting of diplotypes 4 and 5 had a significantly lower median overall survival (9.8 months) compared with the diplotype grouping consisting of diplotypes 1, 2, and 3 (20.0 months, \( P = 0.0017 \); Fig. 1; Table 3). The same analysis was conducted in the docetaxel and thalidomide cohort, where the diplotype grouping of diplotypes 4 and 5 again showed a significantly lower median overall survival (24.0 months) compared with diplotypes 1, 2, and 3 combined (41.9 months, \( P = 0.045 \); Table 3).

### Table 3. Summary of analyses conducted comparing genotype or haplotype to PSA, survival, peripheral neuropathy, and neutropenia after docetaxel

<table>
<thead>
<tr>
<th>Genotype or haplotype</th>
<th>Treatment</th>
<th>Effect</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endpoint—PSA before study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1236C&gt;T</td>
<td>Combined arms</td>
<td>None</td>
<td>NS*</td>
</tr>
<tr>
<td>2677G&gt;T/A</td>
<td>Combined arms</td>
<td>None</td>
<td>NS*</td>
</tr>
<tr>
<td>3435C&gt;T</td>
<td>Combined arms</td>
<td>Lower PSA with ( ABCB1 ) 3435CT genotype</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Diplotype 1-3 versus 4-5</td>
<td>Combined arms</td>
<td>None</td>
<td>NS*</td>
</tr>
<tr>
<td>Diplotype 6-7 versus 8-10</td>
<td>Combined arms</td>
<td>Higher PSA with diplotypes 9-10</td>
<td>0.0004*</td>
</tr>
<tr>
<td><strong>Endpoint—overall survival</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1236C&gt;T</td>
<td>Docetaxel</td>
<td>Lower survival with CT, TT genotypes versus CC</td>
<td>0.0028‡</td>
</tr>
<tr>
<td>2677G&gt;T/A</td>
<td>Docetaxel</td>
<td>Lower survival with GT, GA, TT genotypes versus GG</td>
<td>0.018†</td>
</tr>
<tr>
<td>3435C&gt;T</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS†</td>
</tr>
<tr>
<td>Diplotype 1-3 versus 4-5</td>
<td>Docetaxel</td>
<td>Lower survival with diplotypes 4-5</td>
<td>0.0017‡</td>
</tr>
<tr>
<td>Diplotype 6-7 versus 8-10</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS‡</td>
</tr>
<tr>
<td><strong>Endpoint—peripheral neuropathy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1236C&gt;T</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS†</td>
</tr>
<tr>
<td>2677G&gt;T/A</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS†</td>
</tr>
<tr>
<td>3435C&gt;T</td>
<td>Docetaxel</td>
<td>More rapid neuropathy onset with 2677GT, 2677GA, and 2677TT genotypes compared with 2677GG</td>
<td>0.0070†</td>
</tr>
<tr>
<td>Diplotype 1-3 versus 4-5</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS†</td>
</tr>
<tr>
<td>Diplotype 6-7 versus 8-10</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS†</td>
</tr>
<tr>
<td><strong>Endpoint—Neutropenia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1236C&gt;T</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS‡</td>
</tr>
<tr>
<td>2677G&gt;T/A</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS‡</td>
</tr>
<tr>
<td>3435C&gt;T</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS‡</td>
</tr>
<tr>
<td>Diplotype 1-3 versus 4-5</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS‡</td>
</tr>
<tr>
<td>Diplotype 6-9 versus 10</td>
<td>Docetaxel</td>
<td>None</td>
<td>NS‡</td>
</tr>
<tr>
<td><strong>Combined arms</strong></td>
<td></td>
<td>Combined group trended toward an association with neutropenia</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

*Kruskal-Wallis test or Wilcoxon rank-sum test as appropriate.

† Log-rank test.

‡ Cox model.

§ Fisher’s exact test.

||Cochran-Armitage trend test.
Cox model analyses were done individually within each of the two treatment arms because of the different variables worthy of exploration in the models. For the docetaxel arm, the aforementioned pooling of diplotypes was the sole significant variable that was associated with survival (hazard ratio, 5.85; 95% confidence interval, 1.71-20.10; \( P = 0.0049 \); Table 3). However, for patients treated with docetaxel and thalidomide, the grouped 1236C>T and 2677G>T/A SNPs, along with the diplotype grouping (6), (7) versus (8), (9), (10) were included in an initial Cox model. By backward elimination, the 1236C>T SNP was removed, leaving the 2677G>T transition (hazard ratio, 10.87; 95% confidence interval, 2.07-57.06; \( P = 0.0048 \)) and the aforementioned diplotype grouping (hazard ratio, 5.13; 95% confidence interval, 1.07-24.33; \( P = 0.04 \)); Table 3) as variables that are jointly associated with overall survival in a final Cox model.

Genotype related to docetaxel-induced toxicity. Patients treated with docetaxel alone showed no difference in time to development of neuropathy in a comparison of patients carrying the wild-type \( \text{ABCB1} \) 2677G allele to those patients with at least one variant allele (\( P = 0.68 \); Fig. 2; Table 3). However, patients wild type for the \( \text{ABCB1} \) 2677 allele developed neuropathy significantly slower (1.9 months to onset) than those with at least one variant allele (0.7 months to onset) when examining patients treated with both docetaxel and thalidomide (\( P = 0.0070 \); Fig. 2; Table 3). No other \( \text{ABCB1} \) allele or diplotype was associated with peripheral neuropathy (\( P > 0.05 \); Table 3).

Patients carrying diplotype 10 (carrying only variant alleles at both the 2677 and 3435 SNPs) were also marginally more likely to experience higher grade neutropenia while on study compared with the rest of the population (\( P = 0.053 \); Cochran-Armitage trend test; Table 3). Overall, 3 of 13 patients carrying diplotype 10 experienced a clinically significant grade 3 neutropenia while on study, whereas only 1 of 60 total patients carrying all of the other possible diplotypes experienced similar toxicity. This association is concentrated in those patients receiving both docetaxel and thalidomide, with a similar magnitude of significance (\( P = 0.049 \); Table 3). There is no such association found in the patients who received docetaxel alone (\( P = 1.00 \)) or with any other genotype or diplotype (Table 3). This observation is similar to a previous report where it was found that diplotype 10 is associated with a significantly lower neutrophil count at nadir in patients treated with paclitaxel (18). It is notable that this same strong relationship can be found when using less robust clinical grading systems, such as the ones used in this study.

Pharmacokinetic analysis. To address the question of whether or not \( \text{ABCB1} \) genetic variation is associated with docetaxel plasma concentrations, pharmacokinetic data from 22 patients treated with docetaxel (21) were analyzed as a function of \( \text{ABCB1} \) 1236C>T, 2677G>T/A, and 3435C>T genotypes and diplotypes. There was no statistically significant effect of genotype, either alone or in combination, on the clearance of docetaxel for any allele tested (\( P > 0.34 \); Kruskal-Wallis test; Supplementary Table S1). The pharmacokinetics of thalidomide were not evaluated, given that thalidomide is not a substrate of \( \text{ABCB1} \) (28), and therefore would not be expected to show interindividual variability based on \( \text{ABCB1} \) genotypes.

Discussion

We found that \( \text{ABCB1} \) allelic variation is associated with clinical end points, including overall survival, peripheral neuropathy onset, and neutropenia development after docetaxel-based regimens, but is not associated with docetaxel pharmacokinetics. Our results suggest that variant alleles that are associated with lowered \( \text{ABCB1} \) expression (17) and altered pharmacokinetics should be evaluated in future studies. The time to development of grade 3 neutropenia was significantly longer for patients carrying diplotype 10 (carrying only variant alleles at both the 2677 and 3435 SNPs) compared with docetaxel-treated patients, whereas patients carrying other diplotypes experienced similar toxicity. This finding is consistent with previous reports where it was found that the 1236T polymorphism is associated with significantly increased paclitaxel pharmacokinetics (29). The current literature regarding the pharmacokinetics of docetaxel and its structural analogue paclitaxel in relation to \( \text{ABCB1} \) genotypes is controversial, some finding no relationship between genotypes and pharmacokinetics and others finding such relationships, as reviewed by Marsh et al. (31). Some have found that the 1236T polymorphism is associated with decreased docetaxel clearance in Caucasian patients, although there was no association when other \( \text{ABCB1} \) alleles were combined in a haplotype (32). Our data suggest that none of the aforementioned \( \text{ABCB1} \) alleles or diplotypes are
associated with docetaxel pharmacokinetics, supporting the findings of most other studies in this regard (31).

Others have noted response differences in patients with ovarian cancer treated with paclitaxel. Originally, it was found that individuals carrying ABCB1 2677G>T/A variants had a moderately significant improved progression-free survival after paclitaxel therapy (33). However, a follow-up study in a larger patient population was unable to replicate their observations (34), and a later study found that individuals carrying 2677G>T/A variants actually had a 2.5 times higher risk of death after paclitaxel therapy (35). It is therefore possible that alterations in dosage, schedule, patient selection, and concomitant medications can alter the relationship between survival after taxane treatment and ABCB1 genotype status. Our approach is unique to the above studies in that we used very conservative exploratory statistics and an approach that takes coinheritance of SNPs into account, as has been recommended by some (31).

Given the findings of the above studies, it is interesting that we also found the ABCB1 2677G>T/A polymorphism to be strongly associated with survival in men with AIPC receiving docetaxel, as this is the only nonsynonymous polymorphism of the three studied variants. The corresponding ABCB1 893Ser and 893Thr transitions efflux certain drugs, such as vincristine, with much higher maximal transport rates than the wild-type 893Ala, despite similar protein levels (29). In the context of the present study, these data could suggest that patients carrying ABCB1 2677 variant alleles have an altered tumor disposition toward the taxanes, perhaps due to a more efficient transport of the taxanes of tumor tissues where ABCB1 is found highly expressed.

It is also notable that efficacy is decreased while toxicity is increased in patients carrying variant alleles. This is surprising because tumor cells and normal cells would be expected to have either increased or decreased exposure based on ABCB1 genotype status, resulting in increased efficacy along with increased toxicity and vice versa. However, as advanced prostate tumor cells already overexpress ABCB1 compared with normal tissue (13), it is possible that protein efflux efficiency differences brought on by these polymorphisms are more deterministic of the efflux phenotype rather than expression differences. Thus, if the variant allele is responsible for lower expression (via transcriptional and translational differences) and also increased efflux of the drug (via protein folding differences), it is likely that the drug efflux efficiency is more important when the protein is already up-regulated, as ABCB1 has been shown to be in non–drug-treated prostate tumors. By contrast, in tissues where ABCB1 is expressed at low basal levels (e.g., endothelial and hematopoietic cells), it is likely that these polymorphisms influence drug penetration by altering expression. This hypothesis is borne out by our clinical data and warrants validation in future investigations.

An alternative explanation for the lowered survival in men carrying variant alleles could be that those men had a greater tumor burden before treatment. This is supported by our data showing that men with variant alleles had a higher PSA before receiving docetaxel. There is also biological precedent for this hypothesis, given that the expression and function of ABCB1 is important to modulating intracellular levels of dihydrotestosterone through efflux pathways. Inhibition or lack of ABCB1 expression results in accumulation of dihydrotestosterone and subsequent increases in PSA through androgen receptor signaling mechanisms (36). Thus, variant alleles may be involved in a tumor phenotype in which dihydrotestosterone accumulates during the androgen-dependent stage of prostate cancer, thereby driving androgen receptor signaling even in the presence of androgen deprivation therapy. We have already shown that active transport of testosterone and, potentially, also testosterone metabolites may be an important determinant of androgen deprivation therapy failure by conferring tumor cells the ability to scavenge low levels of testosterone (37, 38). It is therefore also possible that tumor burden after androgen deprivation therapy is also influenced by transport mechanisms through ABCB1, and docetaxel efflux from tumor cells may not truly be associated with docetaxel efficacy.

It is interesting to note that there is a significant increase in time to development of neuropathy when comparing ABCB1 2677 genotype for the docetaxel and thalidomide trial, but not when comparing the same allele in the docetaxel-alone trial. Others have already shown that thalidomide is not transported by ABCB1, and thus, variances in this gene should have no bearing on presence of, and subsequent time to neuropathy development due to, thalidomide (28). However, the toxicity of thalidomide has also been well-established (39). We propose that treatment by thalidomide exposes the patient to a certain threshold level of toxicity and subsequent treatment by docetaxel confers an excess level of toxicity that causes a difference in time to neuropathy development. The influence of ABCB1 SNPs on neuropathy development may therefore be very small in those patients treated with docetaxel alone.

This study is limited by a small sample size. However, it should be noted that, given the high variant allele frequencies of these polymorphisms, the statistical power to detect associations with these SNPs and clinical end points is greatly improved. Furthermore, we used very conservative statistical methods to evaluate these relationships to reduce the chances of spurious findings. This study was also done specifically to explore and report potential genotypes and diplotypes, which may be useful to consider in determining the prognosis for survival in patients with androgen-independent prostate cancer. We have not attempted to determine the significance of these genetic findings in the context of other published prognostic variables, since the number of patients in our trial was very small. We would hope that larger multiinstitutional trials would consider evaluating these variables in similar studies with greater numbers of patients to more definitively determine their role in evaluating prognosis.

In summary, we have identified a patient subset with lowered docetaxel treatment efficacy and also increased toxicity. It is essential that similar studies in larger patient cohorts be investigated in a similar fashion to verify these findings. In addition, as newer treatment options for AIPC become available, taxane treatment should eventually be reconsidered in patients carrying ABCB1 variant alleles.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Tristan M. Sissung, Caitlin E. Baum, John Deeken, et al.


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