Tamoxifen metabolism and efficacy in breast cancer– a prospective multicentre trial

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Abbreviations:
TAS: tamoxifen activity score; CYP: cytochrome P450; ER: oestrogen-receptor; ORR: objective response rate; CB: clinical benefit; PFS: progression-free survival; NM: normal metabolizers; IM: intermediate metabolizers; PM: poor metabolizers; UM: ultra-rapid metabolizers; SNP: single nucleotide polymorphisms; CR: complete response; PR: partial

response; SD: stable disease; OR: odds ratio; QOL: Quality of Life Questionnaire; HER: human epidermal growth factor receptor

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Translational Relevance:
Endoxifen levels vary by the highly polymorphic cytochrome P450 enzymes like CYP2D6. Monitoring and adjusting endoxifen levels in women with breast cancer could be of clinical value if there is an association between endoxifen levels and outcome. Only a prospective study can assess the clinical validity of circulating endoxifen and *CYP2D6* genotyping to predict tamoxifen outcome. The primary objective of this prospective clinical trial was to assess the relationship between serum endoxifen levels, the ‘tamoxifen activity score (TAS)’ and the objective response rate. Secondary endpoints were clinical benefit, progression free survival,

and tolerability. We did not find evidence of a relationship between endoxifen levels and the primary or secondary endpoints. Also, no clear relationship between TAS and the endpoints were found. Therefore, this study does not suggest monitoring of endoxifen neither assessing TAS to be of clinical value in postmenopausal women treated with tamoxifen for endocrine sensitive breast cancer.

Abstract

Purpose: Levels of endoxifen, the most active metabolite of tamoxifen, vary by the highly polymorphic cytochrome P450 (CYP) 2D6 enzyme. We prospectively investigated tamoxifen efficacy by serum endoxifen levels and the tamoxifen activity score (TAS).

Experimental Design: A prospective observational multicentre study including postmenopausal women with an oestrogen-receptor (ER)-positive breast cancer receiving first line tamoxifen, 20mg daily in the neo-adjuvant or metastatic setting recruited between February 2009 and May 2014. The primary endpoint was the objective response rate (ORR) using RECIST criteria 1.0. Secondary endpoints were clinical benefit (CB), progression-free survival (PFS) and tolerability of tamoxifen. The main analysis used logistic regression to relate ORR to serum endoxifen levels after 3 months. Endpoints were also related to other tamoxifen metabolites and to TAS.

Results: Endoxifen levels were available for 247 of all 297 patients (83%) of which 209 with target lesions (85%). Median follow-up time for PFS was 32.5 months, and 62% progressed. ORR and CB were 45% and 84%, respectively. ORR was not related to endoxifen, the odds ratio of ORR was 1.008 per µg/l increase in endoxifen (95% CI 0.971-1.046, *p=0.56*). In general, none of the endpoints was associated with endoxifen levels, tamoxifen metabolites, or TAS.

Conclusion: Under the pre-specified assumptions, the results from this prospective clinical trial do not suggest therapeutic drug monitoring of endoxifen to be of clinical value in postmenopausal women treated with tamoxifen for breast cancer in the neo-adjuvant or metastatic setting.

Clinical Trial number:
NCT00965939

Introduction

In oestrogen-receptor (ER)-positive breast cancer, toxicity and objective response rate (ORR) to tamoxifen which is around 30% in first line metastatic disease, vary (1). One explanation is inter-individual variability in steady state concentrations of tamoxifen and metabolites; the most active is 4-hydroxy-N-desmethyl-tamoxifen or endoxifen. This variability can be explained by genetic variations of cytochrome P450 (CYP) enzymes (2-4) and drugs like selective serotonin- and norepinephrine-reuptake inhibitors inhibiting the catalytic activity of *CYP2D6* (5,6).

The inter-individual variability in steady-state concentrations of tamoxifen and its metabolites (7) can be explained by genetic variations of CYP enzymes (8,9). Germline variations in the gene encoding CYP2D6-isoenzyme can result in low endoxifen levels (10,11). The *CYP2D6* genotype predicts for the metabolic phenotype (12); it classifies tamoxifen users into four different phenotypes (6,13,14). Normal metabolizers (NM); ~70% of Caucasian patients have two wild type alleles and normal CYP2D6 enzyme activity, intermediate metabolizers (IM); 10-15% exhibit 1 non-functional *CYP2D6* variant, poor metabolizers (PM); 4-5% has loss of the two functional alleles, and ultra-rapid metabolizers (UM); 1-2% express multiple copies of *CYP2D6*.

Goetz et al.(15) have been the first to associate the PM genotype with higher disease relapse and reduced metabolic activation of tamoxifen to endoxifen. This was confirmed in several other studies, some using the tamoxifen activity score (TAS), an ordinal score combining metabolic phenotype (NM, IM, PM, and UM) with the effect of interacting drugs (10,16-19). Later observations questioned TAS to predict response to tamoxifen as data have been inconsistent (14,20-23). Controversial results have been all derived from post-hoc or retrospective analyses and poor quality of the primary genetic data. Also, not all PM on tamoxifen have low endoxifen levels and NM sometimes have decreased levels; only about 23-43% of endoxifen variability is believed to be explained by *CYP2D6* phenotypes (10,14). We previously have shown that tamoxifen benefit in PM (24).

The International Tamoxifen Pharmacogenomic Consortium recognised that only prospective studies can assess the clinical validity of circulating endoxifen and *CYP2D6* genotyping to predict tamoxifen outcome (25). We aimed to prospectively investigate the association of serum endoxifen levels with TAS and clinical outcome of tamoxifen in endocrine sensitive disease.

Methods

Patients, setting, design

This multicentre prospective cohort study, CYPTAM-2, enrolled postmenopausal women starting tamoxifen monotherapy in the neo-adjuvant or first-line metastatic setting for an ER-positive breast cancer. Those with secondary metastatic disease were allowed if adjuvant endocrine therapy with tamoxifen monotherapy was stopped for more than 12 months. Patients were recruited between February 2009 and May 2014 in 15 hospitals in Belgium and Switzerland (Supplementary Table S1). Evaluation was discontinued in case of progressive disease or toxicity on tamoxifen. The study was approved by our Ethics Committee (NCT00965939) and all patients gave written informed consent.

Objectives

To study endoxifen levels and TAS with respect to clinical outcome, tolerability and the amount of variation in endoxifen levels being explained by TAS. The primary objective was to assess the relationship between endoxifen plasma concentrations and tamoxifen efficacy in postmenopausal ER-positive patients with breast cancer.

Methodology

Serum samples for tamoxifen and metabolites' assessment were collected after 3 months (+/- 2 months). Blood sample collection was performed at least 12 hours following last tamoxifen intake. Samples were allowed to clot during 30 minutes, under protection of light, and centrifuged during 10 minutes at 3000 rpm. Serum was separated and stored at -80°C until analysis at the laboratory of Clinical Pharmacy and Toxicology at the Leiden University Medical Centre. Tamoxifen, endoxifen, 4-OH-tamoxifen and desmethyl-tamoxifen were determined using a validated high-performance liquid chromatography-tandem mass spectrometry (HPLC LC/MS/MS), as previously described (26). Each analytical run included a calibration curve and quality control samples. An amendment during the study issued the retrieval of a second sample after 6 months (+/- 2 months) to account for missing or failed 3 months sample.

A blood sample for genetic analyses was also collected. Germline DNA was extracted from peripheral blood using the Qiagen DNAeasy kit (Qiagen, Belgium) and genotyped using Sequenom MassARRAY® at the Vesalius Research Centre, as previously described (27). Overall, 33 variants in 5 genes were selected; *CYP2D6*2A* (C1584G), **3A* (rs4986774), **3B* (rs1135824), **4* (rs1800716),**6A* (rs5030655), **6B* (rs5030866), **7* (rs5030867), **8* (rs5030865), **9* (rs5030656), **10* (rs1065852); **11* (rs5030863), **17* (rs28371706), **41* (rs28371725), **2*17* (rs16947). In *CYP2C19*: **2A* (rs4244285), **2B* (rs17878459), **3* (rs4986893), **4* (rs28399504), **5* (rs56337013), **6* (395G>A), **7* (rs72558186), **8* (rs41291556), **9* (rs17884712), **17* (rs11188072). In *CYP2B6*: **4* (rs2279343), **5* (rs3211371), **6* (rs3745274), **22* (rs34223104). In *CYP2C9*: **2* (rs1799853), **3* (rs1057910), **5* (rs28371686), **6* (rs9332131) and *CYP3A5*3* (rs776746).

Two single nucleotide polymorphisms (SNPs) failed genotyping (rs3745274 and rs1799853) and were omitted from analysis. Ten SNPs were 100% wild type. In addition, in 8 SNPs the frequency of the variant allele was <5%. We did not consider them for further analysis. Finally, rs1065852 failed Hardy-Weinberg equilibrium ($p<0.05$) and was removed for further analysis. As a result, 12 SNPs were included in the analysis. *CYP2D6* genotypes were translated to predicted phenotypes (normal, intermediate or poor metabolizer). By definition, the CYP2D6 intermediate metabolizer phenotype predicted by genotype consisted of patients homozygous for a decreased activity allele (e.g. *41/*41) or heterozygous for an absent activity allele (e.g. *1/*4 and *41/*4). Genotype frequencies were in accordance with previous literature in Caucasians (14).

In addition, the predicted phenotype (PM, IM, NM, UM) was corrected for use of well-known drugs that interfere with the metabolism of tamoxifen (28) by multiplying the score with the inhibition factor (0 for strong inhibitor, 0.5 for weak/moderate inhibitor and 1 for no inhibitor) (29). The effect of this corrected score on variation of endoxifen, objective tumour response, CB and PFS was also analysed.

Endpoints

ORR was the primary and CB the main secondary endpoint. Using, RECIST criteria 1.0, ORR was defined as the proportion of patients who achieved a complete response (CR), being the clinical disappearance of tumour or a partial response (PR) being a ≥30% decrease from baseline of unidimensional longest diameter only (size with conventional techniques ≥20 mm; spiral computed tomography ≥10 mm). Patients were assessed at month 3 and 6 and the best response was used. CB was achieved in case of a CR, PR or stable disease (SD) at 6 months.

The same methods of assessment and techniques for detecting lesions at study entry were used to follow up these lesions while on study. All tumour evaluations were performed by the attending physician and a second time by an independent physician of University Hospitals Leuven; discordant results were discussed with a third independent physician to reach consensus. ORR and CB were assumed to be absent in case of death or disease before evaluation of 3 months ORR. As per protocol, the main analysis for ORR and CB excluded patients without target lesion (i.e. bone-only lesions and no other measurable lesions). The main analysis for other secondary endpoints included patients without target lesion.

Efficacy of tamoxifen therapy was also measured through PFS as secondary endpoint defined as the time between start of tamoxifen therapy and the moment of progression. If no progression, patients were censored after last follow up visit. Cut-off date for study termination was nine months after the inclusion of the last patient (Feb. 28, 2015).

In addition, the association between tamoxifen and endoxifen through N-desmethyl-tamoxifen and 4OH-tamoxifen was investigated, as well as the independent relationship of these 4 metabolites and ORR. Steady state serum endoxifen and other metabolites concentrations were measured using HPLC LC/MS/MS.

Tolerability of tamoxifen was assessed with an 8-item health related Quality of Life Questionnaire (HR-QOL, secondary endpoint) (Supplementary Fig. S1). This questionnaire was developed and validated at the University Hospitals Leuven (27), and assessed the severity of menopausal symptoms experienced in the last seven days on a 5-point scale from 1 (not at all) to 5 (intolerable).

Sample size

Under the assumption of a linear relationship between endoxifen levels and ORR with an odds ratio (OR) of 1.49 per 10 nM, a sample size of 200 patients with target lesions would result in a power of 90% at an alpha level of 5% taking 10% dropout rate into account. Using available data on endoxifen concentrations, the above OR was chosen to reflect an improvement from 10% ORR in the lowest endoxifen quartile to 30% in the highest endoxifen quartile assuming the overall ORR would be 18%.

Statistical analysis

For the statistical analysis, we followed the pre-specified analysis plan from the protocol, unless otherwise stated. We used logistic regression to evaluate the relationship of endoxifen with ORR and CB. To examine the functional form of the relationship of endoxifen concentrations with ORR and CB, we used first degree fractional polynomials(29) with a 2 degree of freedom likelihood ratio chi-squared test. In an additional analysis, we repeated this approach with correction for a priori selected confounders: human epidermal growth factor receptor (HER-) 2

positivity and presence of visceral metastasis. We analysed PFS with Cox cause-specific proportional hazards regression, in which we label death prior to progression as a competing event. This deviates from the pre-specified analysis plan, where we stated that Cox proportional hazards regression would be used. We again used first degree fractional polynomials, similar to the analysis for ORR and CB. In the absence of progression or death, follow-up was censored at the time of study termination, loss to follow-up, or therapy switch because of intolerance.

TAS(18) is based on the presence of SNP in relevant genes combined with the effect of well-known drugs that interfere with the metabolism of tamoxifen. Whereas the protocol described that the explained variation in endoxifen by individual SNPs would be analysed, it is more relevant to study explained variation by TAS. This was done with the omega-squared statistic in a regression model of endoxifen on TAS. To assess the relationship between TAS, ORR and CB the logistic regression model was used that included TAS as categorical variable. For PFS, Cox proportional hazards regression was used.

Path analysis was used to investigate the association of tamoxifen with endoxifen through N-desmethyl-tamoxifen and 4OH-tamoxifen, and the association between these four metabolites and ORR. The strength of associations was quantified using omega-squared as a measure of explained variation.

We analysed severity of HR-QOL symptoms at three months using proportional odds logistic regression, with endoxifen and baseline severity as covariates. We graphically assessed the proportional odds assumption.

As per protocol, missing values for ORR, CB, PFS, and tamoxifen and endoxifen concentrations were not imputed. If tamoxifen or endoxifen concentrations were unavailable because of practical issues or refusal, the patient was excluded for the statistical analysis. Missing quality-of-life scores were multiply imputed as per protocol. The method of fully conditional specification was used using IVEware v0.2 (30). Results were compared with results based on (1) a complete case analysis, (2) imputation of worst possible severity for missing values, and (3) imputation of best possible severity for missing values. The last two sensitivity analyses were not pre-specified, but cover the possibility that missing QOL scores reflect very good scores (not worth mentioning) or very bad scores (unwilling to mention).

To check whether it is reasonable to use endoxifen at 6 months when a sample at 3 months is missing, we compared endoxifen levels at 3 and 6 months among patients with levels at both time points. As per protocol, we calculated the difference in mean endoxifen level, and generated a Bland-Altman plot.

Results

Patient characteristics

Twelve of the 309 (4%) patients with breast cancer were excluded due to screen failures (Supplementary Fig. S2). From 247 of the 297 (83%) patients we had endoxifen levels available. 209 (85%) of these had target lesions and considered for the primary and main secondary endpoint as predefined in the study protocol.

Forty-two% of the patients started tamoxifen in the primary metastatic setting; 31% and 27% received tamoxifen as neo-adjuvant and secondary metastatic treatment, respectively. Median age was 72 years (range 48-95) with a median BMI of 27 (range 14.1-50.7). Patient characteristics are listed in Table 1.

Tamoxifen metabolites

The protocol allowed a deviance of 2 months for the sample at 3 months. Hence, 229 of the 247 patients (93%) had a three-month sample, whereas for 18 patients a later sample had to be used.

There were 99 patients with an endoxifen sample at approximately 3 and 6 months, using a cut-off at 4.5 months to distinguish between 3- and 6-month samples. For these patients, we observed that the average endoxifen level was 1.2 µg/l higher at 6 months (95% CI, 0.2 to 2.1). The median level was only 0.4 µg/l higher at 6 months (95% CI, -0.3 to 1.1), due to an outlier with endoxifen levels of 14.2 µg/l at 3 months and 45.5 µg/l at 6 months. Together with the Bland-Altman plot (Supplementary Fig. S3), this suggested that there is no meaningful difference in endoxifen level at 3 and 6 months.

Endoxifen and tamoxifen efficacy

ORR was achieved in 45% (94/208) of the patients after 3 or 6 months on tamoxifen, whereas 1 patient was not evaluable for response. No relationship between endoxifen levels and ORR was found. The OR was 1.008 (95% CI 0.971-1.046, p=0.56). Although endoxifen levels were analysed continuously, Table 3 shows ORR per endoxifen quartile; 48% versus 42% respectively for the highest versus lowest quartile (OR 1.3, 95% CI 0.6-2.9). Adjustment for visceral metastasis and HER-2 positivity resulted in an OR of 1.006 (95% CI 0.967-1.046).

CB was achieved in 84% (170/202), whereas seven patients were not evaluable. No relationship between endoxifen levels and CB was found. The OR was 1.010 (95% CI 0.959-1.064, p=0.12). Table 4, shows CB per endoxifen quartile; 85% versus 79% respectively for the highest versus lowest quartile (OR 1.4, 95% CI 0.5-4.0). Correcting for predefined confounders resulted in an OR of 1.018 (95% CI 0.962-1.076).

After a median follow-up time of 32.5 months, 62% of 247 patients experienced progression on tamoxifen. Again, endoxifen levels were not clearly related to PFS. The hazard ratio (HR) was 0.990 (95% CI 0.968-1.012, p=0.10). The adjusted HR was 0.985 (95% CI 0.962-1.007). Supplementary Fig. S4, shows the Kaplan-Meier curves stratified by endoxifen quartiles.

For ORR, CB, and PFS a per protocol analysis excluding endoxifen samples taken within 12 hours following last tamoxifen intake did not affect the results (n=171 for ORR and CB, n=197 for PFS): the OR for ORR was 1.014 (0.973-1.056), the OR for CB 1.024 (0.963-1.087), and the HR for PFS 0.988 (0.964-1.012). When excluding the neoadjuvant patients for a post hoc analysis of PFS, 71% of 184 had progression after median follow-up of 34 months. The HR was 0.999 (95% CI 0.975-1.022).

TAS

Using TAS, based on the *CYP2D6* phenotype and co-medication (paroxetine, fluoxetine, bupropion, quinidine, cinacalcet, duloxetine, sertraline, terbinafine, amiodarone, and

cimetidine), 9% (22/242) of the patients were classified as PM, 33% (79/242) as NM and 58% (141/242) as IM (Table 5). No UM were present. PM exhibited a median endoxifen level of 4.9 µg/l (range 1.9-13.4 µg/l). IM and NM a median of 11.3 µg/l (range 7.3-17.0 µg/l) and 15.8 µg/l (range 2.9-34.7 µg/l), respectively. TAS explained 19% of the variation in endoxifen levels (95% CI 11-28). In our data, TAS had weak relationships with ORR, CB, and PFS (Table 5-6), although the 95% confidence intervals around the odds ratios and hazard ratios were wide (Table 6).

Association between tamoxifen metabolites and ORR

Tamoxifen and N-desmethyltamoxifen were most abundant (Table 2). All metabolites indicated large interindividual variability. The path analysis suggested that tamoxifen levels explain 68% of the variability in N-desmethyl-tamoxifen and 45% of 4OH-tamoxifen. In turn, N-desmethyl-tamoxifen and 4OH-tamoxifen explain 3% and 65% of the variability in endoxifen. Hence, tamoxifen is mainly related to endoxifen through 4OH-tamoxifen. Neither tamoxifen nor its derivations were meaningfully related to ORR, with explained variations between 0 and 2%.

Tolerability

Most commonly reported symptoms were joint and muscle pain (around 60%) and sleeping problems (around 40%), least commonly reported symptoms were sexual problems (9%) and vaginal dryness (around 18%) (Supplementary Table S2). Only hot flashes were reported more often after 3 months (from 28% to 53% during the day, from 25% to 43% at night).

Because scores >1 were not common, we reduced the responses to three ordinal levels for statistical analysis: 1, 2, and >2. The results of the ordinal regression analysis after multiple imputation of missing values (reported as odds ratios) suggested no clear relationship between endoxifen levels and quality of life (Supplementary Table S2).

Discussion

Given the complexity of *CYP2D6* genotyping and breast cancer outcome, we prospectively studied serum endoxifen levels and ORR as primary outcome together with the TAS based on genotype and interacting drugs studying other endpoints for outcome. None of the chosen endpoints for tamoxifen efficacy were associated with serum endoxifen levels; TAS did not explain the large variability seen in response to tamoxifen and only partially explained the inter-individual variability in endoxifen concentrations.

Some previous studies showed a higher risk of recurrence below an endoxifen threshold of 5.3-5.97 µg/l (10) whereas others showed higher endoxifen concentrations in those with higher risk of recurrence (31). We observed no significant worse outcome in tamoxifen users with low endoxifen levels of <7 µg/l on efficacy endpoints, limiting the use of therapeutic drug monitoring nor TAS. We cannot exclude a smaller effect size as more patients would have been needed with a less optimistic ORR of tamoxifen in first line endocrine therapy. However, only recently, others also have shown that only a fraction of endoxifen is predicted by metabolic phenotype (32).

Several studies have investigated a dose increase from 20 to 40mg of tamoxifen in *CYP2D6* PM or IM patients. This has been shown to significantly elevate endoxifen levels in the majority of patients (33-35), but in our study with even higher doses, serum endoxifen levels in PM could not fully be restored to NM (35). However, judging from our and other results a clear concentration-response effect or threshold for endoxifen does not exist and no full profit is gained by increasing dosage. The low endoxifen levels seen in some PM, together with the presence of active 4-OH tamoxifen and tamoxifen seem to be sufficient to saturate the ER and exert the anticancer effect (36).

Tamoxifen can enhance hot flashes and other menopausal symptoms in a substantial proportion of patients leading to treatment discontinuation (27). Although it has been suggested to be related to endoxifen concentrations (37), this was not confirmed in the current and another recent study (35).

The diverging results can be due to the large heterogeneity in studies reported so far: tissue for genotyping, data on co-medication, diverging assessment of metabolites, and selection of polymorphisms. Furthermore, most of the studies focused on *CYP2D6*, while variants in other genes may also play a role. Although not observed in the present study, it has been suggested that variation in activity of CYP3A, in addition to CYP2D6, appears to considerably influence endoxifen levels (17,38).

Besides the prospective design with prospectively collected endoxifen samples and predefined endpoints and sample size, the current study comprises several other strengths. Peripheral blood was used for germline DNA instead of tumour tissue as loss of heterozygosity of *CYP2D6* has been described in breast tumour (23). A highly selective HPLC LC/MS/MS was used for accurate quantification of tamoxifen and its metabolites. Additionally, data on co-medication were collected and integrated in the metabolic phenotype, which have been shown to improve the ability to predict phenotype (19). A study limitation is that we included three different risk groups of patients for tamoxifen efficacy: neo-adjuvant, primary and secondary metastatic. First, we only included patients where we expected a very high chance of clinical benefit from tamoxifen namely those considered endocrine sensitive independent of tumour stage which also reflects our clinical daily practice. Also, there is no reason to assume that pharmacokinetics of tamoxifen, known to be efficient in endocrine sensitive cases, differs in these patient categories. In addition, excluding those using tamoxifen in the neo-adjuvant setting did not change our results.

We also did not correct for previous adjuvant tamoxifen use in secondary metastatic cases but metastatic relapse after a year adjuvant tamoxifen stop remains a reasonable indication for tamoxifen. Also, we were not able to correct for other factors explaining residual variability

in endoxifen levels, neither did we correct PFS for the Karnofsky Performance Status. Lacking data on adherence can be another limitation as this affects endoxifen (35).

In conclusion, we have shown limited use for sole germline genotyping as a tool to guide tamoxifen treatment. Furthermore, under the pre-specified assumptions of an overall response rate improvement of 20% with higher endoxifen levels, our results do not support TAS or therapeutic drug monitoring on tamoxifen in postmenopausal patients with breast cancer.

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Tables

Table 1. Patient baseline characteristics: all, those with known endoxifen levels, all and per any of 3 treatment groups.

Variable	All (n=297)	Endoxifen (n=247)	Neo-adjuvant (n=63)	Primary metastatic (n=102)	Secondary metastatic (n=82)
Age (years)					
Median (range)	72 (48-95)	72 (48-95)	81 (48-95)	70 (50-88)	67 (50-87)
Missing	0	0	0	0	0
BMI					
Median (range)[a]	26.5 (14.1-50.7)	26.7 (17-51)	26.2 (18.4-42.4)	27.1 (16.6-50.7)	26.2 (17.1-41.1)
Missing	35	25	4	11	10
Endoxifen (µg/L)					
Median (range)	12.1 (0.7-35.1)	12.1 (0.7-35.1)	11.5 (2.2-30.5)	11.8 (1.9-35.1)	12.3 (0.7-34.7)
Missing	50	0	0	0	0
Setting, n (%)					
Neo-adjuvant	79 (27%)	63 (26%)	63 (100%)	0	0
Primary metastatic	126 (42%)	102 (41%)	0	102 (100%)	0
Secondary metastatic	92 (31%)	82 (33%)	0	0	82 (100%)
Missing	0	0	0	0	0
Histology, n (%)[b]					
IDA	196 (82%)	158 (79%)	49 (78%)	83 (81%)	26 (76%)
ILA	41 (17%)	38 (19%)	12 (19%)	18 (18%)	8 (24%)
IDA/ILA	2 (1%)	2 (1%)	1 (2%)	1 (1%)	0
Papillary	1 (<1%)	1 (<1%)	1 (2%)	0	0
Missing	57	48	0	0	48
Grade, n (%)[c]					
1	13 (6%)	12 (7%)	7 (7%)	5 (8%)	0
2	133 (63%)	106 (62%)	63 (66%)	33 (56%)	10 (63%)
3	64 (30%)	53 (31%)	26 (27%)	21 (36%)	6 (38%)
Missing	87	76	6	4	66
Node positive, n (%)[d]	93 (50%)	70 (48%)	45 (64%)	22 (37%)	3 (19%)
Missing	112	101	32	3	66
PR positive, n (%)[e]	226 (89%)	185 (89%)	96 (94%)	57 (92%)	32 (71%)
Missing	43	38	0	1	37
HER-2 positive, n (%)[f]	8 (3%)	8 (4%)	5 (5%)	2 (3%)	1 (3%)
Missing	54	49	1	3	45
Visceral metastasis, n (%)	111 (37%)	92 (37%)	1 (2%)	46 (45%)	45 (55%)
Missing	0	0	0	0	0
Bone only, n (%)	94 (32%)	82 (33%)	1 (2%)	50 (49%)	31 (38%)
Missing	0	0	0	0	0
Adjuvant tamoxifen, n (%)[g]	42/92 (48%)	37 (47%)	NA	NA	37 (47%)
Missing	5	3			3
Time since primary diagnosis (y)					
Median (range)[h]	12.5 (2.1-29.3)	12.8 (3.9-29.3)	NA	NA	12.8 (3.9-29.3)
Missing	4	3			3

a*N=262/297*; b*N=240/297*; c*N=210/297*; d*N=185/297*; e*N=254/297*; f*N=243/297*; g*N=87/92*; h*N=90/92* Q1: quartile 1; BMI: body mass index; *n*: number; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; PR: progesterone receptor; HER-2: human epidermal growth factor receptor 2; NA: not applicable.

Table 2. Steady-state serum concentrations of tamoxifen and its metabolites (*N=247*).

	Median	**Range**
tamoxifen (µg/l)	143.6	15.2-420.5
endoxifen (µg/l)	12.1	0.7-35.1
4-OH-tamoxifen (µg/l)	2.5	0.4-8
N-desmethyltamoxifen (µg/l)	247.1	24.8-537.8

Table 3. ORR stratified by endoxifen level using a quartile split.

Quartile	***N***	**endoxifen range (µg/l)**	**ORR, *n* (%)**	**OR vs first quartile (95% CI)**
1	*51*	0.7-6.9	*21* (42%)	-
2	*53*	7.0-12.0	*26* (49%)	1.4 (0.6-3.0)
3	*53*	12.1-17.7	*22* (42%)	1.0 (0.5-2.2)
4	*52*	18.1-35.1	*25* (48%)	1.3 (0.6-2.9)

ORR: objective response rate; N: number

Table 4. CB stratified by endoxifen level using a quartile split.

Quartile	***N***	**endoxifen range (µg/l)**	**CB, *n* (%)**	**OR vs first quartile (95% CI)**
1	*48*	0.7-6.9	*38* (79%)	-
2	*50*	7.0-12.0	*44* (88%)	1.9 (0.7-5.6)
3	*52*	12.1-17.7	*44* (85%)	1.4 (0.5-4.0)
4	*52*	18.1-35.1	*44* (85%)	1.4 (0.5-4.0)

CB: clinical benefit; N: number

Table 5. Distribution of phenotype corrected score and the associated endoxifen levels.

Corrected score	***N* (%)**	**Median (IQR) endoxifen**	**Range endoxifen**	**ORR, N (%)**
Normal	*79* (33%)	15.8 (11.6-24.2)	2.9-34.7	34/66 (52%)
Intermediate	*141* (58%)	11.3 (7.3-17.0)	0.7-35.1	52/120 (43%)
Poor	*22* (9%)	4.9 (3.1-6.1)	1.9-13.4	8/19 (42%)
Missing value	*5*			

IQR: interquartile range; N: number; ORR: objective response rate.
Results for the primary endpoint (ORR) were based on patients with target lesions only.

Table 6. Predicting clinical outcome using tamoxifen activity score.

Tamoxifen Activity Score	**OR ORR**	**OR CB**	**HR PFS**
Normal vs poor	1.46 (0.52 to 4.22)	1.18 (0.30 to 3.97)	0.82 (0.47 to 1.51)
Intermediate vs poor	1.05 (0.40 to 2.89)	1.65 (0.43 to 5.26)	0.76 (0.45 to 1.36)

Results are presented as odds ratios or hazard ratios with 95% confidence intervals. OR: odds ratio; ORR: objective response rate; CB: clinical benefit; HR: hazard ratio; PFS: progression-free survival. As specified in the protocol, we used only patients with target lesions for ORR and CB, whereas we included patients without target lesions for PFS.