Research Article

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Pharmacogenomics

Patients carrying *CYP2C8*3* have shorter systemic paclitaxel exposure

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Aim: First, evaluate if patients carrying putatively diminished activity *CYP2C8* genotype have longer paclitaxel exposure (e.g., time above threshold concentration of 0.05 μM [$T_{c>0.05}$]). Second, screen additional pharmacogenes for associations with $T_{c>0.05}$. **Methods:** Pharmacogene panel genotypes were translated into genetic phenotypes for associations with $T_{c>0.05}$ (n = 58). **Results:** Patients with predicted low-activity CYP2C8 had shorter $T_{c>0.05}$ after adjustment for age, body surface area and race (9.65 vs 11.03 hrs, β = 5.47, p = 0.02). This association was attributed to *CYP2C8*3* (p = 0.006), not *CYP2C8*4* (p = 0.58). Patients with predicted low-activity SLCO1B1 had longer $T_{c>0.05}$ (12.12 vs 10.15 hrs, β = 0.85, p = 0.012). **Conclusion:** Contrary to previous publications, *CYP2C8*3* may confer increased paclitaxel metabolic activity. *SLCO1B1* and *CYP2C8* genotype may explain some paclitaxel pharmacokinetic variability.

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Paclitaxel is used in the treatment of several solid tumors and has two major dose limiting toxicities; neutropenia and peripheral neuropathy (PN)[1]. Paclitaxel systemic drug concentrations, or exposure, has been associated with these toxicities [2,3] and to efficacy [4]. The exposure parameter most commonly associated with treatment outcomes is 'time above threshold' which is the number of h a patient's systemic plasma concentration remains above 0.05 μM ($T_{c>0.05}$), which has been shown to be predictive of PN. We and other groups have found that another pharmacokinetic (PK) parameter, maximum concentration (C_{max}), was similarly predictive of PN [3,5] and is more conveniently collected, which is a critical advantage for clinical translation.

There is substantial interpatient variability in paclitaxel exposure when receiving standard body surface area (BSA) based dosing, and accurate prediction of exposure could enable dose individualization to optimize efficacy and reduce toxicity [6]. Paclitaxel exposure is likely affected by activity of drug metabolizing enzymes and drug transporters, for which phenotypic activity (i.e., poor [PM], intermediate [IM], normal [NM] or ultra-rapid [UM] metabolizer) can be predicted based on the presence of functional polymorphisms using the standard approach from the Clinical Pharmacogenetics Implementation Consortium [7]. Paclitaxel is primarily metabolized by CYP2C8 through the conversion to 6-hydroxypaclitaxel [8]. The *CYP2C8* genetic polymorphisms, *CYP2C8*3* and *CYP2C8*4*, have been associated with modest decreases in paclitaxel clearance in clinical studies [9,10] and in *in vitro* studies [8,11–16]. *CYP2C8*3* has also been associated with increased paclitaxel-induced PN [17,18]. Based on the available literature, it is expected that *CYP2C8*3* confers decreased activity for paclitaxel metabolism, though this polymorphism confers increased activity for other substrates such as the thiazolidinediones [19,20]. Polymorphisms in the P-gp efflux transporter (*ABCB1*) have also been associated with paclitaxel PK [9,10].

Further research is needed to elucidate the impact of genetic polymorphisms on paclitaxel PK to determine whether pharmacogenetics can be used to inform individualized dosing to optimize therapeutic outcomes. We

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conducted a prospective clinical trial to discover PK, genetic, and other predictive biomarkers of paclitaxel-induced PN in patients with early stage breast cancer. The overall objective of this secondary pharmacogenetic analysis is to identify pharmacogenetic and clinical factors associated with paclitaxel exposure. The primary objective was to determine whether patients with genotype-predicted low-activity CYP2C8 phenotype (i.e., carriers of *CYP2C8*3* or *CYP2C8*4*) have greater paclitaxel exposure, as estimated by a longer $T_{c>0.05}$. A secondary objective of the study was to screen for associations between genotype-predicted phenotypic activity of other genes relevant to paclitaxel metabolism or transport with paclitaxel exposure estimated by $T_{c>0.05}$ or C_{max}.

Methods

Patient cohort

Patients with breast cancer receiving weekly paclitaxel 80 mg/m^2 1-h infusions were enrolled in an observational clinical registry (UMCCC 2014.002, NCT02338115) assessing paclitaxel exposure during the first dose and paclitaxel-induced PN. Detailed information about these patients, treatment, sampling time points and the primary analyses have been previously reported [5]. Briefly, eligible women were >18 years old, had a diagnosis of invasive breast cancer, and were scheduled to receive Cremophor EL-based paclitaxel 80 mg/m^2 1-h infusions weekly for 12 weeks with a curative intent. The study was approved by the University of Michigan IRBMed and conducted in accordance with recognized ethical guidelines including the Declaration of Helsinki and Belmont report. All enrolled subjects signed written informed consent.

Sample & data collection

Patient demographic and treatment information was collected at baseline including age, race, ethnicity and limited medical history. Blood samples were collected at the first paclitaxel infusion for a complete blood count, comprehensive metabolic profile, and extraction of genomic DNA. Additional blood samples were collected within 10 min prior to the end of the first paclitaxel infusion and 16–26 h after infusion for PK analyses. The end of infusion sample was collected from the peripheral vein contralateral to the infusion and the 16–26 h level was collected from a peripheral vein or a properly flushed port. Samples were collected in Na-Heparin tubes, immediately placed on ice, and centrifuged within 10 min of collection. Plasma was transferred to a secondary cryotube and stored at -20°C until analysis.

Pharmacokinetic methodology

Total paclitaxel concentration was measured in plasma samples by liquid chromatography-mass spectroscopy by the University of Michigan College of Pharmacy Pharmacokinetics Core. $T_{c>0.05}$ was calculated using the MyCare™ Dose Exposure Calculator (Saladax Biomedical, Inc, PA, USA). MyCare Dose Exposure Calculator is a PC-based software that uses Phoenix WinNonlin modeling software (Certara USA, Inc., NJ, USA) to determine exposure to therapeutic agents based on a previously published population-PK model [21]. The following parameters were used to calculate $T_{c>0.05}$: absolute dose of paclitaxel (in mg), infusion start and end time, time of sample collection and sample concentration. The concentration determined from the plasma sample collected at the end of infusion was used as C_{max}.

Genotyping & activity phenotype prediction

DNA samples were genotyped for 266 variants in 36 genes relevant to drug metabolism and transport on the iPLEX ADME PGx ProPanel by Agena Bioscience (CA, USA). All genetic information was subjected to appropriate quality control including assessment of sample call rate. Similar to our previous publication [22], each allele was translated into a predicted activity (low, normal and high) based on published data (Supplementary Table 1). Then, each patient's diplotype was translated into a predicted activity phenotype: PM, IM, NM or UM, for analysis (Supplementary Table 2). Hardy Weinberg equilibrium was confirmed for all variants included in significant findings from the primary and secondary analyses using a χ^2 test with simulated p-value based on 10,000 replicates, which was performed using R.

Statistical analysis

All patients with available genetic and exposure ($T_{c>0.05}$ and C_{max}) data were included in the analysis. For the primary analysis, genotype-predicted CYP2C8 activity was tested in a dominant model (PM/IM vs NM) for an association with $T_{c>0.05}$. The secondary analysis was conducted using an additive model for associations between

Table 1. Patient demographics.

Clinical variables	Level	n = 59; mean (SD)/n (%)
Age	Years	51.41 (12.55)
BSA	m^2	1.82 (0.21)
Race	Caucasian	55 (93.2%)
	Other	4 (6.8%)
Alkaline phosphatase	Low/normal	51 (86.4%)
	Elevated	8 (13.6%)
ALT or AST	Normal	53 (89.8%)
	Elevated	6 (10.2%)
Albumin	Low	1 (1.7%)
	Normal	58 (98.3%)
Prior or concurrent chemotherapy	Yes	55 (93.2%)
	No	4 (6.8%)
Prior or concurrent HER2 therapy	Yes	28 (47.5%)
	No	31 (52.5%)

BSA: Body surface area; SD: Standard deviation.

$T_{c>0.05}$ and C_{max} with the following genes with known relevance to paclitaxel metabolism or transport: *CYP3A4, CYP3A5, ABCB1, ABCC2, ABCG2, SLCO1B1* and *SLCO1B3*. Finally, all remaining genes analyzed by the iPLEX ADME chip with no known relevance to paclitaxel metabolism or transport: *COMT, CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2C9, CYP2D6, CYP2E1, DPYD, GSTM1, GSTP1, GSTT1, GSTT2b, NAT1, NAT2, SLC15A2, SLC22A1, SLC22A2, SLC22A6, SLCO2B1, SULT1A1, TPMT, UGT1A1, UGT2B15, UGT2B17, UGT2B7, VKORC1,* were included in a hypothesis generating screen for associations with $T_{c>0.05}$ or C_{max}. For the secondary analysis and the hypothesis generating screen, if the number of patients in an extreme genetic phenotype (PM or UM) was less than three, that phenotype was collapsed into the next phenotype level (i.e., PM/IM or NM/UM). Additional post-hoc analyses were conducted to investigate associations for individual *CYP2C8* genotypes (*CYP2C8*3* or *CYP2C8*4*) with $T_{c>0.05}$. For the analysis of each variant, patients who were heterozygous (i.e., *CYP2C8*1/*3*) or homozygous (i.e., *CYP2C8*3/*3*) for that variant were compared only to patients homozygous for the wild-type genotype (i.e., *CYP2C8*1/*1*) by excluding all carriers of the alternative variant. Clinical variables used in the analysis included age and BSA as continuous variables, race (Caucasian or other), alkaline phosphatase (elevated >116 IU/l), aspartate aminotransferase or alanine aminotransferase (elevated >40 IU/l), albumin (low ≤3.0 g/dl), prior or concurrent pertuzumab/trastuzumab therapy and prior or concurrent chemotherapy as categorical variables. The associations between genetic phenotype or clinical variables and the C_{max} and logarithm of $T_{c>0.05}$ were analyzed using univariate linear regression with an unadjusted $\alpha = 0.05$ (two-sided). None of the clinical covariates was significant in the univariate association, therefore, genetic associations were adjusted for the putatively relevant covariates age, race and BSA. Statistical analysis was performed using SAS v9.4.

Results

Patient demographic, PK, & genetic data

After excluding one patient that received a 3-h paclitaxel infusion, 56 and 58 patients were included in the $T_{c>0.05}$ and C_{max} analyses, respectively (Figure 1). Patient demographic data and relevant baseline laboratory levels are included in Table 1. The mean age of the patients was 51.4 years (standard deviation [SD]: 12.55), mean BSA was 1.82 m^2 (SD 0.21), and 93.2% of patients self-reported as Caucasian (Table 1). The mean $T_{c>0.05}$ and C_{max} was 10.72 h (n = 58, SD 2.73) and 2390.18 ng/ml (n = 56, SD 640.85), respectively.

CYP2C8 diplotype was determined for all patients included in the analysis. For the 57 patients included in the primary analysis, the distribution of *CYP2C8* diplotypes and corresponding genetic phenotypes was: 40 *CYP2C8*1/*1* (NM), 11 *CYP2C8*1/*3* (IM), 3 *CYP2C8*1/*4* (IM), 1 *CYP2C8*3/*3* (PM), 1 *CYP2C8*3/*4* (PM) and 1 *CYP2C8*4/*4* (PM), resulting in a phenotype distribution of 3 PMs (5%), 14 IMs (25%) and 40 NMs (70%). No patients were carriers of *CYP2C8*2, *5, *7* or **8*. The *CYP2C8* and *SLCO1B1* variants were confirmed to be in Hardy–Weinberg equilibrium and the minor allele frequency was similar to that expected for a primarily Caucasian population.

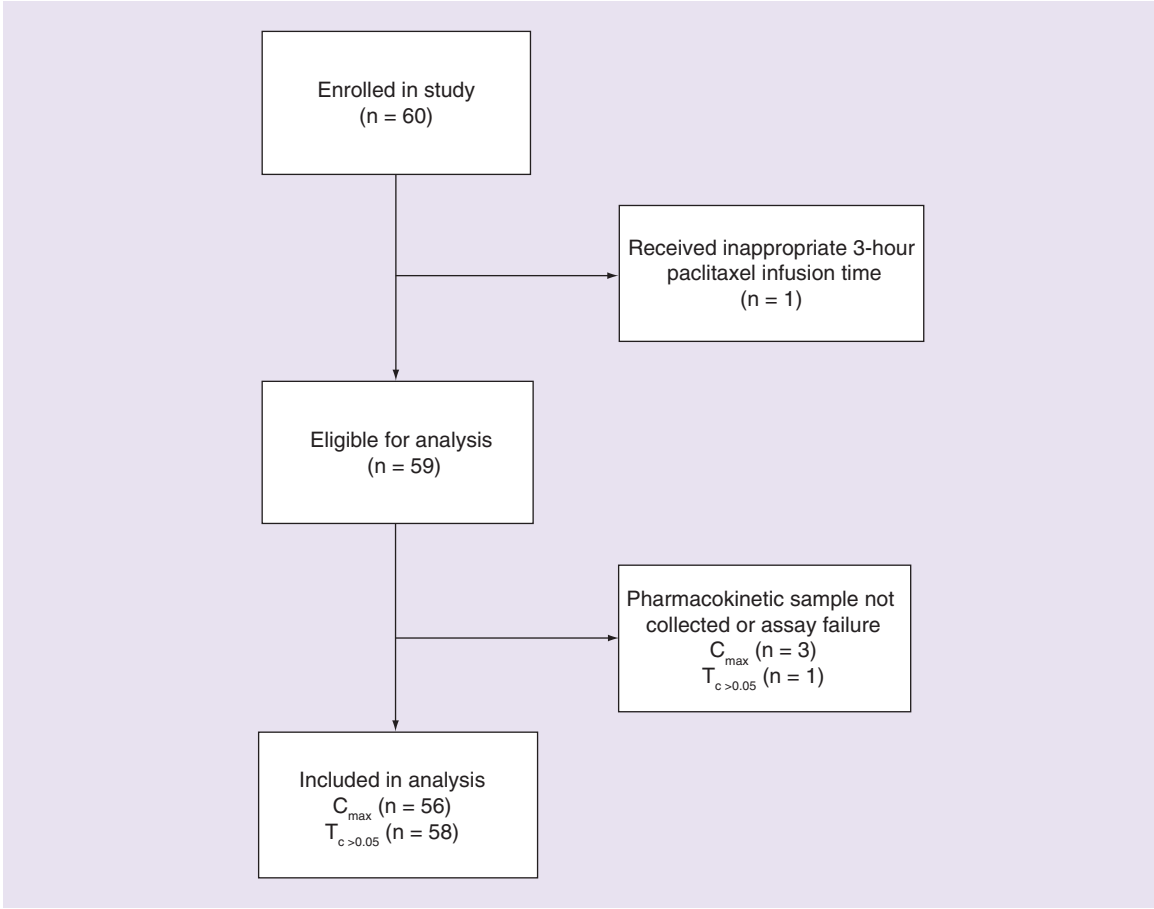

Figure 1. CONSORT Diagram of patient inclusion in the secondary pharmacogenomic analysis. This diagram shows the reasons for exclusion from the parent clinical registry into the secondary pharmacogenomics analysis.

Associations of clinical & genetic variables with $T_{c>0.05}$

In the primary analysis, patients with low-activity CYP2C8 genetic phenotype (PM or IM) had shorter $T_{c>0.05}$ (PM/IM mean = 9.65 h, NM mean = 11.03 h, linear regression β-coefficient = 1.13, 95% CI: 1.01–1.28, p = 0.046, Table 2 & Figure 2 left). None of the clinical variables were associated with $T_{c>0.05}$; however, associations for increasing BSA (β = 1.27, 95% CI: 0.96–1.68, p = 0.10) and Caucasian race (β = 1.22, 95% CI: 0.97–1.53, p = 0.09, Table 3) with longer $T_{c>0.05}$ approached significance. The association with CYP2C8 maintained significance after adjustment for age, BSA and race (β = 5.47, 95% CI: 2.99–9.99, p = 0.020).

In a *post-hoc* analysis to understand the contribution of individual genotypes, the association for low-activity CYP2C8 genetic phenotype was attributable to the *CYP2C8*3* polymorphism (*CYP2C8*1/*3* or *CYP2C8*3/*3* mean = 8.92 h, *CYP2C8*1/*1* mean = 11.03 h, p = 0.0006). Although the numbers for analysis was quite small, there was no association between *CYP2C8*4* and $T_{c>0.05}$ (*CYP2C8*1/*4* or *CYP2C8*4/*4* mean = 10.25 h, **1/*1* mean = 11.03 h, p = 0.58).

In the secondary analysis of genes relevant to paclitaxel metabolism or transport, patients with lower low-activity OATP1B1 genetic phenotype had longer $T_{c>0.05}$ (PM/IM = 12.12 h, NM = 10.15 h, β = 1.17, 95% CI: 1.03–1.33, p = 0.014, Table 2 & Figure 2 right) and this association maintained significance after adjustment for relevant clinical covariates (β = 0.85, 95% CI: 0.75–0.96, p = 0.012). A model containing both *CYP2C8* and *SLCO1B1* maintained independent effects for *SLCO1B1* (*SLCO1B1*: p = 0.01, *CYP2C8*: p = 0.09). In the hypothesis generating screen of genes with no known relevance to paclitaxel PK, patients with lower UGT2B17 activity had shorter $T_{c>0.05}$ (β = 1.13, 95% CI: 1.04–1.22, p = 0.003) and patients with lower CYP2C9 activity had shorter $T_{c>0.05}$ (β = 1.14, 95% CI: 1.03–1.26, p = 0.014, Supplementary Table 3). None of the other genes were associated with $T_{c>0.05}$.

Table 2. Paclitaxel $T_{c>0.05}$ and C_{max} by phenotype predicted metabolizer status.

Gene	Poor	Intermediate	Normal	Ultra-rapid	Univariate association
Primary analysis	**Mean $T_{c>0.05}$ (h) (SD) stratified by phenotype**				**β (95% CI), p-value**
CYP2C8	9.65 (2.06) n = 17		11.03 (2.74) n = 40	†	β = 1.13 (1.01–1.28) p = 0.046*
Secondary analysis	**Mean $T_{c>0.05}$ (h) (SD) stratified by phenotype or genotype**				**β (95% CI), p-value**
*CYP2C8 *1/*3* and **3/*3*	8.92 (1.00) n = 12		11.03 (2.74) n = 40	†	β = 0.82 (0.72–0.94) p = 0.006*
*CYP2C8 *1/*4* and **4/*4*	10.25 (1.71) n = 4		11.03 (2.74) n = 40	†	β = 0.94 (0.76–1.17) p = 0.58
CYP3A4	12.00 (2.83) n = 8		10.52 (2.68) n = 50	†	β = 1.14 (0.97–1.35) p = 0.12
CYP3A5	10.64 (2.72) n = 48	10.83 (2.04) n = 6	– n = 0	†	β = 1.03 (0.85–1.24) p = 0.76
ABCB1	10.91 (3.48) n = 22	10.55 (2.33) n = 20	10.29 (1.33) n = 14	†	β = 0.99 (0.92–1.06) p = 0.73
ABCC2	10.67 (1.53) n = 3	10.83 (3.05) n = 30	10.67 (2.28) n = 18	9.33 (1.37) n = 6	β = 0.96 (0.89–1.04) p = 0.33
ABCG2	– n = 0	9.67 (2.50) n = 9	10.92 (2.74) n = 49	†	β = 0.88 (0.75–1.04) p = 0.14
SLCO1B1	12.12 (3.60) n = 17		10.15 (2.06) n = 41	†	β = 1.17 (1.03–1.33) p = 0.014*
SLCO1B3	10.88 (2.92) n = 40	10.79 (2.39) n = 14	8.67 (1.15) n = 3	†	β = 0.95 (0.86–1.05) p = 0.32
Secondary analysis	**Mean C_{max} (ng/ml) (SD) stratified by phenotype**				**β (95% CI), p-value**
CYP2C8	2187.33 (656.90) n = 15		2455.75 (632.08) n = 40	†	β = 268.42 (-119.46–656.30) p = 0.17
*CYP2C8 *1/*3* and **3/*3*	1985.00 (594.76) n = 12		2455.75 (632.08) n = 40	†	β = -320.75 (-767.56–126.06) p = 0.16
*CYP2C8 *1/*4* and **4/*4*	2550.00 (693.83) n = 4		2455.75 (632.08) n = 40	†	β = 94.25 (-568.47–756.97) p = 0.78
CYP3A4	2250.00 (458.11) n = 7		2410.20 (664.14) n = 49	†	β = -160.20 (-682.31–361.91) p = 0.54
CYP3A5	2413.26 (679.75) n = 46	2226.67 (500.63) n = 6	– n = 0	†	β = -186.59 (-765.50–392.31) p = 0.52
ABCB1	2219.05 (636.73) n = 21	2405.79 (782.05) n = 19	2585.00 (403.94) n = 14	†	β = 183.25 (-36.39–402.88) p = 0.10
ABCC2	2316.67 (560.03) n = 3	2304.67 (672.21) n = 30	2595.00 (683.64) n = 16	2238.33 (353.52) n = 6	β = 58.99 (-172.31–290.30) p = 0.61
ABCG2	– n = 0	2233.75 (517.19) n = 8	2416.25 (660.23) n = 48	†	β = -182.50 (-675.17–310.17) p = 0.46
SLCO1B1	2407.06 (725.44) n = 17		2382.82 (610.46) n = 39	†	β = 24.24 (-352.55–401.03) p = 0.90
SLCO1B3	2390.00 (699.39) n = 38	2432.86 (565.22) n = 14	2143.33 (230.29) n = 3	†	β = -44.27 (-346.45–257.91) p = 0.77

*Statisticaly significant.
†Denotes phenotype groups that did not exist for that gene (See Supplementary Table 2).
SD: Standard deviation.

Associations of clinical & genetic variables with C_{max}

Increasing age was associated with higher C_{max} (β = 15.15, 95% CI: 1.88–28.42, p = 0.026, Table 3). None of the genes from the candidate list or hypothesis generating screen, including *CYP2C8*, were associated with C_{max} (Supplementary Table 3), although the association for patients with lower activity *SLC15A2* genetic phenotype and higher C_{max} approached significance (β = -237.36, 95% CI: -473.72 to -1.00, p = 0.054).

Discussion

Patients carrying putatively diminished-activity *CYP2C8* variants, including *CY2C8*3*, have been reported to have slower paclitaxel clearance [9,10] and greater risk of paclitaxel-induced PN [10,17,18,23]. In this cohort we previously reported that patients with longer $T_{c>0.05}$ had greater risk of PN [5]. Based on these findings, we hypothesized

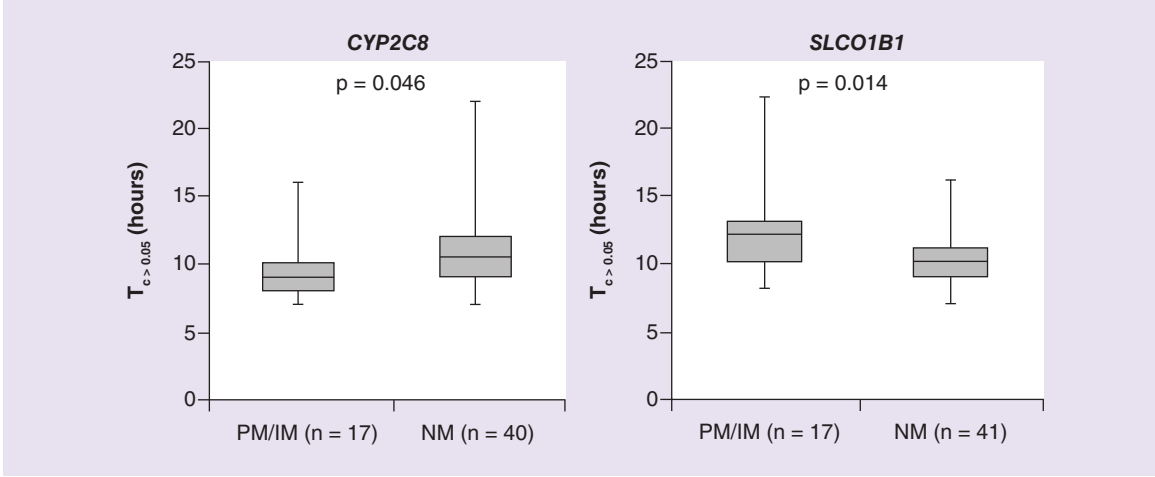

Figure 2. Paclitaxel $T_{c>0.05}$ stratified by genotype-predicted metabolic activity phenotype for *CYP2C8* and *SLCO1B1*.
Paclitaxel $T_{c>0.05}$ stratified by metabolic activity phenotype (PM/IM and NM) for *CYP2C8* (left) and *SLCO1B1* (right). Lower activity CYP2C8 genetic phenotype (PM/IM) was associated with shorter $T_{c>0.05}$ and lower OATP1B1 (*SLCO1B1*) activity was associated with longer $T_{c>0.05}$ in the univariate analyses.
IM: Intermediate metabolizer; NM: Normal metabolizer; PM: Poor metabolizer.

Table 3. Univariate association of clinical variables with paclitaxel $T_{c>0.05}$ and C_{max}.

Clinical variables	Associations with $T_{c>0.05}$		Associations with C_{max}	
	B coefficient (95% CI)	p-value	B coefficient (95% CI)	p-value
Age (years)	1.00 (0.99–1.00)	0.90	15.15 (1.88–28.42)	**0.026**
BSA (m^2)	1.27 (0.96–1.68)	0.10	-262.77 (-1085.39–559.85)	0.52
Caucasian race (vs other)	1.22 (0.97–1.53)	0.09	-387.50 (-1051.95–276.95)	0.25
Elevated alkaline phosphatase (vs normal)	0.90 (0.76–1.06)	0.22	-83.33 (-577.99–411.32))	0.74
Elevated ALT or AST (vs normal)	1.09 (0.90–1.32)	0.38	261.13 (-294.55–816.81)	0.35
Elevated albumin (vs normal)	0.86 (0.55–1.35)	0.52	356.18 (-948.61–1660.98)	0.59
Prior or concurrent chemotherapy (vs none)	1.03 (0.82–1.30)	0.79	-503.46 (-1260.63–253.71)	0.19
Prior or concurrent HER2 therapy (vs none)	1.02 (0.91–1.15)	0.75	136.26 (-208.52–481.03)	0.43

BSA: Body surface area; SD: Standard deviation.

that patients with low-activity CYP2C8 genetic phenotype would have longer $T_{c>0.05}$. Contrary to our primary hypothesis, low-activity CYP2C8 genetic phenotype was associated with significantly shorter $T_{c>0.05}$.

Two human PK studies have reported decreased paclitaxel clearance in patients carrying *CYP2C8 *3* [9,10], one of which only detected the association in patients that were also *ABCB1* 2677G/T heterozygotes [10]. Other studies have found no association between *CYP2C8* genotype or metabolizer phenotype and paclitaxel PK [24,25]. The two alleles classified as low-activity in our cohort were *CYP2C8*3* (rs10509681 and rs11572080) and *CY2C8*4* (rs1058930), with the majority of PM and IM patients carrying the *CYP2C8*3* alleles. *CYP2C8*3* genotype causes two non-synonymous changes (R139K and K399R) and has previously been characterized as diminished paclitaxel metabolic activity in multiple *in vitro* studies [8,11–14]. It is believed that the effect of *CYP2C8*3* may be substrate dependent, as it is well established that *CYP2C8*3* has higher metabolic activity toward rosiglitazone [19,20] and pioglitazone [26]. Contrary to our hypothesis that *CYP2C8*3* confers decreased activity based on the *in vitro* and limited previous *in vivo* studies, our data suggest that *CYP2C8*3* may in fact confer increased activity for paclitaxel metabolism, similar to other substrates. This further complicates interpretation of previous work from our group and others showing that *CYP2C8*3* is associated with greater risk of paclitaxel-induced PN [10,17,18,23], however, these results have not been validated and may be false-positive findings.

*In vitro CYP2C8*4* has been shown to confer variable levels of decreased paclitaxel metabolic activity [13,15,16]. One previous clinical PK study reported decreased paclitaxel clearance [9], whereas others have found no association, possibly due to the small number of *CYP2C8*4* carriers [10,24,25]. Our study included only four carriers of

*CYP2C8*4*, so the marginally shorter exposure was not significantly different from wild-type patients. Considering the *CYP2C8*3* and *CYP2C8*4* results together, it seems that the effect of CYP2C8 polymorphisms on human paclitaxel exposure is modest, at best.

In our analysis, patients with lower OATP1B1 activity, which is encoded by *SLCO1B1*, had longer $T_{c>0.05}$. Low-activity *SLCO1B1* variants indirectly affect hepatic metabolism of various drugs by decreasing OATP1B1 expression at the cell membrane [27] and, consequently, diminishing hepatic uptake of drugs for metabolism [28–30]. Unlike our findings for paclitaxel, longer exposure in patients with low-activity OATP1B1 was as predicted. *In vitro* studies have suggested that *SLCO1B1* likely has modest effects on paclitaxel transport [31,32], but *SLCO1B1* has not been previously examined in humans for its impact on paclitaxel PK. In the hypothesis generating screen, lower CYP2C9 activity was associated with shorter $T_{c>0.05}$, but this association is likely due to linkage disequilibrium between the lower activity *CYP2C9*2* and *CYP2C8*3* alleles [33]. The association between lower UGT2B17 activity and shorter $T_{c>0.05}$ has not been previously observed, and is likely an artifact of the number of statistically uncorrected association tests run.

Our results indicate a modest effect of *CYP2C8* and *SLCO1B1* genotypes on paclitaxel PK in humans. In a prospective clinical trial in patients with non-small-cell lung cancer, randomization to utilizing $T_{c>0.05}$-guided paclitaxel dosing reduced PN without compromising efficacy [34]. Initial dose recommendations in the PK-guided arm were based on BSA, age and sex; incorporating pharmacogenetics into these personalized dosing algorithms could improve their precision and enable further optimization of therapeutic outcomes.

Age has been negatively correlated with clearance in patients receiving a 1-h paclitaxel infusion [35], but not C_{max}. However, in this study we identified a relatively large effect on C_{max}, which was unexpected since age related changes in paclitaxel clearance would not be expected to affect C_{max} following the short 1-h infusions used in this cohort. Further research is needed to determine whether there is some other age-related change that explains the relatively large effect on C_{max} detected in this study. The trend for Caucasian patients to have longer $T_{c>0.05}$ was likely due to the higher allele frequency of *CYP2C8*3* in Caucasian populations compared with Asian and African–American populations, where it is typically rare or absent [36]. Consistent with the trend seen in this cohort, BSA has previously been positively correlated with paclitaxel elimination in a population-PK analysis [37].

The primary limitation of this analysis is its modest size, which may have resulted in reduced statistical power for many of these analyses of genetic and clinical associations. Due to sparse sampling, this analysis used $T_{c>0.05}$, a clinically relevant exposure parameter, rather than the more conventional PK parameters clearance or area under the curve (AUC) [9,10,24,25]. However, $T_{c>0.05}$, is predictive of PN [2,3]. Consequently, determining pharmacogenomic and clinical factors that impact $T_{c>0.05}$ has great potential to positively impact patient outcomes. $T_{c>0.05}$ was chosen as the primary end point due to the expectation that metabolism and active transport would have minimal effect on C_{max} during a 1-h infusion, which is supported by the lack of any pharmacogenetic association in this analysis. Additionally, concomitant medications that induce or inhibit CYP2C8 or other enzymes or transporters could have impacted PK, but information on concomitant medication is not available for this cohort and could not be accounted for in this analysis.

A panel genotyping approach was selected to comprehensively investigate functionally consequential SNPs in genes relevant to drug metabolism and transport, and all genes with known or suspected functional consequence for paclitaxel metabolism or transport were included. However, enzyme activity was not measured directly, and our system for translating genotype to genetic phenotype is limited by the SNPs included on this panel and the current understanding of their functional consequence. Finally, the subjects included in this cohort were primarily Caucasian, which limits the diversity of polymorphisms and patient characteristics represented in the analysis.

Conclusion

In this secondary analysis of female patients with breast cancer receiving weekly 1-h paclitaxel infusions, patients with low-activity CYP2C8 genetic phenotype had shorter $T_{c>0.05}$ and patients with low-activity OATP1B1 genetic phenotype had longer $T_{c>0.05}$. Our findings suggest that *CYP2C8*3* confers increased paclitaxel metabolism, which is contrary to prior studies but is consistent with its established functional impact on for other CYP2C8 substrates. If definitive associations between clinical and pharmacogenetic factors and paclitaxel PK are elucidated, they could potentially be incorporated into personalized dosing strategies to achieve optimal exposure levels that avoid toxicities and/or maximize treatment efficacy.

Future perspective

Clinical, genetic and PK information will be ideally integrated in the future to personalize paclitaxel dosing regimens to reduce the occurrence of dose-limiting toxicities while ensuring drug efficacy. To achieve that goal, discovery of genetic predictors of paclitaxel PK and toxicity are pivotal. Future research is needed to fully elucidate the impact of drug transporters and metabolizing enzymes on paclitaxel exposure. These efforts should be integrated with on-going efforts to determine predictors of toxicity to most accurately create a paclitaxel dosing model.

Summary points

- Based on prior findings, low CYP2C8 activity is expected to lead to longer systemic paclitaxel exposure or time above threshold, $T_{c>0.05}$.
- A comprehensive CYP2C8 genotyping approach was taken including *CYP2C8 *2*, **3*, **4*, **5*, **7* and **8*.
- Contrary to what was expected, patients with low-activity CYP2C8 genetic phenotype had shorter $T_{c>0.05}$.
- *CYP2C8*3* may confer increased activity to metabolize paclitaxel.
- Patients with low-activity CYP2C9 genetic phenotype had shorter $T_{c>0.05}$ likely due to linkage disequilibrium between the low-activity *CYP2C8*3* and *CYP2C9*2* variants.
- In a novel association, low-activity OATP1B1 genetic phenotype patients had longer $T_{c>0.05}$.
- OATP1B1 and CYP2C8 pharmacogenomics may explain some variability in paclitaxel exposure.
- Future studies that verify pharmacogenomic associations with paclitaxel exposure could lead to personalized dosing strategies to optimize efficacy and reduce toxicity.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at:

https://www.futuremedicine.com/doi/suppl/10.2217/pgs-2018-0162

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Ethical conduct of research

The study was approved by the University of Michigan IRBMed and conducted in accordance with recognized ethical guidelines. All enrolled subjects signed written informed consent.

Author contributions

DL Hertz, ML Burness, JJ Griggs, C Van Poznak, AF Schott, DF Hayes, NL Henry contributed to the conception and design of the work. DL Hertz, K Vangipuram, ML Burness, JJ Griggs, C Van Poznak, AF Schott, DF Hayes and NL Henry contributed to the acquisition of the work. DL Hertz, KM Kidwell, AC Robinson, K Vangipuram, LA Marcath contributed to the analysis or interpretation of the data. All authors have contributed to the drafting of the work or revising it critically for content. All authors have reviewed and approve of this manuscript for submission. All authors agree to be accountable for all aspects of the work.

Disclaimer

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