original report

Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study

Anabel Sanchez-Spitman, PharmD[1]; Vincent Dezentjé, MD, PhD[2]; Jesse Swen, PharmD, PhD[1]; Dirk Jan A.R. Moes, PharmD, PhD[1]; Stefan Böhringer, MD, PhD[1]; Erdogan Batman, MD[3]; Edith van Druten, MSN[4]; Carolien Smorenburg, MD, PhD[2,5]; Aart van Bochove, MD[6]; Anneke Zeillemaker, MD, PhD[7]; Lynn Jongen, MSc[8]; Maartje Los, MD, PhD[9]; Patrick Neven, MD, PhD[10]; Hans Gelderblom, MD, PhD[1]; and Henk-Jan Guchelaar, PharmD, PhD[1]

abstract

PURPOSE Tamoxifen is widely prescribed as adjuvant therapy in patients with early-stage breast cancer. It has been postulated that concentrations of endoxifen, the active metabolite of tamoxifen, are a better predictor of tamoxifen efficacy than *CYP2D6* genotypes. Although in a retrospective study, an endoxifen threshold of 5.9 ng/mL for efficacy was described, confirmation based on prospective studies is lacking. The objective of the prospective CYPTAM (The Netherlands National Trial Register: NTR1509) study was to associate endoxifen concentrations and *CYP2D6* genotypes with clinical outcome in patients with early-stage breast cancer receiving tamoxifen.

PATIENTS AND METHODS From February 2008 to December 2010, patients with breast cancer treated with adjuvant tamoxifen were included. Patients could be enrolled up to a maximum of 12 months after tamoxifen initiation. Blood samples were retrieved for *CYP2D6* genotyping and endoxifen measurements by Amplichip (Roche Diagnostics, Indianapolis, IN) and high-performance liquid chromatography–tandem mass spectrometry, respectively. Endoxifen concentrations were analyzed as a continuous variable, classifying patients into quartiles and using an endoxifen threshold of 5.9 ng/mL. Endoxifen concentrations and *CYP2D6* genotypes were associated with relapse-free survival (censored at the time of tamoxifen discontinuation; RFSt) by Cox regression analysis.

RESULTS A total of 667 pre- and postmenopausal patients were enrolled and had received tamoxifen for a median time of 0.37 years (range, 0.23 to 0.6 years) before study entry. No association was found between endoxifen concentrations and RFSt (adjusted hazard ratio, 0.991; 95% CI, 0.946 to 1.038; $P = .691$). Also, neither categorizing endoxifen concentrations into quartiles nor using 5.9 ng/mL as threshold altered these results. In addition, no association was found between *CYP2D6* genotype and RFSt (adjusted hazard ratio, 0.929; 95% CI, 0.525 to 1.642; $P = .799$).

CONCLUSION This prospective clinical study shows no association between endoxifen concentrations or *CYP2D6* genotypes and clinical outcome in patients with early-stage breast cancer receiving adjuvant tamoxifen.

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ASSOCIATED CONTENT
Appendix
Data Supplements
Podcast by Dr Stearns

Author affiliations and support information (if applicable) appear at the end of this article.

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Clinical trial information: Netherlands Trial Register NTR1509.

INTRODUCTION

In estrogen receptor–positive tumors, to block estrogen, endocrine therapy is indicated.[1,2] Tamoxifen and aromatase inhibitors (AIs) are the pillars of endocrine therapy. In the adjuvant setting, tamoxifen can be prescribed as monotherapy or followed by a subsequent switch to an AI after 2 to 3 years of tamoxifen treatment.[1-3] Initially, tamoxifen is metabolized into 4-hydroxy-tamoxifen and N-desmethyl-tamoxifen (NDM-tamoxifen), followed by a biotransformation into the most active metabolite, endoxifen (Fig 1).[4] Also, CYP2D6 is considered the rate-limiting enzyme of the conversion of N-desmethyl-tamoxifen into endoxifen. However, only 23% to 29% of endoxifen concentration intervariability is explained by genetic polymorphisms in *CYP2D6* genotypes.[5,6]

In the study by Goetz et al,[7] the authors described a statistically significant worsening of survival outcomes for patients with breast cancer with *CYP2D6*4/*4* genotype receiving tamoxifen compared with those of other *CYP2D6* genotypes. Since then, many studies have been performed to confirm these findings, but conflicting results have been reported. Because *CYP2D6* genotypes only partially explain the interpatient variability of tamoxifen[8] and endoxifen concentrations,[5,6] attention has been focused on endoxifen concentrations as a predictor for tamoxifen therapy. As a result of their decreased CYP2D6 activity, poor metabolizers (PMs) and intermediate metabolizers (IMs) have lower mean endoxifen concentrations compared with extensive metabolizers (EMs).[8,9] Studies performed in PMs and IMs treated with

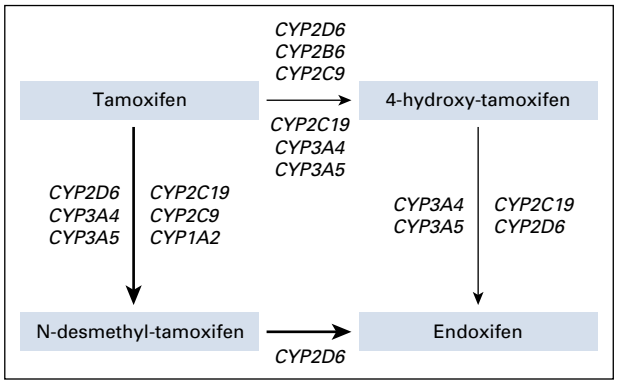

FIG 1. Metabolization of tamoxifen.

increased tamoxifen doses demonstrated that higher endoxifen concentrations were reached, without increasing short-term toxicity or adverse effects.[10,11]

Madlensky et al[12] suggested a threshold of 5.9 ng/mL (15.8 nM), above of which a 26% lower recurrence rate was described (hazard ratio [HR], 0.74; 95% CI, 0.55 to 1.00). However, this threshold has been questioned, because the WHEL (Women's Healthy Eating and Living) study, from which these results were obtained, was not designed to determine such a threshold. Similarly, Saladores et al[13] described another threshold of 5.2 ng/mL for premenopausal women, whereas Helland et al[14] reported a threshold of 3.36 ng/mL.

In several publications, the need for a prospective clinical study specifically designed to explore the relationship between endoxifen concentrations, *CYP2D6* genotypes, and clinical outcome has been underlined.[15] Therefore, the prospective CYPTAM (The Netherlands National Trial Register: NTR1509) study was initiated to properly address the question of whether *CYP2D6* genotypes and endoxifen concentrations are related to clinical outcome in patients with early-stage breast cancer receiving adjuvant tamoxifen therapy.[16] In Figure 2, a CONSORT diagram of the CYPTAM study is shown. The aim of this study was determine the association between *CYP2D6* genotypes or endoxifen concentrations and clinical outcome.

PATIENTS AND METHODS

Study Design and Study Population

From February 2008 to December 2010, women diagnosed with early-stage breast cancer receiving adjuvant tamoxifen were invited to participate in this multicenter prospective study executed in the Netherlands and Belgium. Pre- and postmenopausal patients who were at least 18 years of age and receiving treatment with the standard dose of 20 mg of tamoxifen up to 12 months after initiating tamoxifen treatment were eligible for enrollment. The CYPTAM study was approved by the institutional review board of the Leiden University Medical Center in Leiden, the Netherlands, and recorded in the Netherlands Trial Registry.[16] All the enrolled patients provided written informed consent. Individuals with a history of a previous malignancy within the last 5 years were excluded, with the exception of patients appropriately treated for an in situ cervix carcinoma or basal cell carcinoma. Serum and whole-blood samples were collected for measurements of tamoxifen and its metabolite trough levels and *CYP2D6* genotyping, respectively, once steady-state drug concentration was reached. *CYP2D6* genotyping was performed using the Amplichip CYP450 test (Roche Diagnostics, Indianapolis, IN) to test the major *CYP2D6* alleles in DNA isolated from blood. According to their *CYP2D6* genotypes, all patients were classified into predicted phenotypes, as described by Schroth et al,[17] as ultra-rapid metabolizers (UMs), EMs, heterozygous EMs (hetEMs), IMs, and PMs. More detailed information is described in the Appendix (online only). At enrollment, clinical information was retrospectively collected and registered. From December 2016 until February 2017, information concerning survival outcomes was obtained from the medical records of the patients by professional data managers.

Study Objectives and End Points

Initially, the CYPTAM study was powered to detect a twofold worsening of 3-year relapse-free survival (RFS) of the combined group of PMs, IMs, and hetEMs, compared with EMs and UMs (HR, 2.0). The required sample size was estimated based on the studies of Goetz et al[7] and Gonzalez-Santiago et al,[19] in which HRs of 1.86 and 2.82 were reported, respectively. In total, 650 patients were needed to achieve 80% power at a .05 significance level.

However, since 2007, when the CYPTAM protocol was approved, more evidence suggesting endoxifen concentrations (*v CYP2D6* genotypes only) to be a predictor for tamoxifen efficacy has been published.[12-14] Therefore, before data analysis, the statistical analysis plan was amended, taking into account the role of endoxifen, and approved by the institutional review board.

The primary end point of RFS was defined as the time from study enrollment until locoregional or distant recurrence or second breast cancer. In case of a subsequent switch to an aromatase inhibitor (AI), patients were censored at the time of tamoxifen discontinuation (RFSt). The secondary end points were disease-free survival (DFS), complete RFS (RFSc), complete DFS (DFSc), and overall survival (OS). DFS was defined as the time from study enrollment until locoregional or distant recurrence or second breast cancer or death without recurrence. Patients who switched to an AI were censored at the time of tamoxifen discontinuation (DFSt). RFSc and DFSc differed from RFSt and DFSt in that the complete period of endocrine therapy (tamoxifen and AI use) until an event or loss to follow-up was included in the analysis. OS was defined as the time from study enrollment until death resulting from any cause.

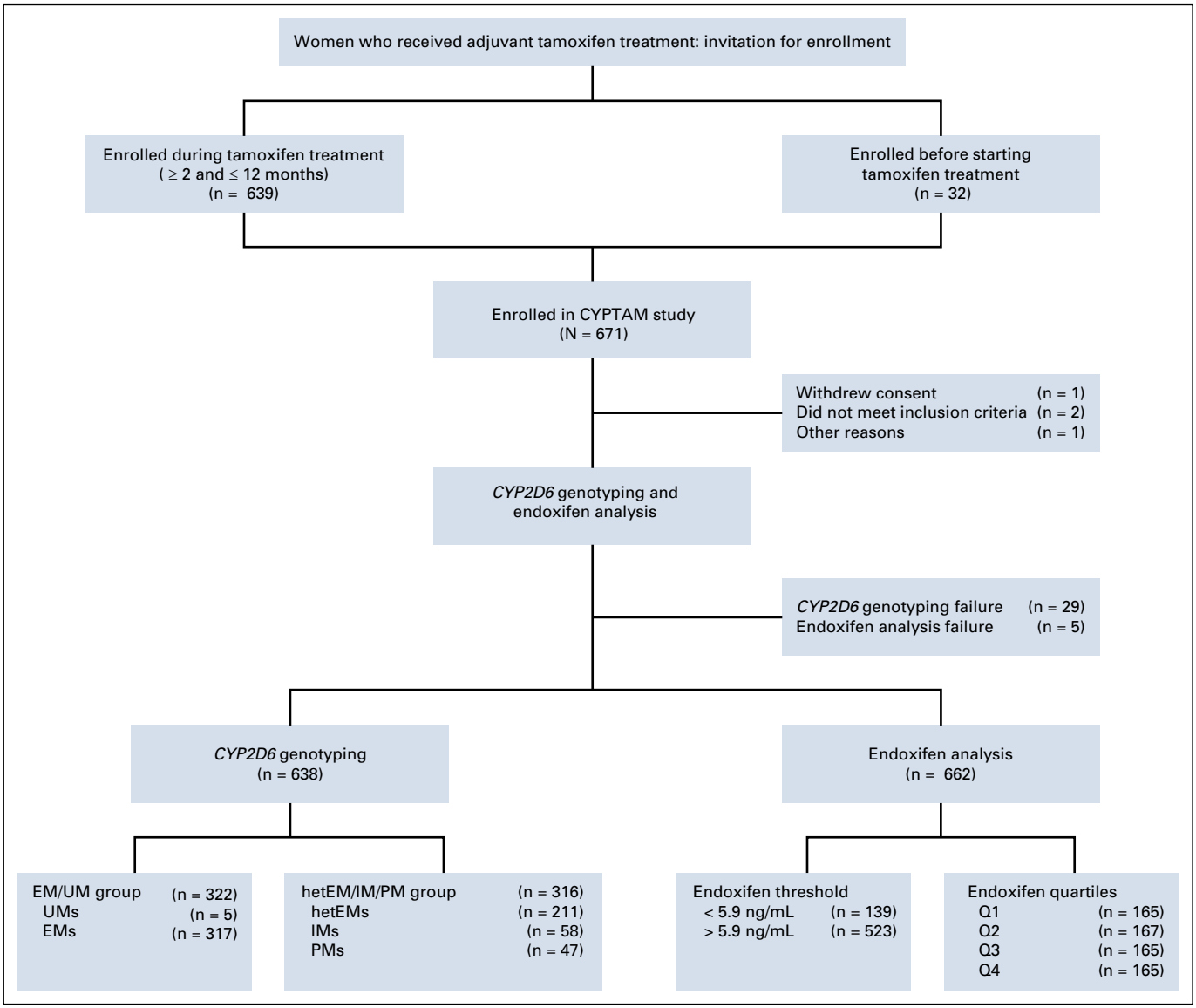

FIG 2. CONSORT diagram. EM, extensive metabolizer; hetEM, heterozygous extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; Q, quartile; UM, ultra-rapid metabolizer.

The primary objective was to determine the association of RFSt with endoxifen concentrations and *CYP2D6* genotypes (UMs and EMs *v* hetEMs, IMs, and PMs). The secondary objectives were to determine the relation between both endoxifen concentrations and *CYP2D6* genotypes (UMs and EMs *v* hetEMs, IMs, and PMs) and DFSt, RFSc, DFSc, and OS. This statistical analysis plan was designed as a gatekeeper analysis for the primary objective. Only in the case of an association between endoxifen and RFSt with $P < .05$ were the remaining objectives considered. Because of the multiple end points, the gatekeeper analysis was selected to avoid false-positive results by multiple testing[20,21] and take into account the biologic and pharmacologic rationales. In addition, an exploratory analysis investigating the association between RFSt and endoxifen concentrations and *CYP2D6* genotypes, accounting for the start of tamoxifen therapy, was performed.

Statistical Methods

To determine the association of RFSt with endoxifen concentrations and *CYP2D6* genotypes, Cox regression and Kaplan-Meier analyses were performed. Endoxifen concentrations were primarily analyzed as a continuous variable in a Cox regression analysis. In addition, other approaches were explored using the endoxifen threshold concentration of 5.9 ng/mL.[12] Also, we examined categorizing patients into quartiles according to the reported endoxifen concentrations. Thereafter, Cox regression analysis and Kaplan-Meier methods were used to investigate any potential associations. All analyses were conducted using SPSS software (version 23.0; SPSS,

Chicago, IL). Statistical significance was delimited as $P < .05$. In the Appendix, the statistical methods used are more extensively reported.

RESULTS

Baseline Characteristics, *CYP2D6* Genotypes, Endoxifen Concentrations, Tamoxifen Therapy, and Follow-Up

A total of 667 pre- and postmenopausal patients who were treated with adjuvant tamoxifen were enrolled in the CYPTAM study. Baseline characteristics of the enrolled patients suggested no differences across all the groups (Table 1), with the exception of progesterone receptor status in the comparison between *CYP2D6*-predicted phenotype groups. All *CYP2D6* genotype distributions were in Hardy-Weinberg equilibrium, with the exception of *CYP2D6*10* (Data Supplement). However, the genotype frequency of *CYP2D6*10* in the study population was in accordance with previous evidence.[22] Also, *CYP2D6* phenotypes and endoxifen concentrations were significantly associated (Data Supplement).

In this study, the median follow-up was 6.4 years (range, 0.10 to 9.30 years), and the median tamoxifen therapy duration was 2.5 years (range, 0.25 to 7.96 years). Representing approximately 66% of the patients, the most frequent endocrine treatment strategy was the initial use of tamoxifen with a subsequent switch to an AI. At enrollment, 95.1% of the patients were already receiving adjuvant tamoxifen, whereas 4.9% of the enrolled patients started tamoxifen therapy at baseline. Before enrollment in the CYPTAM study, the median tamoxifen therapy duration for patients already receiving tamoxifen was 0.37 years (range, 0.23 to 0.60 years).

Clinical Outcome and Endoxifen Concentrations

No significant association was found between endoxifen concentrations, used as continuous variable, and clinical outcome (RFSt) in either univariable (HR, 0.989; 95% CI, 0.945 to 1.035; $P = .627$; Table 2) or multivariable analysis (adjusted HR, 0.991; 95% CI, 0.946 to 1.038; $P = .691$; Table 2). Because no linear association or trend was observed, we explored other approaches. After dividing patients by endoxifen concentration below or above the suggested threshold of 5.9 ng/mL, again no association was observed (adjusted HR, 1.538; 95% CI, 0.719 to 3.290; $P = .267$; Table 2). In the same manner, after dividing patients according to their endoxifen concentrations into quartiles, no associations or trends were found when performing a log-rank test (quartiles: $P = .271$). These results were also verified by Cox regression analysis (Table 2). Similarly, the exploratory analysis investigating the association of RFSt with endoxifen concentrations from the start of tamoxifen therapy (instead of from actual study enrollment) did not alter these results (endoxifen concentrations [continuous variable]: adjusted HR, 0.986; 95% CI, 0.939 to 1.035; $P = .563$; endoxifen threshold of 5.9 ng/mL: adjusted HR, 1.422; 95% CI, 0.664 to 3.043; $P = .365$).

Clinical Outcome and *CYP2D6*-Predicted Phenotypes

Kaplan-Meier analysis did not reveal any differences in RFSt between UMs EMs versus hetEMs, IMs, and PMs (log-rank $P = .944$). In Figure 3, a Kaplan-Meier survival analysis is shown. Of note, an important decrease in the number of patients after the third year of treatment was observed, which is explained by the preplanned switch to an AI that took place in almost 66.0% of patients in the CYPTAM study. In line with the results noted in the log-rank test, Cox regression did not indicate significant differences in RFSt between *CYP2D6* phenotype groups in either univariable (HR, 1.020; 95% CI, 0.589 to 1.767; $P = .944$; Table 3) or multivariable analysis (adjusted HR, 0.929; 95% CI, 0.525 to 1.642; $P = .799$; Table 3). In addition, the exploratory analysis investigating the association between RFSt and *CYP2D6* genotype from the start of tamoxifen therapy (instead of from actual study enrollment) did not alter these findings (UMs and EMs *v* hetEMs, IMs, and PMs: adjusted HR, 0.962; 95% CI, 0.545 to 1.700; $P = .894$).

Gatekeeper Statistical Analysis Plan: Secondary Objectives

According to the gatekeeper analytic design chosen, no additional analyses of the secondary objectives were required, because we failed to find an association in the primary objective. However, we performed sensitivity analyses of the secondary objectives as an exploratory analysis. None of the secondary objectives were found to be statistically significant when analyzing the association between endoxifen concentrations or *CYP2D6* genotypes and the different end points (RFSt, DFSt, RFSc, DFSc, and OS; Data Supplement). Menopausal status was analyzed by categorizing patients by age (premenopausal: age $\leq$ 45 years; perimenopausal: age 45 to 55 years; postmenopausal: age $\geq$ 55 years). Similarly, no significant differences were observed (Data Supplement).

DISCUSSION

In this first prospective study, to our knowledge, low-activity *CYP2D6* genotypes and low endoxifen concentrations were not associated with a worse clinical survival outcome. In our opinion, these results are highly representative of clinical practice, because they illustrate the effect of tamoxifen on survival outcome based on the endocrine treatment strategy of switching to an AI after 2 or 3 years of tamoxifen treatment, widely used in the adjuvant setting in early-stage breast cancer. Also, the CYPTAM population had comparable survival and event rates to those observed in larger studies[23] and in clinical practice.[24]

A high number of clinical studies have assessed the putative association of low-activity *CYP2D6* genotypes with tamoxifen efficacy. In an attempt to resolve this ongoing controversy, the International Tamoxifen Pharmacogenomics Consortium[25] performed a meta-analysis with a

TABLE 1. Baseline Characteristics of CYPTAM Patients

Characteristic	No. (%)					
	CYP2D6-Predicted Phenotypes			Endoxifen Analysis, ng/mL		
	UMs/EMs (n = 322)	hetEMs/IMs/PMs (n = 316)	*P**	< 5.9 (n = 139)	> 5.9 (n = 523)	*P**
Age at enrollment, years			.061			.219
Mean	57.0	55.4		55.3	56.7	
SD	11.5	10.4		10.3	11.3	
Tumor stage			.566			.052
T1	163 (50.6)	175 (55.4)		77 (55.4)	277 (53.0)	
T2	142 (44.1)	122 (38.6)		61 (43.9)	211 (40.3)	
T3/T4	13 (4.0)	14 (4.4)		1 (0.7)	27 (5.2)	
Not specified	4 (1.2)	5 (1.6)		0 (0.0)	8 (1.5)	
Nodal stage			.188			.726
N0	148 (46.0)	152 (48.1)		68 (48.9)	247 (47.2)	
N1	141 (43.8)	117 (37.0)		52 (37.4)	213 (40.7)	
N2	23 (7.1)	30 (9.5)		15 (10.8)	42 (8.0)	
N3	8 (2.5)	16 (5.1)		4 (2.9)	19 (3.6)	
Not specified	2 (0.6)	1 (0.3)		0 (0.0)	2 (0.4)	
Histologic classification			.776			.887
Ductal adenocarcinoma	250 (77.6)	239 (75.6)		105 (75.5)	399 (76.3)	
Lobular adenocarcinoma	40 (12.4)	47 (14.9)		21 (15.1)	73 (14.0)	
Other	30 (9.3)	29 (9.2)		13 (9.4)	49 (9.4)	
Not specified	2 (0.6)	1 (0.3)		0 (0.0)	2 (0.4)	
Histologic grade			.872			.893
1	43 (13.4)	44 (13.9)		17 (12.2)	76 (14.5)	
2	186 (57.8)	173 (54.7)		80 (57.6)	297 (56.8)	
3	90 (28.0)	95 (30.1)		41 (29.5)	145 (27.7)	
Not specified	3 (0.9)	4 (1.3)		1 (0.7)	5 (1.0)	
Progesterone receptor status			.036			.297
Positive	255 (79.2)	251 (79.4)		112 (80.6)	415 (79.3)	
Negative	58 (18.0)	64 (20.3)		27 (19.4)	99 (18.9)	
Not specified	9 (2.8)	1 (0.3)		0 (0.0)	9 (1.7)	
HER2 receptor status			.899			.961
0+	190 (59.0)	192 (60.8)		85 (61.2)	320 (61.2)	
1+	86 (26.7)	80 (25.3)		35 (25.2)	131 (25.0)	
2+	16 (5.0)	19 (6.0)		8 (5.8)	27 (5.2)	
3+	28 (8.7)	24 (7.6)		11 (7.9)	43 (8.2)	
Not specified	2 (0.6)	1 (0.3)		0 (0.0)	2 (0.4)	
FISH			.754			.667
Positive (amplification)	30 (9.3)	26 (8.2)		14 (10.1)	45 (8.6)	
Negative	290 (90.1)	289 (91.5)		125 (89.9)	476 (91.0)	
Not specified	2 (0.6)	1 (0.3)		0 (0.0)	2 (0.4)	

(continued on following page)

TABLE 1. Baseline Characteristics of CYPTAM Patients (continued)

Characteristic	No. (%)					
	CYP2D6-Predicted Phenotypes			Endoxifen Analysis, ng/mL		
	UMs/EMs (n = 322)	hetEMs/IMs/PMs (n = 316)	P*	< 5.9 (n = 139)	> 5.9 (n = 523)	P*
Surgery			.864			.978
Mastectomy	146 (45.3)	150 (47.5)		65 (46.8)	243 (46.5)	
Breast conserving	174 (54.0)	164 (51.9)		73 (52.5)	277 (53.0)	
Not specified	2 (0.6)	2 (0.6)		1 (0.7)	3 (0.6)	
Surgery axilla			.857			.897
Sentinel node procedure only	156 (48.4)	160 (50.6)		67 (48.2)	263 (50.3)	
Axillary lymph node dissection	164 (50.9)	154 (48.7)		71 (51.1)	257 (49.1)	
Not specified	2 (0.6)	2 (0.6)		1 (0.7)	3 (0.6)	
Adjuvant radiotherapy			.092			.256
Yes	210 (65.2)	231 (73.1)		104 (74.8)	356 (68.1)	
No	110 (34.2)	84 (26.6)		35 (25.2)	165 (31.5)	
Not specified	2 (0.6)	1 (0.3)		0 (0.0)	2 (0.4)	
Adjuvant chemotherapy			.608			.500
Yes	193 (59.9)	200 (63.3)		90 (64.7)	315 (60.2)	
No	127 (39.4)	115 (36.4)		49 (35.3)	206 (39.4)	
Not specified	2 (0.6)	1 (0.3)		0 (0.0)	2 (0.4)	
Trastuzumab treatment			.746			.510
Yes	31 (9.6)	25 (7.9)		12 (8.6)	46 (8.8)	
No	288 (89.4)	288 (91.1)		127 (91.4)	472 (90.2)	
Not specified	3 (0.9)	3 (0.9)		0 (0.0)	5 (1.0)	
Reason for tamoxifen discontinuation, years						
Adverse effects						
Median	1.72	1.99		1.77	1.86	
SD	1.33	1.15		1.16	1.28	
Regular switch to AI						
Median	2.60	2.60		2.55	2.60	
SD	0.87	1.00		0.92	0.94	
Disease recurrence						
Median	2.30	2.09		1.78	2.28	
SD	1.20	1.13		0.69	1.18	
Other						
Median	4.96	4.48		4.23	4.78	
SD	1.44	1.71		1.61	1.56	

Abbreviations: AI, aromatase inhibitor; EM, extensive metabolizer; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; hetEM, heterozygous extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; SD, standard deviation; UM, ultra-metabolizer.

*In all cases, no missing data were included when comparing groups.

large number of patients, in which a small effect was observed, with CYP2D6 PMs being associated with worse survival (HR, 1.25; 95% CI, 1.06 to 1.47; $P = .009$). Because of strict selection criteria and the exclusion of large studies such as the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial,[26] the BIG (Breast International Group) 1-98 trial,[27] and the TEAM (Tamoxifen and Exemestane in Early Breast Cancer) trial,[28] this small effect has been questioned. At first, the ATAC and BIG 1-98 trials were ruled out because of the use of tumor as a source of DNA. Even so, a study by our group used tumor-derived DNA but rejected loss of heterozygosity by studying microsatellite

TABLE 2. Cox Proportional Hazards Model of RFSt

		Univariable Analysis			Multivariable Analysis*		
Endoxifen Analysis	**No. of Patients**	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Endoxifen, ng/mL (as continuous variable)	662	0.989	0.945 to 1.035	.627	0.991	0.946 to 1.038	.691
Endoxifen threshold, ng/mL							
< 5.9	139	1.000	Reference		1.000	Reference	
> 5.9	523	1.426	0.673 to 3.021	.354	1.538	0.719 to 3.290	.267
Endoxifen concentration by quartile, ng/mL							
Q1 (< 6.6)	165	1.000	Reference	.282	1.000	Reference	.187
Q2 (6.6-10.3)	167	1.820	0.851 to 3.891	.123	1.986	0.909 to 4.340	.085
Q3 (10.3-14.1)	165	1.314	0.583 to 2.959	.510	1.331	0.580 to 3.059	.500
Q4 (> 14.1)	165	0.977	0.415 to 2.302	.957	0.950	0.399 to 2.262	.907
CYP2D6-predicted phenotypes							
UMs/EMs	322	1.00	Reference		1.00	Reference	
hetEMs/IMs/PMs	316	1.020	0.589 to 1.767	.944	0.929	0.525 to 1.642	.799

Abbreviations: EM, extensive metabolizer; hetEM, heterozygous extensive metabolizer; HR, hazard ratio; IM, intermediate metabolizer; PM, poor metabolizer; Q, quartile; RFSt, relapse-free survival (censored at the time of tamoxifen discontinuation); UM, ultra-metabolizer.

*Adjusted for human epidermal growth factor receptor 2/neu status, histologic grade and classification, tumor size, and nodal stage.

markers flanking the *CYP2D6* gene.[28] Another major concern about the studies published is their retrospective nature, which may have introduced bias. In addition, none of these studies was specifically designed for the purpose of determining such an association, and this may have affected data retrieval.

Many studies have shown that low-activity *CYP2D6* genotypes result in lower endoxifen concentrations compared with EMs.[12] As a consequence, endoxifen concentrations have been postulated to be a predictor of tamoxifen efficacy.[29] In a few retrospective studies, patients with low endoxifen concentrations were associated with worse survival outcomes.[12-14] Madlensky et al[12] suggested a threshold for endoxifen of 5.9 ng/mL, above of which patients had at least a 26% lower chance of relapse (HR, 0.74; 95% CI, 0.55 to 1.00). Following this approach, we also divided our cohort into two groups (below and above 5.9 ng/mL). In our comparison, no significant differences were found. However, we also explored the possibility of dividing our cohort into quartiles, but again, no statistical associations were observed. In line with our results, a recent study analyzed the role of endoxifen therapeutic drug monitoring in the locally advanced and metastatic settings.[30] Remarkably, the objective response rate in the lowest quartile (0.7 to 6.9 μg/L) was not significantly different compared with that of the higher quartiles.

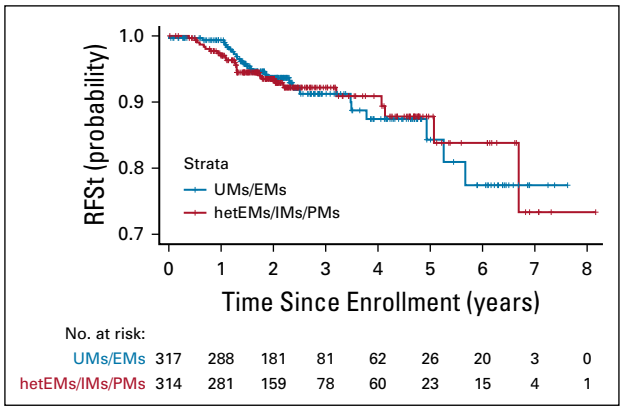

FIG 3. Kaplan-Meier curve for relapse-free survival (censored at the time of tamoxifen discontinuation; RFSt). EM, extensive metabolizer; hetEM, heterozygous extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer.

In this study, the sample size of 650 patients was based upon the studies of Goetz et al[7] and Gonzalez-Santiago et al,[19] and we assumed an HR of 2.0. Therefore, we cannot formally exclude a smaller effect (HR < 2.0) of *CYP2D6* genotypes or endoxifen concentrations on clinical outcome.

In the last 10 years, Dutch guidelines have recommended a switch to an AI after 2 or 3 years of tamoxifen therapy for postmenopausal women.[1,2] We are aware that the complete follow-up is relatively short, considering the possibility of disease recurrence in luminal breast cancer up to 20 years after diagnosis.[31] In the CYPTAM study, in approximately 66.0% of patients, the possible detrimental effect of low endoxifen levels may have been weakened because of switching to an AI. However, because we censored patients at the time of tamoxifen discontinuation, potential effect modification by AI use was avoided, because only survival time during tamoxifen therapy was counted, and we could separate the effect of tamoxifen exposure from the effect of AI exposure. Along the same lines, we also examined RFSc,

TABLE 3. Cox Proportional Hazards Model of RFSt: Univariable and Multivariable Analyses

		Univariable Analysis			Multivariable Analysis*		
Variable	**No. of Patients**	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age at enrollment, years	666	1.017	0.994 to 1.040	.146			
Tumor size							
T1	356	1.000	Reference	.316	1.00	Reference	.416
T2	274	1.529	0.880 to 2.657	.132	1.122	0.626 to 2.013	.698
T3/T4	28	1.419	0.424 to 4.745	.570	0.474	0.123 to 1.826	.260
Nodal status							
N0	317	1.000	Reference	.053	1.00	Reference	.249
N1	266	1.610	0.867 to 2.988	.131	1.714	0.909 to 3.233	.096
N2	57	2.388	1.029 to 5.542	.043	2.240	0.956 to 5.248	.063
N3	24	3.342	1.23 to 9.081	.018	2.797	0.959 to 8.157	.060
Grade							
1	94	1.000	Reference	.420	1.00	Reference	.115
2	378	0.899	0.409 to 1.977	.792	0.621	0.275 to 1.404	.252
3	188	1.330	0.580 to 3.051	.500	1.115	0.474 to 2.625	.803
HER status							
Negative	605	1.000	Reference		1.00	Reference	
Positive	59	1.402	0.634 to 3.101	.404	1.402	0.578 to 3.403	.455
Histologic classification							
Ductal classification	508	1.000	Reference		1.000	Reference	
Lobular classification	94	3.435	1.927 to 6.121	< .001	4.015	2.014 to 8.003	< .001
Other	62	1.139	0.403 to 3.222	.806	1.125	0.339 to 3.738	.848
Progesterone status							
Negative	127	1.000	Reference				
Positive	530	0.630	0.337 to 1.175	.146			
Surgery							
Mastectomy	310	1.00	Reference				
Breast conserving	352	1.523	0.879 to 2.640	.134			
Surgery axilla							
Sentinel node procedure	333	1.00	Reference				
Axillary lymph node dissection	329	1.523	0.879 to 2.640	.134			
Chemotherapy							
No	257	1.000	Reference				
Yes	407	1.084	0.613 to 1.916	.781			
Radiotherapy							
No	202	1.000	Reference				
Yes	462	0.793	0.455 to 1.383	.414			
CYP2D6-predicted phenotypes							
UMs/EMs	322	1.00	Reference		1.00	Reference	
hetEMs/IMs/PMs	316	1.020	0.589 to 1.767	.944	0.929	0.525 to 1.642	.799

Abbreviations: EM, extensive metabolizer; HER, human epidermal growth factor receptor; hetEM, heterozygous extensive metabolizer; HR, hazard ratio; IM, intermediate metabolizer; PM, poor metabolizer; Q, quartile; RFSt, relapse-free survival (censored at the time of tamoxifen discontinuation); UM, ultra-metabolizer.

*Adjusted for HER2/neu status, histologic grade and classification, tumor size, and nodal stage.

including during AI therapy and after stopping endocrine therapy, enabling the inclusion of later recurrences. This latter analysis is specifically useful, because it reflects the common practice of postmenopausal patients starting with tamoxifen and subsequently switching to an AI after 2 to 3 years of treatment. Therefore, whether these results could be extrapolated to other tamoxifen regimens remains uncertain, as does the impact of these results in the long term.

Menopausal status is clinically relevant but was not available for a majority of women in our study. To evaluate any influence of menopausal status on outcome, we used age group status as a proxy. Although correcting for menopausal status only slightly modified our results, we still failed to find an association between *CYP2D6* genotypes or endoxifen concentrations and clinical outcome.

A potential limitation of the study could be the study design, with a risk of selection bias resulting from the inclusion of patients who started with tamoxifen therapy up to 12 months before study enrollment. As a consequence, early recurrences may have been missed. However, the analysis shows that the association of RFSt, starting from the beginning of tamoxifen treatment, only slightly modified our results and did not yield a statistically significant association. Therefore, we believe selection bias is minimal. Similarly, the missed percentage of early recurrences is deemed low (< 1%).[32] Indeed, our recurrence rate during the first year (2.1%) is highly similar to those reported in larger studies,[28,32] indicating a minimal effect of early recurrences. Moreover, 2 months of tamoxifen therapy is required to assure steady-state concentrations before the effect of tamoxifen can be evaluated, and therefore, any early recurrence before this time would be unlikely to be explained by tamoxifen exposure. Overall, we expect that selection bias as a result of the chosen study design was minimal and unlikely to have changed our results or conclusions. Also, no testing for tamoxifen adherence could possibly be a limitation, because it was not directly measured. Although data on tamoxifen adherence were lacking, information on persistence was well recorded. A comparison of mean endoxifen concentrations between patients who discontinued tamoxifen earlier because of adverse effects and those who did not stop did not significantly differ (adverse effects: 11.17 ng/mL *v* no adverse effects: 10.96 ng/mL; $P = .335$).

A potential limitation of this study could be the lack of accurate information on concomitant CYP2D6 inhibitor use during tamoxifen therapy. This concomitant use could bias the analysis between *CYP2D6* genotypes and RFSt, because it could have led to misclassification of *CYP2D6* phenotypes. However, the influence of potent CYP2D6 inhibitors was estimated to be low, because concomitant CYP2D6 inhibitors are infrequently prescribed in the Netherlands,[33] especially in the last decade, because of greater awareness of the potential interactions among prescribers. According to the Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2D6 and tamoxifen therapies,[34] potent CYP2D6 inhibitors are contraindicated during tamoxifen therapy.

A more accurately predicted *CYP2D6* phenotype by genotype and comedication is still merely a proxy for endoxifen concentration. When determining the relation between endoxifen concentrations and clinical outcomes, we are only interested in steady-state endoxifen concentrations, regardless of the cause of lower endoxifen concentrations (eg, genotype and concomitant CYP2D6 inhibitor use). The lack of information regarding comedication would be unlikely to have influenced the association between endoxifen concentrations and clinical outcomes.

In conclusion, the results of this prospective, specifically designed study do not support *CYP2D6* genotyping to guide tamoxifen treatment in the adjuvant setting. Similarly, our data do not justify therapeutic drug monitoring based on endoxifen concentrations in patients with breast cancer receiving tamoxifen.

AFFILIATIONS

[1]Leiden University Medical Center, Leiden, the Netherlands
[2]Netherlands Cancer Institute/Antoni van Leeuwenhoek, Amsterdam, the Netherlands
[3]Alrijne Leiden, Leiden, the Netherlands
[4]Reinier de Graaf Gasthuis, Delft, the Netherlands
[5]Medical Center Alkmaar, Alkmaar, the Netherlands
[6]Zaans Medisch Centrum, Zaandam, the Netherlands
[7]Alrijne Leiderdorp, Leiderdorp, the Netherlands
[8]Katholieke Universiteit Leuven, Leuven, Belgium
[9]St Antonius Ziekenhuis, Nieuwegein, the Netherlands
[10]University Hospitals Leuven, Leuven, Belgium

CORRESPONDING AUTHOR

Henk-Jan Guchelaar, PharmD, PhD, Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Albinusdreef 2 2300 RC Leiden, the Netherlands; e-mail: h.j.guchelaar@lumc.nl.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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AUTHOR CONTRIBUTIONS

Conception and design: Vincent Dezentjé, Hans Gelderblom, Henk-Jan Guchelaar

Financial support: Hans Gelderblom

Administrative support: Anabel Sanchez-Spitman, Vincent Dezentjé, Lynn Jongen

Provision of study material or patients: Vincent Dezentjé, Jesse Swen, Carolien Smorenburg, Aart van Bochove, Anneke Zeillemaker, Lynn Jongen, Hans Gelderblom

Collection and assembly of data: Anabel Sanchez-Spitman, Vincent Dezentjé, Jesse Swen, Dirk Jan A.R. Moes, Erdogan Batman, Edith van Druten, Carolien Smorenburg, Aart van Bochove, Anneke Zeillemaker, Lynn Jongen, Maartje Los, Patrick Neven

Data analysis and interpretation: Anabel Sanchez-Spitman, Vincent Dezentjé, Jesse Swen, Dirk Jan A.R. Moes, Stefan Böhringer, Maartje Los, Patrick Neven, Hans Gelderblom, Henk-Jan Guchelaar

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REFERENCES

1. Burstein HJ, Temin S, Anderson H, et al: Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: American Society of Clinical Oncology clinical practice guideline focused update. J Clin Oncol 32:2255-2269, 2014
2. Senkus E, Kyriakides S, Ohno S, et al: Primary breast cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 26(Suppl 5): v8-v30, 2015
3. Knowledge Institute of Medical Specialists: Dutch Breast Cancer - Hormonal Therapy - Guideline - Richtlijnendatabase. https://richtlijnendatabase.nl/en/richtlijn/breast_cancer/adjuvant_systemic_therapy/hormonal_therapy.html
4. Stearns V, Johnson MD, Rae JM, et al: Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst 95:1758-1764, 2003
5. Jin Y, Desta Z, Stearns V, et al: CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 97:30-39, 2005
6. Sanchez-Spitman AB, Moes DA, Gelderblom H, et al: The effect of rs5758550 on CYP2D6*2 phenotype and formation of endoxifen in breast cancer patients using tamoxifen. Pharmacogenomics 18:1125-1132, 2017
7. Goetz MP, Rae JM, Suman VJ, et al: Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. J Clin Oncol 23:9312-9318, 2005
8. Mürdter TE, Schroth W, Bacchus-Gerybadze L, et al: Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. Clin Pharmacol Ther 89:708-717, 2011
9. Borges S, Desta Z, Li L, et al: Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: Implication for optimization of breast cancer treatment. Clin Pharmacol Ther 80:61-74, 2006
10. Hertz DL, Deal A, Ibrahim JG, et al: Tamoxifen dose escalation in patients with diminished CYP2D6 activity normalizes endoxifen concentrations without increasing toxicity. Oncologist 21:795-803, 2016
11. Dezentjé VO, Opdam FL, Gelderblom H, et al: CYP2D6 genotype- and endoxifen-guided tamoxifen dose escalation increases endoxifen serum concentrations without increasing side effects. Breast Cancer Res Treat 153:583-590, 2015
12. Madlensky L, Natarajan L, Tchu S, et al: Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. Clin Pharmacol Ther 89: 718-725, 2011
13. Saladores P, Mürdter T, Eccles D, et al: Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics J 15:84-94, 2015
14. Helland T, Henne N, Bifulco E, et al: Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Res 19:125, 2017
15. Goetz MP, Ingle JN: CYP2D6 genotype and tamoxifen: Considerations for proper nonprospective studies. Clin Pharmacol Ther 96:141-144, 2014
16. The CYPTAM study: Effect of CYP2D6 genotype on pharmacokinetics and clinical outcome in tamoxifen treated breast cancer patients. http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=1509
17. Schroth W, Goetz MP, Hamann U, et al: Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. JAMA 302:1429-1436, 2009
18. Teunissen SF, Rosing H, Koornstra RH, et al: Development and validation of a quantitative assay for the analysis of tamoxifen with its four main metabolites and the flavonoids daidzein, genistein and glycitein in human serum using liquid chromatography coupled with tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 877:2519-2529, 2009
19. Gonzalez-Santiago S, Zárate R, Haba-Rodríguez J, et al: CYP2D6*4 polymorphism as blood predictive biomarker of breast cancer relapse in patients receiving adjuvant tamoxifen. J Clin Oncol 25, 2007 (suppl; abstr 590)
20. Dmitrienko A, Tamhane AC: Gatekeeping procedures with clinical trial applications. Pharm Stat 6:171-180, 2007
21. Dmitrienko A, Tamhane AC, Wang X, et al: Stepwise gatekeeping procedures in clinical trial applications. Biom J 48:984-991, 2006
22. Bradford LD: CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics 3:229-243, 2002
23. van de Velde CJ, Rea D, Seynaeve C, et al: Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): A randomised phase 3 trial. Lancet 377: 321-331, 2011
24. Chen L, Linden HM, Anderson BO, et al: Trends in 5-year survival rates among breast cancer patients by hormone receptor status and stage. Breast Cancer Res Treat 147:609-616, 2014
25. Province MA, Goetz MP, Brauch H, et al: CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. Clin Pharmacol Ther 95:216-227, 2014
26. Rae JM, Drury S, Hayes DF, et al: CYP2D6 and UGT2B7 genotype and risk of recurrence in tamoxifen-treated breast cancer patients. J Natl Cancer Inst 104: 452-460, 2012

27. Regan MM, Leyland-Jones B, Bouzyk M, et al: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: The Breast International Group 1-98 trial. J Natl Cancer Inst 104:441-451, 2012
28. Dezentjé VO, van Schaik RH, Vletter-Bogaartz JM, et al: CYP2D6 genotype in relation to tamoxifen efficacy in a Dutch cohort of the tamoxifen exemestane adjuvant multinational (TEAM) trial. Breast Cancer Res Treat 140:363-373, 2013
29. Binkhorst L, Mathijssen RH, Jager A, et al: Individualization of tamoxifen therapy: Much more than just CYP2D6 genotyping. Cancer Treat Rev 41:289-299, 2015
30. Neven P, Jongen L, Lintermans A, et al: Tamoxifen metabolism and efficacy in breast cancer: A prospective multicentre trial. Clin Cancer Res 24:2312-2318, 2018
31. Pan H, Gray R, Braybrooke J, et al: 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. N Engl J Med 377:1836-1846, 2017
32. Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Aromatase inhibitors versus tamoxifen in early breast cancer: Patient-level meta-analysis of the randomised trials. Lancet 386:1341-1352, 2015
33. Dezentjé VO, van Blijderveen NJ, Gelderblom H, et al: Effect of concomitant CYP2D6 inhibitor use and tamoxifen adherence on breast cancer recurrence in early-stage breast cancer. J Clin Oncol 28:2423-2429, 2010
34. Goetz MP, Sangkuhl K, Guchelaar HJ, et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 and tamoxifen therapy. Clin Pharmacol Ther 103:770-777, 2018

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study

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Other Relationship: Daiichi Sankyo

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APPENDIX

Collaborating hospitals and local investigators: Leiden University Medical Center, Gelderblom and V.O. Dezentjé; Alrijne Leiden, R. Vree and E. Batman; Reinier de Graaf Groep, E.M. Maartense; Noordwest Ziekenhuisgroep, C. Smorenburg; St Antonius Ziekenhuis, M. Los; Rijnland Ziekenhuis, A.M. Zeillemaker; Zaans Medisch Centrum, A. Van Bochove; Waterland Ziekenhuis, J.A.C. Brakenhoff; IJsselland Ziekenhuis, D.F.S. Kehrer; Spaarne Gasthuis, P. Kuijer; Meander Medisch Centrum, H.J. Bloemendal; Tergooi Ziekenhuizen, S.A. Luijkx; HMC Bronovo Ziekenhuis, N.I. Weijl; Langeland Ziekenhuis, Goossens; Diakonessenhuis Utrecht, D. Ten Bokkel Huinink; HAGA Ziekenhuis, J.W.S. Merkus; St Antonius Ziekenhuis, M.J. Verhaar; Groene Hart Ziekenhuis, A. Van der Torren; Amstelland Ziekenhuis, A. H. Baan; and UZ Leuven (also on behalf of the other Belgian hospitals), P. Neven.

Tamoxifen and Metabolite Measurements and *CYP2D6* Genotyping

Steady-state trough levels of tamoxifen and its metabolites were measured with high-performance liquid chromatography–tandem mass spectrometry.[18]

The considered *CYP2D6*-predicted phenotypes are: ultra-rapid (duplication of fully active alleles), extensive (with two fully active alleles, nowadays indicated as normal metabolizers), heterozygous extensive (with one fully active allele and one nonactive allele), intermediate (with two low activity alleles or a combination of one low activity allele and one inactive allele), and poor metabolizer (with two inactive alleles). Alleles with decreased *CYP2D6* activity were **9*, **10*, **17*, **29*, **36*, **41*, **10xN*, **17xN*, and **41xN*, whereas *CYP2D6* inactive alleles were **3*, **4*, **5*, **6*, **7*, **8*, **11*, **14A*, **15*, **19*, **20*, **40*, and **4xN*.

Statistical Methods and Analysis

The distribution of the relapse-free survival (censored at the time of tamoxifen discontinuation) end point was estimated overall using Kaplan-Meier analysis, whereas differences between *CYP2D6* genotypes and endoxifen concentrations were assessed by a log-rank test. Fisher's exact test was used to evaluate baseline characteristics between *CYP2D6* phenotype groups and endoxifen groups. To evaluate differences in allele frequencies of *CYP2D6* and deviations from Hardy-Weinberg equilibrium, χ^2 tests were carried out.

Cox regression analyses were performed in two steps. First, a univariable analysis was carried out, and then a multivariable analysis was performed. Only covariables with $P < .1$ in the univariable analysis were included in the multivariable analysis. In any case, histologic grade, tumor size, nodal status, and human epidermal growth factor receptor 2/neu status were used in the multivariable analysis because of their known clinical relevance.

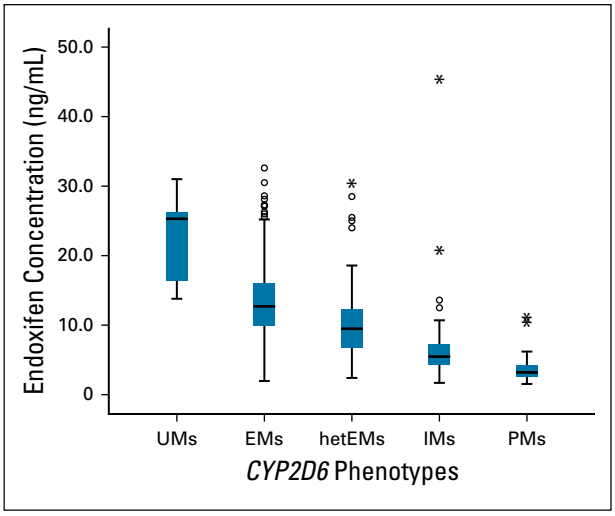

FIG A1. Endoxifen concentration in *CYP2D6* phenotypes. (*) A total of 42.3% of the variance of endoxifen serum concentration is explained by *CYP2D6*-predicted phenotypes. EMs, extensive metabolizers; hetEMs, heterozygous extensive metabolizers; IMs, intermediate metabolizers; PMs, poor metabolizers; UMs, ultra-metabolizers.