ORIGINAL RESEARCH ARTICLE

Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer

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Abstract

Background Endoxifen is the most important active metabolite of tamoxifen. Several retrospective studies have suggested a minimal or threshold endoxifen systemic concentration of 14–16 nM is required for a lower recurrence rate. The aim of this study was to investigate the feasibility of reaching a predefined endoxifen level of ≥ 16 nM (5.97 ng/mL) over time using therapeutic drug monitoring (TDM).

Methods This prospective open-label intervention study enrolled patients who started treatment with a standard dose of tamoxifen 20 mg once daily for early breast cancer. An outpatient visit was combined with a TDM sample at 3, 4.5, and 6 months after initiation of the tamoxifen treatment. The tamoxifen dose was escalated to a maximum of 40 mg if patients had an endoxifen concentration < 16 nM. The primary endpoint of the study was the percentage of patients with an endoxifen level ≥ 16 nM at 6 months after the start of therapy compared with historical data, in other words, 80% of patients with endoxifen levels ≥ 16 nM with standard therapy.

Results In total, 145 patients were included. After 6 months, 89% of the patients had endoxifen levels ≥ 16 nM, compared with a literature-based 80% of patients with endoxifen levels ≥ 16 nM at baseline (95% confidence interval 82–94; $P =$ 0.007). In patients with an affected CYP2D6 allele, it was not always feasible to reach the predefined endoxifen level of ≥ 16 nM. No increase in tamoxifen-related adverse events was reported after dose escalation.

Conclusion This study demonstrated that it is feasible to increase the percentage of patients with endoxifen levels ≥ 16 nM using TDM. TDM is a safe strategy that offers the possibility of nearly halving the number of patients with endoxifen levels < 16 nM.

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1 Introduction

Tamoxifen significantly reduces the risk of disease recurrence and mortality in patients with hormone receptor-positive breast cancer [1–3]. In premenopausal women, tamoxifen monotherapy (preferably with ovarian suppression) is indicated for a period of 5 years, whereas postmenopausal women are often prescribed a sequential treatment of tamoxifen followed by an aromatase inhibitor [4, 5]. Despite adjuvant endocrine treatment, the disease returns within 5 years in 11–23% of patients and within 15 years in approximately 30% [6, 7].

Tamoxifen is a prodrug that is mainly metabolized by cytochrome P450 (CYP)-2D6 and -3A4 into its main active metabolites (Fig. 1). The two main active metabolites (4-hydroxy-tamoxifen and endoxifen) have a 30–100

Key Points

Endoxifen is the most important active metabolite of tamoxifen. Several studies have suggested a minimal or threshold endoxifen systemic concentration of 14–16 nM is required for a lower recurrence rate.

We studied 145 patients who started with tamoxifen treatment in the adjuvant setting. This study demonstrated the feasibility of therapeutic drug monitoring (TDM)-guided tamoxifen dosing.

TDM offers the possibility of safely halving the number of patients with subtherapeutic endoxifen levels without introducing additional toxicity. Therefore, physicians are encouraged to implement TDM of tamoxifen in clinical practice, pending further prospective evidence of the role of TDM in recurrence-free and overall survival.

Fig. 1 Main metabolism pathway of tamoxifen into its most active metabolite, endoxifen. *CYP* cytochrome P450

times greater binding affinity for the estrogen receptor than does tamoxifen. In addition, endoxifen achieves a five to ten times higher plasma concentration than 4-hydroxy-tamoxifen. Therefore, endoxifen is regarded as the most important metabolite [8–11]. The complex metabolic profile contributes to a high observed interindividual variability in endoxifen concentrations [12].

In several studies, the endoxifen concentration was inversely associated with the risk of breast cancer recurrence. In a retrospective analysis, Madlensky et al. [13] reported a related endoxifen threshold of 16 nM. This study included 1370 pre- and postmenopausal patients with early breast cancer who were treated with tamoxifen in the adjuvant setting for 5 years. Patients with an endoxifen concentration below the lowest quintile (< 16 nM) showed a 26% lower disease-free survival rate than patients with endoxifen levels > 16 nM [13]. In a smaller study among 306 premenopausal patients, the endoxifen levels were divided into quartiles. Compared with endoxifen concentrations in the highest quartile (i.e., > 35 nM), endoxifen concentrations in the lowest quartile (< 14 nM) were associated with an almost two times higher risk of distant recurrence [14]. At the current standard dose of tamoxifen 20 mg once daily (QD), approximately 20–24% of patients have an endoxifen level < 16 nM [13, 15].

Given this exposure–response relationship, dose optimization may reduce the risk of a recurrence of breast cancer. Dose optimization based on *CYP2D6* genotype has been attempted since approximately 39% of the variability in endoxifen concentration can be explained by *CYP2D6* genotype [16]. However, conflicting results have been reported regarding the association between *CYP2D6* genotyping and
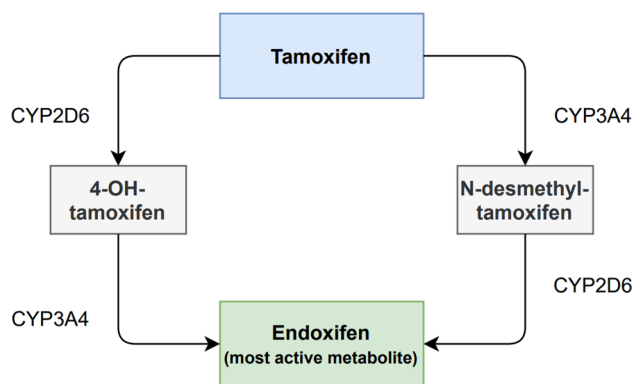
recurrence rate [17–22]. These conflicting results underline the importance of considering other factors that may influence the variability in endoxifen concentration, including concomitant medication, dietary or food supplements, adherence, age, body mass index (BMI), hormonal status, and circadian rhythm [23–27]. Therefore, therapeutic drug monitoring (TDM)-guided dose individualization appears to be a valid method to optimize the endoxifen level [28]. TDM is a tool commonly used to select the right dose of a drug for individuals based on plasma concentrations of the drug or active metabolite [29]. The aim of this prospective study was to investigate the feasibility of increasing the proportion of patients reaching a prespecified endoxifen threshold concentration using TDM. Although the exact threshold of the endoxifen level is currently unknown, we opted for the highest threshold value as described in the literature (≥ 16 nM) to minimize the risk of underdosing [14].

2 Patients and Methods

2.1 Study Design and Population

The TOTAM (TDM Of TAMoxifen) trial was an open-label single-arm intervention study performed at the Erasmus University Medical Center in Rotterdam, the Netherlands, approved by the institutional review board, and registered in the Netherlands Trial Registry (http://www.trialregister.nl; NL6918). Patients who started treatment with a standard dose of tamoxifen 20 mg QD for early breast cancer and who were able and willing to give written informed consent were eligible for participation in this trial. Exclusion criteria were patients receiving tamoxifen for a period longer than 3 months, a starting dose higher than 20 mg QD, a prior diagnosis of endometrial cancer (≤ 3 years prior), or a diagnosis of advanced or metastatic breast cancer.

A hospital visit was combined with TDM samples at 3, 4.5, and 6 months after initiation of the tamoxifen treatment. A hospital visit included registration of comedication or supplements, registration of adverse events using the US National Cancer Institute's Common Terminology Criteria for Adverse Events version 5 (CTCAEv5), and monitoring of drug adherence using the Morisky Medication Adherence Scale, a widely used self-report questionnaire resulting in a high (score 8), medium (score 6–7), or low (score < 5) adherence rate [30, 31]. Endoxifen trough concentrations at steady-state were sampled and processed to plasma. For quantification of tamoxifen and endoxifen, a validated liquid chromatography tandem mass spectrometry method was used [32]. After laboratory analysis, both tamoxifen and endoxifen concentrations were evaluated by a pharmacist or clinical pharmacologist. Escalation of tamoxifen dose was advised in patients with a high adherence score and a measured endoxifen level < 16 nM. Before increasing the tamoxifen dose, the investigator discussed possible individual factors leading to an endoxifen level below the threshold. Only in patients with nonadherence or potential drug–drug interactions that could be avoided was the tamoxifen dose not initially escalated. In other cases, the tamoxifen dose was escalated to a maximal daily dose of 40 mg as described in the tamoxifen drug label. Patients with an endoxifen level in the range of 12 to < 16 nM were advised to escalate to 30 mg, whereas patients with endoxifen levels < 12 nM were advised to escalate to the maximal daily dose of 40 mg. *CYP2D6* genotyping was performed using the Infiniti test (Autogenomics; Carlsbad, CA, USA) and the Quantstudio test (ThermoFisher Scientific; Waltham, MA, USA). Variation in the *CYP2D6* gene is responsible for alterations in enzyme activity compared with wild type [33]. *CYP2D6* phenotype was assayed in the laboratory on the genetic variants *2–10, *12, *14, *17, *29, and *41; thereafter, patients were classified into four phenotypes based on enzyme function, including ultrarapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM). Classification and interpretation were based on the Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and tamoxifen therapy [33].

2.2 Primary Endpoint and Statistical Analysis

The primary endpoint was the percentage of patients with an endoxifen level ≥ 16 nM at 6 months after initiation of adjuvant tamoxifen treatment. The secondary endpoint was the incidence of tamoxifen-related adverse events (CTCAEv5) after dose escalation. This study was powered to demonstrate that 90% of the patients would have endoxifen levels ≥ 16 nM at 6 months after start of tamoxifen by means of TDM-guided dose individualization. For the sample size calculation, we assumed that 80% of the patients had endoxifen levels ≥ 16 nM without TDM-guided dose individualization, as retrieved from literature data [13, 15]. To test this hypothesis with a two-sided α of 0.05, a power of 80%, and applying a continuity correction, at least 118 evaluable patients were required. Patients were defined as evaluable if TDM samples were taken at 3, 4.5, and 6 months after start with tamoxifen treatment. Secondary, the influence of the covariate age categorized as ≤ 45 or ≥ 55 years at the start of tamoxifen treatment on endoxifen exposure was tested using a t-test. The association between *CYP2D6* phenotype and BMI with baseline levels of endoxifen were tested by means of analysis of variance. Statistical analysis was performed using SPSS statistics software (version 26, IBM; Armonk, NY, USA).

3 Results

Between January 2018 and June 2019, a total of 145 women with early breast cancer who were treated with adjuvant tamoxifen were enrolled in this trial. To compensate for withdrawals in the period to reach the primary endpoint, more than the required 118 patients were included for the primary endpoint analysis. A total of 136 (94%) participants were evaluable for the primary endpoint analysis as 9 of the 145 patients were switched to an aromatase inhibitor in the meantime (five because of subtherapeutic endoxifen concentrations and four because of tamoxifen-related toxicity). The median age of the patients was 57 years (range 46–66). Prior to tamoxifen treatment, most patients underwent surgery in combination with either radiotherapy, (neo)-adjuvant chemotherapy, or a combination thereof. *CYP2D6* was successfully genotyped in all patients and conformed to Hardy–Weinberg equilibrium distribution ($P < 0.05$). Table 1 provides an overview of the relevant baseline characteristics.

The primary objective of the study was reached by means of TDM-guided dose individualization. After 6 months, 121 of 136 (89%) patients had an endoxifen concentration above the threshold of 16 nM (95% confidence interval [CI] 82–94; $P = 0.007$). In total, 130 of 145 (90%) participants reached the target concentration or successfully switched to an aromatase inhibitor within the study period of 6 months (Fig. 2). The pharmacokinetic profile of subtherapeutic endoxifen levels over time is depicted in Fig. 3A, and the endoxifen profile of all patients stratified by *CYP2D6* phenotype is presented in Fig. 3B.

At the first TDM visit (T1) after 3 months of tamoxifen treatment, 30 (21%) patients had an endoxifen level below the threshold of 16 nM. The tamoxifen dose was escalated to 30 or 40 mg in 27 patients, and three patients received no dose escalation at the discretion of the physician. The mean ± standard deviation concentration of tamoxifen and

Table 1 Baseline characteristics of the 145 TOTAM study participants

Characteristics	Value
Age, years	57.0 (46.0–66.0)
BMI, kg·m^{-2}	25.9 (22.9–28.7)
Tumor stage	
T1	67 (46)
T2	67 (46)
T3/4	11 (8)
Nodal stage	
N0	79 (55)
N1	49 (34)
N2	12 (8)
N3	5 (3)
Histologic classification	
Ductal adenocarcinoma	100 (69)
Lobular adenocarcinoma	34 (23)
Mucinous carcinoma	4 (3)
Other[a]	7 (5)
Histologic grade	
1	20 (14)
2	94 (65)
3	31 (21)
PR status	
0%	16 (11)
1–10%	14 (10)
> 10%	115 (79)
HER2 status	
Positive	13 (9)
Negative	132 (91)
Prior treatment	
Surgery	
Mastectomy	63 (43)
Lumpectomy	82 (57)
Radiotherapy	
Yes	106 (73)
No	39 (27)
Neoadjuvant chemotherapy	
Yes	28 (19)
No	117 (81)
Adjuvant chemotherapy	
Yes	32 (22)
No	113 (78)
CYP2D6 predicted phenotype[b]	
UM	1 (1)
EM	92 (63)
IM	41 (28)
PM	11 (8)
Comedications	
CYP2D6 inhibitors	
Weak[c]	1 (1)
Moderate[d]	1 (1)

Table 1 (continued)

Characteristics	Value
Potent[e]	1 (1)
CYP3A4 inhibitors	
Weak[f]	18 (12)
Moderate[g]	1 (1)
Potent	0 (0)
Morisky Medication Adherence Scale	
High adherence	132 (91)
Medium adherence	7 (5)
Low adherence	6 (4)

Data are presented as *N* (%) or median (interquartile range). Baseline is visit 1 (T1), 3 months after starting tamoxifen therapy

BMI body mass index, *CYP* cytochrome P450, *EM* extensive metabolism, *HER2* human epidermal growth factor receptor 2, *IM* intermediate metabolizer, *PM* poor metabolizer, *PR* progesterone receptor, *UM* ultra-rapid metabolizer

[a]Papillary lesions and inflammatory breast cancer

[b]CYP2D6 phenotype was assayed in the laboratory and genetic variants, including *2–10, *12, *14, *17, *29 and *41. Classification and interpretation were based on the Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and tamoxifen therapy[33]

[c]Citalopram

[d]Sertraline

[e]Quinidine

[f]Esomeprazole, omeprazole, pantoprazole

[g]Diltiazem

endoxifen at T1 was 315 ± 99 and 27.7 ± 14.8 nM, respectively (Table 2). At the second TDM visit (T2) after 4.5 months of tamoxifen treatment, 18 patients (tamoxifen dose 20 mg [*n* = 6], 30 mg [*n* = 3], and 40 mg [*n* = 9]) had an endoxifen level < 16 nM. In seven of these patients, the tamoxifen dose was escalated after T2. At T2, the mean endoxifen level of the five patients who had an endoxifen level ≥ 16 nM at T1 and < 16 nM at T2 was 14.4 nM (range 13.8–15.4). Finally, 6 months after initiation of tamoxifen therapy (T3), 15 of 136 (11%) patients (tamoxifen dose 20 mg [*n* = 8] and 40 mg [*n* = 7]) had an endoxifen level < 16 nM.

Within the first 6 months of tamoxifen treatment, 31 patients were escalated to a dosage of tamoxifen 30 or 40 mg with one or two dose-escalation steps. After dose escalation, 21 (68%) of these patients reached the threshold. Of the ten patients with subtherapeutic endoxifen concentrations, five (50%) switched to an aromatase inhibitor within 6 months, three (30%) switched to an aromatase inhibitor after 6 months, and two (20%) continued tamoxifen treatment with 40 mg QD. Four participants (mean endoxifen level 23 ± 4.9 nM) switched to an aromatase inhibitor because of tamoxifen-related adverse events while receiving tamoxifen
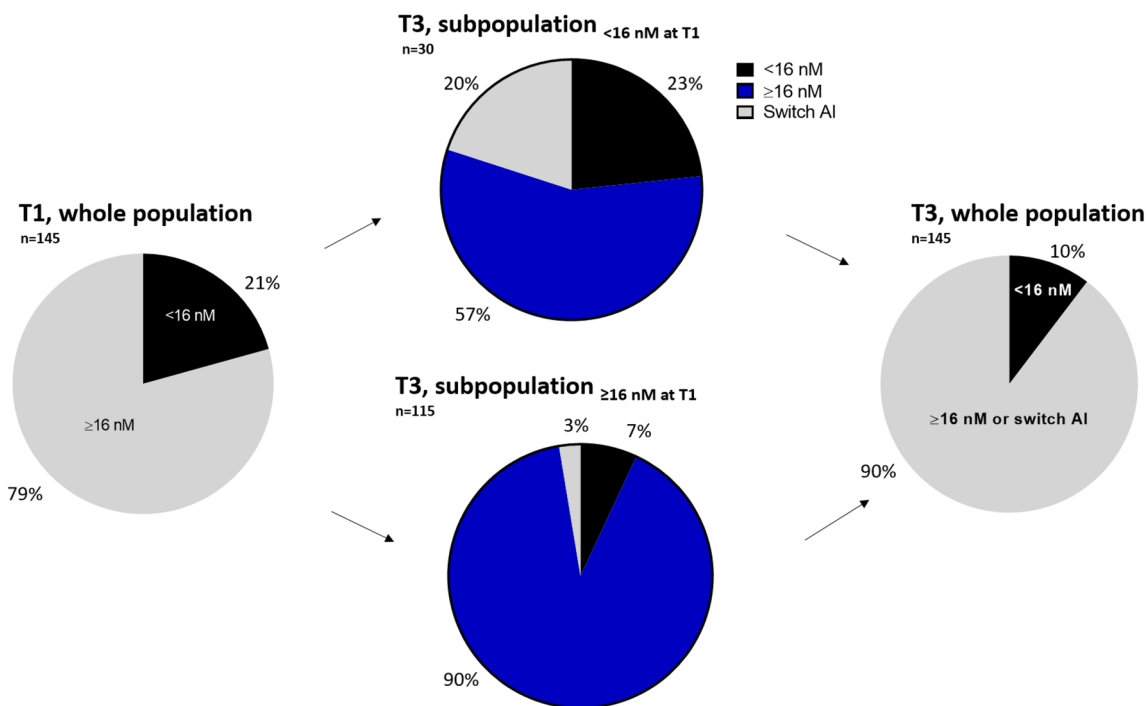
Fig. 2 Therapeutic drug monitoring over time ($n = 145$). Endoxifen concentrations (nM) were stratified based on threshold: (1) < 16 nM or (2) ≥ 16 nM. *AI* aromatase inhibitor (i.e., letrozole, anastrozole, or exemestane), *T1* 3 months after start with tamoxifen, *T3* 6 months after start with tamoxifen

20 mg QD (Table 3). In our study population, age and BMI had no clear effect on tamoxifen metabolism ($P = 0.27$ and $P > 0.60$, respectively). *CYP2D6* phenotype (PM, IM, and EM) had a statistically significant effect on endoxifen exposure ($P < 0.001$), with PMs having the lowest systemic endoxifen concentrations (Table 4).

Low endoxifen levels were found in the majority of patients with an IM or PM *CYP2D6* phenotype. At T1, 7% of the patients with an EM phenotype had an endoxifen level < 16 nM, whereas 34% of the IMs and 100% of the PMs were below this target. Also, 8% of the participants (with a mean endoxifen concentration of 19.1 nM [range 16.6–19.9]) declined below the threshold during the relatively short study period, despite having a preliminary measurement > 16 nM. After dose escalation, 100% of the EMs, 79% of the IMs, and 36% of the PMs reached the pharmacokinetic target of ≥ 16 nM. The endoxifen concentrations also increased linearly from 10.5 ± 3.3 to 21.8 ± 10.4 nM after dose escalation to tamoxifen 40 mg QD. In the nonescalated group ($n = 106$), stable endoxifen concentrations over time were observed compared with T1, with mean ± SD endoxifen levels of 32.3 ± 12.5 and 31.9 ± 13.6 nM at baseline and 6 months after initiation of treatment, respectively (Table 2). In the nonescalated group, a relatively low intra-individual variability of 19% was found.

The most commonly reported tamoxifen-related adverse events were hot flashes (61%), arthralgia (19%), fatigue (11%), vaginal dryness (8%), and mood swings (6%) (all CTCAE grade 1) during the first 6 months of this clinical trial. Low-grade toxicity adverse events were often persistent and perceived as limiting. Four patients experiencing these events discontinued their tamoxifen treatment (between T1 and T3). After dose escalation, no increase in tamoxifen-related adverse events, severe or serious adverse events, or treatment discontinuation were reported. Morisky adherence scores in our population were 91%, 5%, and 4% for high, medium, and low adherence, respectively; these results imply a high adherence rate in our study population (Table 1). The use of concomitant CYP2D6 or CYP3A4 inhibitors was limited in the study population. Only two patients used a moderately potent CYP2D6 inhibitor (i.e., sertraline 50 mg QD and quinidine 200 mg twice daily), and one patient used a moderately potent CYP3A4 inhibitor (i.e., diltiazem 120 mg three times daily). One patient used a weak CYP2D6 inhibitor (i.e., citalopram), and 18 patients used a weak CYP3A4 inhibitor (i.e., esomeprazole, omeprazole, or pantoprazole).

a

b
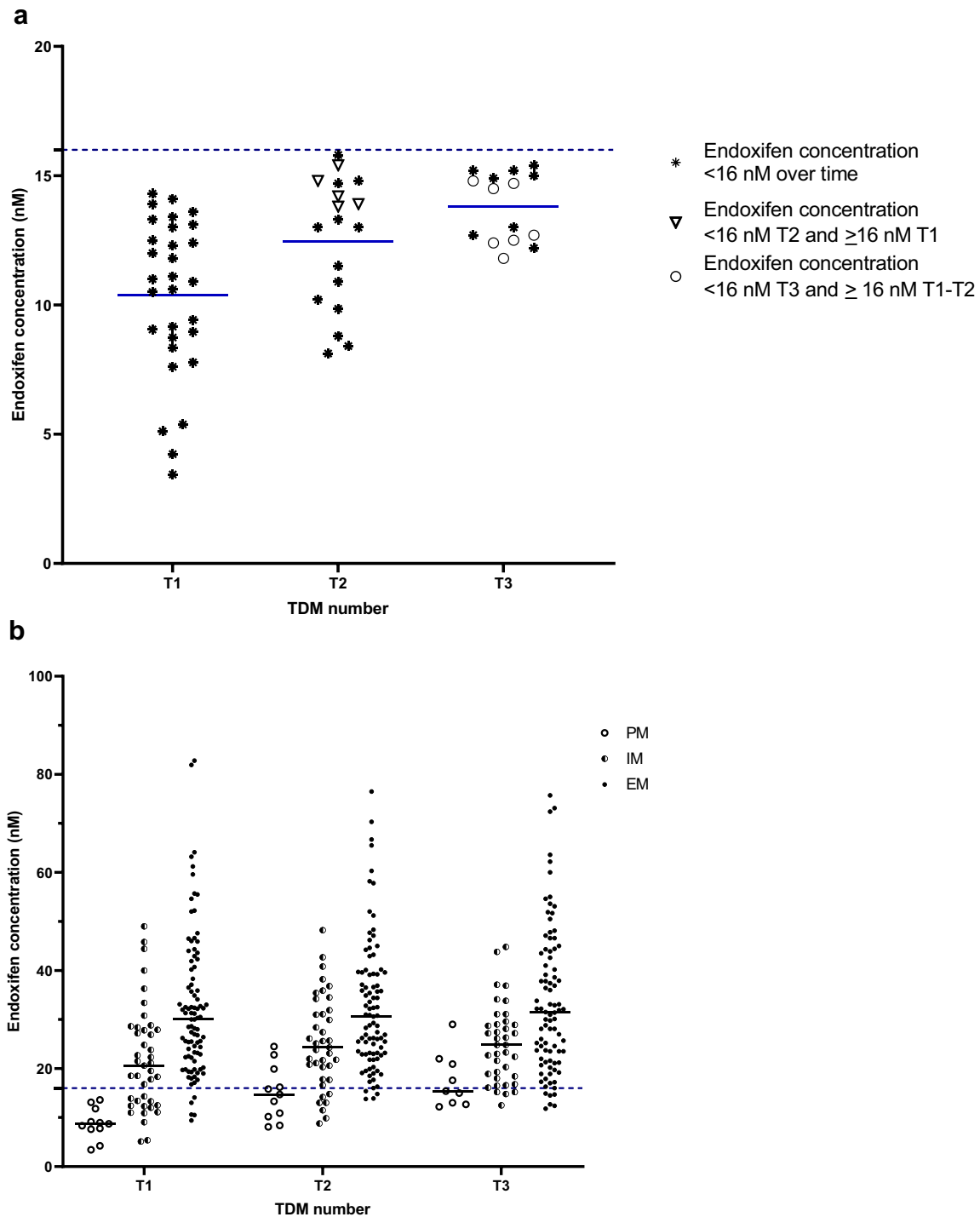
Fig. 3 (**A**) Pharmacokinetic profile of endoxifen levels below threshold, 3 months ($n = 30$), 4.5 months ($n = 18$) and 6 months ($n = 15$) after start with tamoxifen treatment. (**B**) Pharmacokinetic profile of endoxifen levels stratified based on *CYP2D6* phenotype, 3 months ($n = 145$), 4.5 months ($n = 141$), and 6 months ($n = 136$) after start with tamoxifen treatment. The horizontal line represents the mean endox-

ifen concentration and the horizontal dashed line represents the pre-defined endoxifen threshold (≥ 16 nM). *EM* extensive metabolizer, *IM* intermediate metabolizer, *PM* poor metabolizer, *T1* 3 months after start with tamoxifen, *T2* 4.5 months after start with tamoxifen, *T3* 6 months after start with tamoxifen

Table 2 Main pharmacokinetic results with tamoxifen in 145 TOTAM study participants

Concentration (nM)	3 months (T1)	4.5 months (T2)	6 months (T3)
Tamoxifen			
TOTAM cohort[a]	315 ± 99	345 ± 154	347 ± 165
20 mg cohort b	315 ± 99	303 ± 104	306 ± 124
Endoxifen			
TOTAM cohort[a]	27.7 ± 14.8	29.4 ± 13.1	30.2 ± 13.6
20 mg cohort b	32.3 ± 12.5	32.3 ± 12.6	31.9 ± 13.6

Data are presented as mean ± standard deviation

[a]TOTAM cohort, patients with a tamoxifen dose in the range of 20–40 mg once daily (n = 145 [T1], n = 141 [T2], and n = 136 [T3]); [b]20 mg cohort (n=106), patients with a tamoxifen dose of 20 mg once daily during the whole study period (T1–T3). *T1* 3 months after starting tamoxifen, *T2* 4.5 months after starting tamoxifen, *T3* 6 months after starting tamoxifen

4 Discussion

The TOTAM study demonstrated that TDM-guided dose individualization led to a statistically significant and clinically relevant increase in the number of patients with endoxifen levels above the predefined threshold of 16 nM after 6 months of treatment. By using TDM-guided dose individualization for 3 months, nearly 50% of patients with an initial endoxifen level below the predefined threshold reached endoxifen levels ≥16 nM. Therefore, our study offers tools for applying TDM of tamoxifen in clinical practice.

Dose escalation resulted in a significant increase of both tamoxifen and endoxifen concentrations (independent of CYP2D6 status), which is consistent with previous data [12, 34–38]. An Australian dose-escalation study had a higher percentage of patients achieving the threshold than in our data: 94 versus 76% at baseline [15]. However, it should be noted that the defined threshold differed (> 15 vs. ≥ 16 nM), the dose of tamoxifen was increased to a maximum of 60 mg (instead of 40 mg), and the tamoxifen dose was increased in patients with endoxifen levels < 30 nM.

Stratification based on the *CYP2D6* genotype showed differences in mean endoxifen levels both before and after dose escalation. After dose escalation, the predefined endoxifen target was achieved in 100% of EMs and 79% of IMs but only 36% of PMs. This result implies that, to achieve therapeutic endoxifen concentrations early in treatment, it is advisable to anticipate—if available—the *CYP2D6* genotype status. Our results indicate that PMs might benefit from a starting dose of tamoxifen 40 mg QD combined with

Table 3 Therapeutic drug monitoring over time (n = 145)

Variable	T1: 3 months	T2: 4.5 months	T3: 6 months
Tamoxifen users	145 (100)	141 (97)	136 (94)
Endoxifen concentration ≥ 16 nM	115 (79)	123 (87)	121 (89)[a]
≥ 16 nM resulting from dose escalation		15/27 (56)	5/7 (71)
Endoxifen concentration < 16 nM	30 (21)	18 (13)	15 (11)
< 16 nM without dose escalation		1 (5)	6 (40)
< 16 nM despite dose escalation		12 (67)	2 (13)
< 16 nM T2 but ≥ 16 nM T1		5 (28)	–
< 16 nM T3 but ≥ 16 nM T2		–	7 (47)
Tamoxifen dose (mg)			
10		2 (1)	2 (2)
20	145 (100)	112 (80)	108 (79)
30		6 (4)	4 (3)
40		21 (15)	22 (16)
Switch aromatase inhibitor[b], reason		4 (3)	9 (6)
Endoxifen concentration < 16 nM without dose escalation		2 (1.5)	2 (1)
Endoxifen concentration < 16 nM after dose escalation		–	3 (2)
Toxicity with tamoxifen 20 mg		2 (1.5)	4 (3)
Toxicity with tamoxifen 30–40 mg		–	–

Data are presented as n (%) or n/N (%) unless otherwise indicated

T1 3 months after starting tamoxifen, *T2* 4.5 months after starting tamoxifen, *T3* 6 months after starting tamoxifen

[a]Evaluable population (n = 136) compared with a literature-based 80% of patients with endoxifen levels ≥ 16 nM without TDM-guided dosing (95% confidence interval 82–94; P = 0.007)

[b]Anastrozole, letrozole, or exemestane

Table 4 Pharmacokinetic results of tamoxifen after dose escalation and stratified on *CYP2D6* phenotype

Endoxifen concentration (nM)	Before dose escalation (T1 or T2)	After dose escalation (T2 or T3)
Dose escalation		
+10 mg, *n* = 7	12.4 ± 1.3	20.7 ± 9.1
+20 mg, *n* = 24	10.5 ± 3.3	21.8 ± 10.4
CYP2D6 phenotypes		
EM, *n* = 92	32.5 ± 14.5	–
< 16 nM	6/92 (6.5)	0/6 (0)
IM, *n* = 41	21.9 ± 10.8	–
< 16 nM	14/41 (34.1)	3/14 (21)
PM, *n* = 11	8.8 ± 3.2	–
< 16 nM	11/11 (100)	7/11 (64)

Data are presented as mean ± standard deviation or *n*/*N* (%)

EM extensive metabolizer, *IM* intermediate metabolizer, *PM* poor metabolizer, *T1* 3 months after starting tamoxifen, *T2* 4.5 months after starting tamoxifen, *T3* 6 months after starting tamoxifen

TDM and that IMs might benefit from the standard dose combined with TDM. For most extensive metabolizers, tamoxifen 20 mg QD might be sufficient without TDM. An ongoing proof-of-concept study in CYP2D6 IMs and PMs is combining tamoxifen with probenecid (a uridine glucuronyl transferase inhibitor), aiming to reduce the conversion of endoxifen in inactive metabolites (http://www.trialregister.nl, study number NL8444). Another ongoing trial is evaluating the effect of supplementation of Z-endoxifen according to *CYP2D6* genotype or plasma levels to reach a predefined endoxifen threshold (NCT03931928). However, a switch to an aromatase inhibitor might also be a valid option for the subgroup of patients with persistently low endoxifen levels after a tamoxifen dose adjustment to 40 mg.

Also in patients with adequate CYP2D6 function, at least one TDM sample is advisable for every patient treated with tamoxifen [16, 39]. Multiple factors other than *CYP2D6* genotype can contribute to lower endoxifen levels, including concomitant CYP2D6 inhibitors, adherence, menopausal status, and decreased absorption [23, 40]. However, the low incidence of concomitant use of CYP2D6 inhibitors and the median age of 57 years in our analysis meant that only the *CYP2D6* phenotype was highly predictive for endoxifen exposure at baseline. Despite the relatively low intra-individual variability of 19% (as found in our study), 8% of the participants fell below the threshold somewhere during the relatively short study period, even though TDM sample 1 or 2 was > 16 nM (mean endoxifen concentration 19.1 nM [range 16.6–19.9]). This suggests that patients with endoxifen levels in the range of 16–20 nM should be monitored more frequently than patients with endoxifen levels > 20 nM at the first measurement at 3 months after the start of treatment. Long-term (2 years of follow-up) intra-individual

data collection in the TOTAM study for tamoxifen pharmacokinetics is ongoing. These data will ultimately represent the pharmacokinetic profile during the first 24 months of treatment with tamoxifen in this cohort of patients.

No increase in the degree or severity of toxicity was observed after the tamoxifen dose was increased. Although the absolute number of patients with increased doses was relatively low, the toxicity data are in agreement with that in the literature. Prospective studies have shown no correlation between tamoxifen dose and incidence of side effects [34, 35, 41, 42]. A longer follow-up should reveal whether this also applies to rare or long-term side effects, such as the risk of developing endometrial carcinoma, deep vein thrombosis, and pulmonary embolism [43–45]. Given the likelihood of these serious side effects, a conscious decision was made to increase the dose up to the maximal registered dose of tamoxifen 40 mg QD.

In our study, the degree of adherence was high: 91% of the population received the highest Morisky medication adherence score. Both the adherence questionnaire and tamoxifen levels > 100 nM indicated that participants in this study were highly motivated to adhere to tamoxifen treatment. Another contributor to this high degree of tamoxifen adherence could be the serial TDM, including active counseling by a pharmacist or medical oncologist. However, in real life, the degree of adherence fluctuates between 41 and 88%, so the relevance of TDM can be greater outside the context of a clinical trial or a longer follow-up period [46, 47]. In the literature, discontinuation of tamoxifen therapy is mostly observed during the first year of treatment [46]. For example, a recent study demonstrated that TDM was a useful tool for detecting nonadherence (tamoxifen level < 100 nM) in an early stage of treatment [46–48]. As a result of earlier research, almost all concomitant moderate and strong CYP2D6 inhibitors are included in the medication monitoring system of Dutch pharmacies as a monitoring signal [49]. Therefore, minimal use of concomitant CYP2D6 or CYP3A4 inhibitors was noticed in our population.

A methodological strength of our study is the design, with repeated measures of tamoxifen TDM samples compared with mostly single sampling as described in the literature [35]. However, the intensive monitoring could also, paradoxically, be considered a potential limitation. The intensive monitoring strategy potentially positively contributed to the adherence and motivation of patients for tamoxifen treatment. Another strength of our trial was the inclusion of a real-life population of tamoxifen users as well as (1) both pre- and postmenopausal patients, (2) evaluation of an approved on-label dosage (maximum tamoxifen 40 mg QD), and (3) evaluation of a switch to an aromatase inhibitor.

The proportion of patients with breast cancer with subtherapeutic endoxifen levels is determined based on the threshold of 16 nM as first reported by Madlensky et al.

[13]. If the true value of this threshold is lower, this could imply that a lower proportion of treatment is categorized as subtherapeutic before TDM. Based on this, the uncertainty regarding the effectiveness of tamoxifen below the current threshold of 16 nM is an important limitation underlying the presented outcome.

Conflicting results have been reported for an endoxifen exposure–response relationship for adjuvant tamoxifen treatment. A preclinical study in mice showed an association between endoxifen levels and tumor growth, and a xenograft model found a dose-dependent association between concentration and degree of gene expression in an MCF7 cell line [50, 51]. These preclinical data support the findings of the retrospective analyses by Madlensky et al. [13] and Saladores et al. [14] with an endoxifen threshold in the range of 14–16 nM. In contrast to these findings, the CYPTAM trial, in which 667 women were treated with tamoxifen, found no association between *CYP2D6* genotype and endoxifen concentration in relapse-free survival [52]. However, the design of the study had some limitations, and power was insufficient to conclude that there was no exposure–response relationship. Therefore, an exposure–response relationship remains disputed [53–57]. A prospective randomized controlled TDM study could provide clarity. However, such a trial is probably impossible, since it would require many thousands of patients to participate and a follow-up period of more than a decade [54]. To break out of this potential dead end, physicians are encouraged to implement TDM in clinical practice in the meantime and pending further prospective data. In our opinion, TDM of endoxifen is the most suitable approach for tamoxifen precision dosing because several factors could affect endoxifen concentrations. TDM is mainly recommended in patients with polypharmacy (many concomitant drugs or food supplements), premenopausal patients, and patients diagnosed with an affected CYP2D6 allele. Importantly, an infrastructure to quantify endoxifen concentrations in human plasma is easy to implement in clinical practice.

5 Conclusion

The TOTAM study clearly demonstrated the feasibility of TDM in personalizing tamoxifen treatment in patients with breast cancer. This strategy offers the possibility of safely halving the number of patients with endoxifen levels < 16 nM without introducing additional toxicity.

Declarations

Conflict of interest C. Louwrens Braal, Agnes Jager, Esther Oomen–de Hoop, Justin D. Westenberg, Koen M.W.T. Lommen, Peter de Bruijn, Mijntje M. Vastbinder, Quirine C. van Rossum–Schornagel, Martine F. Thijs–Visser, Robbert J. van Alphen, Liesbeth E.M. Struik, Hanneke J.M. Zuetenhorst, Ron H.J. Mathijssen, and Stijn L.W. Koolen have no potential conflicts of interest that might be relevant to the contents of this manuscript.

Ethics approval The prospective clinical study was approved by the local ethics committee (Erasmus MC, Rotterdam; MEC 17-548) and was registered in the Dutch Trial Registry (NL6918).

Consent Written informed consent was obtained from all patients participating in the TOTAM study.

Availability of data and material Available upon reasonable request.

Author contributions All authors participated in data analysis and interpretation and the writing and editing of the manuscript. Creation of figures and tables: LB, AJ, RM, and SK.

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