

ARTICLE

The Influences of Adherence to Tamoxifen and *CYP2D6* Pharmacogenetics on Plasma Concentrations of the Active Metabolite (Z)-Endoxifen in Breast Cancer

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Tamoxifen efficacy in breast cancer is suspected to depend on adherence and intact drug metabolism. We evaluated the role of adherence behavior and pharmacogenetics on the formation rate of (Z)-endoxifen. In 192 Brazilian patients, we assessed plasma levels of tamoxifen and its metabolites at 3, 6, and 12 months of treatment (liquid-chromatography tandem mass spectrometry), adherence behavior (Morisky, Green, and Levine medication adherence scale), and cytochrome P450 2D6 (*CYP2D6*) and other pharmacogene polymorphisms (matrix-assisted laser-desorption-ionization time of flight) mass spectrometry, real-time polymerase chain reaction). Adherence explained 47% of the variability of tamoxifen plasma concentrations ($P < 0.001$). Although *CYP2D6* alone explained 26.4%, the combination with adherence explained 40% of (Z)-endoxifen variability at 12 months ($P < 0.001$). The influence of low adherence to not achieving relevant (Z)-endoxifen levels was highest in patients with noncompromised *CYP2D6* function (relative risk 3.65; 95% confidence interval 1.48–8.99). As a proof-of-concept, we demonstrated that (Z)-endoxifen levels are influenced both by patient adherence to tamoxifen and *CYP2D6*, which is particularly relevant for patients with full *CYP2D6* function.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Poor patient adherence to tamoxifen is associated with reduced clinical efficacy. cytochrome P450 2D6 (*CYP2D6*) is a key factor of tamoxifen metabolism, however, genetic variants only partially explain the variability of plasma concentrations of the active metabolite (Z)-endoxifen.

WHAT QUESTION DID THIS STUDY ADDRESS?

We studied the influence of patient adherence behavior and *CYP2D6* phenotype (and other pharmacogenes) on plasma metabolite concentrations. We investigated whether (Z)-endoxifen concentrations depended on either factor alone or in combination.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Adherence and *CYP2D6* status are independent determinants of tamoxifen and (Z)-endoxifen plasma levels. Their combined influence is particularly relevant for patients with full *CYP2D6* function.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The dual monitoring of tamoxifen (surrogate for adherence) and (Z)-endoxifen (surrogate for clinical response) plasma levels could be a strategy to improve tamoxifen outcome in the future.

One-third of patients with early breast cancer with estrogen receptor (ER)-positive tumors treated with the selective estrogen receptor modulator tamoxifen either relapse or die from the disease in the following decade.^{1,2} Improvement of tamoxifen efficacy requires a better knowledge of factors determining outcome of which the medication-

taking behavior (i.e., adherence), is a strong suspect.^{3,4} Discontinuation and nonadherence to tamoxifen are frequent and result in increased mortality.^{5–9} A large study using automated pharmacy records showed that 31% of patients discontinued therapy, and among those who continued, only 70% adhered at 4.5 years with only 50% being

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fully adherent.¹⁰ Nonadherence gradually evolves over time from 10% in the first year to > 50% in the fifth year^{6,8,10,11} being most pronounced in young women being below 40 years of age and women older than 75 years.¹⁰ Ten-year survival rates significantly differed between patients who continued treatment (82%) compared with patients who did not adhere (78%).¹¹ Recently, the BIG I-98 trial reported a considerably reduced disease-free survival (hazard ratio 1.45; 95% confidence interval (CI) 1.09–1.93) for patients who ceased protocol-assigned endocrine treatment.¹²

To assess drug adherence, commonly used prescription-refill patterns infer that the medication is taken exactly as prescribed, yet, partial adherence or actual use of the medication cannot be controlled.¹³ In contrast, an objective surrogate of tamoxifen adherence is the monitoring of drug concentrations, including the parent drug and its active metabolite (Z)-endoxifen. (Z)-endoxifen can be easily measured in the patients' plasma, however, individual concentrations depend on pharmacogene polymorphisms, particularly those of the liver enzyme cytochrome P450 2D6 (CYP2D6).¹⁴ Polymorphisms are responsible for different phenotypes, which aside from individuals with normal CYP2D6 function (extensive metabolizer (EM)), comprise individuals with absent (poor metabolizer (PM)), reduced (intermediate metabolizer (IM)), and increased (ultrarapid metabolizer (UM)) CYP2D6 activity. Among Europeans, the frequency of PM and IM individuals is 9% and 40%, respectively. In Brazil, the observed PM frequency is 2.5% with an increased 4% frequency in patients with ER-positive breast cancer.^{15,16}

For patients receiving tamoxifen therapy, CYP2D6 genotyping allows the prediction of (Z)-endoxifen plasma concentrations.^{17–19} A putative threshold of 5.9 ng/mL has been proposed in the Women's Healthy Eating and Living (WHEL) study suggesting that a minimal concentration threshold is required above which (Z)-endoxifen is more effective against the recurrence of breast cancer and below which patients are at higher risk for recurrence,²⁰ likely due to incomplete inhibition of ER-dependent growth signaling.²¹ Although several outcome studies demonstrated the importance of CYP2D6 genotyping for the prediction of the risk-to-relapse in adjuvant and metastatic settings,^{22–25} other studies did not confirm this association,^{26–29} the reason why current clinical guidelines do not support the use of CYP2D6 genotypes for predicting tamoxifen response.³⁰ Therefore, it is important to identify confounders that may mask the tamoxifen CYP2D6 association, with tamoxifen adherence being a prime candidate.¹³ The combined analysis of adherence behavior and tamoxifen metabolism may shed new light on this important issue, particularly because first evidence has been reported of an increased effect of CYP2D6 genotype on patient outcome when adjusted for adherence to tamoxifen therapy.³¹ Here, we present a prospective study of mainly patients with early breast cancer from Brazil, in which interview-informed tamoxifen adherence together with CYP2D6 metabolizer status have been measured in order to evaluate their combined contribution to the lowering of the patients' plasma (Z)-endoxifen concentrations to potentially subtherapeutic levels. The study demonstrates how patients with breast cancer of underserved patient populations can contribute

valuable pharmacokinetic and pharmacogenetic information in the field of breast cancer biomarker research.

MATERIALS AND METHODS

Patients and study design

Patients were consecutively recruited at the Erasto Gaertner Hospital, Curitiba, Southern Brazil, a national reference center for oncology treatment. Between April 2014 and June 2017, 192 patients with ER-positive breast cancer treated with tamoxifen were included to investigate the relevance of drug adherence on the plasma levels of active tamoxifen metabolites. According to recommendations for tumor marker studies (REMARK),³² inclusion criteria were defined as women aged 18 years or older who were diagnosed with any stage, histologic, or molecular subtype of ER-positive breast cancer, and who started daily treatment with 20 mg tamoxifen for an intended 5 years of therapy. Exclusion criteria were age beyond 82 years and patients unable to complete the study schedule and questionnaire. Patients were followed during the first year of treatment at months 3, 6, and 12 for the assessment of adherence to tamoxifen intake based on interview, measurement of plasma levels of tamoxifen and its metabolites, and assessment of CYP2D6 metabolizer status (and other relevant drug metabolizing enzymes (DMEs)) based on genotypes. Ethical approval was obtained from the Brazilian National Commission of Ethical Research. All patients provided written informed consent. Study size calculation (99.9% power) revealed a minimum of 42 patients required to detect an association of (Z)-endoxifen variability with CYP2D6 polymorphism, based on the prevalence of IM and PM patients of 40% in Brazil³³ and an expected effect size of $R^2 = 0.4$.¹⁷

Assessment of adherence to tamoxifen therapy

Tamoxifen adherence was assessed using the Morisky, Green, and Levine Medication Adherence Scale questionnaire, a structured four-item self-reported adherence measure validated for a wide range of diseases, including cancer.³⁴ This questionnaire has been successfully used in patients with low literacy and its feasibility was previously confirmed in our hospital for inpatients and outpatients with cancer.³⁵ Four trained pharmacists performed the questionnaire-based interviews at 3, 6, and 12 months after starting tamoxifen therapy and included the following questions: (i) Do you ever forget to take your medicines?; (ii) Are you careless at times about taking your medicines?; (iii) When you feel better do you sometimes stop taking your medicine?; and (iv) Sometimes, if you feel worse when you take the medicine, do you stop taking it? For each patient and visit "yes" (1 point) or "no" (0 point) answers were documented and the four-item scores summed up to define three adherence levels: high (0), medium (1–2), and low adherence (3–4). Information on concomitant medication and self-reported adverse events during tamoxifen treatment were recorded during all visits.

Genotyping and quantification of tamoxifen and its active metabolites from plasma

Genomic DNA obtained from peripheral blood mononuclear cells was genotyped for CYP2D6 polymorphisms as

Table 1 Adherence behavior to tamoxifen treatment (20 mg daily) of Brazilian patients with breast cancer in relation to their demographic and clinical characteristics

Characteristic	Overall distribution (n = 192)	Adherence at 3 months (n = 163)			Adherence at 6 months (n = 173)			Adherence at 12 months (n = 170)					
		High, n (%)	Medium/low, n (%)	OR 95% CI	High, n (%)	Medium/low, n (%)	OR 95% CI	High, n (%)	Medium/low, n (%)	OR 95% CI			
Ethnicity, ^a n (%)													
White	139 (72.4)	89 (74.8)	30 (25.2)	0.804 (0.37–1.73)	0.689	98 (79.7)	25 (20.3)	2.019 (0.97–4.20)	0.078	78 (62.9)	46 (37.1)	0.904 (0.45–1.84)	0.859
Black	11 (5.7)	31 (70.5)	13 (29.5)			33 (66.0)	17 (34.0)			30 (65.2)	16 (34.8)		
Pardo	37 (19.3)												
Asian/Indian	5 (2.6)												
Educational level, ^b n (%)													
None or incomplete basic	68 (35.4)	46 (76.7)	14 (23.3)	0.777 (0.37–1.62)	0.582	48 (77.4)	14 (22.6)	1.157 (0.56–2.41)	0.853	40 (67.8)	19 (32.2)	1.331 (0.68–2.59)	0.503
Basic	50 (26.0)	74 (71.8)	29 (28.2)			83 (74.8)	28 (25.2)			68 (61.3)	43 (38.7)		
Upper secondary	53 (27.6)												
University (Bachelor/ Master/Doctoral)	21 (10.9)												
Age at diagnosis, median (range), years	51.5 (24–82)	54 (33–82)	47 (28–76)		< 0.001	54 (24–82)	49 (32–77)		0.007	56 (24–82)	48 (28–79)		< 0.001
< 65	145 (75.5)	87 (69.0)	39 (31.0)	0.270 (0.09–0.82)	0.018	94 (71.8)	37 (28.2)	2.913 (1.03–7.98)	0.038	71 (55.9)	56 (44.1)	4.86 (1.92–12.34)	< 0.001
65	47 (24.5)	33 (89.2)	4 (10.8)			37 (88.1)	5 (11.9)			37 (86.0)	6 (14.0)		
Menopausal status, n (%)													
Premenopausal	127 (66.1)	74 (66.7)	37 (33.3)	0.261 (0.10–0.67)	0.004	81 (70.4)	34 (29.6)	2.623 (1.13–6.12)	0.025	60 (53.6)	52 (46.4)	4.160 (1.92–9.04)	< 0.001
Postmenopausal	65 (33.9)	46 (88.5)	6 (11.5)			50 (86.2)	8 (13.8)			48 (82.8)	10 (17.2)		
ECOG performance status, n (%)													
0–1	187 (97.4)	116 (73.0)	43 (27.0)	1.371 (1.25–1.51)	0.574	126 (75.0)	42 (25.0)	1.333 (1.22–1.46)	0.337	103 (62.4)	62 (37.6)	1.602 (1.42–1.80)	0.160
2–4	5 (2.6)	4 (100.0)	0 (0.0)			5 (100.0)	0 (0.0)			5 (100.0)	0 (0.0)		
Clinical stage at diagnosis, ^c n (%)													
0 (<i>in situ</i>)	2 (1.0)	93 (72.1)	36 (27.9)	0.670 (0.27–1.67)	0.513	104 (75.4)	34 (24.6)	1.103 (0.46–2.66)	1.000	85 (61.6)	53 (38.4)	1.593 (0.69–3.70)	0.314
IA/IB	56 (29.2)												
IIA/IIIB	92 (47.9)												
IIIA/IIIB/IIIC	33 (17.2)	27 (79.4)	7 (20.6)			27 (77.1)	8 (22.9)			23 (71.9)	9 (28.1)		
IV	9 (4.7)												
Concomitant medications, ^d n (%)													
< 3 medications	122 (63.6)	92 (71.3)	37 (28.7)	1.877 (0.72–4.91)	0.274	107 (77.0)	32 (23.0)	1.393 (0.60–3.22)	0.504	92 (62.2)	56 (37.8)	0.616 (0.23–1.67)	0.477
3 or more medication	70 (36.5)	28 (82.4)	6 (17.6)			24 (70.6)	10 (29.4)			16 (72.7)	6 (27.3)		
Self-reported adverse events													
Yes	155 (80.7)	60 (69.8)	26 (30.2)	0.654 (0.32–1.33)	0.287	66 (70.2)	28 (29.8)	1.970 (0.95–4.08)	0.076	63 (55.8)	50 (44.2)	2.976 (1.42–6.22)	0.004
No	37 (19.3)	60 (77.9)	17 (22.1)			65 (82.3)	14 (17.7)			45 (78.9)	12 (21.1)		

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; OR, odds ratio.

P: significance value for Fisher's exact test for qualitative variables and Mann–Whitney test for quantitative variable (age).

^aBlack, Pardo, and Asian/Indian were grouped to be tested against white. In Brazil, Pardo is an ethnic/skin color category used by the Brazilian Institute of Geography and Statistics (IBGE) in the Brazilian censuses. The term "pardo" is commonly used to refer to Brazilians of mixed ethnic ancestries. Pardo Brazilians represent a wide range of skin colors and backgrounds. They are typically a mixture of white Brazilian, Afro-Brazilian, and Native Brazilian. Indian refers to Native Brazilian. ^bBasic, upper secondary and university levels were grouped to be tested against no instruction. ^c*In situ*, IA/IB and IIA/IIIB were grouped to be tested against IIIA/IIIB/IIIC and IV. ^dConcomitant medication in this analysis was assessed to evaluate the impact of polypharmacy in adherence behavior. The main prescribed pharmacologic classes were antihypertensive drugs (29.2%, 29.4%, and 26.3% for 3, 6, and 12 months, respectively) and nonopioid analgesics and nonsteroidal anti-inflammatory drugs (13.2%, 16.0%, and 22.0% for 3, 6, and 12 months, respectively). Strong cytochrome P450 2D6 (CYP2D6) inhibitors were near 1.0% of all concomitant medications for all time points (0.5%, 0.9%, and 1.2% for 3, 6, and 12 months, respectively).

previously described.²⁴ A CYP2D6 enzyme activity score (AS) was assigned to genotypes (diplotypes) based on allele scores of 0 (PM), 0.5 (IM), 1 (EM), and 2 (UM)^{36,37} and CYP2D6 phenotypes were deduced from AS: PM (0), IM (0.5 to 1.0), EM (1.5 to 2.0), and UM (3.0). Other DME gene polymorphisms included CYP2C9*2 and *3, CYP2C19*2 and *17, and CYP3A4*22 and CYP3A5*3 (**Supplemental Material**).

Heparinized plasma samples were obtained at months 3, 6, and 12 after the start of tamoxifen therapy. Plasma levels of tamoxifen and the inactive major metabolite DM-Tam as well as active metabolites (Z)-endoxifen and (Z)-4-OH-Tam were measured by liquid chromatography tandem mass spectrometry as previously described.¹⁷

Statistical analysis

DME genotype frequencies were tested for Hardy–Weinberg Equilibrium. Parametric and nonparametric tests were applied to determine whether tamoxifen and its metabolite concentrations as well as metabolic ratio's (MRs) differ between DME genotypes and adherence behavior. Multiple linear regression modeling was applied to evaluate the contribution of factors to the variability of plasma concentrations of tamoxifen, (Z)-endoxifen, DM-Tam, and (Z)-4-OH-Tam, as well as the respective MRs. Relative risk (RR) and 95% CIs were calculated at the 12-month time point to evaluate the risk of patients not achieving a previously proposed clinical threshold of 5.9 ng/mL (15.8 nM) (Z)-endoxifen.²⁰ All *P* values were two-sided, and values < 0.05 were considered statistically significant (details provided in **Supplemental Material**).

RESULTS

Patient adherence to treatment

Demographic and clinical characteristics together with adherence assessment at specific time points are given in **Table 1** and **Table S1**. Adherence was assessed for 163 patients at month 3 (85%), 173 patients at month 6 (90%), and 170 patients at month 12 (89%). Median age at diagnosis was 51.5 years (range 24–82 years); 127

patients (66%) were premenopausal. At 3 and 6 months, 74–76% of patients showed high adherence rates to tamoxifen treatment, which dropped to 63% at 12 months (**Figure 1a**). Low adherence was not observed during the first 3 months but increased to 10.6% at 12 months (**Figure 1a**; *P* < 0.05). High adherence at 12 months was more prevalent in patients without reported adverse events compared with those who reported adverse events (**Figure 1b**; *P* < 0.05). With the exception of age at diagnosis, menopausal status, and self-reported adverse events at 12 months, patient and tumor characteristics did not differ between adherence subgroups across time points (**Table 1**). Adverse events at 3, 6, and 12 months were reported for 53%, 54%, and 66% of patients, respectively (**Table 1**), with hot flashes being most frequent (62%, 65%, and 68%, respectively). Others included edema of the inferior members (17%, 25%, and 20%), fatigue (14%, 25%, and 30%), and nausea/vomiting (15%, 12%, and 12%) at 3, 6, and 12 months, respectively.

Genotype frequencies and enzyme AS

Genotypes met Hardy–Weinberg Equilibrium with the exception of CYP2D6*29 (**Table S2**). Although allele frequencies differed significantly between ethnic groups (*P* < 0.001; **Table S2**), ethnicity was not a prognostic factor for DME phenotypes or plasma concentration of tamoxifen and its major metabolites. DME phenotypes/AS predicted from 17 polymorphic loci tested in the 192 patients are summarized in **Figure S1**.

Association between treatment adherence and plasma concentrations of tamoxifen and its metabolites

At 3 months of treatment, patients with good adherence had 26% higher tamoxifen concentrations than patients with medium adherence (318 ± 97 nM vs. 236 ± 115 nM; *P* < .001; **Figure 2a**). Multiple linear regression analysis showed that 16–21% of the variability of tamoxifen levels at months 3 and 6 were explained by adherence to treatment and age at diagnosis (*P* < 0.001; **Table 2**). At

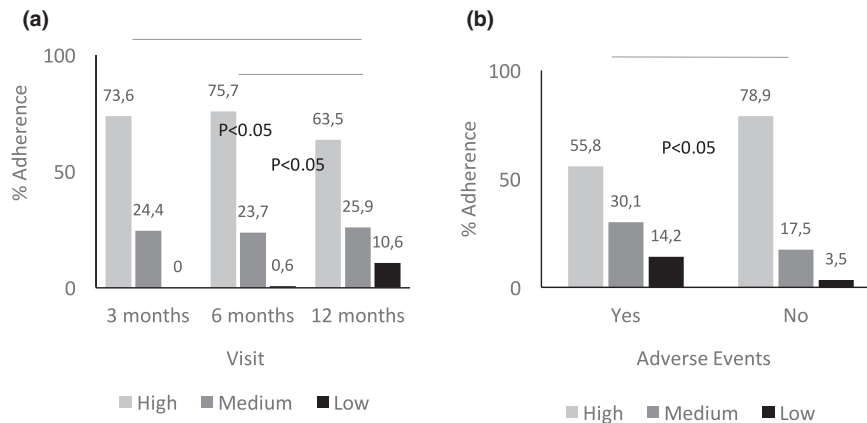


Figure 1 Adherence levels at consecutive time points after starting tamoxifen therapy of 20 mg daily. **(a)** 3 months (*n* = 163); 6 months (*n* = 173); and 12 months (*n* = 170). **(b)** 12 months, stratified by the occurrence of self-reported adverse events: presence of adverse events (Yes; *N* = 113); absence of adverse events (No; *N* = 57). Categories high (light grey), medium (dark grey), and low (black) are given as % for each group; *P* values refer to contingency Chi-square tests of a difference between visit **a** or grouping by adverse events **b**.

12 months, tamoxifen plasma levels strongly correlated with adherence ($r = 0.70$; $P < 0.001$) with tamoxifen levels being highest in patients with high adherence (389 ± 99 nM) compared with those with low or medium adherence (157 ± 67 nM and 258 ± 61 nM; $P < 0.001$; **Figure 2a**). In multivariate analyses, adherence was confirmed as the sole determinant that explained 47% of tamoxifen plasma concentration variability ($P < 0.001$; **Table 2**).

Similar associations with adherence were obtained for tamoxifen metabolite (Z)-endoxifen (**Figure 2b**). In subgroup analysis of patients with functional CYP2D6 (EM/EM) treatment adherence was significantly correlated with tamoxifen metabolites at all three time points (**Figure 2c**, data shown for months 3 and 12).

Association between genotypes of pharmacogenes and plasma concentrations of tamoxifen and its metabolites

Plasma concentrations of all tamoxifen metabolites were affected by CYP2D6 phenotype with a strongest effect for (Z)-endoxifen (**Table S3**). CYP2D6 AS demonstrated gene-dose effects on both (Z)-endoxifen and MR of *N*-desmethyltamoxifen (DM-Tam)/(Z)-endoxifen confirming the importance of CYP2D6 for the bioactivation of tamoxifen to (Z)-endoxifen via DM-Tam (**Figure S2**; $P < 0.001$). CYP2D6 deficient PM patients (PM/PM) had 4.5 to 5.5 times lower (Z)-endoxifen concentrations than EM patients with functional CYP2D6 (EM/EM). The mean concentrations of PM compared with EM patients at 3, 6, and 12 months were 6.5 ± 2.7 nM vs. 29.6 ± 12.9 nM; 7.2 ± 3.3 nM vs. 30.3 ± 14.5 nM; and

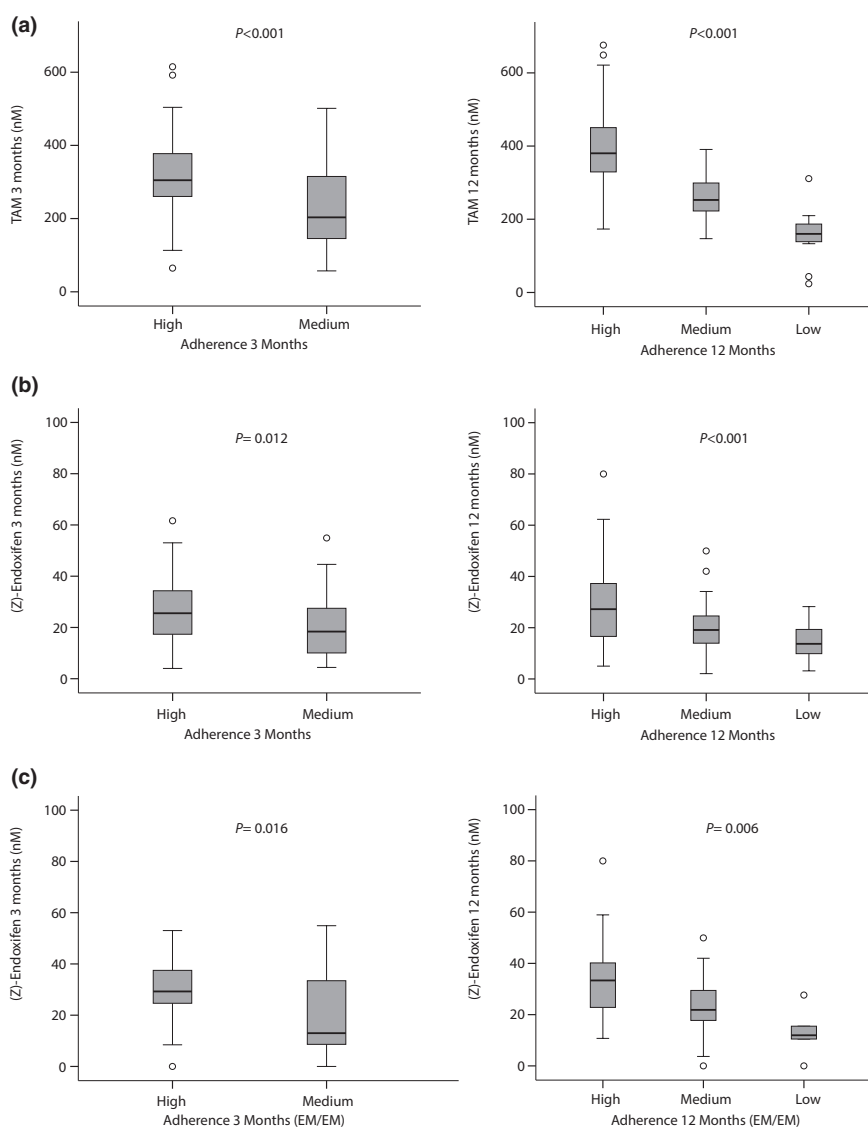


Figure 2 Influence of treatment adherence on tamoxifen and (Z)-endoxifen plasma concentration at 3 months ($n = 156$ patients) and 12 months ($n = 139$ patients). Plasma concentrations are presented as boxplots with boxes representing medians, 25% and 75% percentiles, and whiskers defined by the 5th and 95th percentiles and extreme values outside the whiskers. **(a)** TAM: Tamoxifen (parent drug). **(b)** (Z)-endoxifen. **(c)** (Z)-endoxifen for the subgroup of cytochrome P450 2D6 (CYP2D6). Extensive metabolizer (EM/EM) patients at 3 months ($n = 67$) and 12 months ($n = 55$). P values refer to test for different plasma concentrations between adherence categories. Data at 6 months not shown.

Table 2 Summary of multiple linear regression models for tamoxifen and selected metabolites

Metabolite/time point	r ²	F	P value ^a	Parameters in the model
Tam 3 months	0.212	19.750	< 0.001	Medium adherence; age at diagnosis
Tam 6 months	0.161	9.520	< 0.001	Low and medium adherence; age at diagnosis
Tam 12 months	0.471	39.130	< 0.001	Low and medium adherence
DM-Tam 3 months	0.412	8.995	< 0.001	CYP2D6 PM/IM, IM/IM, EM/PM, EM/IM; medium adherence; age at diagnosis; Asian/Indian ethnicity
DM-Tam 6 months	0.217	4.384	< 0.001	CYP2D6 PM/PM, EM/PM; low and medium adherence; age at diagnosis
DM-Tam 12 months	0.427	10.451	< 0.001	CYP2D6 PM/IM, EM/PM, EM/IM; low and medium adherence; age at diagnosis
Z-Endo 3 months	0.345	7.370	< 0.001	CYP2D6 PM/PM, PM/IM, EM/PM; medium adherence; Asian/Indian ethnicity
Z-Endo 6 months	0.322	8.421	< 0.001	CYP2D6 PM/PM, PM/IM, EM/PM, IM/IM; medium adherence
Z-Endo 12 months	0.403	10.877	< 0.001	CYP2D6 PM/PM, PM/IM, EM/PM, IM/IM; low and medium adherence
Metabolic ratio				
DM-Tam/Z-Endo 3 months	0.556	30.002	< 0.001	CYP2D6 PM/IM, IM/IM, EM/PM, EM/IM
DM-Tam/Z-Endo 6 months	0.510	20.195	< 0.001	CYP2D6 PM/IM, IM/IM, EM/PM, EM/IM
DM-Tam/Z-Endo 12 months	0.575	15.540	< 0.001	CYP2D6 PM/IM, EM/PM, EM/IM; black ethnicity

Cytochrome P450 2D6 (CYP2D6) diplotypes PM/PM, two null alleles; DM-Tam, N-desmethyltamoxifen; EM/IM, one normal and one reduced activity allele; EM/PM, one normal and one null activity allele; EM/UM, patient with gene duplications of alleles with normal activity; F, F-test (ANOVA) for the model; IM/IM, two reduced activity alleles; PM/IM, one null activity and one reduced activity allele; r², model's coefficient of determination; Tam, tamoxifen; Z-Endo, (Z)-endoxifen.

^aSignificance value of F-test (analysis of variance) for the proposed model.

5.4 ± 1.1 nM vs. 30.3 ± 14.5 nM. In multivariate analyses, CYP2D6 phenotype was a significant predictor of tamoxifen metabolites and MRs across all time points (Table 2).

Due to the local prescription practice there were few patients with concurrent comedication of strong CYP2D6 inhibitors. Although three of the four patients taking a strong CYP2D6 inhibitor (fluoxetine) showed low levels of (Z)-endoxifen (6–15 nM), CYP2D6 inhibitor use was not significantly associated with tamoxifen metabolite concentrations. Among other covariates, age at diagnosis was positively associated with increased tamoxifen and DM-TAM concentrations, whereas DMEs other than CYP2D6 had little influence (Table S3).

Combined analysis of factors influencing (Z)-endoxifen concentrations

Adherence and CYP2D6 phenotype were jointly associated with (Z)-endoxifen concentrations across all time points (Table 2). The additive effect of adherence and CYP2D6 phenotype was most evident at 12 months. Although CYP2D6 as a single factor explained 26.4% of (Z)-endoxifen variability (P < 0.001), the explained variability increased to 40% when adherence was included in multivariate analysis (P < 0.001; Table 2). We next evaluated whether adherence or CYP2D6 polymorphisms had a stronger effect on (Z)-endoxifen concentrations based on standardized beta coefficients (Table 3). Across all time points, severe

Table 3 Evaluation of contributing variables based on linear regression coefficients for the prediction of (Z)-endoxifen plasma variability

Variable	3 Months		6 Months		12 Months			
	St beta coef. ^a	P value ^b	Variable	St beta coef. ^a	P value ^b	Variable	St beta coef. ^a	P value ^b
CYP2D6 PM/IM	-0.353	< 0.001	CYP2D6 PM/IM	-0.378	< 0.001	CYP2D6 PM/PM	-0.374	< 0.001
CYP2D6 PM/PM	-0.312	< 0.001	CYP2D6 PM/PM	-0.307	< 0.001	CYP2D6 PM/IM	-0.342	< 0.001
CYP2D6 EM/PM	-0.249	0.001	Adherence-medium	-0.266	< 0.001	Adherence-low	-0.337	< 0.001
Adherence-medium	-0.189	0.008	CYP2D6 EM/PM	-0.203	0.010	CYP2D6 EM/PM	-0.208	0.007
CYP2D6 IM/IM	-0.119	0.103	CYP2D6 IM/IM	-0.173	0.016	Adherence-medium	-0.206	0.004
CYP2D6 EM/IM	-0.146	0.055	CYP2D6 EM/IM	-0.134	0.083	CYP2D6 IM/IM	-0.136	0.050
Black	-0.092	0.206	Adherence-low	-0.110	0.114	CYP2D6 EM/IM	-0.137	0.069
Brown/mixed	-0.052	0.456	CYP2D6 EM/UM	-0.010	0.887	CYP2D6 EM/UM	0.140	0.051
Asian/Indian	0.144	0.042						
CYP2D6 EM/UM	0.102	0.156						

Black, Brown/Mixed, Asian/Indian refers to the non-European ethnicities included in the study cohort; cytochrome P450 2D6 (CYP2D6) diplotypes EM/IM, one normal and one reduced activity allele; EM/PM, one normal and one null activity allele; EM/UM, patient with gene duplications of alleles with normal activity; IM/IM, two reduced activity alleles; PM/IM, one null activity and one reduced activity allele; PM/PM, two null alleles; St, standard.

^aVariables are listed according to their relevance in the model according to standardized beta coefficients. ^bOnly variables with P < 0.05 (t-test) are relevant in the model.

CYP2D6 impairment ($AS \leq 0.5$; PM/PM and PM/IM) was the strongest determinant of (Z)-endoxifen variability. The second strongest effect resulted from low or medium treatment adherence at 6 and 12 months with an effect size equal or greater to that resulting from a CYP2D6 $AS > 0.5$ (IM and EM; **Table 3**). As a putative surrogate predictor of clinical response, we used plasma concentrations of (Z) endoxifen and evaluated the relevance of adherence vs. CYP2D6 phenotype based on the RR of not achieving the threshold plasma concentration of 5.9 ng/mL (Z)-endoxifen (**Figure 3**). The risk to not achieve the threshold was highest in patients with CYP2D6 PM phenotype (RR 4.1; 95% CI 3.04–5.50), followed by patients with either CYP2D6 IM phenotype (RR 2.54; 95% CI 1.41–4.60) or low treatment adherence (RR 2.44; 95% CI 1.40–4.25). In subgroup analyses in patients with noncompromised CYP2D6 function (EM phenotype), low adherence showed to be a strong risk factor of not achieving clinical threshold concentrations (RR 3.65; 95% CI 1.48–8.99). This association was less pronounced in patients with reduced CYP2D6 activity (IM phenotype; RR 2.19; 95% CI 1.21–3.97).

DISCUSSION

We provide first evidence that the ability to achieve clinically relevant (Z)-endoxifen plasma concentrations during breast cancer tamoxifen treatment is cooperatively influenced by treatment adherence behavior, CYP2D6 genomic background, and pharmacokinetic capacity. Conjointly, this could improve the prediction of active tamoxifen metabolite levels (Z-endoxifen) and possibly clinical efficacy,^{17,20,38} a high priority goal in personalized endocrine treatment. Previous attempts to predict the variability of (Z)-endoxifen levels relied on pharmacokinetic and pharmacogenomic knowledge,^{17,38,39} which has been implemented into therapeutic recommendations based on CYP2D6 genotyping.¹⁴ Although a recent drug monitoring study showed that tamoxifen metabolites may be predictive of side effects, such as nausea and vaginal dryness,⁴⁰ clinical tamoxifen outcome studies as of yet provided controversial results possibly due to the influence of unidentified confounders.^{22–24,26–28} Because it is well known that women stop taking their medication before completing the standard 5-year regimen, we investigated patients' adherence behavior during the first year of tamoxifen treatment in relation to their metabolic capacity for achieving relevant (Z)-endoxifen concentrations. Our observed 12-month adherence rate is considerably lower than the 1-year tamoxifen adherence reported by Dezentje *et al.*⁴¹ (93%) based on prescription refill data. The latter assumed that prescription-refilling patterns correspond to patient medication-taking behavior and that the medication is taken exactly as prescribed independent of patients' beliefs or concerns about treatment. A 90% 1-year adherence rate was also reported by Makubate *et al.*⁴² in a retrospective cohort of endocrine-treated breast cancers that declined to 51% in the fifth year, and that demonstrated an association of low adherence (< 80%) with poor survival. Our prospective study used the Morisky, Green, and Levine Medication Adherence Scale questionnaire in a pharmacist-guided interview to assess adherence rates,

a tool previously used in an oncology setting in Brazil^{35,43} and which is considered reliable based on the validation of self-report questionnaires in relation to medication-monitoring devices.⁴⁴ Notably, Morisky's selected adherence scale is an easy to perform and fast questionnaire suitable for our prospective setting with 35% of patients with low-literacy.^{13,45} The questionnaire provided us with the benefit to retrieve valuable tamoxifen adherence behavior and pharmacokinetic information from a health disparity cancer population that otherwise would not be accessible. The high observed tamoxifen nonadherence rate within the first year may reflect the socioeconomic condition of this population and the high percentage of young women (two-thirds) known to be at increased risk to stop medication.¹⁰

We confirmed that CYP2D6 phenotype is a strong determinant of plasma (Z)-endoxifen levels in that increasing CYP2D6 allele activity correlated with increasing plasma metabolite concentrations. Our data are sound as the prevalence of reduced-function and null-function alleles compares with those reported from Brazil³³ and are in line with population admixture of Europeans, Native Americans, Africans, and Asians, with our institution serving as a national reference center for oncology treatment. (Z)-endoxifen plasma levels were in the range of those reported by others,^{15,17} and, as expected, CYP2D6 polymorphism only partially predicted its variability.¹⁹ Potential confounders, such as DME polymorphisms or strong CYP2D6 inhibitors, do not play a significant role as the former showed no effect and the latter were infrequently used by our patients given the low prescription rates at our hospital.

Of note, the observed effect of adherence behavior on active metabolite levels depended on CYP2D6 functionality. Although PM status was still the most important predictor of reduced (Z)-endoxifen concentrations, and the risk to not achieve sufficient concentration due to low-adherence was moderate across all patients (RR 2.44; 95% CI 1.40–4.25), low-adherence was a strong predictor in EM patients (RR 3.65; 95% CI 1.48–8.99). The latter clearly indicates an independent influence of low-adherence resulting in suboptimal concentrations of active tamoxifen metabolites. An immediate consequence from this finding is that EM and IM patients who are now in their ensuing year(s) of the 5-year treatment must be encouraged to adhere to tamoxifen, as the expected benefit will depend on their own authority. Known barriers to prevent tamoxifen adherence are manifold and include low recurrence risk perception, side effects, age extremes, medication cost, ethnicity, educational level, lack of social support, and suboptimal patient physician communication.^{3,4,13}

Importantly, we confirmed the relevance of age on tamoxifen adherence that patients at 65 years of age or older showed better adherence to tamoxifen than younger patients. Moreover, multivariate modeling identified age as an independent predictor of parent drug levels (i.e., tamoxifen). Previous findings by others showed that plasma concentrations of tamoxifen and its metabolites increase with age,⁴⁶ which, according to our findings, may be explained at least in part by patient adherence behavior. Given the strong predictive value of adherence for the variability of tamoxifen plasma concentrations we propose that drug monitoring is a powerful surrogate to assess tamoxifen adherence for which we identified a threshold concentration of 157 nM (± 67 nM) to stratify patients into low vs.

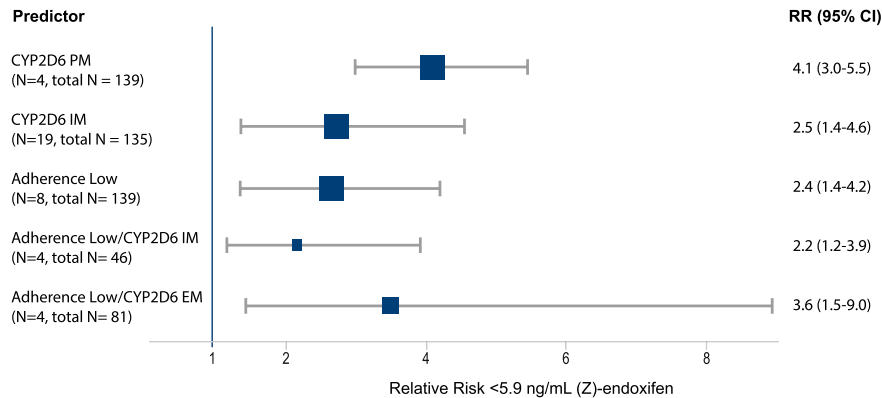


Figure 3 Forest plot for the relative risk of not achieving the clinical threshold of a (Z)-endoxifen concentration ≥ 5.9 ng/mL (15.8 nM) at 12 months. RR estimates are given as squared symbols with size weighted by the number of included patients. 95% CI, 95% confidence interval; EM, extensive metabolizer; IM, intermediate metabolizer; N, number of patients positive for predictor variable and in total analyses; PM, poor metabolizer; RR, relative risk.

medium/high tamoxifen adherent. This is particularly relevant to young patients, as their risk to discontinue tamoxifen is among the highest^{4,10} with tamoxifen currently being the sole standard-of-care treatment in this patient group.

Our study provides a first link between poor patient adherence to tamoxifen and the risk to not achieve relevant (Z)-endoxifen plasma concentrations. However, the study is not without limitations. We are aware that the Morisky, Green, and Levine Medication Adherence Scale scoring system does not quantitatively capture adherence, yet we consider this approach appropriate as it allowed us to assess tamoxifen adherence in this patient group with a high proportion of illiteracy. Moreover, the putative threshold of 5.9 ng/mL (Z)-endoxifen²⁰ has not been prospectively validated and controversies on the CYP2D6—endoxifen relationship for the prediction of tamoxifen efficacy have not been finally resolved, which currently limits the clinical utility of our findings.^{29,47–52}

In conclusion, our proof-of-concept study suggests that the dual monitoring of tamoxifen plasma levels as surrogate marker for adherence and (Z)-endoxifen formation as surrogate marker for clinical response could be a strategy to improve patients' chances to avoid recurrence and premature death in the future.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Figure S1.

Figure S2.

Table S1.

Table S2.

Table S3.

Supplementary Material.

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