Tamoxifen pharmacogenetics and pharmacokinetics in early breast cancer

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6 CHAPTER

CYP2C19 genotypes and tamoxifen therapy: effect on metabolism and early-breast cancer relapse

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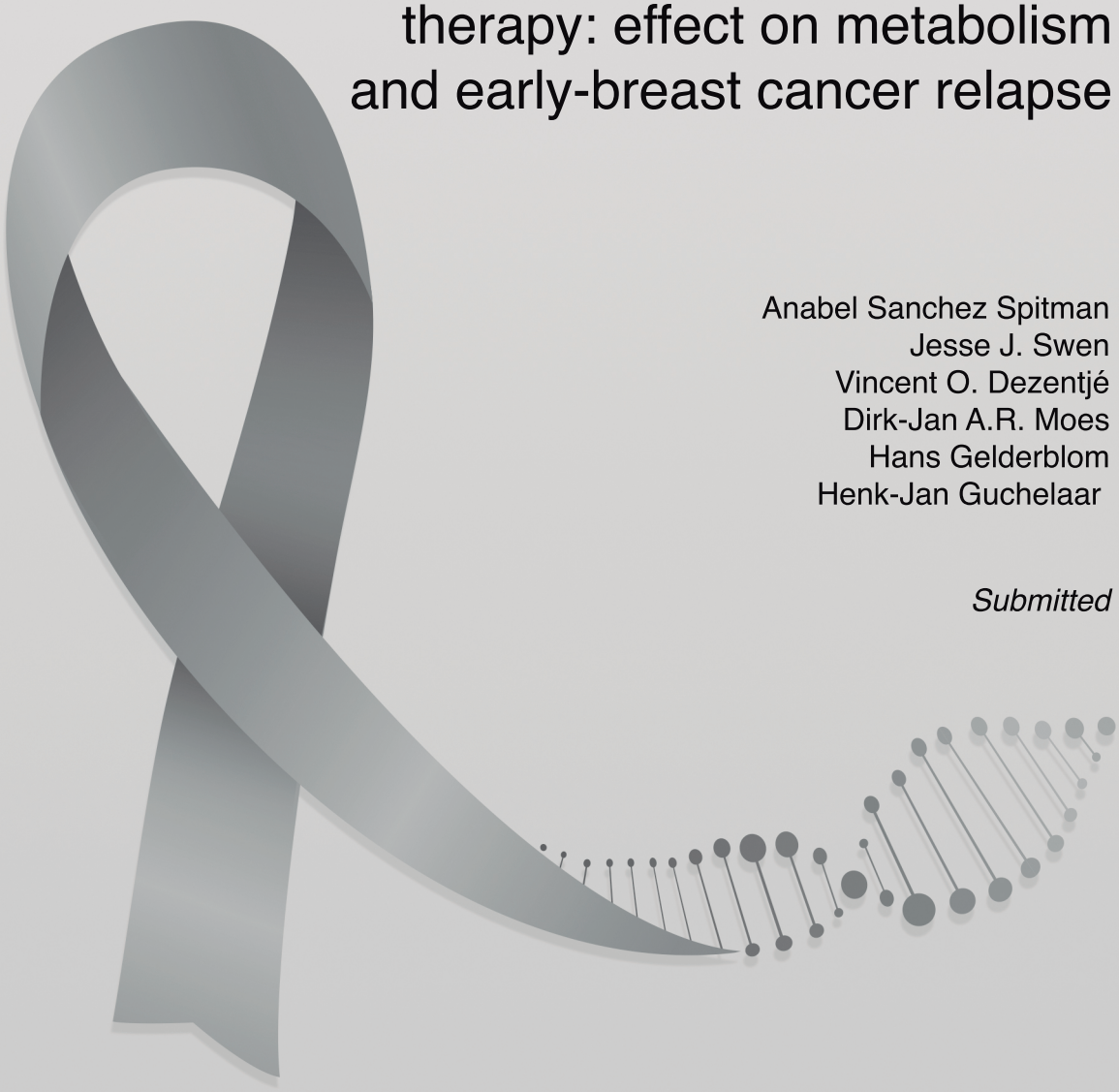
Submitted



Abstract

Background

*CYP2C19*2* and *CYP2C19*17* might influence tamoxifen variability. We aim to investigate the effect of *CYP2C19*2* and *CYP2C19*17* on tamoxifen concentrations and metabolic ratios (MRs) and breast cancer recurrence in a large cohort of Caucasian women.

Methods

Genetic variants, tamoxifen and metabolites concentrations, baseline characteristics, and breast cancer recurrence from the CYPTAM study (NTR1509) were used. *CYP2C19*2* and *CYP2C19*17* were evaluated as independent alleles and as groups based on *CYP2D6* genotypes (high, intermediate and low activity). Classification tree analyses (CTAs) were conducted to assess the levels of interactions per polymorphism (CYP2D6, *CYP2C19*2* and *CYP2C19*17*) on concentrations.

Outcomes

Only significant differences (p-value <0.05) in mean concentrations and MR were obtained when comparing tamoxifen activity groups (high, intermediate and low activity). In terms of recurrence (HR), *CYP2C19*2* failed to find an association (Hazard Ratio (HR) for heterozygous: 1.090 (95% Confidence Interval (CI): 0.595-1.994, p-value: 0.666; HR for homozygous: 0.759 (95% CI: 0.101-5.700, p-value: 0.789), whereas for *CYP2C19*17*, HR for hetero- and homozygous was 0.881 (CI: 0.478-1.625, p-value: 0.686) and 1.797 (CI:0.598-5.397, p-value:0.296), respectively. CTAs showed a significant relationship between *CYP2D6* and endoxifen (p-value<0.0001).

Conclusions

CYP2C19 polymorphisms do not have a significant impact on tamoxifen metabolism or breast cancer relapse.

Introduction

Worldwide, breast cancer is still the most frequent malignity in women[1,2], and accounted for 571000 deaths in 2015[1]. Since the majority of newly diagnosed breast cancer cases are estrogen-receptor positive, endocrine therapy with tamoxifen or aromatase inhibitors is recommended[3,4].For many years, tamoxifen has been prescribed as monotherapy or with subsequent switch to an aromatase inhibitor after two or three years of endocrine therapy[3,4]. In the adjuvant scenario, tamoxifen therapy decreases mortality and disease recurrences of breast cancer[5], whilst in the metastatic setting prolonged survival outcomes has been observed[6]. Unfortunately, there is a high variability in tamoxifen response, and about 30 % of patients using adjuvant tamoxifen still will have a disease relapse[5].

Tamoxifen is a competitive estrogen receptor antagonist and is metabolized into its primary metabolites, N-desmethyl-tamoxifen (NDM-tamoxifen) and 4-hydroxy-tamoxifen, followed by a second conversion into endoxifen (**Figure 6.1**)[7]. Both 4-hydroxy-tamoxifen and endoxifen have similar anti-estrogenic potencies[8], but endoxifen is reported as the active metabolite, as it is found in 5-10 times higher concentrations than 4-hydroxy-tamoxifen[9].

In tamoxifen metabolism, the limiting step in the transformation to endoxifen is regulated by CYP2D6 enzyme. Although many studies have associated genetic variants in *CYP2D6* gene with clinical outcome[10], many other researchers have reported null-association between survival outcome and decreased CYP2D6 enzyme activity [11]. Since *CYP2D6* only partly contributes to explaining the 42.3% variability of endoxifen concentrations[12] and 68.7 % of endoxifen formation (metabolic ratio (MR) of NDM-tamoxifen/endoxifen)[13], *CYP2D6* genotyping has not been implemented in the daily clinical practice in order to predict tamoxifen efficacy. However, other polymorphisms in other drug-metabolizing enzymes involved in tamoxifen metabolism might also have an impact in the endoxifen formation and potentially in clinical outcome.

CYP2C19 gene is highly polymorphic[14], and it plays multiple roles in the tamoxifen pathway (**Figure 6.1**). Several polymorphisms in the gene encoding the CYP2C19 enzyme have been described. While *CYP2C19*17* variant leads to an increased enzymatic activity, other variants e.g. *CYP2C19*2* and *CYP2C19*3*[14] genotypes have a decreased enzyme activity. Regarding the role of these *CYP2C19* genotypes and tamoxifen metabolism, several studies have been published. Lim and colleagues reported no association between tamoxifen and its metabolites concentration levels and *CYP2C19* genotypes. In line with these outcomes, Mürdter *et al.* failed to find an association regarding *CYP2C19* genotypes and endoxifen, 4-hydroxy-tamoxifen and NDM-tamoxifen concentrations or MRs. In contrast, Gjerde *et al.* observed a higher 4-hydroxy-tamoxifen formation in *CYP2C19*17* carriers[15]. Interestingly, Lim and colleagues reported in a recent study an association of *CYP2C19*2* with norendoxifen,

also named 4-hydroxy-N,N-didesmethyltamoxifen[16]. Norendoxifen is an active metabolite of tamoxifen, which is the result of the direct de-methylation of endoxifen. In contrast to endoxifen and tamoxifen, Lu *et al.* characterized this metabolite as dual aromatase inhibitor and selective estrogen-receptor modulator[17] which may lead to an interesting novel drug[18].

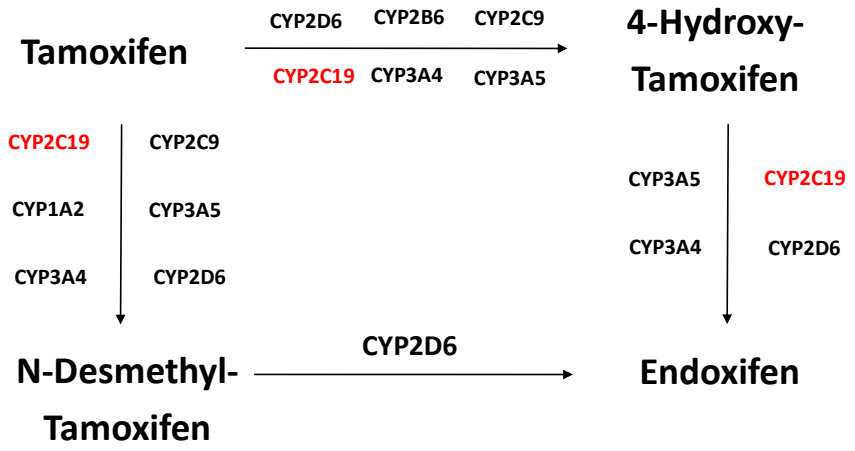


Figure 6.1. Tamoxifen metabolism

Also, the relationship between *CYP2C19* genotypes and breast cancer recurrence has been examined, yet contradictory results have also been published. Schroth and colleagues described a more favorable survival outcome for *CYP2C19*17* carriers (Hazard Ratio (HR):0.45; 95 % Confidence Interval (CI): 0.21-0.92; p-value: 0.03)[19]. Similarly, a meta-analysis described improved survival outcomes in *CYP2C19*17* carriers [20]. However, Moyer failed to find an association between clinical outcome and *CYP2C19*17* genotype (HR: 0.93; 95 % CI: 0.64-1.37; p-value: 0.667)[21]. In line with Moyer, these results were recently ratified by Damkier and colleagues after analyzing the publicly available dataset of the International Tamoxifen Pharmacogenomics Consortium (ITPC)[22]. In this heterogeneous group, homo- and heterozygotes of the *CYP2C19*17* variant were not associated with better survival outcome.

In the same manner, *CYP2C19*2* genotype has been studied, and conflicting results were found again. Schaik and colleagues reported better clinical outcomes in the advanced setting (HR:0.72; 95 % CI:0.57-0.90; p-value: 0.004) in a cohort of 499 patients[23]. In the same line, Beelen observed better survival results in adjuvant tamoxifen-treated group (HR: 0.26; 95 % CI:0.12-0.55; p-value: 0.001)[24], which is accordance with Ruiter and colleagues[25]. In contrast, Damkier showed again no association between *CYP2C19*2* genotype and breast cancer outcomes in a larger group of patients[22]. Due to this large variety in results, we aimed to investigate the

role of *CYP2C19*2* and *CYP2C19*17* on tamoxifen metabolism and the associations of these two *CYP2C19* variants with breast cancer survival outcomes in the large cohort of the prospective CYPTAM study[26], which enrolled 667 Caucasian pre- and post-menopausal patients diagnosed with early-breast cancer receiving adjuvant tamoxifen.

Methods

Study objectives

The primary objective of this study was to investigate the impact of *CYP2C19*2* and *CYP2C19*17* on the concentrations and MRs of tamoxifen, NDM-tamoxifen, 4-hydroxy-tamoxifen and endoxifen. However, tamoxifen metabolism is complex and mainly determined by *CYP2D6*, and accounting only for *CYP2C19*2* and *CYP2C19*17* would not be of significant value, since these genotypes have a minor effect on tamoxifen variability [9,27,28]. Therefore, following the approach of Schroth[29] and Damkier[22], an analysis taking into account the overall tamoxifen enzymatic activity groups (low, intermediate and high) was performed (**Table 6.1**).

Table 6.1. Overall Tamoxifen enzymatic activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*2* and *CYP2C19*17*

		CYP2D6	***CYP2C19*17***
Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*17* genotype	**High activity**	EM/EM	Yes
	Intermediate activity	EM/EM	No
		EM/IM	Yes
		EM/PM	Yes
	Low activity	EM/IM	No
		EM/PM	No
		IM/IM	Yes or No
		IM/PM	Yes or No
		PM/PM	Yes or No
		CYP2D6	***CYP2C19*2***
Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*2* genotype	**High activity**	EM/EM	No
	Intermediate activity	EM/EM	Yes
		EM/IM	No
		EM/PM	No
	Low activity	EM/IM	Yes
		EM/PM	Yes
		IM/IM	Yes or No
		IM/PM	Yes or No
		PM/PM	Yes or No

*Ultra-metabolizers (UM) were treated as extensive metabolizers (EM). EM: extensive metabolizers; IM: intermediate metabolizer; PM: poor metabolizer.

The secondary objective was to assess the effect of these two *CYP2C19* variants with breast cancer outcomes in a large cohort of Caucasian patients diagnosed with early-breast cancer receiving adjuvant tamoxifen. In the core CYPTAM study, the selected primary endpoint was relapse-free survival (RFS), which was defined as the time from enrolment to loco-regional or distant relapse or second breast cancer. In case of a switch to an aromatase inhibitor, patients were censored at the moment of tamoxifen discontinuation[26].

Study design and population

To research the influence of *CYP2C19*2* and *CYP2C19*17* variants on tamoxifen metabolism and survival outcomes, whole blood and serum samples and clinical information and follow-up regarding pre- and post-menopausal female patients encompassed in the CYPTAM study were used. Concisely, from February 2008 till December 2010, a total of 667 patients were enrolled in this study, which comprises research from 25 hospitals from Belgium and The Netherlands. The primary objective was to associate *CYP2D6* predicted phenotypes and endoxifen serum concentration to breast cancer recurrence[26]. In this study, female individuals diagnosed with early-breast cancer and starting 20 mg QD tamoxifen as adjuvant endocrine therapy, were eligible to participate in this observational study. Also, patients were allowed to participate if they were receiving tamoxifen for no longer than twelve months. Exclusion criteria were pregnancy, breast-feeding and earlier malignancy, with the exception of adequately treated in-situ cervix carcinoma and basal-cell carcinoma. The study protocol was approved by the Institutional Review Board of the Leiden University Medical Center (The Netherlands) and registered in the Netherlands Trial Registry (NTR1509). All encompassed female individuals gave written informed consent. For this pharmacogenetic study, the CYPTAM population was analysed, which is described in more detail elsewhere[12,30].

Quantification analysis and Genotyping

Serum and whole blood specimens were collected for quantification analysis of tamoxifen and its metabolites and genotyping, respectively. Samples were retrieved after at least two-month of tamoxifen therapy in order to assure steady-state concentrations. Also, a minimum of twelve hours after the last intake was required for steady state trough concentrations.

Quantification of tamoxifen and its metabolites concentrations were performed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)[31]. *CYP2D6* Genotyping was performed with Amplichip CYP450 test (Roche Diagnostic, Indianapolis, USA). In accordance with their *CYP2D6* genotypes, all individuals were classified in predicted phenotypes, as defined by Schroth *et al* [19,32]. More comprehensive description about *CYP2D6* predicted phenotypes is outlined

elsewhere[33]. In the same manner, *CYP2C19* genotyping was performed with Amplichip CYP450 test (Roche Diagnostic, Indianapolis, USA) for *CYP2C19*2* and TaqMan7500 (Applied Biosystems, Nieuwerkerk a.d. IJssel, The Netherlands) SNP Genotyping Assays for *CYP2C19*17*. The reference genotype was the wild-type *CYP2C19*1*, whereas the analysed variants were *CYP2C19*2* and *CYP2C19*17.*

Due to the low allele frequency of other *CYP2C19* genotypes in the Caucasian population, no other genotypes were assessed in this study. For instance, *CYP2C19*3* variant has a reported frequency of occurrence of 0.04 %, while it has an allele frequency of 5-11% in Asian population groups[34,35]. Therefore, only the two most common of *CYP2C19* variants among Caucasians, *CYP2C19*2* and *CYP2C19*17*, were investigated.

Statistical analysis

To evaluate the role of *CYP2C19*2* and *CYP2C19*17* on tamoxifen metabolism, concentrations and metabolic ratios of tamoxifen, endoxifen, NDM-tamoxifen and 4-hydroxy-tamoxifen were used. In this case, MRs were considered as concentration of substrate divided by metabolite concentration. To assess differences between groups, analysis of variance (ANOVA) test were carried out. Also, multiple linear regression analyses were performed to investigate the contributions of these *CYP2C19* genotypes to the total explained variability of MRs and concentrations of tamoxifen, endoxifen, NDM-tamoxifen and 4-hydroxy-tamoxifen.

For the second objective, Cox regression was carried to analyse whether RFS varied across all groups (Hazard Ratio: HR). When in the univariable analysis, a p-value below 0.1 was obtained, this covariate was adopted in the multivariable analysis. In addition, the following covariates were fitted in the multivariate analysis due to their known clinical relevance: tumor and nodal stage, histological classification and grade, and Her2 receptor status.

At the same time, we conducted an exploratory examination by performing classification tree analyses in order to determine the levels of interactions by polymorphisms (*CYP2D6*, *CYP2C19*2* and *CYP2C19*17*) on the effect endoxifen concentrations. More detailed information about how these type of analyses are performed is described elsewhere[36]. All statistical analyses were performed with IBM SPSS for Windows, Version 23.0. Statistical significance was accepted for p-values below 0.05.

Results

Allele frequencies and distributions: *CYP2C19*2* and *CYP2C19*17*

The genotype distributions of *CYP2C19*2* and *CYP2C19*17* variants are described in **Table 6.2.** In this study, both genotypes were found to be in consistency with Hardy-Weinberg equilibrium (*CYP2C19*2*: χ^2=0.518, p-value=0.472; *CYP2C19*17*: χ^2=0.135, p-value=0.713). Also, a summary of the overall tamoxifen activity groups depending on *CYP2C19* variant and *CYP2D6* predicted phenotypes is described. Of note, *CYP2D6* ultra-rapid metabolizers (n= 5) were sorted out as extensive metabolizers for the formation of the overall tamoxifen activity groups.

Table 6.2. Genotype distribution and frequency in the study population.

		CYP2D6	****17***	**Total patients (N)**	**Frequency (%)**
Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*2* genotype	**High activity**	**EM/EM**	**Yes**	155	24.4
	Intermediate activity	**EM/EM**	**No**	281	44.3
		EM/IM	**Yes**		
		EM/PM	**Yes**		
	Low activity	**EM/IM**	**No**	198	31.2
		EM/PM	**No**		
		IM/IM	**Yes or No**		
		IM/PM	**Yes or No**		
		PM/PM	**Yes or No**		
		CYP2D6	****2***		
Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*17* genotype	**High activity**	**EM/EM**	**No**	79	12.8
	Intermediate activity	**EM/EM**	**Yes**	249	40.3
		EM/IM	**No**		
		EM/PM	**No**		
	Low activity	**EM/IM**	**Yes**	290	46.9
		EM/PM	**Yes**		
		IM/IM	**Yes or No**		
		IM/PM	**Yes or No**		
		PM/PM	**Yes or No**		
			Variants	**Total patients (N)**	**Frequency (%)**
***CYP2C19*2* genotype**			****1/*1***	465	71.1
			****1/*2***	170	26.0
			****2/*2***	19	2.9
***CYP2C19*17* genotype**			****1/*1***	391	61.8
			****1/*17***	211	33.3
			****17/*17***	31	4.9

Study population

From the CYPTAM study, data from 667 female patients were used: a comprehensive overview of the clinical demographics of the enrolled CYPTAM individuals has been previously described. [12,26,30,37]. For this pharmacogenetics sub-analysis from the core CYPTAM cohort, the baseline demographics of the overall tamoxifen enzymatic activity groups (low, intermediate and high) of *CYP2C19*2* and *CYP2C19*17*, are described in **Table 6.3**. At baseline, no differences were observed, concerning tumor and nodal stage, histological classification and grade, progesterone and HER2 receptor status, type of surgery and axillar surgery, adjuvant chemotherapy and radiotherapy and trastuzumab treatment. Also, an overview of the baseline characteristics of the study patients by *CYP2C19*2* and *CYP2C19*17* alleles is listed as **Supplementary Table 6.1**.

Associations of tamoxifen metabolism and *CYP2C19* genotypes

When the overall tamoxifen activity groups (high, intermediate and low) were compared**,** statistically significant differences in mean concentrations of endoxifen, NDM-tamoxifen and 4-hydroxy-tamoxifen were observed in both cases (corrected for the *CYP2D6* predicted phenotypes and *CYP2C19*2* or *CYP2C19*17* alleles)(**Figure 6.2** and **6.3**). In contrast, tamoxifen mean concentrations did not differ when comparing the overall tamoxifen activity groups (high, intermediate and low). At the same time, significant variations in all MRs were observed in both analyses. In **Figure 6.2** and **6.3**, mean concentrations and MRs of the overall tamoxifen activity groups (high, intermediate and low) are illustrated.

In contrast, when *CYP2C19*2* or *CYP2C19*17* alleles were independently evaluated, mean concentrations and MRS of tamoxifen, endoxifen, NDM-tamoxifen and 4-hydroxy-tamoxifen were comparable and no statistical differences were observed when comparing homozygotes, heterozygotes and wild-type (**Supplementary Table 6.2**).

To study the additional effect of *CYP2C19*2* and *CYP2C19*17* to the explained variance of endoxifen concentrations and MRs, these variants were fitted in a multiple regression model in which previously *CYP2D6* predicted phenotypes and concentrations of tamoxifen and its metabolites were already assessed[12]. When both *CYP2C19* genotypes were introduced in the model, the explained variability of the concentration levels of tamoxifen and its metabolites barely improved. For instance, in the case of endoxifen concentrations, the explained variability varied from 0.423 to 0.425 (p-value 0.362) and 0.427 (p-value: 0.881) when *CYP2C19*2* and *CYP2C19*17* were fitted in the model, respectively. In contrast, the explained variance of the MRs tamoxifen/4-hydroxy-tamoxifen and 4-hydroxy-tamoxifen/endoxifen slightly, yet statistically significantly, increased, varying the improvements of the predictability (R^2) between 0.008 and 0.02, respectively. In **Supplementary Table 6.3**, a summary of *CYP2C19* variants covariate analysis is presented.

Table 6.3. Baseline clinical characteristics of the CYPTAM patients according to *CYP2D6* predicted phenotypes and *CYP2C19*2* and *CYP2C19*17* genotypes groups

			Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*2* genotype				Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*17* genotype			
			High activity (N=155)	Intermediate activity (N=281)	Low activity (N=198)	P-value	High activity (N=79)	Intermediate activity (N=249)	Low activity (N=290)	P-value
Age at enrolment (years): Mean (SD)			56.0 (11.3)	56.1 (10.9)	56.5 (11.1)	0.869	56.9 (11.6)	55.8 (10.2)	56.3 (11.5)	0.689
Tumor stage	**T1**	**N**	76	149	108	0.340	39	127	163	0.343
		%	22.8%	44.7%	32.4%		11.9%	38.6%	49.5%	
	T2	**N**	66	113	85		32	106	114	
		%	25.0%	42.8%	32.2%		12.7%	42.1%	45.2%	
	T3/T4	**N**	9	15	4		6	13	9	
		%	32.1%	53.6%	14.3%		21.4%	46.4%	32.1%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Nodal stage	**N0**	**N**	70	120	106	0.343	35	116	136	0.523
		%	23.6%	40.5%	35.8%		12.2%	40.4%	47.4%	
	N1	**N**	60	132	64		29	104	117	
		%	23.4%	51.6%	25.0%		11.6%	41.6%	46.8%	
	N2	**N**	15	18	22		9	19	27	
		%	27.3%	32.7%	40.0%		16.4%	34.5%	49.1%	
	N3	**N**	8	10	6		6	8	9	
		%	33.3%	41.7%	25.0%		26.1%	34.8%	39.1%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Histological classification	**Ductal adenocarcinoma**	**N**	112	211	159	0.601	59	190	227	0.926
		%	23.2%	43.8%	33.0%		12.4%	39.9%	47.7%	
	Lobular adenocarcinoma	**N**	25	42	23		12	35	35	
		%	27.8%	46.7%	25.6%		14.6%	42.7%	42.7%	

table continues

	Other	**N**	16	27	16		8	22	27	
		%	27.1%	45.8%	27.1%		14.0%	38.6%	47.4%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Histological grade	**G1**	**N**	20	43	21	0.143	12	35	38	0.402
		%	23.8%	51.2%	25.0%		14.1%	41.2%	44.7%	
	G2	**N**	95	159	106		44	149	153	
		%	26.4%	44.2%	29.4%		12.7%	43.1%	44.2%	
	G3	**N**	37	77	69		22	63	95	
		%	20.2%	42.1%	37.7%		12.2%	35.0%	52.8%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Progesterone Receptor Status	**Positive**	**N**	115	234	154	0.077	62	201	229	0.696
		%	22.9%	46.5%	30.6%		12.6%	40.9%	46.5%	
	Negative	**N**	35	43	44		14	43	59	
		%	28.7%	35.2%	36.1%		12.1%	37.1%	50.9%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
HER2 receptor status	**0**	**N**	98	170	115	0.476	51	152	167	0.355
		%	25.6%	44.4%	30.0%		13.8%	41.1%	45.1%	
	1+	**N**	34	75	53		13	66	81	
		%	21.0%	46.3%	32.7%		8.1%	41.3%	50.6%	
	2+	**N**	6	18	10		5	12	17	
		%	17.6%	52.9%	29.4%		14.7%	35.3%	50.0%	
	3+	**N**	15	17	20		10	17	24	
		%	28.8%	32.7%	38.5%		19.6%	33.3%	47.1%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
FISH	**Positive (amplification)**	**N**	17	19	20	0.243	11	18	26	0.198
		%	30.4%	33.9%	35.7%		20.0%	32.7%	47.3%	
	Negative	**N**	136	261	178		68	229	263	
		%	23.7%	45.4%	31.0%		12.1%	40.9%	47.0%	

table continues

	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Surgery	**Mastectomy**	**N**	76	131	88	0.649	40	108	135	0.526
		%	25.8%	44.4%	29.8%		14.1%	38.2%	47.7%	
	Breast conserving	**N**	77	149	109		39	139	153	
		%	23.0%	44.5%	32.5%		11.8%	42.0%	46.2%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Surgery axilla	**Sentinal node procedure only**	**N**	71	133	109	0.157	33	125	148	0.302
		%	22.7%	42.5%	34.8%		10.8%	40.8%	48.4%	
	Axillary lymph node dissection	**N**	82	147	88		46	122	140	
		%	25.9%	46.4%	27.8%		14.9%	39.6%	45.5%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Adjuvant Radiotherapy	**Yes**	**N**	100	195	142	0.433	52	173	204	0.710
		%	22.9%	44.6%	32.5%		12.1%	40.3%	47.6%	
	No	**N**	53	85	56		27	74	85	
		%	27.3%	43.8%	28.9%		14.5%	39.8%	45.7%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Adjuvant Chemotherapy	**Yes**	**N**	99	177	118	0.580	48	163	170	0.228
		%	25.1%	44.9%	29.9%		12.6%	42.8%	44.6%	
	No	**N**	54	103	80		31	84	119	
		%	22.8%	43.5%	33.8%		13.2%	35.9%	50.9%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Trastuzumab Therapy	**Yes**	**N**	18	18	20	0.136	12	18	25	0.091
		%	32.1%	32.1%	35.7%		21.8%	32.7%	45.5%	
	No	**N**	134	260	178		66	228	264	
		%	23.4%	45.5%	31.1%		11.8%	40.9%	47.3%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	

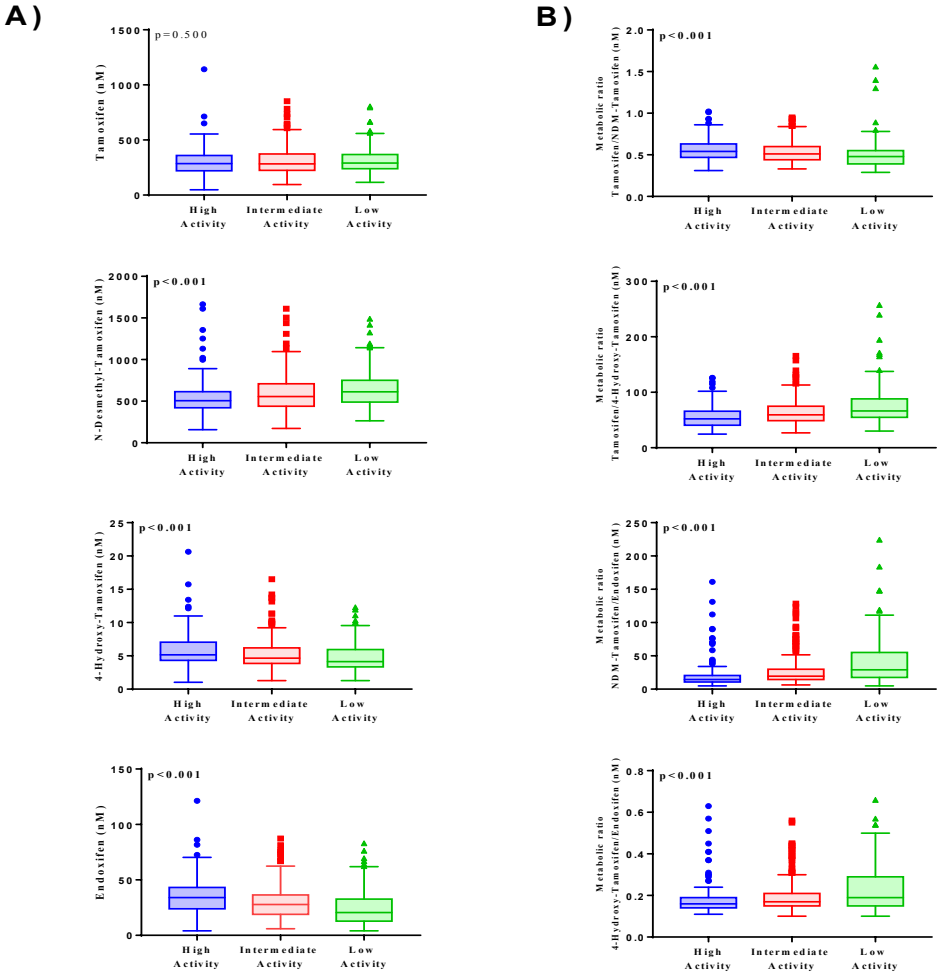


Figure 6.2. Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*2* genotype

A) Association between High, Intermediate and Low activity groups and their effect on the concentrations of tamoxifen and its metabolites.

B) Association between High, Intermediate and Low activity groups and their effect on the metabolic ratios of tamoxifen and its metabolites.

Breast cancer recurrence and *CYP2C19* genotypes

For the *CYP2C19*2* genotype, analysing the association between the overall tamoxifen enzymatic activity groups (high, intermediate and low) with RFS, no differences in terms of HR were found. In the multi-variable analysis, a HR of 1.191 (95 % CI: 0.538-2.636, p-value: 0.666) and 1.346 (95 % CI: 0.538-2.636, p-value: 0.404) for the intermediate

and high activity group, respectively were obtained. In the same manner, no significant variations for the *CYP2C19*17* genotype across the overall tamoxifen enzymatic activity groups were found. In this case, adjusted HRs for the intermediate and high were 0.819 (95 % CI: 0.302-2.220, p-value: 0.695) and 1.369 (95 % CI: 0.736-2.548, p-value: 0.321), respectively.

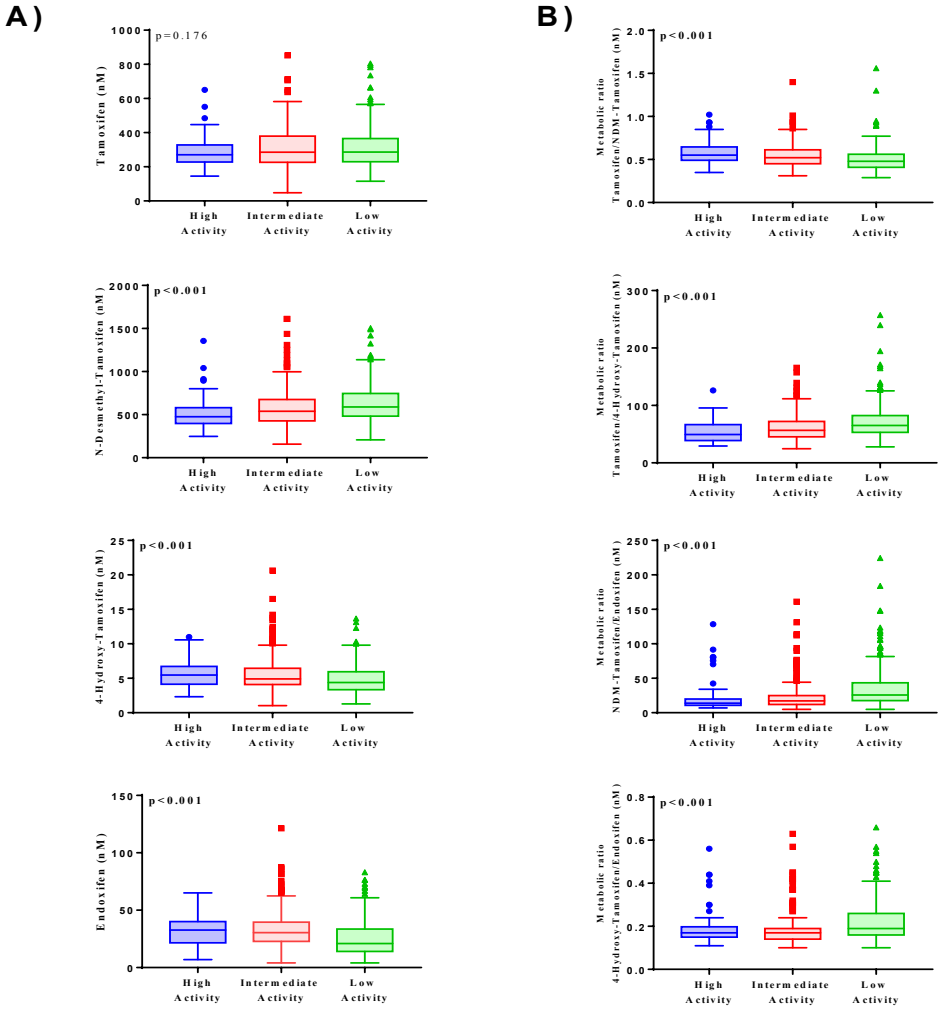


Figure 6.3. Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*17* genotype.

A) Association between High, Intermediate and Low activity groups and their effect on the concentrations of tamoxifen and its metabolites.

B) Association between High, Intermediate and Low activity groups and their effect on the metabolic ratios of tamoxifen and its metabolites.

For the *CYP2C19*2* genotype, uni- and multi-variable analysis did not reveal differences in terms of RFS between homozygotes and heterozygotes. In these cases, adjusted HRs for associations between *CYP2C19*1/*2* and *CYP2C19*2/*2* compared to the wild-type and RFS were 1.090 (95 % CI: 0.595-1.994) and 0.759 (95 % CI: 0.101-5.700), respectively.

In the same line, no differences in RFS were observed for the *CYP2C19*17* carriers and non-carriers. In contrast, adjusted HRs for *CYP2C19*17* homozygotes and heterozygotes were 1.797 (95 % CI: 0.598-5.397, p-value: 0.296) and 0.881 (95 % CI: 0.478-1.625, p-value:0.686), respectively. A summary of the Cox regression analysis of all CYP2C19 genotypes is presented in **Table 6.4**.

Table 6.4. Cox proportional hazard ratios for *CYP2C19*2* and *CYP2C19*17* genotypes, and comparison of activity groups according to **CYP2D6** predicted phenotypes and *CYP2C19*2* and *CYP2C19*17* genotype.

		Univariable analysis		Multivariable analysis*		
	N	**HR**	**95 % CI**	**p-value**	**HR**	**95 % CI**	**p-value**
Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*2* genotype							
Low activity group	198	1.000	Reference	(0.804)	1.000	Reference	(0.704)
Intermediate activity group	281	1.133	0.531-2.147	0.747	1.191	0.538-2.636	0.666
High activity group	155	1.246	0.648-2.398	0.510	1.346	0.669-2.708	0.404
Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*17* genotype							
Low activity group	290	1.000	Reference	(0.582)	1.000	Reference	(0.458)
Intermediate activity group	249	0.832	0.313-2.208	0.711	0.819	0.302-2.220	0.695
High activity group	79	1.271	0.707-2.285	0.423	1.369	0.736-2.548	0.321
***CYP2C19*2* genotype**							
No *CYP2C19*2* variant	465	1.000	Reference	(0.797)	1.000	Reference	(0.921)
CYP2C19*1/*2	170	1.201	0.668-2.159	0.541	1.090	0.595-1.994	0.781
CYP2C19*2/*2	19	0.796	0.109-5.819	0.822	0.759	0.101-5.700	0.789
CYP2C19*17 genotype							
No *CYP2C19*17* variant	391	1.000	Reference	(0.404)	1.000	Reference	(0.481)
CYP2C19*1/*17	211	0.842	0.463-1.531	0.573	0.881	0.478-1.625	0.686
CYP2C19*17/*17	31	1.787	0.632-5.048	0.273	1.797	0.598-5.397	0.296

Classification Tree analyses

As an exploratory analysis, we conducted different Classification Tree Analyses (CTA) to evaluate the levels of interactions between *CYP2D6* predicted phenotypes and *CYP2C19*2* and *CYP2C19*17* genotypes on endoxifen concentrations. The first CTA was performed with the only focus on the *CYP2D6* predicted phenotypes and endoxifen concentration. In this CTA, patients were subdivided in only one level of the CTA with three different groups of *CYP2D6* phenotypes that statistically different (EM/UM *verus* hetEM *versus* IM/PM; p-value<0.001) (**Figure 6.4**). In contrast, when *CYP2C19*2* and *CYP2C19*17* were added into the first CTA (*CYP2D6* predicted phenotypes and endoxifen concentrations), no other levels of the CTA were achieved.

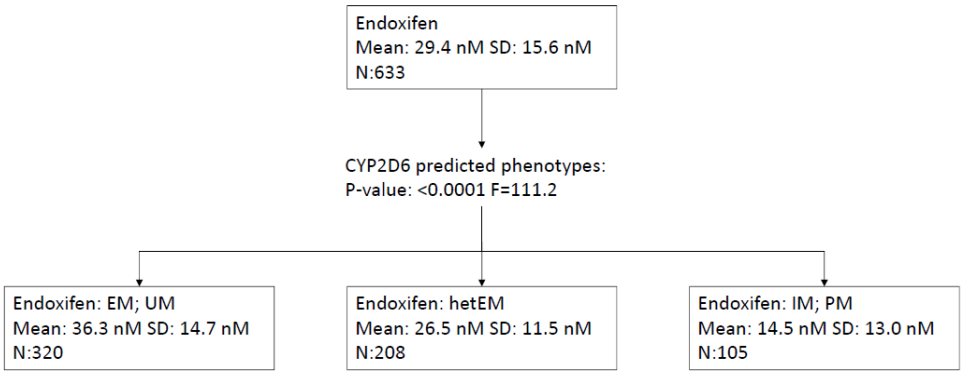


Figure 6.4. Classification Tree analyses for endoxifen concentrations and *CYP2D6* predicted phenotypes. EM: extensive metabolizer; hetEM: heterogenous extensive metabolizer; IM: intermediate metabolizer; N: number of individuals. PM: poor metabolizer; SD: standard deviation; UM: ultrarapid metabolizer.

Discussion

In this study, we assessed the effect of *CYP2C19* genotypes on tamoxifen metabolism and efficacy in an extensive cohort of Caucasian breast cancer patients receiving tamoxifen as adjuvant endocrine therapy. In our study, we failed to find any differences in mean concentrations of tamoxifen, endoxifen, 4-hydroxy-tamoxifen and NDMA-tamoxifen when comparing both *CYP2C19*2* and *CYP2C19*17* genotypes to their wild-type. Interestingly, an analysis accounting for *CYP2D6* predicted phenotypes and *CYP2C19* genotypes, in which the overall tamoxifen enzymatic activity was categorized as high, intermediate and low activity, resulted in statistically significant differences in mean concentrations of endoxifen, NDM-tamoxifen, and 4-hydroxy-tamoxifen and their corresponding MR. In contrast, tamoxifen mean concentrations were comparable across all the groups. At the same time, we found a lack of association between

CYP2C19 variant alleles and RFS, when accounting for *CYP2D6* predicted phenotypes and *CYP2C19*2* or *CYP2C19*17* or when comparing both genotypes independently to wild-type.

Tamoxifen has a complex metabolic pathway and many different enzymes are implicated in its activation into endoxifen. Yet, the most relevant enzyme of tamoxifen metabolism is CYP2D6, but it only partially contributes to explaining the inter-variability in endoxifen concentrations between patients. Therefore, many studies have been conducted to find other potentials sources which could clarify the high variability in concentration levels and response to therapy between patients, such as *CYP2C19* genotypes and its **17* and **2* variants.

According to Scroth and colleagues, the *CYP2C19*17* with its higher functioning genotypes has been correlated with improved clinical outcome[29]. In theory, tamoxifen may be more easily metabolized into its active metabolites, e.g. endoxifen, mainly due to the higher enzymatic activity among *CYP2C19*17* carriers[15,29]. Consequently, a higher exposure to the anti-estrogenic activity of tamoxifen and its metabolites could be expected, which potentially may clarify why *CYP2C19*17* patients may have an increased survival outcome. However, we failed to find such an association of improved clinical survival in our study, which is in line with Damkier *et al*[22]. Interestingly, this hypothesis of higher exposure to anti-estrogenic activity due to higher concentration levels of tamoxifen active metabolites was not seen in our study. Instead, no significant differences in mean concentrations or MRs were obtained when comparing *CYP2C19*17* hetero- and homozygous to the wild-type. At the same time, adding *CYP2C19*17* variant to the multiple regression model, barely improved the inter-patient variability (e.g. R^2 for endoxifen concentrations varied from 0.423 to 0.427). Although the hypothesis of a prognostic marker for tamoxifen efficacy of only one variant may be tempting, we believe our results do not support that *CYP2C19*17* might be it.

Likewise, for the *CYP2C19*2* allele, Van Schaik and colleagues[23], Beelen *et al* [24]and Ruiter and colleagues[25], found improved survival outcomes in the metastatic setting and in the adjuvant scenario. In this case, the decreased enzymatic activity of *CYP2C19*2* may probably lead to a lower exposure to antiestrogenic activity of tamoxifen and its metabolites, due to the potentially lower concentration levels, and therefore, a worsened clinical outcome. Nevertheless, all of these studies reported improved survival outcomes. A potential explanation for this increased clinical outcome among *CYP2C19*2* carriers may be due to the increased transformation from endoxifen into norendoxifen, which has a dual antiendocrine mechanism of action[16]. However, we did not find a statistically significant variations in mean concentration levels or MRs between *CYP2C19*2* homo- or heterozygous in comparison with the wild-type. In this case, our results are again in agreement with Damkier and colleagues, still the main advantage of our study might rely on the use of concentration levels.

Following the approach of Schroth *et al* of creating a new combined variable accounting for *CYP2D6* predicted phenotypes and *CYP2C19* genotypes[29], we also assessed the differences in mean concentrations, MRs and clinical outcome. In this case, we found statistically significant lower mean concentrations (with the exception of tamoxifen) and higher MRs in the low activity group of *CYP2C19*2*, whereas mean concentrations of 4-hydroxy-tamoxifen and endoxifen in the high activity group of *CYP2C19*17* were significantly higher. To evaluate the rationale after this variable, we conducted a CTA. Interestingly, we failed to find any improvement in the prediction of endoxifen concentrations when *CYP2C19*2* or *CYP2C19*17* were fitted in the corresponding models, whereas only when *CYP2D6* predicted phenotypes were introduced, significant differences were observed. Our interpretation is that the use of *CYP2C19* genotypes only in order to predict endoxifen concentrations, might lack of usefulness in the clinical setting, and that *CYP2D6* genotypes might have the most relevant role in the prediction of endoxifen concentrations. Due to differences in mean concentrations and metabolic ratios when correcting for *CYP2D6* predicted phenotypes, we hypothesize that *CYP2C19*2* and *CYP2C19*17* might help to compensate the formation of endoxifen and 4-hydroxy-tamoxifen in the case of low CYP2D6 enzymatic activity. Yet, this effect did not translate in better clinical outcomes.

A limitation of our study might be our sample size of 667 patients compared to the cohort of 2102 female patients of the ITPC. However, we believe that our study was sufficiently powered to replicate the results of Damkier and colleagues[22], with the main advantage of the use of concentrations or MRs.

To conclude, we have shown that *CYP2C19* polymorphisms have no impact on concentration levels and MRs of tamoxifen, endoxifen, 4-hydroxy-tamoxifen and NDM-tamoxifen, or clinical outcomes in breast cancer patients treated with adjuvant tamoxifen. Therefore, *CYP2C19* genotypes might not be clinically decisive for patients with early-breast cancer treated with adjuvant tamoxifen.

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Supplementary Table 6.1. Baseline clinical characteristics of the CYPTAM patients according to *CYP2C19*2* and *CYP2C19*17* genotypes

			*CYP2C19*2* genotype				*CYP2C19*17* genotype			
			**1/*1*	**1/*2*	**2/*2*	P-value	**1/*1*	**1/*17*	**17/*17*	P-value
			N=465	N=170	N=18		N=391	N=211	N=31	
Age at enrolment (years): Mean (SD)			55.72 (10.90)	58.17 (11.57)	54.55 (9.67)	0.038	56.35 (11.37)	56.21 (10.67)	57.29 (10.30)	0.880
Tumor stage	**T1**	**N**	244	89	12	0.678	211	112	16	0.863
		%	70.7%	25.8%	3.5%		62.2%	33.0%	4.7%	
	T2	**N**	191	74	6		160	86	12	
		%	70.5%	27.3%	2.2%		62.0%	33.3%	4.7%	
	T3/T4	**N**	22	6	0		14	11	2	
		%	78.6%	21.4%	0.0%		51.9%	40.7%	7.4%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Nodal stage	**N0**	**N**	213	85	13	0.311	181	103	18	0.098
		%	68.5%	27.3%	4.2%		59.9%	34.1%	6.0%	
	N1	**N**	190	66	4		167	76	9	
		%	73.1%	25.4%	1.5%		66.3%	30.2%	3.6%	
	N2	**N**	40	15	0		30	22	1	
		%	72.7%	27.3%	0.0%		56.6%	41.5%	1.9%	
	N3	**N**	19	4	1		10	10	3	
		%	79.2%	16.7%	4.2%		43.5%	43.5%	13.0%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Histological classification	**Ductal adenocarcinoma**	**N**	352	131	13	0.753	297	167	22	0.751
		%	71.0%	26.4%	2.6%		61.1%	34.4%	4.5%	
	Lobular adenocarcinoma	**N**	63	27	3		55	24	6	
		%	67.7%	29.0%	3.2%		64.7%	28.2%	7.1%	
	Other	**N**	47	12	2		36	20	3	

table continues

		%	77.0%	19.7%	3.3%		61.0%	33.9%	5.1%	
	Not specified	N	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Histological grade	G1	N	62	24	3	0.757	57	29	4	0.907
		%	69.7%	27.0%	3.4%		63.3%	32.2%	4.4%	
	G2	N	271	92	9		216	124	16	
		%	72.8%	24.7%	2.4%		60.7%	34.8%	4.5%	
	G3	N	125	54	6		114	56	10	
		%	67.6%	29.2%	3.2%		63.3%	31.1%	5.6%	
	Not specified	N	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Progesterone Receptor Status	Positive	N	373	132	15	0.589	308	173	24	0.496
		%	71.7%	25.4%	2.9%		61.0%	34.3%	4.8%	
	Negative	N	84	37	3		77	34	7	
		%	67.7%	29.8%	2.4%		65.3%	28.8%	5.9%	
	Not specified	N	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
HER2 receptor status	0	N	292	97	10	0.608	231	130	22	0.141
		%	73.2%	24.3%	2.5%		60.3%	33.9%	5.7%	
	1+	N	108	51	5		111	44	6	
		%	65.9%	31.1%	3.0%		68.9%	27.3%	3.7%	
	2+	N	24	8	2		18	16	0	
		%	70.6%	23.5%	5.9%		52.9%	47.1%	0.0%	
	3+	N	38	14	1		28	21	3	
		%	71.7%	26.4%	1.9%		53.8%	40.4%	5.8%	
	Not specified	N	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
FISH	Positive (amplification)	N	42	14	1	0.838	31	22	3	0.598
		%	73.7%	24.6%	1.8%		55.4%	39.3%	5.4%	

table continues

	Negative	**N**	420	156	17		357	189	28	
		%	70.8%	26.3%	2.9%		62.2%	32.9%	4.9%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Surgery	**Mastectomy**	**N**	220	73	10	0.451	178	97	14	0.994
		%	72.6%	24.1%	3.3%		61.6%	33.6%	4.8%	
	Breast conserving	**N**	241	96	8		209	113	17	
		%	69.9%	27.8%	2.3%		61.7%	33.3%	5.0%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Surgery axilla	**Sentinal node procedure only**	**N**	227	90	9	0.671	198	100	20	0.202
		%	69.6%	27.6%	2.8%		62.3%	31.4%	6.3%	
	Axillary lymph node dissection	**N**	234	79	9		189	110	11	
		%	72.7%	24.5%	2.8%		61.0%	35.5%	3.5%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Adjuvant Radiotherapy	**Yes**	**N**	320	121	8	0.065	270	147	21	0.976
		%	71.3%	26.9%	1.8%		61.6%	33.6%	4.8%	
	No	**N**	142	49	10		118	64	10	
		%	70.6%	24.4%	5.0%		61.5%	33.3%	5.2%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	
Adjuvant Chemotherapy	**Yes**	**N**	294	95	11	0.206	231	132	20	0.700
		%	73.5%	23.8%	2.8%		60.3%	34.5%	5.2%	
	No	**N**	168	75	7		157	79	11	
		%	67.2%	30.0%	2.8%		63.6%	32.0%	4.5%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	

table continues

Trastuzumab Therapy	**Yes**	**N**	42	14	1	0.838	30	23	3	0.404
		%	73.7%	24.6%	1.8%		53.6%	41.1%	5.4%	
	No	**N**	418	155	17		358	186	28	
		%	70.8%	26.3%	2.9%		62.6%	32.5%	4.9%	
	Not specified	**N**	0	0	0		0	0	0	
		%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	

Supplementary Table 6.2. Overview of Mean (SD) concentration levels and metabolic ratios of tamoxifen. endoxifen. 4-hydroxy-tamoxifen and NDM-tamoxifen by *CYP2C19*2* and *CYP2C19*17* genotypes. activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*2* and *CYP2C19*17* genotypes and *CYP2C19* combined gentoypes. SD: standard deviation; MR: metabolic ratio.

		Tamoxifen	NDM-Tamoxifen	4-Hydroxy-Tamoxifen	Endoxifen	MR Tamoxifen / NDM-Tamoxifen	MR Tamoxifen / 4-Hydroxy-Tamoxifen	MR 4-Hydroxy-Tamoxifen / Endoxifen	MR NDM-Tamoxifen / Endoxifen
		Mean (SD) (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
***CYP2C19*2* genotype**	****1/*1***	308.72 (122.36)	601.00 (226.14)	5.24 (2.33)	29.21 (15.98)	0.52 (0.13)	63.96 (26.12)	0.20 (0.08)	28.97 (24.62)
	****1/*2***	320.62 (123.93)	607.49 (222.95)	5.11 (2.18)	29.47 (15.83)	0.54 (0.15)	68.02 (28.12)	0.20 (0.10)	30.69 (30.96)
	****2/*2***	303.84 (105.45)	606.48 (245.04)	4.84 (2.07)	32.35 (15.70)	0.51 (0.09)	68.21 (23.71)	0.17 (0.07)	24.72 (19.16)
	p-value	0.538	0.948	0.66	0.712	0.27	0.21	0.217	0.581
***CYP2C19*17* genotype**	****1/*1***	307.03 (115.80)	595.99 (213.82)	5.08 (2.36)	29.25 (16.92)	0.52 (0.13)	66.30 (27.14)	0.20 (0.08)	29.76 (26.51)
	****1/*17***	315.07 (125.16)	606.27 (240.03)	5.32 (2.07)	29.16 (13.86)	0.54 (0.16)	63.15 (25.54)	0.20 (0.08)	27.79 (22.86)
	****17/*17***	285.16 (80.81)	558.57 (159.31)	5.31 (1.90)	29.77 (14.24)	0.53 (0.14)	57.47 (17.24)	0.20 (0.08)	26.26 (20.64)

table continues

	p-value	0.381	0.519	0.436	0.980	0.664	0.105	0.906	0.548
Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*2* genotype	**High activity**	302.93 (125.40)	549.08 (219.10)	5.88 (2.68)	35.96 (17.38)	0.56 (0.13)	55.48 (19.57)	0.18 (0.07)	20.83 (21.47)
	Intermediate activity	311.58 (122.77)	596.59 (223.28)	5.17 (2.15)	29.21 (14.25)	0.53 (0.12)	63.77 (21.18)	0.20 (0.08)	26.19 (20.25)
	Low activity	318.40 (119.48)	653.54 (221.72)	4.69 (1.96)	24.34 (14.90)	0.50 (0.16)	74.26 (31.98)	0.23 (0.10)	40.08 (32.79)
	p-value	0.500	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Activity groups according to *CYP2D6* predicted phenotypes and *CYP2C19*17* genotype	**High activity**	285.26 (90.48)	512.08 (182.17)	5.67 (1.93)	32.68 (13.31)	0.58 (0.13)	53.74 (18.89)	0.19 (0.08)	21.25 (21.39)
	Intermediate activity	312.20 (122.46)	583.92 (228.08)	5.50 (2.52)	33.17 (16.59)	0.55 (0.13)	60.87 (22.32)	0.18 (0.07)	23.33 (20.69)
	Low activity	311.88 (119.83)	631.60 (216.91)	4.74 (1.98)	24.89 (14.48)	0.50 (0.14)	71.27 (28.88)	0.22 (0.09)	36.10 (29.54)
	p-value	0.176	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Supplementary Table 6.3. Summary of *CYP2C19* genotypes covariate analysis. Ln(Tamoxifen)= natural log of tamoxifen concentration; Ln(Endoxifen)= natural log of endoxifen concentration; Ln(4-Hydroxy-Tamoxifen)= natural log of 4-hydroxy-tamoxifen concentration; Ln(NDM-Tamoxifen)= natural log of NDM-tamoxifen concentration. MR= Metabolic ratio. Ln(MR Tamoxifen/NDM-Tamoxifen)= natural log of MR Tamoxifen/NDM-Tamoxifen; Ln(MR Tamoxifen/4-hydroxy-tamoxifen)= natural log of MR Tamoxifen/4-hydroxy-tamoxifen; Ln(MR 4-Hydroy-Tamoxifen/Endoxifen)= natural log of MR 4-Hydroy-Tamoxifen/Endoxifen; Ln(MR NDM-Tamoxifen/Endoxifen)= natural log of MR NDM-Tamoxifen/Endoxifen

		R^2	p-value
Ln Tamoxifen	***CYP2D6***	0.003	0.169
	CYP2C19*2	0.006	0.171
	CYP2C19*17	0.002	0.972
Ln Endoxifen	***CYP2D6***	0.423	<0.001
	CYP2C19*2	0.425	0.362
	CYP2C19*17	0.427	0.881
Ln 4-Hydroy-Tamoxifen	***CYP2D6***	0.127	<0.001
	CYP2C19*2	0.127	0.485
	CYP2C19*17	0.134	0.112
Ln NDM-Tamoxifen	***CYP2D6***	0.138	<0.001
	CYP2C19*2	0.142	0.598
	CYP2C19*17	0.141	0.922
Ln MR Tamoxifen/NDM-Tamoxifen	***CYP2D6***	0.218	<0.001
	CYP2C19*2	0.223	0.183
	CYP2C19*17	0.225	0.833
Ln MR Tamoxifen/4-hydroxy-tamoxifen	***CYP2D6***	0.219	<0.001
	CYP2C19*2	0.228	0.021
	CYP2C19*17	0.227	0.046
Ln MR 4-Hydroy-Tamoxifen/Endoxifen	***CYP2D6***	0.449	<0.001
	CYP2C19*2	0.459	0.009
	CYP2C19*17	0.469	0.028
Ln MR NDM-Tamoxifen/Endoxifen	***CYP2D6***	0.570	<0.001
	CYP2C19*2	0.576	0.605
	CYP2C19*17	0.582	0.939