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A prospective study on the effect of CYP2D6 genotype and pharmacokinetics on clinical outcome in tamoxifen treated early breast cancer patients (CYPTAM study)

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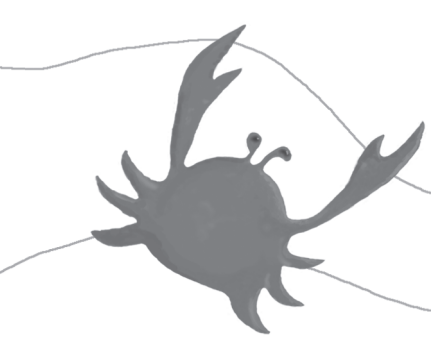

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ABSTRACT

Background In tamoxifen treated breast cancer patients absent or reduced CYP2D6 activity results in lower blood concentrations of the active metabolite endoxifen, potentially impairing tamoxifen's clinical benefit. Endoxifen levels have never been prospectively correlated with tamoxifen efficacy.

Methods In a prospective multicenter study (CYPTAM: NTR1509) in The Netherlands and Belgium tamoxifen treated early breast cancer patients were included aiming to relate CYP2D6 predicted phenotypes and endoxifen serum concentrations to disease free survival (primary aim) and recurrence free and overall survival (secondary aim). Additionally, the CYP2D6 phenotypes (ultrarapid (=UM), extensive (=EM), heterozygous extensive (=hetEM), intermediate (=IM) and poor metabolizer (=PM)) were correlated with endoxifen serum concentrations.

Results In the prospective CYPTAM study 671 patients were enrolled. The median (range) endoxifen serum concentration for each phenotype was: UM: 67.7 nM (43.9-83.0); EM: 33.7 nM (7.2-87.3); hetEM: 25.3 nM (6.5-76.3); IM: 15.0 nM (4.6-121.3); PM: 9.1 nM (5.1-27.6). The CYP2D6 phenotypes were correlated with endoxifen concentrations ($p<0.001$); 43% of the variance of endoxifen levels was explained by CYP2D6 phenotype. The hetEM phenotype was identified as a distinct phenotype with a different mean endoxifen level than the other phenotypes ($p<0.001$).

Conclusions The different CYP2D6 phenotypes are significantly associated with endoxifen concentrations, although not all variance is explained by CYP2D6 phenotype alone. Therefore, endoxifen serum concentration may be a better predictor of tamoxifen efficacy. Final results of the current study addressing the association between CYP2D6 phenotypes, serum endoxifen levels and clinical outcome after three years of follow-up are awaited.

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INTRODUCTION

In the last decade great effort has been put in investigating the relation between the Cytochrome P450 2D6 (CYP2D6) activity and tamoxifen efficacy. These investigations were encouraged by the first observations that CYP2D6 genotype and concomitant CYP2D6 inhibitor use led to significant lower endoxifen plasma concentrations in tamoxifen users.[1, 2] Endoxifen is considered the most important active tamoxifen metabolite based on in vitro experiments and the observation that in tamoxifen users plasma concentrations of endoxifen are 10-fold higher than those of the equally potent metabolite 4-hydroxytamoxifen.[1, 3-6] In a retrospective study endoxifen serum concentrations were associated with breast cancer survival.[7] An endoxifen threshold effect was suggested. However, the borderline significant results should be interpreted with caution, especially because the study was not designed for that purpose. The direct effect of endoxifen levels on clinical outcome in tamoxifen treated breast cancer patients has never been prospectively tested.

CYP2D6 genetic variants leading to absent or decreased enzymatic activity have been studied in relation to tamoxifen efficacy as a proxy for endoxifen concentration, because CYP2D6 is mainly responsible for endoxifen formation. An advantage of testing CYP2D6 genotype rather than endoxifen concentration is that genotyping can be performed in advance of tamoxifen treatment. Nonetheless, the data from mainly retrospective studies addressing the relation between CYP2D6 genotype and tamoxifen efficacy are conflicting.[8-15] An important issue remains that despite the significant association between CYP2D6 genotypes and endoxifen plasma concentration, the correlation coefficient is low. The explained variance in the first study by Jin et al. was 23%, although this was based on determination of only four CYP2D6 alleles.[2] Schroth et al. subsequently demonstrated that the effect size of the association between the CYP2D6 predicted phenotype and tamoxifen efficacy was increased when the number of tested CYP2D6 alleles was expanded.[16]

Recently, two large CYP2D6 association studies were criticized because tumor tissue was used as a source of DNA to genotype CYP2D6.[14, 15] Loss of heterozygosity in tumor tissue may have led to false CYP2D6 genotype assignment. Therefore, blood is the preferred source of DNA when germline genetic variants of CYP2D6 are investigated.[17]

A prospective multicenter documentation study in The Netherlands and Belgium was initiated in 2008 aiming to relate CYP2D6 predicted phenotypes and endoxifen serum concentrations to disease free survival (primary aim) and recurrence free and overall survival (secondary aim) in tamoxifen treated early breast cancer patients (CYPTAM study: NTR1509[18]). CYP2D6 genotyping was performed using the Amplichip CYP450 test (Roche Diagnostics, Indianapolis, US) to test a broad range of CYP2D6

alleles on whole blood. As an additional analysis, the CYP2D6 predicted phenotypes were related to steady state serum tamoxifen and tamoxifen metabolite (including endoxifen) levels.

METHODS

Study population

From February 2008 early breast cancer patients who were using tamoxifen as part of an adjuvant treatment were included in a multicenter study (CYPTAM) in The Netherlands and Belgium aiming to relate CYP2D6 predicted phenotype and endoxifen serum concentrations to breast cancer survival.[18] Pre- and postmenopausal women (≥ 18 years) with hormone receptor positive early breast cancer treated with tamoxifen with maximum treatment duration of one year were eligible for the study if they were willing and able to give informed consent. Patients were ineligible if they had a medical history of another malignancy within the previous 5 years (except adequately treated in situ carcinoma of cervix or basal cell carcinoma). Concomitant use of a CYP2D6 inhibitor was permitted.

Endpoints

Primary endpoint was disease free survival (DFS) defined as the time from study enrollment to locoregional or distant recurrence, second breast cancer, or death without recurrence. Secondary endpoints were 1) recurrence free survival (RFS) defined as the time from study enrollment to locoregional or distant recurrence or second breast cancer and 2) overall survival (OS) defined as the time from study enrollment to death by any cause.

CYP2D6 predicted phenotype

CYP2D6 genotyping was performed using the Amplichip CYP450 test (Roche Diagnostics, Indianapolis, US) to test the major (33) CYP2D6 alleles on whole blood. CYP2D6 genotypes were translated to predicted phenotypes (ultrarapid (UM), extensive (EM), heterozygous extensive (hetEM), intermediate (IM) or poor metabolizer (PM)). By definition, the CYP2D6 IM phenotype was predicted by a genotype consisting of two decreased activity alleles (e.g. *41/*41) or one absent and one decreased activity allele (e.g. *41/*4). The hetEM phenotype was defined as a genotype with one absent and one normal activity allele (e.g. *1/*4).[16] Additionally, concomitant use of a CYP2D6 inhibitor could reclassify the CYP2D6 phenotype predicted by genotype.[19]

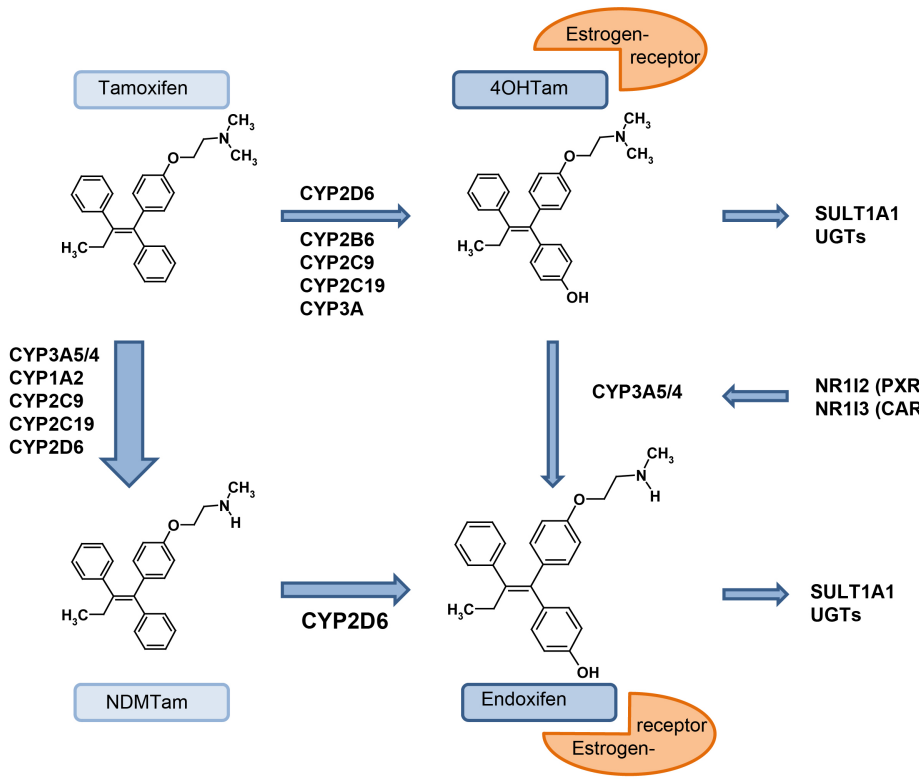

Figure 11.1 Tamoxifen metabolism. Abbreviations: 4OHTam, 4-hydroxytamoxifen; CYP, cytochrome P450 isoenzyme; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase; NDMTam, N-desmethyltamoxifen; NR1, nuclear receptor subfamily 1; PXR, pregnane X receptor; CAR, constitutive androstane receptor.

Tamoxifen and metabolite measurement

Serum concentrations of tamoxifen and its metabolites were measured by using a high performance liquid chromatography-tandem mass spectrometry (HPLC LC/MS/MS) assay for the detection of tamoxifen and the metabolites N-desmethyltamoxifen (NDMTam), 4-hydroxytamoxifen (4OHTam) and 4-hydroxy-N-desmethyltamoxifen (endoxifen) in human serum (Figure 11.1). The HPLC LC/MS/MS assay was developed and validated for the purpose of the CYPTAM study at the laboratory of Clinical Pharmacy and Toxicology at the Leiden University Medical Center and is similar to a previously described method.[20]

Study design

After enrollment in the CYPTAM documentation study whole blood and serum samples were retrieved for CYP2D6 genotyping and measurement of tamoxifen and its metabolites after ≥ 2 months of tamoxifen use, ensuring steady state endoxifen levels. The time interval between serum sample retrieval and the last tamoxifen intake had to be more than 12 hours to obtain endoxifen trough levels. All patients were subsequently informed of their CYP2D6 phenotype by their own physician. The reason for this was to enable patients with a PM or IM phenotype to participate in a separate genotype-directed dose escalation study. Patients were followed in time to meet the clinical endpoints. Information regarding patient and tumor characteristics, previous and subsequent therapies, start and stop dates of the tamoxifen therapy, concomitant use of other medication and information needed for the clinical endpoints will be retrieved afterwards using the Dutch cancer registry, the patient's medical record and information from the community pharmacies. This information will be collected by trained data managers and registered on case record forms. The current study was approved by the central medical ethics review board of the Leiden University Medical Center in Leiden, The Netherlands. All patients gave written informed consent before entering the study.

Statistical methods

In order to detect a two-fold worse 3-years disease free survival (DFS) of the combined group of PMs hetEMs and IMs compared to EMs and UMs (hazard ratio=2.0), 650 patients should be enrolled to achieve 80% power at a 0.05 significance level. For comparison of proportions and means, χ^2 statistics and the Student's t-test or one-way ANOVA will be used respectively. Cox regression analysis will be used to assess whether DFS, RFS and OS differ with respect to age at diagnosis, surgical procedure, tumor size, grade, nodal status, adjuvant chemotherapy, adjuvant radiotherapy, the CYP2D6 predicted phenotypes and endoxifen serum concentrations. The distributions of DFS, RFS and OS will be estimated overall using the Kaplan-Meier method. A log-rank test will be used to assess the association between the CYP2D6 phenotypes, serum endoxifen concentration categories and the clinical outcome of interest.

The contributions of the various CYP2D6 predicted phenotypes to the total variability of serum tamoxifen and tamoxifen metabolite concentrations and the metabolic ratio of N-desmethyltamoxifen and endoxifen were determined using linear regression analysis. For this purpose, tamoxifen and tamoxifen metabolite serum levels were log transformed.

RESULTS

From February 2008 until December 2010 a total of 671 patients were enrolled in the CYPTAM study from 19 participating hospitals in The Netherlands and 6 hospitals in Belgium, two of which were academic hospitals. From 656 out of 671 patients whole blood samples were retrieved. Serum samples were collected from 664 patients. In 642 out of 656 patients (97.9%) successful genotyping led to classification into a CYP2D6 phenotype. CYP2D6 phenotyping resulted in 5 UMs (0.8%), 319 EMs (49.7%), 212 hetEMs (33.0%), 59 IMs (9.2%) and 47 PMs (7.3%). From 635 of these 642 patients serum samples were available to analyze tamoxifen and metabolite serum concentrations. The baseline patient and tumor characteristics, previous and subsequent therapies, tamoxifen start and stop dates, information on co-medication as well as data needed for the different endpoints are currently being collected by trained data managers.

As an additional analysis the CYP2D6 phenotypes were related to the steady state serum endoxifen trough concentrations. The mean tamoxifen and metabolite serum concentrations according to CYP2D6 phenotype are shown in Table 11.1 and Figure 11.2. The mean (SD; range) endoxifen serum concentration for each phenotype was: UM: 60.4 nM (19.3; 37.0-83.0); EM: 35.9 nM (14.3; 5.3-87.3); hetEM: 26.4 nM (11.5; 6.5-81.4); IM: 18.3 nM (15.8; 4.6-121.3); PM: 9.7 nM (4.7; 4.1-27.6).

Table 11.1 CYP2D6 phenotypes in relation to tamoxifen and metabolite serum concentration

	Tamoxifen (nM)			NDM-tamoxifen (nM)		
CYP2D6 phenotype	Mean (median)	SD	Range	Mean (median)	SD	Range
UM	374.8 (383.0)	95.8	270.0-519.5	509.3 (499.3)	102.2	399.2-674.6
EM	301.2 (278.3)	110.9	48.2-735.1	533.5 (498.9)	191.1	157.2-1418.5
hetEM	313.9 (282.9)	127.9	95.2-1141.6	620.6 (569.5)	214.7	207.8-1664.6
IM	341.7 (323.0)	150.1	133.6-805.1	758.5 (714.7)	247.7	292.6-1611.2
PM	316.4 (287.6)	123.5	115.1-576.3	775.8 (715.3)	245.4	380.7-1325.1
	Endoxifen (nM)			4-OH-tamoxifen (nM)		
	Mean (median)	SD	Range	Mean (median)	SD	Range
UM	60.4 (67.7)	19.3	37.0-83.0	8.9 (9.0)	2.3	5.9-12.1
EM	35.9 (34.0)	14.3	5.3-87.3	5.8 (5.4)	2.3	1.0-16.5
hetEM	26.4 (25.4)	11.5	6.5-81.4	4.8 (4.4)	1.9	1.3-15.7
IM	18.3 (14.5)	15.8	4.6-121.3	4.5 (3.9)	2.6	1.6-20.6
PM	9.7 (8.57)	4.7	4.1-27.6	3.7 (3.6)	1.3	1.3-9.0

UM, ultrarapid metabolizer; EM, extensive metabolizer; hetEM, heterozygous extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer

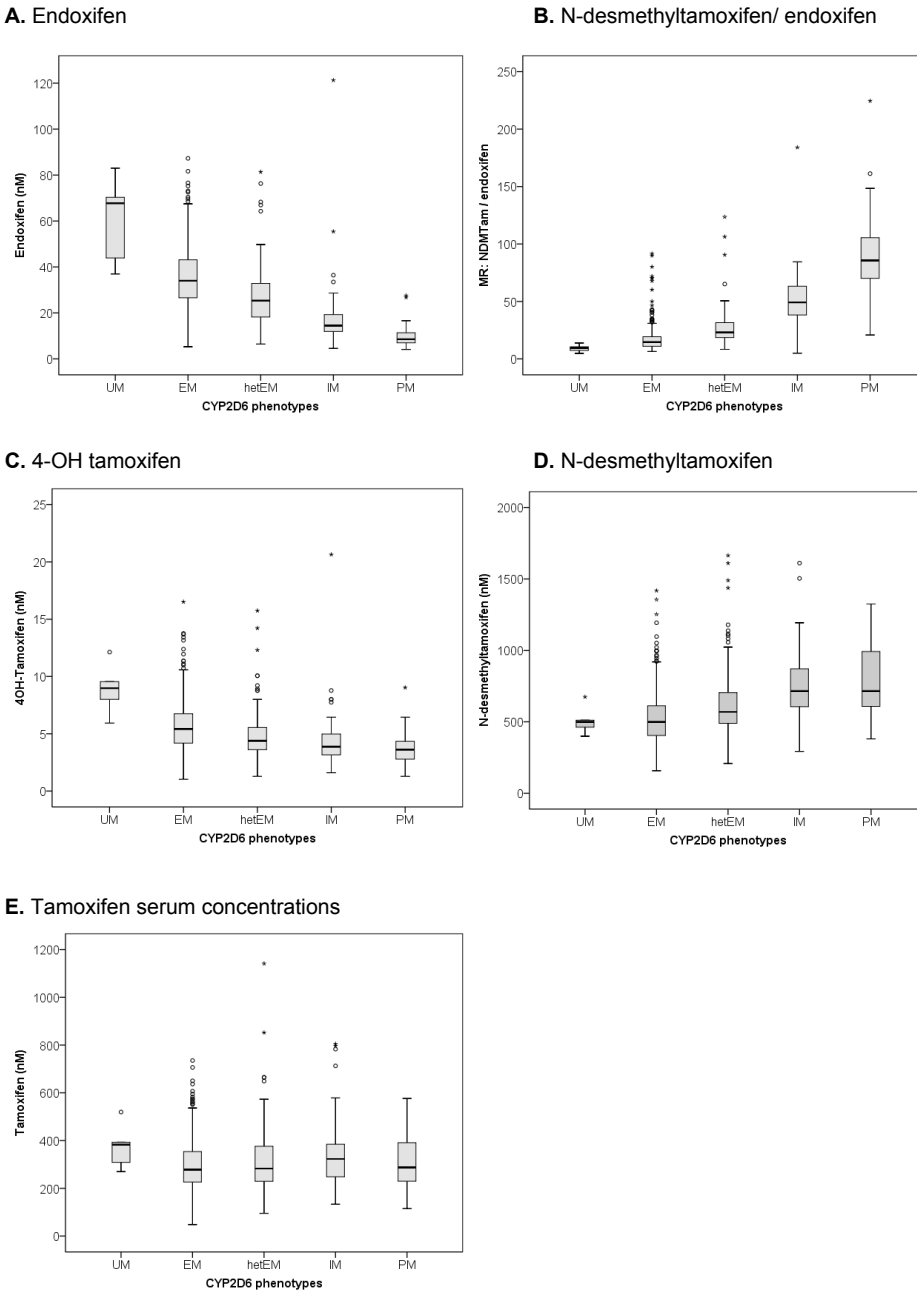

Figure 11.2 CYP2D6 predicted phenotypes in relation to (**A**) endoxifen serum concentrations; (**B**) metabolic ratio of N-desmethyltamoxifen / endoxifen; (**C**) 4-hydroxytamoxifen serum concentrations; (**D**) N-desmethyltamoxifen serum concentrations; (**E**) Tamoxifen serum concentrations. UM, ultrarapid metabolizer; EM, extensive metabolizer; hetEM, heterozygous extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; MR, metabolic ratio

Table 11.2 Linear regression analysis of CYP2D6 phenotypes and serum concentrations of tamoxifen and its metabolites

		R	R^2	p-value
Tamoxifen		0.11	0.01	0.12
	LN (Tamoxifen)	0.10	0.01	0.15
NDMTam		0.37	0.14	<0.001
	LN (NDMTam)	0.38	0.15	<0.001
4-OHTam		0.33	0.11	<0.001
	LN (4-OHTam)	0.37	0.14	<0.001
Endoxifen		0.54	0.29	<0.001
	LN (Endoxifen)	0.66	0.43	<0.001
NDMTam/endoxifen		0.77	0.59	<0.001
	LN (NDMTam/endoxifen)	0.76	0.57	<0.001

R, correlation coefficient; R^2, explained variance; NDMTam, N-desmethyltamoxifen; 4-OHTam, 4-hydroxytamoxifen

The CYP2D6 phenotypes were significantly associated with endoxifen, 4-hydroxytamoxifen and N-desmethyltamoxifen concentrations ($p<0.001$); 43% of the variance of (log transformed) endoxifen levels and 59% of the variance of N-desmethyltamoxifen/endoxifen ratio was explained by CYP2D6 phenotype (Table 11.2 and Figure 11.2). The correlation between the CYP2D6 phenotypes and 4-hydroxytamoxifen and N-desmethyltamoxifen was small with an R^2 of 14% and 15% respectively (Table 11.2). No correlation was found between the CYP2D6 phenotypes and tamoxifen serum concentrations. The hetEM phenotype was identified as a distinct phenotype with a different mean endoxifen level compared to the other phenotypes ($p<0.001$).

DISCUSSION

The ongoing research investigating the relation between tamoxifen metabolism and clinical outcome is timely and important, despite the previously reported conflicting data. Critics state that some data from retrospective association studies may not be valid because of the use of tumor blocks as source of DNA potentially leading to false CYP2D6 genotype assignment.[15, 17] In addition, in some studies the association may have been underestimated because only a limited number of CYP2D6 alleles was investigated.[8, 21-23] Other criticism concerns the retrospective nature of most studies: many potentially confounding factors such as concomitant CYP2D6 inhibitor use are not or incompletely documented. To encounter this criticism, we initiated a prospective documentation study in which potentially confounding factors are well documented, a broad range of CYP2D6 alleles was tested

and whole blood was used to genotype instead of tumor tissue. Furthermore, this is one of the first prospective studies addressing the direct relation between endoxifen and tamoxifen efficacy.

In the current study a total of 671 patients were enrolled. In 642 patients the predicted CYP2D6 phenotypes will be related to DFS, RFS and OS; in 664 patients the endoxifen levels will be related to the same clinical endpoints. Of note, the sample size calculation for the primary aim of the current study was based on the studies by Goetz et al. (HR=1.86) and unpublished results from Gonzalez-Santiago et al. (HR=2.82) with the assumption of a larger effect size than reported by Goetz because of a broader CYP2D6 allelic coverage.[8, 24] We assumed a two-fold increased risk for disease recurrence in PMs, IMs and hetEMs compared to EMs and UMs (HR=2.0), which may be an overestimation given the results of more recent studies.[14, 15, 25, 26]

In the current study we detected a small correlation between the CYP2D6 phenotypes and 4-hydroxytamoxifen (R^2=14%) and N-desmethyltamoxifen (R^2=15%) respectively. This is explained by the moderate contribution of CYP2D6 to 4-hydroxytamoxifen formation and the accumulation of N-desmethyltamoxifen in case of an impaired CYP2D6 mediated biotransformation from N-desmethyltamoxifen to endoxifen (Figures 11.1 and 11.2). However, 43% of the variation of the (log transformed) serum endoxifen concentration was explained by the CYP2D6 phenotype (Table 11.2). Furthermore, we distinguished the heterozygous extensive metabolizer (e.g. *1/*4) phenotype as a separate CYP2D6 phenotype with a mean endoxifen level in between those observed in IMs and EMs ($p<0.001$). The explained variance is higher than the previously observed 23% by Jin et al., probably because of the broader allelic coverage using the Amplichip CYP450 test. Of note, in the study by Jin et al. endoxifen concentrations were not log-transformed. When the CYP2D6 phenotypes were related to the metabolic ratio between N-desmethyltamoxifen and endoxifen, the explained variance was even better (57%; Table 11.2). This exemplifies the importance of CYP2D6 as main enzyme involved in the hydroxylation of N-desmethyltamoxifen leading to endoxifen formation. Nonetheless, the residual variance of the endoxifen levels is not explained by the CYP2D6 predicted phenotype. A partial explanation is that endoxifen not only results from hydroxylation of N-desmethyltamoxifen by CYP2D6, but also from N-demethylation of 4-hydroxytamoxifen. Furthermore, CYP2D6 activity is not only influenced by CYP2D6 genotype but also by epigenetic and environmental factors.[27] Concomitant use of a CYP2D6 inhibitor influences CYP2D6 activity and must be accounted for when translating the genotype to a predicted CYP2D6 phenotype. Data managers are currently collecting data for the CYPTAM study concerning CYP2D6 inhibitor use. Thus, in the current analysis concomitant CYP2D6 inhibitor use was not accounted for. Nonetheless, the influence of concomitant CYP2D6 inhibitor use is expected to be small: previously, we demonstrated

that in the Dutch population only 7.6% of the early breast cancer patients was regularly using a CYP2D6 inhibitor during tamoxifen treatment.[28]

The final results of this study and similar ongoing prospective studies addressing the relation between CYP2D6 phenotypes, endoxifen concentrations and clinical outcome are awaited. A positive association between endoxifen levels and clinical outcome is important as it may lead to the possibility of therapeutic drug monitoring, as well as the further development of endoxifen as primary endocrine therapeutic for breast cancer.[29] An additional positive association between CYP2D6 predicted phenotypes and tamoxifen efficacy may lead to a pre-treatment selection of those patients that we should withhold tamoxifen therapy or treat with a higher daily tamoxifen dose guided by endoxifen levels. The long term safety and efficacy of an increased tamoxifen dose should of course first be ensured.

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St. Antonius Ziekenhuis; Los
Rijnland Ziekenhuis; Zeillemaker
Zaans Medisch Centrum; Van Bochove
Waterland Ziekenhuis; Brakenhoff
IJsselland Ziekenhuis; Kehrer
Kennemer Gasthuis; Kuijer
Meander Medisch Centrum; Bloemendal
Tergooi Ziekenhuizen; Luijkx
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