Prospective assessment of CYP2D6 by genotyping, phenotyping and measurement of tamoxifen, 4-hydroxy-tamoxifen and endoxifen in breast cancer patients treated with tamoxifen.

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Background

Tamoxifen (tam) is a widely used endocrine therapy in the treatment of early and advanced stage breast cancer in women and men. It is a pro-drug having weak affinity with the estrogen receptor and needs to be converted to its main metabolite, endoxifen (endox), to have full anticancer activity.

Cytochrome 2D6 (CYP2D6) plays a major role in the metabolism of tamoxifen to endoxifen. It is genetically highly polymorphic and its activity influences profoundly the synthesis of endoxifen and potentially the efficacy of tamoxifen treatment.

Genotyping is currently the most widely used approach in studies and also in clinical practice to categorize patients as poor- (PM), intermediate- (IM), extensive- (EM) and ultra rapid-metabolizers (UM). Some clinicians already use genotyping in order to tailor the endocrine therapy of their patients.

Owing to the large inter-individual variations in concentrations of the active moiety due to genetic and non-genetic influences renders the predictive value of the test uncertain for an individual patient. A significant number of patients classified as EM or IM by genotyping have indeed relatively low endoxifen levels similar to PMs[1]. This suggests that genotyping is probably not the optimal method for predicting endoxifen levels.

Tamoxifen and its main metabolites

**Factors influencing endoxifen level**
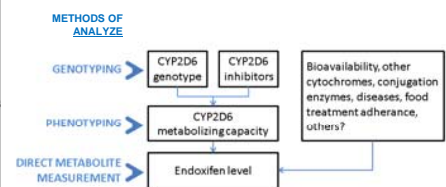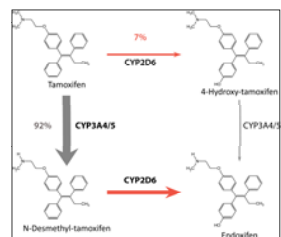
Objective

To assess if the correlation between genotyping and endoxifen levels is strong enough to use this test in individual patients and what is the value of CYP2D6 and CYP3A4/5 phenotyping?

We present here a substudy of a larger ongoing trial studying tamoxifen metabolism in breast cancer patients (NCT00963209).

Materials & Methods

Study design:

≥ 4 months

Tamoxifen 20 mg/day

Inclusion

Tamoxifen and metabolites
CYP2D6 genotyping
CYP2D6 and CYP3A4/5 phenotyping
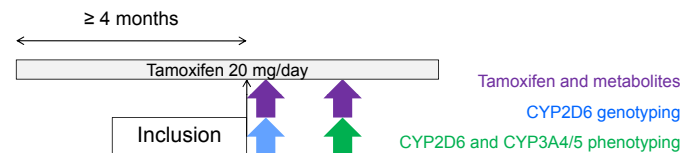
Blood sampling: 2x 5 mL in EDTA tubes
Samples were centrifuged without delay: cellular pellet was used for genetics analyses and plasma for determination of levels of tamoxifen and its metabolites by LC tandem MS; and stored frozen (-80°C).
The analyses were done centrally in our laboratories:

➤**Tamoxifen and Metabolites levels in plasma**
An ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was used for the simultaneous measurements of tamoxifen and its metabolites[2].

➤**CYP2D6 genotyping analyses**
alleles *3, *4, *5, *6, *XN

➤**CYP2D6 phenotyping test**
The dextromethorphan test was used for the determination of CYP2D6[3].

➤**CYP3A4/5 phenotyping test**
midazolam metabolic ratio[4] was used for the determination of CYP3A4 activity.

Eligibility criteria
–Signed informed consent.
–Breast cancer patients treated with tamoxifen for ≥ 4 months
– No history of deep venous thrombosis or pulmonary embolism.
– No history of endometrial carcinoma.
– No known history of vaginal bleeding, endometriosis, endometrial hyperplasia, endometrial hypertrophy and/or polyps.
– No pregnancy nor lactating women.
– No known allergy to midazolam or dextromethorphan

Treatment adherence assessment
Each patient completed an anonymized questionnaire to report semi-quantitatively her treatment adherence.

Statistical considerations
The CYP2D6 genotypes were categorized into four groups and scored on the basis of the number of functional alleles (PM = homozygous loss of functional allele, IM = heterozygous loss of functional allele, EM = homozygous reference allele, UM= multiple functional alleles).
Correlations between log-transformed trough levels of tamoxifen, its metabolites, and the ratios endoxifen/N-desmethyltamoxifen, N-desmethyltamoxifen/tamoxifen with CYP2D6 genotypes, phenyotypes and CYP3A4/5 phenotypes were analyzed by univariate and multivariate linear regression analyses and analyses of variance. Comparison between models were performed based the adjusted R^2 and the likelihood ratio test, at a significance level of $p=0.05$. All data analyses were conducted with Stata statistical software (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP).

Results

57 patients were included in the principal trial. 39 of them, with trough levels measured > 12 h post-dose, were eligible for these analyzes.

7 patients were treated with CYP2D6 inhibitors (3 citalopram, 1 escitalopram, 1 venlafaxine, 1 risperidone, 1 paroxetine).

Table 1: Patients characteristics

Age Median (range)		52 years (32 - 78)
Race	Caucasian	37
	African	1
	Indian	1
Premenopausal		27
Postmenopausal		12
Tumor histology	Ductal invasive	25
	Lobular invasive	8
	Other invasive subtypes	5
	Ductal in situ	1
Treatment adherence		> 95%

Table 2: Plasma levels of tamoxifen and its metabolites:

	Geometric mean (nM)	CV (%)	Median (nM)	Range (nM)
Tam	372	44	351	187-1070
N-D-Tam	804	42	729	383-1779
4-OH-Tam	5.8	50	5.8	2.6-18.8
Endoxifen	63.3	84	72.2	14.1-302

Table 3: How do CYP2D6 genotype or phenotype and CYP3A4/5 phenotype influence concentrations of tamoxifen and its metabolites (linear regression analyses):

Correlation to CYP2D6 genotype:

	Tam	N-D-Tam	4-OH-Tam	Endox	Endox/N-D-Tam	4-OH-Tam/Tam	N-D-Tam/Tam
R^2 (%)	6	12	16	33	47	50	3
P	0.13	0.027	0.012	0.0001	<0.0001	<0.0001	0.27

Correlation to CYP2D6 phenotype (dextromethorphan/dextrorphan ratio) :

	Tam	N-D-Tam	4-OH-Tam	Endox	Endox/N-D-Tam	4-OH-Tam/Tam	N-D-Tam/Tam
R^2 (%)	2	8	8	39	55	26	6
P	0.35	0.08	0.1	<0.0001	<0.0001	0.0013	0.13

Correlation to CYP3A4/5 phenotype (1'-OH-midazolam/midazolam ratio) :

	Tam	N-D-Tam	4-OH-Tam	Endox	Endox/N-D-Tam	4-OH-Tam/Tam	N-D-Tam/Tam
R^2 (%)	36	12	23	12	1	1	31
P	0.0001	0.036	0.003	0.034	0.51	0.56	0.0003

Multivariate analyses
Performed to assess the correlation between endoxifen level and the independent variables (CYP2D6 genotype and phenotype and CYP3A4/5 phenotype):
• Only CYP2D6 and CYP3A4/5 phenotypes influenced significantly endoxifen level.
• Suggests no additional statistical predictive value of CYP2D6 genotype in addition to CYP2D6 phenotype on endoxifen (p = 0.36) and endoxifen/N-D-Tam ratio (p = 0.17).
• An additional effect of CYP3A4 phenotype was observed, increasing the predictive value of endoxifen levels (p < 0.0001, adjusted R^2 = 50%).

Figure1: Genotyping vs. endoxifen level and endoxifen/N-D-tamoxifen ratio
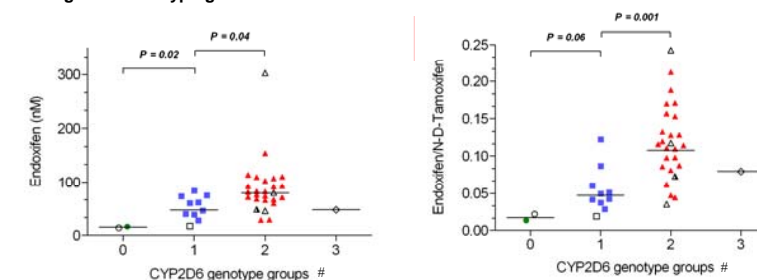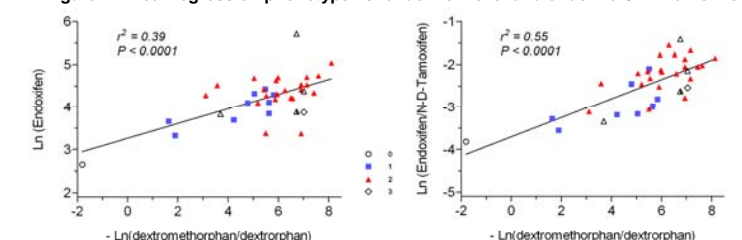

Figure 2: linear regression phenotype vs. endoxifen level and endoxifen/N-D-Tamoxifen ratio

0: PM (n=2); 1: IM (n=10); 2: EM (n=26); 3: UM (n=1); empty figures = patients with CYP2D6 inhibitors; filled figures = patients without CYP2D6 inhibitors

Conclusions

▪ **Our data confirm a significant correlation between plasma levels of endoxifen and CYP2D6 activity defined by genotyping or by phenotyping.**

▪ **Following the multivariate analyses, phenotyping appears slightly superior to genotyping in the prediction of endoxifen's level.**

▪ **Due to the large overlap between EM and IM, the predictive value of genotyping seems uncertain for an individual patient.**

▪ **Our results are consistent with a potential superiority of monitoring the active metabolites themselves rather than genetic or phenotypic surrogates**

▪ **These conclusions are based on a limited number of patients. The study assessing tamoxifen metabolism and the impact of tamoxifen dose on endoxifen level is ongoing.**

References

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