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*CYP3A4*22* genotype and systemic exposure affect paclitaxel-induced neurotoxicity

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Abstract

PURPOSE—Paclitaxel is used for the treatment of several solid tumors and displays a high inter-individual variation in exposure and toxicity. Neurotoxicity is one of the most prominent side-effects of paclitaxel. This study explores potential predictive pharmacokinetic and pharmacogenetic determinants for the onset and severity of neurotoxicity.

EXPERIMENTAL DESIGN—In an exploratory cohort of patients (n=261) treated with paclitaxel, neurotoxicity incidence and severity, pharmacokinetic parameters and pharmacogenetic variants were determined. Paclitaxel plasma concentrations were measured by HPLC or LC-MS/MS, and individual pharmacokinetic parameters were estimated from previously developed population pharmacokinetic models by non-linear mixed effects modeling (NONMEM). Genetic variants of paclitaxel pharmacokinetics tested were *CYP3A4*22*, *CYP2C8*3*, *CYP2C8*4*, and *ABCB1 3435 C>T*. The association between *CYP3A4*22* and neurotoxicity observed in the exploratory cohort was validated in an independent patient cohort (n=239).

RESULTS—Exposure to paclitaxel ($_{log}$AUC) was correlated with severity of neurotoxicity (P <0.00001). Female *CYP3A4*22* carriers were at increased risk of developing neurotoxicity (P = 0.043) in the exploratory cohort. *CYP3A4*22* carrier status itself was not associated with pharmacokinetic parameters (CL, AUC, C_{max}, or $T_{>0.05}$) of paclitaxel in males or females. Other genetic variants displayed no association with neurotoxicity. In the subsequent independent validation cohort, *CYP3A4*22* carriers were at risk of developing grade 3 neurotoxicity (odds ratio = 19.1; P = 0.001).

CONCLUSIONS—Paclitaxel exposure showed a relationship with the severity of paclitaxel-induced neurotoxicity. In this study, female *CYP3A4*22* carriers had increased risk of developing

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severe neurotoxicity during paclitaxel therapy. These observations may guide future individualization of paclitaxel treatment.

Keywords

paclitaxel; pharmacokinetics; *CYP3A4*22*; neurotoxicity; pharmacodynamics

INTRODUCTION

Paclitaxel is a highly active anti-microtubular agent used for the treatment of various solid tumors and has a large inter-patient variability in pharmacokinetics and toxicity (1). Neurotoxicity is frequently observed during paclitaxel treatment and is often dose-limiting. The degree of neurotoxicity is highly variable between individual patients (2, 3). Axonal degeneration and demyelization are the primary underlying causes of this neurotoxicity (4).

Genetic variants in enzymes involved in paclitaxel metabolism could contribute to inter-individual differences in toxicity and efficacy of paclitaxel treatment. Paclitaxel is metabolized by cytochrome 450 (CYPs) enzymes CYP2C8 and CYP3A4 (5, 6). Recently, a new intron 6 single nucleotide polymorphism (SNP), encoding the *CYP3A4*22* variant allele, was discovered. This variant allele is associated with decreased CYP3A4 hepatic mRNA levels and consequently lower enzymatic activity (7). *In vivo*, the *CYP3A4*22* variant allele was shown to be associated with altered therapeutic parameters in several CYP3A4 metabolized drugs (e.g., tacrolimus, simvastatin, and cyclosporine) (8–10).

The majority of patients treated with paclitaxel will develop peripheral neurotoxicity in the course of their treatment (11). The incidence and severity of neurotoxicity has been associated with pharmacokinetic exposure parameters such as area under the curve (AUC), and time above total paclitaxel concentrations of 0.05 μmol/L ($T_{>0.05}$) (12). Mielke *et al* studied the association between paclitaxel pharmacokinetics and neurotoxicity in 24 patients and found that drug exposure (AUC x weeks of paclitaxel therapy) was higher in the group that developed neurotoxicity (12). Furthermore, Green *et al* showed in 23 patients that paclitaxel pharmacokinetics and severity of neurotoxicity were correlated (13). Studies in larger cohorts on the relationship between paclitaxel exposure and neurotoxicity have not been published so far.

The aim of the current study was to evaluate the influence of several SNPs in genes encoding drug metabolizing enzymes and transporters on the pharmacokinetics of paclitaxel and development and severity of sensory neuropathy. In addition, we aimed to further clarify potential associations between paclitaxel pharmacokinetic parameters and the development and severity of peripheral neuropathy in a large cohort of patients.

PATIENTS AND METHODS

Patients

Exploratory and validation cohort—A exploratory cohort of cancer patients treated with paclitaxel for different tumor types within a prospective trial in which pharmacokinetics, pharmacodynamics and pharmacogenetics was studied (registered at www.trialregister.nl as NTR2311, ethics board study number MEC 03.264) were included in the exploratory cohort (n=261). The influence of genetic variants on the pharmacokinetics and frequency and severity of paclitaxel-induced neurotoxicity were studied. The findings were subsequently validated in an independent cohort of paclitaxel-treated patients (n=239) from whom whole blood for DNA analysis and neurotoxicity data were available (ethics board study number MEC 02.1002; this study involves a large data set of cancer patients

who provided blood for DNA analysis for pharmacogenetic purposes). In this validation cohort the association between *CYP3A4*22* carrier status and development and severity of neuropathy were studied.

The inclusion criteria for the exploratory cohort were (i) histological or cytological confirmed diagnosis of cancer treated with paclitaxel, (ii) age 18 years or older, (iii) WHO performance score 0–1 and (iv) adequate hematopoietic, hepatic and renal functions. The use of CYP3A4 and CYP2C8 inducers or inhibitors was not allowed. In the validation cohort, patients were included if whole blood and neurotoxicity data were available. The trials were approved by the Ethics Board of the Erasmus University Medical Center and supported by the Dutch Cancer Society. All patients provided written informed consent prior to study participation.

Neurotoxicity—During the entire treatment course with paclitaxel, neurotoxicity was graded by the treating physician according to National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE) criteria version 2–4. During each hospital visit the highest neurotoxicity score of the previous cycle was assessed. In both cohorts the highest neurotoxicity score during paclitaxel treatment was used in the analyses.

Pharmacokinetic analysis—Paclitaxel pharmacokinetics, using a validated limited sampling strategy, were assessed in up to three treatment cycles for each patient in the exploratory cohort. Pharmacokinetic sampling was allowed during any treatment cycle. Lithium heparin was used as anticoagulant for all samples. Paclitaxel was quantitated by a validated UV detection HPLC method (14) or by a validated LC-MS/MS method (15).

Individual pharmacokinetic parameters were calculated based on measured plasma samples and a previously developed population pharmacokinetic model for paclitaxel (16–18). Individual pharmacokinetic parameters were estimated as Empirical Bayes estimates within the non-linear mixed-effect modeling software NONMEM version 7 (Icon Development Solutions, Ellicott City, MD). AUCs were obtained by integrating the predicted concentration-time profile up to 96 h after start of the infusion. The time above 0.05 μmol/L ($T_{>0.05}$) was predicted for each patient.

Genotyping—Genomic DNA was isolated from 200 μL EDTA whole blood using MagnaPure LC (Roche Diagnostics GmbH, Mannheim, Germany). Genotyping was performed using TaqMan® (Applied Biosystems, Carlsbad, CA) assays for *CYP2C8*3* (rs10509681, C_25625782_20, 1196A>G), *CYP2C8*4* (rs1058932, C_361406_1, 792C>G), *ABCB1 3435 C>T* (rs1045642, C_7586657_20) and *CYP3A4*22* (rs35599367, C_59013445_10, intron 6 C>T), using 20 ng genomic DNA on the ABI PRISM 7500® fast real-time PCR Systems (Applied Biosystems) according to the manufactures instructions. Assays were validated by sequencing.

Expression of CYP3A4 in human dorsal root ganglia—Human dorsal root ganglia isolated from the lumbar position 4 (L4) were obtained from the National Disease Research Interchange (NDRI) and RNA was extracted using the RNEasy mini kit (Qiagen). Expression of *CYP3A4* was measured by qRT-PCR using SYBR green and the gene specific primers (Forward: 5′-CACAGATCCCCCTGAAATTAAGCTTA-3′; Reverse: 5′-AAAATTCAGGCTCCACTTACGGTG-3′). Gene expression was determined by C_t relative to the housekeeping gene, *GAPHD,* which was measured using a gene specific TaqMan probe (HS02758991_g1; Applied Biosystems).

Statistical analysis

Data are presented as medians with ranges, unless stated otherwise. To test whether patients with different grades of neurotoxicity had different PK parameters, the Kruskal-Wallis test was used. To study the relationship between genetic variants and severity of neurotoxicity, the Fisher exact test was used. To test the association between severity of neurotoxicity and *CYP3A4*22* carrier status, logistic regression was performed. The analysis was performed separately for males and females because of the reported gender difference in paclitaxel pharmacokinetic parameters (19). Also, to correct for different dosing regimens, the analysis was stratified on weekly and 3-weekly schedules of paclitaxel. To test if all studied genetic variants were in Hardy-Weinberg equilibrium, the chi-square test was used. A *P*-value below 0.05 was considered statistically significant. All statistical analyses were performed with SPSS (Armonk, NY) version 20.0 and Stata (StataCorp, College Station, TX), release 12.

RESULTS

Patients

Exploratory cohort—In the exploratory cohort, 261 patients (135 male, 126 female) were included. Median age was 61 years (range: 18–82 years) and 96% of patients were of Caucasians (Table 1). Esophageal cancer was the main diagnosis (46%) in this cohort. Patients were treated with a median dose of 180 mg paclitaxel during each cycle (range: 75–560 mg). The median cumulative dose in this cohort was 975 mg (range: 280–3,910 mg). In 7 patients genotyping could not be performed due to poor DNA quality.

Validation cohort—In the validation cohort, 239 patients (129 male, 110 female) were included. Median age was 63 years (range: 24–83 years) and 95% of patients were Caucasians. Most patients in this cohort were diagnosed with esophageal cancer (64%; Table 1). Patients were treated with a median dose of 165 mg paclitaxel during each cycle (range: 70–480 mg). The median cumulative dose in this cohort was 1,140 mg (range: 200–2,975 mg). In 2 patients genotyping could not be performed due to poor DNA quality.

Paclitaxel dose—Patients in both cohorts received paclitaxel weekly or every 3 weeks in different combination regimens. Also patients receiving chemotherapy in combination with radiotherapy, as a preoperative regimen for resectable esophageal cancer, were included.(20) These patients received a weekly dose of 50 mg/m^2. The cumulative dose of paclitaxel did not differ between CYP3A4*22 carriers and non-carrier in both cohorts together ($P = 0.30$). In the training set, the cumulative dose did not differ between CYP3A4*22 carriers and non-carriers in males ($P = 0.93$) and females ($P = 0.66$). In the validation cohort, the cumulative dose was also not significantly different between CYP3A4*22 carriers and non-carriers, both in males ($P = 0.66$) and females ($P = 0.12$).

Association pharmacokinetic parameters and development of toxicity

Exploratory cohort—Systemic exposure (AUC) of paclitaxel was significantly associated with severity of neurotoxicity in both females and males ($P \leq 0.001$; Table 2). Also, $T_{>0.05}$ and the maximum observed concentration after administration (C_{max}) were significantly associated with neurotoxicity ($P \leq 0.001$; Table 2). Paclitaxel exposure ($_{log}$AUC) and development and severity of neurotoxicity showed a relationship ($R = 0.52$; $P < 0.000001$).

Influence of genetic variants on neurotoxicity—All tested genetic variants were in Hardy-Weinberg equilibrium (Suppl table 1).

Exploratory cohort—In the exploratory cohort neurotoxicity was observed in 106 of 261 patients (41%). There were significantly more females than males who developed neurotoxicity (67% *versus* 33%; $P < 0.0001$). In this cohort, severity of neurotoxicity was differently distributed between female *CYP3A4*22* carriers and non-carriers ($P = 0.043$), while male *CYP3A4*22* carriers and non-carriers had an even distribution of neurotoxicity ($P = 0.90$; Table 3). The other tested SNPs showed no association with severity of neurotoxicity (Table 4). *CYP3A4*22* carrier status in both males and females was not associated with pharmacokinetic parameters (unbound CL, AUC, $T_{>0.05}$ and C_{max}) of paclitaxel (data not shown). There was no influence of *CYP2C8*3* or *CYP2C8*4* carrier status on pharmacokinetics of paclitaxel (data not shown). Furthermore, the *ABCB1 3435C>T* SNP was also not associated with paclitaxel pharmacokinetics (data not shown). Cumulative dosages of patients with grade 3 neurotoxicity are summarized in Table 5.

Validation cohort—To confirm the relationship observed in the exploratory cohort between *CYP3A4*22* carrier status and severity of neurotoxicity, we studied this association in an independent validation cohort. In this cohort, 113 of 239 patients (47%) developed neurotoxicity. Significantly more females than males developed neurotoxicity (65% *versus* 29%; $P < 0.0001$). In this cohort, in both females and males, the grade of neurotoxicity was differently distributed in *CYP3A4*22* carriers than in *CYP3A4*22* non-carriers ($P = 0.036$ and $P = 0.025$, respectively; Table 3). The risk of developing grade 3 neurotoxicity was higher in *CYP3A4*22* carriers than in non-carriers (odds ratio = 19.1; $P = 0.001$; 95% confidence interval = 3.3–110), confirming the observation in females in the exploratory cohort and showing this time a comparable effect in males. Cumulative dosages of patients with grade 3 neurotoxicity are summarized in Table 5.

Additional exploratory analysis

Grade 3 neurotoxicity may be a result of the cumulative dose of paclitaxel, and is a reason to discontinue paclitaxel treatment. Therefore we also performed an exploratory Cox regression analysis in patients of both cohorts together because of the small number of neurotoxicity grade 3, taking cumulative dose into account. In this analysis, the occurrence of grade 3 neurotoxicity was included as the event, while the cumulative dose of paclitaxel was included as the time-to-event variable. The prognostic impact of CYP3A4*22 was then evaluated, adjusted for cohort and gender. Again, neurotoxicity grade 3 was more often seen in *CYP3A4*22* carriers (hazard ratio = 22.1, 95% confidence interval = 4.7–105, $P < 0.001$).

Expression of CYP3A4 in human dorsal root ganglia

We found that CYP3A4 was expressed in human dorsal root ganglia in two separate patient samples as demonstrated by amplified products that were detected by qRT-PCR (Supplemental Figure 1). CYP3A4 transcripts were expressed with a C_t value of 28.71 ± 0.074 in dorsal root ganglia of patient 1 and 28.27 ± 0.009 in the dorsal root ganglia of patient 2, relative to the control gene, GAPDH, which was expressed with a C_t value of 21.96 ± 0.008 in dorsal root ganglia of patient 1 and 25.40 ± 0.090 in the dorsal root ganglia of patient 2.

DISCUSSION

In this study, we showed that systemic exposure to paclitaxel was highly correlated with the development of (severe) neurotoxicity. Importantly, systemic exposure to paclitaxel measured during one course is already predictive for both development and severity of neuropathy in males and females. This result is in line with the study of Mielke *et al* who observed that the time above the threshold of 0.05 μmol/L paclitaxel was associated with

development of neuropathy (12) and the study of Green *et al*, reporting a relationship between paclitaxel exposure and neurotoxicity (13).

In addition, we showed that females carrying the reduced function *CYP3A4*22* variant allele had an increased risk of developing severe neurotoxicity. This was demonstrated in our exploratory cohort, and subsequently confirmed in the independent validation cohort. Interestingly, in the exploratory cohort only female carriers of *CYP3A4*22* were found to have an increased risk of neurotoxicity, whereas in the validation cohort there was an increased risk of grade 3 neuropathy in both males and females carrying the *CYP3A4*22* allele. It should be noted that the low incidence of grade 3 neurotoxicity in our cohort makes the absolute risk of developing neurotoxicity during paclitaxel treatment difficult to interpret. The lack of statistical significance in the male *CYP3A4*22* carriers in the exploratory cohort could possibly be explained by the fact that there were no male patients with grade 3 neurotoxicity in this cohort. Because of the observed discrepancy between exploratory and validation cohorts, it is not yet possible to present a conclusion on the risk of neurotoxicity for male *CYP3A4*22* carriers.

Recently, it was shown that taxane-induced neuropathy is not a pharmacodynamic marker of treatment outcome (21). Therefore, a predictive marker for neuropathy during paclitaxel therapy could be of particular clinical usefulness. *CYP3A4*22* carrier status has the potential to aid medical oncologists in selecting female patients sensitized to development of neurotoxicity during paclitaxel therapy. It would be clinically relevant to predict grade 3 (or higher) neurotoxicity because this toxicity often leads to dose reductions or preliminary discontinuation of paclitaxel therapy. For a patient in whom severe neurotoxicity should absolutely be avoided (*e.g.* those with disabling peripheral neurological disorders, or those with pre-existing neuropathy from previous chemotherapy), pre-treatment knowledge of the *CYP3A4*22* carrier status might help choosing the appropriate (chemo-) therapy for an individual patient. If alternative drugs are available, these patients should preferably not be exposed to paclitaxel.

In this study, systemic pharmacokinetic parameters did not differ between *CYP3A4*22* carriers and non-carriers. This is in contrast with altered tacrolimus pharmacokinetics observed in *CYP3A4*22* carriers (9) and increased cholesterol reduction in simvastatin treated patients who are CYP3A4*22 carriers (8). It is also in contrast to the increased risk of delayed graft function and worse creatinine clearance in cyclosporine-treated kidney patients who carry the *CYP3A4*22* allele (10). This discrepancy could possibly be due to the fact that CYP3A4 in the liver is only a minor elimination pathway of paclitaxel when compared to CYP2C8, which indeed has a 2.3-fold greater metabolite production than CYP3A4 (22). However, none of the CYP2C8 SNPs nor *ABCB1 C3435T* showed an association with paclitaxel pharmacokinetics or the development of paclitaxel-induced neuropathy in our study. These findings are in line with several other pharmacogenetic studies in paclitaxel treated patients (1, 23). Bergmann and colleagues also did not find an association between *CYP2C8*3*, and *ABCB1 C3435T* and sensory neuropathy and overall survival in ovarian cancer patients (24). More recently, these authors reported that paclitaxel clearance was 11% lower in *CYP2C8*3* carriers than in non-carriers (25). In our study, we did not observe pharmacokinetic differences between patients, also not in a subgroup analysis of ovarian cancer patients (data not shown). We also could not confirm the findings by Leskala *et al* and Green *et al*, who reported an association between *CYP2C8*3* and neurotoxicity in patients treated with paclitaxel (13, 26). Because of the discrepancy in results in these studies, the potential of genetic variants to predict individual paclitaxel pharmacokinetics is still under debate. We are currently performing a large study associating 1,936 relevant SNPs in drug metabolizing enzymes and transporters (DMET) with paclitaxel pharmacokinetics to elucidate this issue further.

A higher systemic exposure to paclitaxel could not explain the higher incidence of neurotoxicity seen in CYP3A4*22 carriers. Therefore, a possible explanation might be that the effect of the CYP3A4*22 SNP is not systemic but localized in the peripheral neurons. Gosh and colleagues suggested a potential cytoprotective role for CYP3A4*22 in central nerves (27, 28). It was already known that CYP3A4 is expressed by endothelial cells in the blood brain barrier (27), but these authors observed that CYP3A4 was expressed in approximately 75% of neurons of epileptic brain tissue (28). In CYP3A4 transfected cells, incubated with toxic concentrations of carbamazepine, a remarkably reduced cell death was observed, suggesting a cytoprotective effect of CYP3A4 (28). In the current study, we found that CYP3A4 is also expressed in peripheral nerves, in particular dorsal root ganglia, and this could explain the possible cytoprotective mechanism against toxic CYP3A4 substrates, such as paclitaxel. This localization of CYP3A4 in peripheral neurons provides a potential mechanistic explanation for the observation that female carriers of the *CYP3A4*22* variant allele, which is associated with reduced CYP3A4 function, have a higher risk to develop severe neuropathy in our study. The observation that CYP3A4*22 is also expressed in peripheral neurons is only an indication that CYP3A4 might protect against neurotoxicity during paclitaxel therapy. It is, however, too early to provide a mechanistic explanation for our observations. Further research into the underlying biological principles of this potential protective role of CYP3A4 is needed.

Unfortunately, multivariate analyses were not warranted in both cohorts because of the relatively low incidence of grade 3 neurotoxicity. Therefore, we cannot exclude the possibility that the effect of *CYP3A4*22* on neurotoxicity is influenced by confounders. Therefore, our preliminary findings have to be validated in future research to explore the clinical potential of CYP3A4*22 as a marker for development of neurotoxicity.

Recently, several other SNPs identified in large genome wide association studies (GWAS) were associated with paclitaxel-induced neuropathy. Schneider *et al*, presented results of an interim analysis of their E5103 phase III trial, comparing chemotherapy plus concurrent bevacizumab, or chemotherapy with concurrent and sequential bevacizumab, as adjuvant treatment for early stage breast cancer (29). They found that SNPs in RWDD3 (rs2296308) and TECTA (rs1829) were associated with the time of first reporting ≥grade 2 neuropathy. Not much is known about RWDD3 or TECTA, but involvement of TECTA in sensoric hear loss and cellular stress has been suggested (30), making an association with the development of neuropathy biologically plausible. Bergmann *et al* aimed to validate these findings in an independent cohort but could not confirm any association between these SNPs and time to neurotoxicity (31). In another GWAS, Baldwin *et al* found a SNP in FGD4 (rs10771973) to be associated with the onset of peripheral neuropathy and validated this finding in an independent European and African American cohort (32). In this study, there was also evidence that two other markers in EPHA5 (rs7349683) and FZD3 (rs7001034) were associated with onset or severity of paclitaxel-induced neuropathy.

Neurotoxicity is not only a side effect attributable to taxanes, but is also frequently seen in treatments with several other drugs metabolized by CYP3A4. For example, bortezomib and thalidomide, used for the treatment of multiple myeloma, have incidences of grade ≥3 neurotoxicity of 8% and 5%, respectively (33). Also, vincristine (34) and ixabepilone (35) have been reported to frequently cause severe peripheral neuropathy. Therefore, further clinical research should elucidate the possible effects of *CYP3A4*22* carrier status on the development of neurotoxicity during treatment with these agents.

In conclusion, we identified a relationship between *CYP3A4*22* carrier status in women and occurrence of neurotoxicity during paclitaxel therapy. In our study, female carriers of *CYP3A4*22* had an increased risk of neurotoxicity, although paclitaxel pharmacokinetics

profiles were similar to those of non-carriers. This novel SNP could potentially be used as a predictive factor for paclitaxel-induced neurotoxicity in females, but further research is necessary to confirm our preliminary findings. Also, the predictive value of *CYP3A4*22* carrier status in other CYP3A4-metabolized drugs remains to be established.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

The chemotherapeutic agent paclitaxel is known for its small therapeutic window and large inter-individual variability in metabolism and toxicity profile. Peripheral neuropathy is a severe adverse event frequently seen during paclitaxel therapy. Pharmacogenetic and pharmacokinetic determinants have been suggested as predictive factors for this severe toxicity and could therefore potentially identify patients at risk. However, contradictory findings have been reported on the influence of genetic variants on the development of neurotoxicity. Also, the influence of pharmacokinetics on this potentially dose-limiting side-effect has not been studied in large cohorts of patients before. Furthermore, associations between the newly discovered *CYP3A4*22* polymophism and development of neurotoxicity during paclitaxel therapy has not been explored yet. More knowledge of factors that may predict neurotoxicity prior to taxane treatment could ultimately help choosing the appropriate therapy and dose for the individual patient.

Table 1

Patient characteristics[a]

	Exploratory cohort	Validation cohort
Characteristic		
Number of patients	261	239
Median age, years (range)	61 (18–82)	63 (24–83)
Gender, N (%)		
Male	135 (52)	129 (54)
Female	126 (48)	110 (46)
Ethnicity, N (%)		
Caucasian	250 (96)	226 (95)
Other	10(4)	8(4)
Unknown	1 (0)	5(2)
Primary tumor site, N (%)		
Esophagus	121 (46)	152 (64)
Ovary	39(15)	36(15)
Cervix	18(7)	6(3)
Endometrial	15(6)	6(3)
Breast	13(5)	26(11)
Lung	12(5)	2(1)
Head/Neck	10(4)	1 (0)
A(CUP)	9(3)	4(2)
Other	24(9)	6(3)

[a]Continuous data are given as median with range in parentheses, and categorical data are given as number of patients with percentage of the total population in parentheses.

Abbreviations: N, number; A(CUP), (adenoma)carcinoma of unknown origin.

Table 2

Associations between pharmacokinetic parameters and neurotoxicity

	Neurotoxicity CTCAE				P-value[b]
PK parameters[a]	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	
Weekly schedule (N)	109	29	3	-	
AUC (ngxh/ml)	2.3 (1.3–11.7)	5.0 (1.6–11.3)	6.1 (5.6–6.2)	-	0.001
$T_{>0.05}$ (h)	7.6 (3.5–29.3)	12.9 (4.0–30.4)	15.2 (13.7–16.3)	-	0.007
C_{max} (ng/mL)	1,394 (174–7,860)	2,896 (850–4,656)	4,370 (3,500–4,431)	-	<0.0001
3-Weekly schedule (N)	46	57	15	2	
AUC (ngxh/ml)	11.6 (2.9–26.1)	13.9 (3.5–25.4)	14.1 (8.8–24.8)	16.5 (15.9–17.0)	0.003
$T_{>0.05}$ (h)	21.4 (8.3–31.8)	24.6 (12.7–32.8)	23.8 (18.2–32.1)	29.0 (25.3–32.7)	0.005
C_{max} (ng/mL)	3,211 (495–8,420)	4,346 (496–8,313)	3,991 (1,922–8,713)	5,033 (4,180–5,886)	0.001
Female (N)	55	57	12	2	
AUC (ngxh/ml)	4.5 (1.3–21.0)	13.3 (3.3–24.7)	13.5 (6.1–20.5)	16.5 (15.9–17.0)	<0.00001
CL (L/h)	466 (157–696)	394 (228–654)	402 (191–636)	193 (186–200)	0.003
$T_{>0.05}$ (h)	14.8 (3.7–29.3)	22.9 (9.2–32.2)	23.4 (15.2–28.9)	29.0 (25.3–32.7)	<0.00001
C_{max} (ng/mL)	1,602 (673–7,107)	3,989 (886–7,726)	4,401 (2,706–6,143)	5,033 (4,180–5,886)	<0.00001
Male (N)	100	29	6	-	
AUC (ngxh/ml)	2.5 (1.4–26.1)	6.2 (1.6–25.4)	13.7 (5.6–24.8)	-	0.0001
CL (L/h)	539 (142–1,037)	541 (273–886)	494 (278–550)	-	0.3
$T_{>0.05}$ (h)	8.3 (3.5–31.8)	15.4 (4.0–32.8)	22.9 (13.7–32.1)	-	0.001
C_{max} (ng/mL)	1,409 (174–8,420)	3,916 (496–8,313)	3,694 (1,922–8,713)		0.0001

[a] Data are represented as median with ranges

[b] P-values <0.05 represents differentially distributed pharmacokinetic values between grades of neurotoxicity and are calculated with the Kruskal-Wallis test

Abbreviations: CTCAE, National Cancer Institute's Common Terminology Criteria for Adverse Events version 2–4; PK, pharmacokinetic; N, number; AUC, area under the curve; CL, clearance; $T_{>0.05}$, time above 0.05 μmol/L; C_{max}, maximum concentration.

Table 3

Association between CYP3A4*22 and neurotoxicity[a]

	No. of patients	Neurotoxicity CTCAE grade 0	Neurotoxicity CTCAE grade 1	Neurotoxicity CTCAE grade 2	Neurotoxicity CTCAE grade 3	P-value[b]
Exploratory Cohort	254					
Female	122					
C/C	105	50 (48)	46 (44)	8(8)	1(1)	
C/T+T/T	17	4(24)	8 (47)	4(24)	1(6)	0.043
Male						
C/C	114	85 (75)	24(21)	5(4)	-	
C/T + T/T	18	13 (72)	4(22)	1(6)	-	0.90
Validation Cohort	237					
Female	110					
C/C	98	30(31)	53 (54)	13(13)	2(2)	
C/T+T/T	12	6 (50)	4(33)	-	2(17)	0.036
Male	127					
C/C	113	80 (71)	28(25)	5(4)	-	
C/T + T/T	14	8 (57)	4(29)	-	2(14)	0.025

P-values are calculated with the chi-square test

[a] All data are represented as absolute number with percentage in parentheses, unless stated otherwise

[b] P-values < 0.05 represents differentially distributed neurotoxicity scores between non-carriers and carriers of the variant allele and are calculated with the Fisher exact test.

Abbreviations: CTCAE, National Cancer Institute's Common Terminology Criteria for Adverse Events version 2–4

Table 4

Associations between polymorphisms and neurotoxicity[a]

Gene and Variant	No. of patients	Neurotoxicity CTCAE				*P*-value[b]
		Grade 0	Grade 1	Grade 2	Grade 3	
*CYP2C8*3*	254					
Female	122					
*1/*1	92	41	40	9	2	
*1/*3 + *3/*3	30	13	14	3	-	1.0
Male	132					
*1/*1	105	79	21	5	-	
*1/*3 + *3/*3	27	19	7	1	-	0.84
*CYP2C8*4*	250					
Female	119					
*1/*1	108	47	48	11	2	0.65
*1/*4	11	7	4	-	-	
Male	131					
*1/*1	119	88	25	6	-	
*1/*4	12	10	2	-	-	1.0
ABCB1 3435 C>T	255					
Female	122					
C/C	30	16	13	1	-	
C/T	57	23	27	6	1	
T/T	35	15	14	5	1	0.69
Male	133					
C/C	36	26	8	2	-	
C/T	63	43	17	3	-	
T/T	34	30	3	1	-	0.24

[a] All data are represented as absolute number with percentage in parentheses, unless stated otherwise

[b] P-values < 0.05 represents differentially distributed neurotoxicity scores between non-carriers and carriers of the variant allele and are calculated with the Fisher exact test.

Abbreviations: CTCAE, National Cancer Institute's Common Terminology Criteria for Adverse Events version 2–4

Table 5

Patients with grade 3 neurotoxicity

Patient ID	Cohort	Gender	Age	Tumor type	CYP3A4*22	Cumulative Dose
1	Training	Female	54	Lung	CT	960
2	Training	Female	65	Ovarium	CC	2880
3	Validation	Male	64	Esophagus	CT	1060
4	Validation	Male	70	Esophagus	CT	1940
5	Validation	Female	25	Breast	CC	710
6	Validation	Female	46	Breast	CC	2635
7	Validation	Female	62	Breast	CT	1305
8	Validation	Female	71	Esophagus	CT	1760