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ORIGINAL ARTICLE

Pharmacologic and phenotypic study of docetaxel in patients with ovarian or primary peritoneal cancer

William C. Zamboni · Austin J. Combest · Julie A. DeLoia · Robert P. Edwards · Arlene S. Bridges · Beth A. Zamboni · Christine M. Walko · Angela Y. C. Yu · Thomas C. Krivak · Joseph L. Kelley

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Abstract
Purpose The objectives of this study were to determine whether the midazolam clearance predicted docetaxel pharmacokinetics, CA-125 change, and response and to assess the impact of cytochrome P450 (CYP) 3A5 and ATP-binding cassette, subfamily B, member 1 (ABCB1) genotypes on docetaxel pharmacokinetics and pharmacodynamics in ovarian or primary peritoneal cancer patients.
Methods Thirty-four patients with advanced ovarian and primary peritoneal cancer were administered docetaxel at 75 mg/m^2 as a 1-h infusion in combination with carboplatin IV over 30 min at a target AUC of 5 mg/ml min. Cycles were repeated every 21 days for 6 cycles. Midazolam was administered at 2 mg as a 30-min IV infusion the day prior to cycle one of docetaxel administration. Pharmacokinetic studies of docetaxel and CYP3A5 and ABCB1 genotype studies were performed.
Results There was an inverse relationship between midazolam clearance (CL) and CA-125 level after cycle 6 where a higher midazolam CL was associated with a CA-125 <10 U/ml ($P = 0.007$) and CA-125 <15 U/ml ($P = 0.048$). The CA-125 categories were associated with response achieved (complete response/partial response) (CR/PR), stable disease (SD), and progressive disease (PD) at the end of therapy ($P = 0.0173$). Docetaxel CL was not related to midazolam CL or genotype. Docetaxel exposure and genotypes were not related to toxicity or response ($P > 0.05$).
Conclusions The midazolam CL predicted CA-125 levels and response that was independent of other factors including docetaxel pharmacokinetics. Future studies need to evaluate the mechanism for the relationship between midazolam CL and response in patients with ovarian cancer.

W. C. Zamboni (✉) · A. J. Combest · C. M. Walko · A. Y. C. Yu
Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, 1013 Genetic Medicine Building, CB 7361, Chapel Hill, NC 27599, USA
e-mail: zamboni@unc.edu

W. C. Zamboni · C. M. Walko
UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA

W. C. Zamboni
Carolina Center of Cancer Nanotechnology Excellence, Chapel Hill, NC 27599, USA

W. C. Zamboni · C. M. Walko
Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27598, USA

W. C. Zamboni
North Carolina Biomedical Innovation Network, Chapel Hill, NC, USA

J. A. DeLoia · R. P. Edwards · T. C. Krivak · J. L. Kelley
Division of Gynecologic Oncology, Department of Obstetrics, Gynecology and Reproductive Sciences, Magee Womens Research Institute, Pittsburgh, PA 15213, USA

A. S. Bridges
Department of Pathology, UNC School of Medicine, Chapel Hill, NC 27599, USA

B. A. Zamboni
Department of Mathematics, Carlow University, Pittsburgh, PA 15213, USA

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Introduction

Ovarian cancer remains the most lethal of the gynecologic cancers [1]. The chemotherapeutic management of advanced stage primary epithelial ovarian cancer consists of a platinum agent in combination with a taxane analog. The combination of intravenous (IV) carboplatin at an AUC of 5–7.5 and paclitaxel at a dose of 175 mg/m^2 every 21 days was recommended by the Contemporary Management of Ovarian Cancer Workshop for patients with newly diagnosed ovarian cancer [2]. However, with this regimen, neurotoxicity became evident after repeated paclitaxel administration. To circumvent this adverse event and the associated result of treatment discontinuation, new combinations are under investigation. One combination that was previously studied by Vorobiof et al. was docetaxel at a dose of 75 mg/m^2 IV and carboplatin IV at an AUC of 5 in a phase II study in 37 patients with advanced ovarian cancer [3]. An overall response rate of 89% was documented. The primary toxicities reported were leucopenia and thrombocytopenia. Grade 2 or grade 3 neuropathy occurred in only three patients [3]. In another large randomized trial conducted by Vasey et al. of paclitaxel and carboplatin compared with docetaxel and carboplatin, there was a similar response rate in both arms. Patients in the docetaxel arm had similar progression-free survival to those in the paclitaxel arm [4]. The differences were the treatment-related toxicities with the paclitaxel arm having more neurotoxicity, whereas the docetaxel arm had more myelosuppression [4]. Thus, the combination of docetaxel and carboplatin is a viable option in the treatment of patients with newly diagnosed ovarian cancer. It is, however, no longer being actively pursued for first-line treatment but is considered an option if a patient has experienced hypersensitivity to paclitaxel or its excipients or if the patient has a medical history of neuropathy.

Interpatient pharmacokinetic variability of docetaxel that may result in unpredictable efficacy and toxicity remains a challenge for clinicians. A biomarker that would predict which patients may respond and/or be at greater risk for developing toxicity to docetaxel treatment is needed. This would potentially enable clinicians to better tailor a chemotherapy regimen for each patient. A number of biomarkers have been studied to predict efficacy and toxicity in ovarian cancer (CA-125) as well as for the pharmacokinetics of docetaxel (pharmacogenetics and midazolam clearance (CL) [1, 5–12].

A phenotypic probe is an agent used to measure enzyme activity in order to predict the pharmacokinetics and pharmacodynamics of another drug such as docetaxel. The probe drug should undergo similar metabolism as the drug of interest. Phenotyping has been used to predict the wide interpatient variability of docetaxel pharmacokinetics. Midazolam is metabolized by both CYP3A4 and CYP3A5 and is not a substrate for P-glycoprotein (ABCB1) making it a viable phenotypic probe for docetaxel metabolism and CL [5]. Midazolam has been used as a phenotyping probe for docetaxel CL in an Asian population [6]. The authors showed a correlation between midazolam CL and docetaxel CL ($P = 0.0005$; $R = 0.6$) [6]. In another study, five patients with malignant solid tumors with the haplotype CYP3A4/5*2 (patients expressing at least one CYP3A4*1B and one CYP3A5*1A allele) had a 46% increased midazolam CL compared to non-carriers ($P = 0.02$) [7]. However, no prior studies have assessed these factors in patients with ovarian cancer. In addition, no studies have evaluated the relationship between midazolam and docetaxel pharmacokinetics and pharmacodynamics [response and toxicity (e.g., CA-125, complete response/partial response (CR/PR), stable disease (SD), and progressive disease) (PD)] in patients with ovarian cancer.

Several studies have evaluated variability in docetaxel pharmacokinetics and pharmacodynamics as related to genotype. However, no prior studies have evaluated the effects of pharmacogenomics on docetaxel PK in patients with ovarian cancer. Four separate studies have assessed docetaxel pharmacokinetics and pharmacogenetic variation in CYP3A [6, 8, 10, 12]. The studies revealed mixed results in regards to pharmacogenetic variation and docetaxel pharmacokinetics; however, it is still thought that common genetic variations in these genes correlate with docetaxel metabolism and pharmacokinetics. Patients homozygous or heterozygous for the functional CYP3A5*1 allele are expected to have higher systemic CL of docetaxel [13]. Results from a previous study showed that the presence of the CYP3A5*1A allele resulted in a 49% increase in docetaxel CL in Caucasian patients [13]. Caucasian patients expressing one CYP3A5*1 allele are also more likely to have a variant CYP3A4 allele (CYP3A4*1B), which is also associated with increased docetaxel CL. This allele was associated with a 64% increase in docetaxel CL [13]. Having one or more of these variant alleles could result in decreased exposure of docetaxel and a lower likelihood of response. Patients having the CYP3A5*3/*3 genotype (non-expressors) are more likely to have a decreased docetaxel CL. This could potentially result in increased docetaxel exposure and an increased incidence of severe neutropenia and other associated side effects in Caucasian patients. However, in a large study (SCOTROC1 substudy; $n = 914$), Marsh et al. found no relationship between CYP3A4*1B or CYP3A5*3C polymorphism and outcome or response in ovarian cancer patients treated with either

docetaxel or paclitaxel with a platinum agent [9]. Another previous study in Asian patients showed no correlation between any CYP3A5 genotype and docetaxel CL [6]. A possible reason for this is that Asians do not express the functional CYP3A4*1B allele that has been correlated with CYP3A5*1 content in Caucasians [13].

The efflux transporter P-gp, which is encoded by ABCB1, is involved in the resistance of many drugs and has been shown to influence docetaxel tumor exposure [10]. P-gp has been demonstrated to play a role in the efflux of docetaxel in tumor cells in vivo [11]. ABCB1 is polymorphic and may further explain some of the variability seen with docetaxel CL, toxicity, and response. Patients with a high expression of P-gp efflux activity in tumors are expected to have a lower intratumor drug concentrations and less likelihood for response. However, liver P-gp has not been associated with docetaxel CL in Caucasian patients [13]. Isla and colleagues found no relationship between ABCB1 genetic polymorphism and response or survival in non-small cell lung cancer (NSCLC) patients treated with docetaxel. However, the authors did find a significant relationship between ABCB1 polymorphism and toxicity (diarrhea) [14]. The SCOTROC1 substudy found no relationship between ABCB1 polymorphism observed and outcome or response [9]. Neither of the studies evaluated docetaxel or midazolam pharmacokinetics, which may have an impact on docetaxel response and toxicity.

In this study, we evaluated the midazolam CL as a phenotypic probe for docetaxel pharmacokinetics and pharmacodynamics (CA-125 and response) in patients with newly diagnosed ovarian cancer. The objectives of this study were to determine whether the midazolam CL predicted docetaxel pharmacokinetics, CA-125 change, and response and to assess the impact of CYP3A5 and ABCB1 genotypes on docetaxel pharmacokinetics and pharmacodynamics in ovarian or primary peritoneal cancer patients.

Materials and methods

Eligibility

This research project was approved by the Institutional Review Board of the University of Pittsburgh (IRB #0409102). All patients gave written informed consent prior to their inclusion in the study. Women between the ages of 18–77 with stage IC to IV epithelial ovarian or primary peritoneal cancer were eligible for this study. Patients were eligible with either optimal or suboptimal cytoreduction or in the setting of neoadjuvant therapy when histologic confirmation of disease was obtained. A minimal neutrophil count of >1,500/mm^2, hemoglobin >8 g/dL, platelet count >100,000/mm^3, and bilirubin, AST, ALT, and alkaline phosphatase within normal range were required. Exclusion criteria included mucinous tumors, borderline tumors, mixed mesodermal tumors, fallopian tube carcinomas, ECOG performance status of 3–4, prior chemotherapy or radiation for the disease process, neutrophil counts of <1,500/mm^3, platelet <100,000/mm^3, creatinine >1.25 upper limit of normal, uncontrolled diabetes melittus, concurrent or previously treated malignancy within the prior 5 years, previous hypersensitivity reaction to either docetaxel or carboplatin, and peripheral neuropathy >NCCTC grade 2.

Dose and administration

Pharmacokinetic, phenotyping, and pharmacogenetic studies were performed on cycle 1 only. Midazolam was administered at 2 mg IV over 30 min on the day prior to docetaxel. Docetaxel was administered at 75 mg/m^2 over 1 h, and carboplatin was administered as a 30 min IV infusion at a AUC of 5 mg/ml min. Cycles were repeated every 21 days.

Sample collection and analysis

For midazolam, blood samples were obtained from an IV site not used for midazolam administration, immediately prior to and at the end of the midazolam infusion and at 5, 15, 30, 60, 120, 240, 300, and 360 min after midazolam administration. Blood samples were collected in 10-ml EDTA tubes and centrifuged within 2 h of collection at 1,200×g, at 4°C × 15 min. Plasma was collected and stored at −80°C until analyzed.

For docetaxel pharmacokinetic studies, blood samples were obtained from a site other than the site of docetaxel administration at prior to administration, at 30 min after the start of the infusion, 5 min before the end of the infusion, and at 0.25, 0.5, 1, 1.5, 2, 3, 6, and 24 h following the infusion. Blood samples were collected in 4-ml lithium heparin tubes and centrifuged at 1,200×g × 5 min. Plasma was collected and stored at −80°C until analyzed.

Analytical methods

Docetaxel samples were analyzed by two different methods at two different laboratories. Samples from patients 1 to 25 were analyzed at The University of Pittsburgh Cancer Institute (UPCI) via liquid chromatography–mass spectrometry (LC–MS). Samples from patients 26 to 34 were analyzed at The University of North Carolina at Chapel Hill (UNC) via liquid chromatography/tandem mass spectrometry (LC–MS/MS) [15, 16]. Samples from patient 12 were run at both institutions for cross validation. The LC–MS and LC–MS/MS results for each sample from patient 12 were within

±10%. All midazolam samples were analyzed at UNC via LC–MS/MS [17].

At UPCI, docetaxel samples were prepared for LC–MS analysis using a previously described solid-phase extraction method [15]. Paclitaxel was used as the internal standard. The lower limit of quantitation (LLOQ) of docetaxel was 0.3 ng/ml. At UNC, docetaxel samples were prepared for LC–MS/MS analysis using a previously described liquid–liquid extraction method [16]. Paclitaxel was also used as the internal standard. The LLOQ of docetaxel was 1.2 ng/ml. Midazolam samples were analyzed with an existing LC–MS/MS assay at UNC [17]. Alprazolam was used as the internal standard. The LLOQ of midazolam was 1.5 ng/ml.

Pharmacokinetic methods

Noncompartmental pharmacokinetic analysis was performed using WinNonlin Software Version 5.0 (Pharsight Corp., Mountain View, CA). Area under the concentration versus time curve from 0 to infinity ($AUC_{0-\infty}$) and CL for docetaxel and midazolam were calculated using standard equations using WinNonlin. The average percent extrapolation of the AUC value for docetaxel was less than 15% for all patients and thus an AUC of 0 to infinity was reported. Volume of distribution (Vd) and half-life ($t_{½}$) were also calculated using standard equations using WinNonlin.

Pharmacogenomic methods

Genotyping of ABCB1, CYP3A5*3, and CYP3A5*6 was done by polymerase chain reaction (PCR) and pyrosequencing, as described previously [18]. DNA isolation was performed on 10 ml of whole blood collected in an EDTA vacutainer tube using the Purgene Genomic DNA Purification Kit (Gentra, Minneapolis, MN) as per the manufacturer's instructions.

Primer sequences used were as follows: CYP3A5*3 forward biotinylated primer 5′-CCCACGTATGTACCACCCAGC-3′ and of the CYP3A5*3 reverse primer 5′-ATTAGGGTGTGACACAGCAAGA-3′, CYP3A5*6 forward biotinylated primer 5′-TCTTTGGGGCCTACAGCATG-3′ and of the CYP3A5*6 reverse primer 5′-AAAGAAATAATAGCCCACATACTTATTGAGAG′, MDR1 (ABCB1) c3435t forward primer 5′-GAGCCCATCCTGTTTGACTG-3′ and of the MDR1 c3435t biotinylated reverse primer 5′-GCATGTATGTTGGCCTCCTT-3′, and MDR1 g2677at forward biotinylated primer 5′-CTGGACAAGCACTGAAAGATAAGA-3′ and a MDR1 g2677at reverse primer 5′-AGCATAGTAAGCAGTAGGGAGTAACA-3′ (IDT DNA, Coralville, IA). Pyrosequencing was completed using the PSQ96 MA and the SQA reagent kit (Biotage, Uppsala, Sweden).

Pharmacodynamics

Clinical response was evaluated at cycle 3 and cycle 6 of chemotherapy. The clinical response evaluations included comprehensive physical examination, CT scans of the abdomen and pelvis, and CA-125 levels. Response and progression were evaluated based on response evaluation criteria in solid tumors (RECIST) [19]. Toxicity was assessed by NCI common toxicity criteria (http://www.cancer.gov). Blood samples were tested once weekly during each cycle and analyzed for complete blood count (CBC) with differential and platelet count. Chemistry, electrolyte, and CA-125 assessment were conducted within three days of each new cycle.

Statistical methods

Hardy–Weinberg equilibrium was determined using HWSIM (http://krunch.med.yale.edu/hwsim). For each of the genes evaluated, association between genotype and docetaxel pharmacokinetic or pharmacodynamic variables were evaluated with the exact Kruskal–Wallis test. Each genotype was treated as a distinct group. The a priori level of significance was $P < 0.05$. Because this was an exploratory study intended to be hypothesis-generating, no corrections for multiple comparisons were made. The relationship between genotypes and midazolam CL as related to response and toxicity was evaluated using the same comparisons and analyses. Comparisons between CA-125 groupings in categories of <10, 10–20, and >20 U/ml were based on a previously published study that found significance at these levels [22]. Due to the small sample size of this study, the CA-125 groupings were also expanded to <15, 15–30, and >30 U/ml for further hypothesis testing.

Results

Patient characteristics and enrollment

A summary of the patients characteristics are included in Table 1. Pharmacokinetic analysis of midazolam and docetaxel was performed on 30 and 31 patients, respectively. Twenty-nine patients had corresponding midazolam and docetaxel pharmacokinetic data. Pharmacogenomic analysis was performed on 26 patients. Twenty-five of these 26 patients had corresponding docetaxel pharmacokinetic data and genotyping data.

Pharmacodynamics: response

There was an inverse relationship between midazolam CL and CA-125 after cycle 6, where a higher midazolam CL was associated with a lower CA-125 value at <10, 10–20,

Table 1 Patient characteristics

	Number of cases
Age	
Mean, range	58.9 (41–74) years
Eligibility	
Total	34
Withdrawal	3
Progression prior to therapy	1
Hypersensitivity to taxotere	1
Race	
Caucasian	32
African American	1
Stage distribution	
IC	1
II A	1
II B	0
II C	1
III A	1
III B	1
III C	20
IV	2
Unstaged	6
Number of cycles (mean, range)	5.9 (3–8)
Total cycles	188
Cytoreduction	
Optimal	20 (62.5%)
Suboptimal	8 (25.0%)
Not accessible	3 (12.5%)
Toxicity events	
Neutropenia grade 3–4 events	34 (18%)
Neuropathy grade 3–4 events	0 (0%)
Diarrhea grade 3–4 events	1 (3%)
Nausea grade 3–4 events	1 (3%)
Neutropenia grade 3–4/pt	9 (25%)
Status at end of therapy	
Complete clinical response	16
Partial response	1
Stable disease	10
Progression	2

and >20 U/ml ($P = 0.007$) (Fig. 1a). After cycle 6, there also was an inverse relationship between midazolam CL and CA-125 at <15, 15–30, and >30 U/ml, with a higher midazolam CL associated with a lower CA-125 ($P = 0.048$) (Fig. 1b).

The mean ± SD CA-125 at baseline was 293 ± 433 U/ml, 463 ± 579 U/ml, and 1452 ± 1937 U/ml for patients with CR/PR, SD, and PD, respectively (Fig. 1c). The mean ± SD CA-125 at nadir was 13.2 ± 10.5 U/ml, 33.5 ± 36.7 U/ml, and 58.1 ± 36.7 U/ml for patients with CR/PR, SD, and PD, respectively (Fig. 1d). Of the patients with nadir CA-125 of <10 U/ml, 41% (10/17), 30% (3/10), and 0% (0/2) had CR/PR, SD, and PD, respectively. Of the patients with nadir CA-125 levels <15 U/ml, 76% (13/17), 40% (4/10), and 0% (0/2) had CR/PR, SD, and PD, respectively. CA-125 nadir when grouped into categories <10, 10–20, and >20 U/ml did not significantly predict response; however, when grouped into categories <15, 15–30, and >30 U/ml, the relationship between CA-125 and response was significant ($P = 0.0173$) (Table 2). There was no relationship between any genotype observed and response (Table 3).

Pharmacokinetics

The mean ± SD docetaxel AUC was 3,085 ± 1,050 ng/ml h. Mean ± SD docetaxel CL was 26.8 ± 8.3 l/h/m^2 or 47.1 ± 16.5 l/h. Mean ± SD elimination *t*½ for docetaxel was 6.0 ± 3.1 h. The mean ± SD docetaxel *V*d was 392 ± 230 l. The mean ± SD midazolam CL was 268 ± 121 ml/min. There was no correlation between docetaxel CL and midazolam CL ($R^2 = 0.05$) (Fig. 2).

Polymorphisms were analyzed for CYP3A5*6, CYP3A5*3, ABCB1 C3435T, and ABCB1 G2677AT in 26 patients. Five patients expressed the CYP3A5*1/*3 genotype, and 21 patients expressed the CYP3A5*3/*3 genotype. No patients expressed the CYP3A5*6 allele. In patients expressing the CYP3A5*1/*3 genotype, the mean ± SD docetaxel CL was 25.6 ± 8.7 l/h/m^2. The mean ± SD docetaxel CL for patients expressing the CYP3A5*3/*3 genotype was 28.6 ± 7.9 l/h/m^2. For patients expressing the CYP3A5*1/*3 genotype, the mean ± SD midazolam CL was 263 ± 131 ml/min. Patients expressing CYP3A5*3/*3 genotypes had a mean ± SD midazolam CL of 261 ± 113 ml/min. There was also no observed difference in docetaxel CL with any ABCB1 genotype expressed in the present study.

Pharmacodynamics: toxicity

No patient experienced any grade of neurotoxicity on cycle 1. Grade 1 neurotoxicity occurred in 13 patients on any cycle. Grade 2 neurotoxicity occurred in one patient. Fifty-five percent (11/20) of CYP3A5*3/*3 expressors and 60% (3/5) of CYP3A5*1/*3 expressors experienced neurotoxicity during the study. Grade 2 neurotoxicity occurred in one patient who was a CYP3A5*3/*3 expressor. Thirteen patients (out of 25 with available genotype and toxicity data) had neutropenia grade 2 or higher on cycle 1. Fifty-five percent (11/20) of CYP3A5*3/*3 patients and 40% (2/5) of CYP3A5*1/*3 patients experienced grade 2 or higher neutropenia on cycle 1 (Table 4). There was no relationship between ABCB1 or CYP3A5 genotypes and neutropenia (Table 4).

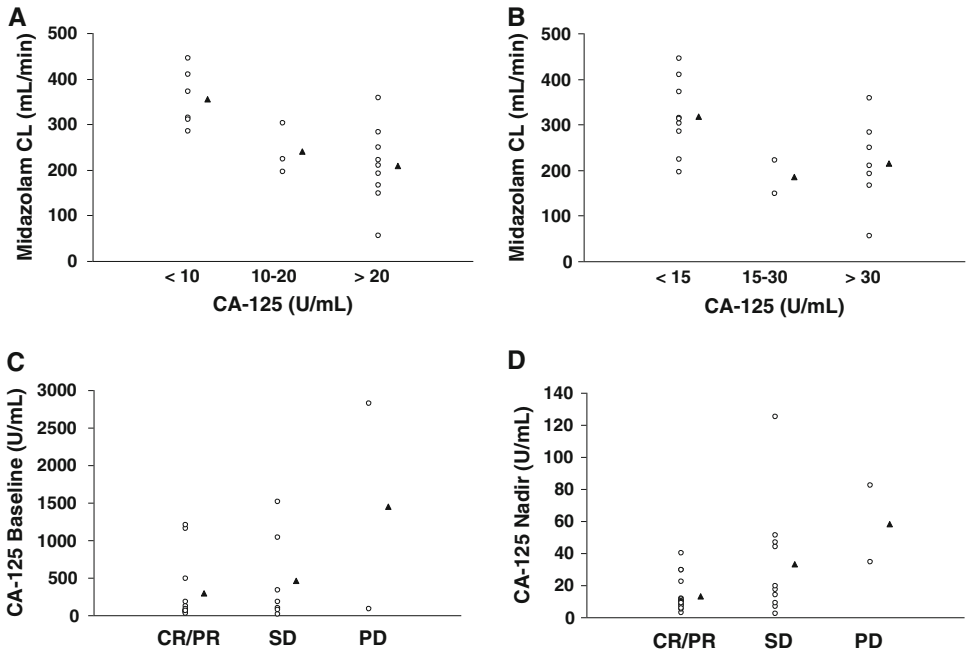

Fig. 1 **a** The relationship between midazolam clearance (CL) and CA-125 (U/ml) following cycle 6. Individual patients are represented by the *open circles* ($N = 18$). The *triangles* represent mean midazolam clearance. The mean ± SD midazolam clearance for patients having CA-125 levels <10, 10–20, and >20 U/ml was 356 ± 62.6, 241 ± 55.6, and 209 ± 85.5 ml/min, respectively ($P = 0.007$). **b** The relationship between midazolam clearance (CL) and CA-125 (U/ml) following cycle 6. Individual patients are represented by the *open circles* ($N = 19$). The *triangles* represent mean midazolam clearance. The mean ± SD midazolam clearance for patients having CA-125 levels <15, 15–30, and >30 U/ml was 318 ± 81.1, 185 ± 51.9, and 216 ± 95.0 ml/min, respectively ($P = 0.048$). **c** The relationship between CA-125 at baseline for patients who had CR/PR, SD, and PD. Individual patients are represented by *open circles* ($N = 21$). The *solid triangles* represent mean CA-125 level at baseline. Mean ± SD baseline CA-125 for patients with CR/PR, SD, and PD was 293 ± 433, 463 ± 579, and 1,452 ± 1,937 U/ml, respectively. **d** The relationship between CA-125 at nadir for patients who had CR/PR, SD, and PD. Individual patients are represented by *open circles* ($N = 29$). The *solid triangles* represent mean CA-125 level at nadir. Mean ± SD CA-125 at nadir for patients with CR/PR, SD, and PD was 13.2 ± 10.5, 33.5 ± 36.7, and 58.1 ± 36.7 U/ml, respectively

Table 2 Relationship between CA-125 nadir and response

CA-125 (U/ml)	Response	Stable disease	Progression	*P* value
<15	13	4	0	
15–30	3	2	0	
>30	1	4	2	0.0173
<10	10	3	0	
10–20	3	3	0	
>20	4	4	2	0.1018

Table 3 Relationship between genotype and response

Genotype	*N*	NED/PR	Stable/ persistent	Progression
CYP3A5*3				
C/C	19	12	6	1
C/T	5	3 (NED) 1 (PR)	0	1
Total	24	16	6	2
ABCB1 C3435T				
C/C	5	4	1	0
C/T	13	7 (NED) 1 (PR)	3	2
T/T	6	4	2	0
Total	24	16	6	2
ABCB1 G2677AT				
G/G	6	5	1	0
G/T or G/A	12, 1	6 (NED) 1 (PR)	4	1 (G/A) 1 (G/T)
T/T	5	4	1	0
Total	24	16	6	2

Discussion

This is the first study evaluating factors affecting docetaxel pharmacokinetics and pharmacodynamics in patients with ovarian cancer. In our study, there was an inverse relationship between midazolam CL and reduction in CA-125 after cycle 6 ($P = 0.007$). This corresponded with a relationship between CA-125 and response when stratified into different groups (Table 2). The higher the midazolam CL, the greater the predicted response as measured by CA-125 following cycle 6. Our study did not find that CA-125 levels <10 U/ml predicted for response; however, when grouped in

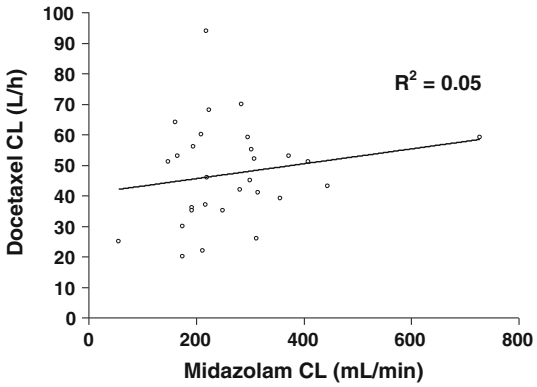

Fig. 2 Correlation between midazolam and docetaxel clearance. Individual patients are represented by the *open circles*. The best fit line is represented by the *line*. The R^2 value was 0.05

Table 4 Relationship between genotype and neutropenia on cycle 1

Genotype	*N*	Grade 1	Grade 2	Grade 3	Grade 4
CYP3A5*3					
C/C	20	0	1	2	8
C/T	5	0	0	0	2
Total	25	0	1	2	10
ABCB1 C3435T					
C/C	6	0	0	0	4
C/T	13	0	1	1	3
T/T	6	0	0	1	3
Total	25	0	1	2	10
ABCB1 G2677AT					
G/G	6	0	0	0	4
G/T or G/A	12, 1	0	1	2	4
T/T	5	0	0	0	2
Total	24	0	1	2	9

categories of <15, 15–30, and >30 U/ml, CA-125 significantly predicted response ($P = 0.0173$). The CA-125 categories were expanded due to our small sample size and were meant to be hypothesis-generating. This relationship was independent of docetaxel CL or AUC, suggesting that midazolam CL may be a measure of CA-125 production or metabolism. The underlying mechanism(s) whereby midazolam CL and CA-125 changes are correlated with response after treatment with docetaxel and carboplatin in patients with ovarian cancer is unknown.

CA-125 is frequently used as a biomarker for ovarian cancer and is elevated in more than 80% of patients with ovarian cancer [1]. CA-125 is encoded by the Mucin-16 (MUC16) gene and is thought to originate from peritoneal, ovarian, and amniotic sources [20, 21]. Although useful as a biomarker in ovarian cancer, measurement can be variable depending on the patient's disease stage, analytical imprecision, and human error. In one previous study, CA-125 levels were stratified into three groups: ≤10, 11–20, and 21–30 U/ml [22]. The study found that median survival was 2,968, 537, and 537 days, in patients with CA-125 nadir levels of ≤ 10, 11–20, and 21–30 U/ml, respectively ($P < 0.001$). There have been many studies linking CA-125 with response, but the stratification of CA-125 levels remains unclear [22–24]. Despite the fact that CA-125 is considered normal at <35 U/ml, the prior study linking median survival to CA-125 levels <10 U/ml warranted further investigation.

The mechanism associated with midazolam prediction of CA-125 changes needs to be determined and was beyond the scope of this study. In addition, it is currently unclear whether midazolam CL is associated with CA-125 changes after other types of treatment for ovarian cancer or only for docetaxel and carboplatin treatment that was evaluated in this trial. Our results linking midazolam CL and changes in CA-125 and response warrant further investigation.

We found no relationship between midazolam CL and docetaxel CL as previously reported in other studies [6]. We also observed a higher average docetaxel CL in this study than observed in previous studies [6, 13]. The small sample size of our present study, relatively non-diverse patient population (1 African American and 32 Caucasians), and low number of patients presenting with CYP3A5*1 allele ($n = 5$) are possible reasons for the lack of a correlation between the genotyping results and docetaxel pharmacokinetics as seen in a previous study [13].

We evaluated the effect of CYP3A5 and ABCB1 genotypes and docetaxel pharmacokinetics and pharmacodynamics. Although some evidence suggests CYP3A5 polymorphism plays a role in docetaxel pharmacokinetics, the prevailing evidence of ABCB1 polymorphism on drug disposition, toxicity, or response is still largely negative. A recent study by Baker et al. found no relationship between docetaxel CL and any ABCB1 polymorphism evaluated [13]. The SCROTROC1 trial found no relationship between ABCB1 genotype polymorphism and outcome or response. Findings from both studies are consistent with our study. Isla and colleagues found no relationship between ABCB1 genetic polymorphism and response or survival in NSCLC; however, the authors did find a significant relationship between ABCB1 polymorphism and toxicity (diarrhea) [12]. It was a limitation of our study that CYP3A4 was not evaluated; however, since CYP3A4 and CYP3A5 polymorphism are covariates of each other, it would be unlikely that evaluating CYP3A4 genotype would have changed the results of our study.

Alterations in tumor genetics and tumor drug delivery, as well as differences in tumor vascularity and capillary permeability, are all factors that may explain why the genotypes observed in our study did not correlate with response

or toxicity. There may also be other factors that should also be considered that cause the wide interpatient variability of docetaxel pharmacokinetics including non-genetic factors, such as body composition (% body fat, body mass index), fat content as well as other genetic factors.

Conflict of interest Research funding was provided by Sanofi-Aventis Oncology.

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