Tamoxifen pharmacogenetics and pharmacokinetics in early breast cancer

Sanchez Spitman, A.B.

Cover Page

Universiteit Leiden

Leiden University Repository

The handle https://hdl.handle.net/1887/3142390 holds various files of this Leiden University dissertation.

Author: Sanchez Spitman, A.B.
Title: Tamoxifen pharmacogenetics and pharmacokinetics in early breast cancer
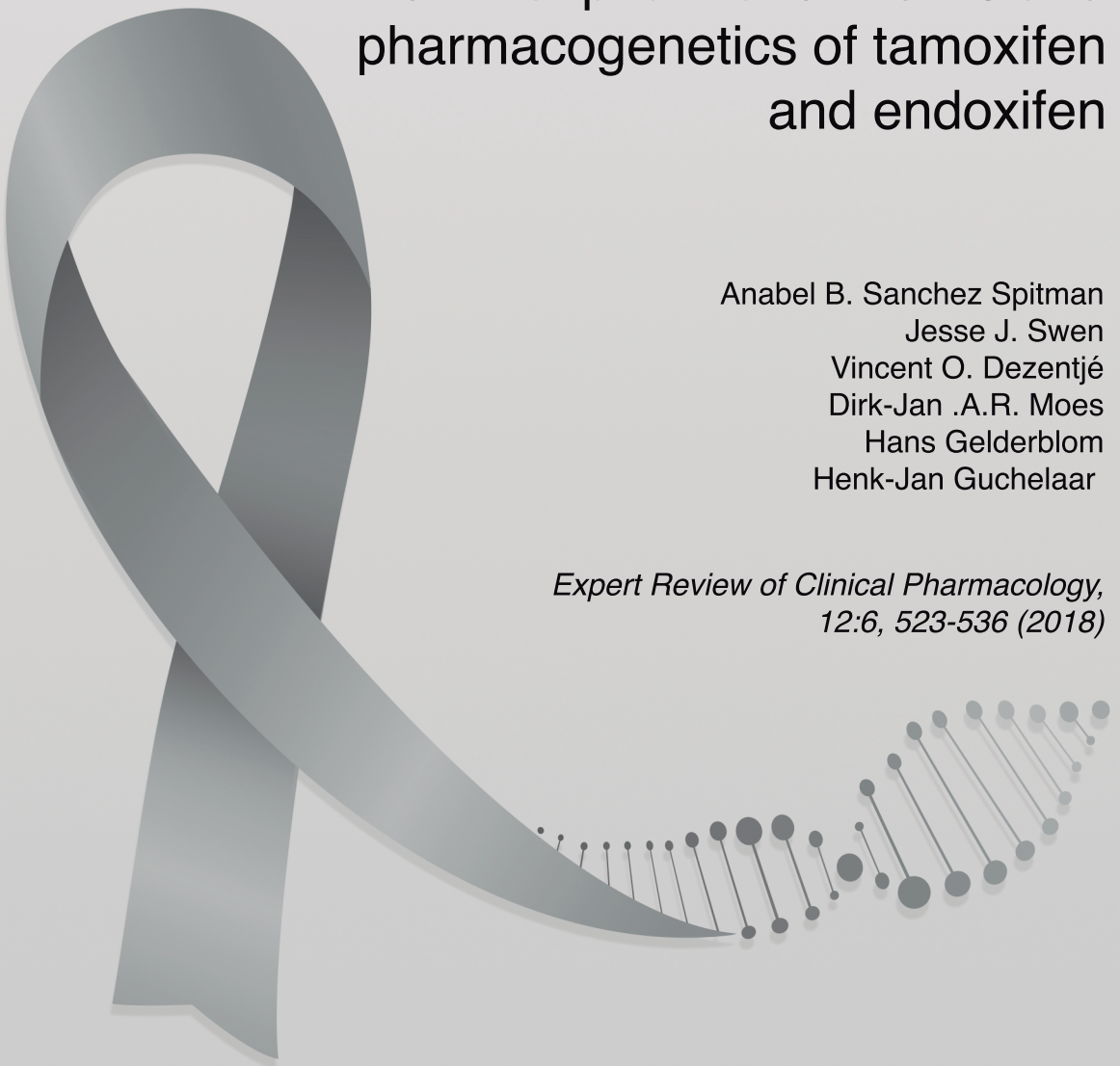**Issue Date**: 2021-02-18

Clinical pharmacokinetics and pharmacogenetics of tamoxifen and endoxifen

Anabel B. Sanchez Spitman
Jesse J. Swen
Vincent O. Dezentjé
Dirk-Jan .A.R. Moes
Hans Gelderblom
Henk-Jan Guchelaar

Abstract

Introduction

Tamoxifen dominates the anti-estrogenic therapy in the early and metastatic breast cancer setting. Tamoxifen has a complex metabolism, being mainly metabolized by CYP2D6 into its 30–100 times more potent metabolite, endoxifen. Recently, a phase I study in which endoxifen as an orally z-endoxifen hydrochloride has been successfully evaluated.

Areas covered

The principal pharmacogenetic and non-genetic differences in the pharmacology of tamoxifen and endoxifen are evaluated. To this end, references from PubMed, Embase or Web of Science, among others, were reviewed As non-genetic factors, important differences and similarities such age, or adherence to tamoxifen therapy are comprehensively illustrated. Additionally, since CYP2D6 genotypes are considered the main limitation of tamoxifen, many studies have investigated the association between the worsened clinical outcomes in patients with non-functional CYP2D6 genotypes. In this review, an overview of the research on this field is presented. Also, a summary describing the literature about individualizing tamoxifen therapy with endoxifen concentrations and its limitations is listed.

Expert opinion

z-endoxifen hydrochloride is only investigated in the metastatic setting, still more research is required before its place in therapeutics is known. Similarly, monitoring tamoxifen efficacy based on endoxifen concentrations might not be overall recommended due to the limited evidence available.

Introduction

Breast cancer is a heterogeneous disease with disparate clinical outcomes. Globally, breast cancer is the most frequent cancer diagnosed among women, accounting for around 25 % of the newly diagnosed cancers in female patients[1]. According to the World Health Organization, approximately 570.000 deaths in 2015 were related to breast cancer worldwide, which represented around 15 % of all cancer deaths in women[2]. Nearly 60-75 % of newly diagnosed breast cancer cases are estrogen-receptor positive (ER)[3], and in these cases, endocrine therapy with e.g. tamoxifen or aromatase inhibitors is prescribed.

For more than 40 years, tamoxifen monopolized the antiestrogenic therapy in the early and metastatic breast cancer setting. Tamoxifen is a key element of endocrine therapy prescribed in breast cancer patients, whilst it also is a drug with a very complex metabolism. Tamoxifen is mainly metabolized by different cytochrome P-450 enzymes into its primary metabolites, N-desmethyl-tamoxifen (NDM-tamoxifen) and 4-hydroxy-tamoxifen. Thereafter a second transformation from its primary metabolites, into the active metabolite endoxifen, occurs (**Figure 2.1**)[4].
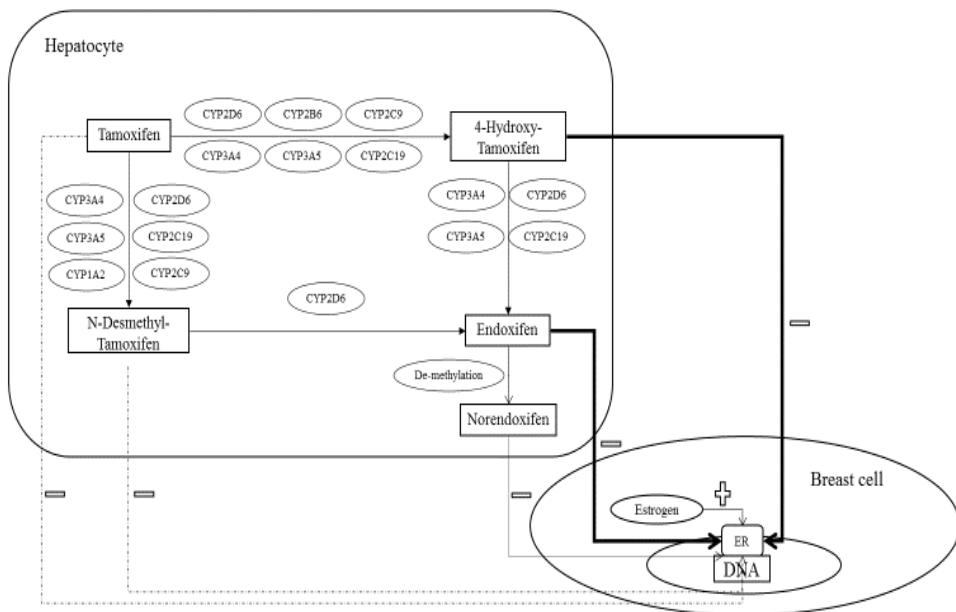

Figure 2.1. Tamoxifen metabolism

For pre-menopausal women, continuous tamoxifen is the preferred choice of treatment, whilst post-menopausal women can be switched to aromatase inhibitors

after two or three years [5-7]. In the adjuvant treatment setting tamoxifen therapy during 5 years has demonstrated to reduce mortality and disease recurrence[8-10]. Yet, as more studies with longer follow-up are published, it is apparent that extended duration of tamoxifen therapy up to 10 years is more beneficial in reducing mortality and disease recurrence in high risk disease[11]. In the metastatic setting, treatment with tamoxifen has also been investigated, showing longer survival rates and tumour reduction[12], but still poorer results compared to the adjuvant practice. However, in early breast cancer roughly 30 % of breast cancer patients will have a disease recurrence within fifteen years after treatment, indicating a wide variability in clinical response to tamoxifen treatment[10]. Both non-genetic (age[13], gender[14]) and genetic factors have been described to influence this high interpatient variability in response to tamoxifen. In this latter case, the most studied factor has been the variation in CYP2D6 gene encoding the CYP2D6 metabolic liver enzyme [15,16]. While it is almost present in all tamoxifen metabolic transformations, CYP2D6 is also the only enzyme which converts NDM-tamoxifen into endoxifen. Therefore, CYP2D6 is considered the critical enzyme of tamoxifen metabolism[15]. At the same time, many researches have analysed the clinical implications of CYP2D6 and its relationship with tamoxifen efficacy[17]. While some studies describe the importance of *CYP2D6* genotyping due to the poorer clinical outcome among patients with none or decreased CYP2D6 enzymatic activity[18-20], other authors have failed to find such an association[21]. Consequently, the use of *CYP2D6* genotyping for predicting tamoxifen efficacy, has not generally been implemented in the daily clinical practice. In the search for an alternative in order to predict tamoxifen efficacy, monitoring endoxifen concentrations have been proposed[22].

Recently, a phase I study in which endoxifen was orally administrated to hormone-resistant metastatic breast cancer patients was published[23]. In this study, endoxifen presented an acceptable toxicity and a high anti-estrogenic exposure, whilst also clinical antitumor outcome was observed. According to the authors, the main advantage of endoxifen compared to tamoxifen is the fact that CYP2D6 metabolism is avoided, and consequently, the anti-estrogenic effect of endoxifen is unaffected by the CYP2D6 enzyme. Tamoxifen has been the preferred choice of oral anti-estrogenic therapy for pre- and post-menopausal women in the adjuvant setting, but endoxifen, its active metabolite, may be an interesting alternative of treatment in the future. In order to summarize these differences, a literature search in PubMed, Embase, Web of Science and Cochrane Library (until 30/11/2018) was performed. The aim of this review is to evaluate both the principal genetic and non-genetic differences in the pharmacology of tamoxifen and endoxifen.

Tamoxifen metabolic pathway

Generally, a description of tamoxifen metabolism mainly follows two pathways via 4-hydroxylation and N-de-methylation, into 4-hydroxy-tamoxifen and NDM-tamoxifen, respectively. Thereafter, both metabolites are finally transformed into the most potent

secondary metabolite endoxifen (**Figure 2.1**)[4]. Still tamoxifen metabolic pathway is more complicated than only these two parallels pathways, since many newly tamoxifen metabolites have been discovered. Therefore, tamoxifen pathway is becoming more challenging to interpret. An example of the complexity of tamoxifen pathway was recently described by Johanning and colleagues, were estrogen-like tamoxifen metabolites were recently described. Authors suggest a broader metabolic pathway for tamoxifen were all these estrogen-like metabolites are included.

The transformation from tamoxifen into NDM-tamoxifen represents around 92 % of tamoxifen metabolism, whilst the pathway through 4-hydroxy-tamoxifen accounts for 7 %[4]. Endoxifen and 4-hydroxy-tamoxifen are estimated to have comparable anti-estrogenic effect, which is around 30- to 100-fold more active in comparison to tamoxifen[24]. However, since endoxifen is found in around 5 to 10 times higher concentrations compared to 4-hydroxy-tamoxifen[25], endoxifen is considered the most relevant metabolite of tamoxifen.

Tamoxifen is largely metabolised to different, either active or inactive, metabolites, by many enzymes. For instance, several phase I enzymes, including different cytochrome P450 enzymes and flavin-monooxygenases (FMOs), and phase II enzymes, such as sulfotransferases (SULTs) and uridine-5'-diphospho-glucuronosyl-transferases (UGTs).

Recently, a fifth and also active metabolite of tamoxifen, norendoxifen, has been identified [26]. Norendoxifen is the product of the de-methylation of endoxifen or the product of the hydroxylation of di-desmethyltamoxifen[27], and it slightly differs from the other tamoxifen metabolites since it also has the capacity to inhibit CYP19A1 (aromatase). Consequently, norendoxifen would have a dual mechanism of action: aromatase inhibition and estrogen receptor inhibition[26,28,29]. Yet, to our best knowledge, this metabolite has not been commercialized at present[28].

CYP2D6: limiting factor in tamoxifen metabolism

CYP2D6 enzymatic activity has been repeatedly reported as one of the essential elements of tamoxifen metabolism[15,17,30-32]. CYP2D6 is involved in the transformation of tamoxifen into 4-hydroxy-tamoxifen and NDM-tamoxifen, and in the conversion of 4-hydroxy-tamoxifen into endoxifen, and from NDM-tamoxifen into endoxifen. While many enzymes are involved in this complex metabolic pathway, CYP2D6 is the only enzyme responsible for the biotransformation from NDM-tamoxifen into endoxifen[15].

The *CYP2D6* gene is a highly polymorphic gene, for which currently more than 100 different polymorphisms has been described[33]. All of these variations are mainly the result of differences in one single nucleotide polymorphisms (SNP), gene amplifications or deletions. While some of these variants encode a non-functional CYP2D6 enzyme,

others encode for CYP2D6 with decreased enzymatic activity. Some typical examples of *CYP2D6* inactive alleles are **3, *4,*5, *6, *7,*8 *11,*12, *13,*14A, *15, *19, *20,*40*, whereas some cases of *CYP2D6* alleles with decreased activity are **9,*10, *17, *29, *36, *41.*

The most common allelic variant among Caucasians with non-functional *CYP2D6* activity is *CYP2D6*4* with an allele frequency of nearly 20 %[34,35]. In contrast, *CYP2D6*10* and its decreased activity is the most frequent allele among Asians, since it is found in almost 40 % of this population[35]. In the same manner, another relatively important variant with decreased reduced activity in the African-American population is *CYP2D6*17* allele, with an allele frequency around 22 %[36].According to the combination of these alleles, individuals can be categorized into four principal predicted phenotypes: ultra-rapid metabolizers (UM, duplication of fully active alleles), normal metabolizers (NM, with two fully active alleles, which also in the past used to be called extensive metabolizers, EM), intermediate metabolizers (IM, with two low activity alleles or a combination of one low activity allele and one inactive allele) and poor metabolizers (PM, with two non-functional alleles). In addition to this classification, some authors also contemplate a fifth phenotype named heterozygous extensive (hetEM), which consists of a combination of one fully functional active allele and an inactive allele[32]. The enzymatic activity resulting from the hetEM genotype is in between the activity of IM and NM.

Another similar strategy for individualizing tamoxifen therapy is the classification of patients in five different groups according to the *CYP2D6* gene activity score (AS)[37]. The functionality of each allele is assigned an activity value as follows: 1 for fully functional alleles, 0.5 for alleles with decreased activity, and 0 for non-functional alleles. In the same manner, the combination of both alleles leads to five CYP2D6 phenotypes: PM (AS:0); IM (AS:0.5); IM or NM (AS:1.0); NM (AS:1.5-2.0); UM (AS:>2.0).

CYP2D6 genetics and tamoxifen outcomes

Since the publication of Goetz and colleagues[38], the role of polymorphisms in *CYP2D6* in clinical outcomes of women receiving tamoxifen as adjuvant therapy has been an ongoing controversy due to the contradictory results of studies. Theoretically, patients with reduced and inactive CYP2D6 enzymatic activity and using e.g. adjuvant tamoxifen, reach lower endoxifen concentrations, and consequently, lower exposure to anti-estrogenic therapy, with the consequence of a higher chance of relapse. Based on this hypothesis, PM and IM would be therefore more likely to have worsened clinical outcomes, since both phenotypes attain up to 74 % and 60 % lower endoxifen concentrations respectively, compared to EM[30]. This continuous discussion regarding poorer clinical outcomes for PM and IM receiving tamoxifen therapy may be difficult to clarify due to a great number of differences across studies. A few examples might be such the source of DNA for genotyping (tumour-tissue or blood), tamoxifen

treatment duration (5 years or 10 years, or even shorter treatments), the study design (retrospective or prospective), the different endpoints analysed (relapse-free survival, disease-free survival, overall survival, among others), different quality of clinical cohorts, long-enough follow-up, and the number of **CYP2D6** allelic variants which were analysed. Consequently, a comparison between studies is extremely challenging

Many studies have been published since the study of Goetz and colleagues in 2005, with different outcomes. In 2007, another relevant study by Schroth and colleagues[20] studied 1325 patients from Germany and US who were diagnosed with breast cancer and receiving tamoxifen treatment between 1986 and 2005. According to the authors, hetEM, IM and PM patients had worsened disease-free survival (HR:1.29, 95 % CI: 1.03-1.61), compared to EM.

In an attempt to uniformly investigate this potential relationship among patients with decreased enzymatic CYP2D6 activity and worsened clinical outcome, a meta-analysis performed by the International Tamoxifen Pharmacogenomics Consortium including 4973 patients treated with adjuvant tamoxifen were analysed by using three beforehand defined inclusion criteria. In the primary analysis, in which the inclusion criteria were the most restricted, Province and colleagues reported a poorer survival outcome in PM patients (Hazard Ratio (HR): 1.25; 95 % Confidence interval: 1.06-1.47; p-value: 0.009) among post-menopausal patients receiving 20 mg/day of adjuvant tamoxifen for 5 years. Because of the strict inclusion criteria, this first analysis included 1996 individuals. In contrast, in both criteria 2 and 3 analyses, where less rigorous inclusion conditions were applied, no differences in clinical outcomes between groups were observed. [39].

Still, this meta-analysis has been largely commented, since important studies with large number of enrolled patients e.g. ATAC[40], BIG 1-98[41], or TEAM[42] studies were not included. However, these studies also might have important limitations for the inclusion in this meta-analysis. For instance, the TEAM study might not have included in this meta-analysis due to the follow-up time of 2.5 years[42], whilst the ATAC[40] and BIG 1-98[41] trials analysed tumour tissue, which could potentially lead to loss of heterozygosity.

Of note, the ATAC[40] and BIG 1-98[41] studies have been criticized since potentially wrong outcomes were obtained. In these studies, formalin-fixed paraffin-embedded tissues were analysed, but important deviations from Hardy Weinberg Equilibrium were not taken into consideration when interpreting their results. In this case, several reports analysed this problem[15,43-46], and the clinical implications and consequences of these results. If "loss of heterozygosity" takes place, individuals could potentially be misclassified in "wrong" **CYP2D6** genotype and confusing outcomes could be expected. In contrast to these studies, the TEAM trial ruled out loss of heterozygosity by analysing microsatellite markers with a high frequency of heterozygosity (D22S423, D22S276

and D22S2284) and found near to the *CYP2D6* gene[42]. In this case, authors did not find any statistically significant differences regarding disease-free survival among several *CYP2D6* genotypes.

In the same line, a prospective specifically designed for investigating the clinical effect of *CYP2D6* genotyping on 667 early-breast cancer female patients receiving adjuvant endocrine therapy with tamoxifen also did not find an association of poorer clinical outcome among patients with decreased or non-functional CYP2D6 genotype[47] (Adjusted HR: 0.929; 95 % CI 0.525-1.642; p=0.799).

In an effort to uniformly interpret clinical evidence, a recent clinical guideline written by the Clinical Pharmacogenetics Implementation Consortium (CPIC) has been published in order to consistently give detailed recommendations based on the most current literature[37]. Briefly, this CPIC guideline regarding tamoxifen therapy recommends to initiate tamoxifen therapy with the standard dose of 20 mg in UM (AS:>2.0) and NM (AS:1.5-2.0). In the case of NM or IM (AS:1.0) and IM (AS:0.5), this guideline suggests to consider alternative endocrine therapy e.g. aromatase inhibitor for post-menopausal patients, and in pre-menopausal women, the use of aromatase inhibitor with ovarian function suppression. Yet, in these cases, if the use of aromatase inhibitor is contraindicated, the authors suggests to consider the use of 40 mg of tamoxifen. For PM (AS:0), alternative hormonal therapy e.g. aromatase inhibitor should be preferably considered. Of note, no higher doses of tamoxifen (40 mg/day) are recommended in PM (AS:0), since endoxifen concentrations do not normalize (compare to NM).

Therefore, the potential association between decreased or lacking CYP2D6 enzyme activity and worsened clinical outcome, still remains unclear. Consequently, CYP2D6 genotyping has not been commonly adopted in the clinical practice for tamoxifen treatment. Nonetheless, since tamoxifen metabolism is highly complex, it is debatable that tamoxifen therapy mainly depends on *CYP2D6* genotypes. As a consequence, there seems to be a trend to take the focus off *CYP2D6*, since it only partly clarifies tamoxifen inter-patient variability, and paying more attention to other tamoxifen metabolites, especially endoxifen. As CYP2D6 is only partially predictive of endoxifen concentration levels, it is only logical to assume that endoxifen concentrations are better predictive of tamoxifen efficacy than solely CYP2D6 genotypes.

Tamoxifen and endoxifen

Tamoxifen and endoxifen pharmacokinetics
In **Table 2.1,** all these differences and comparisons are summarized. Tamoxifen is normally formulated as tamoxifen citrate, whilst endoxifen, is orally formulated as z-endoxifen hydrochloride. Elimination half-life of tamoxifen is 5-7 days on average. In contrast, endoxifen's half-life varies between 49.0 and 68.1 hours for the dose of 20 mg and 160 mg[23], respectively. Also, minor difference in Tmax and Cmax have been

reported. While tamoxifen has a longer Tmax (4-7 hours), endoxifen as z-endoxifen hydrochloride requires a shorter time to reach Tmax (2-4 hours). Also, differences in Cmax values are reported: when a 20 mg single dose of tamoxifen is administered a Cmax value of 40 ng/ml is reached[48], whilst a single dose of 20 mg and 160 mg of z-endoxifen hydrochloride reaches Cmax of 64.8 and 635 ng/ml, respectively[23].

Table 2.1. Pharmacokinetics parameters of tamoxifen and endoxifen.

		Tmax	Cmax (after one single dose)	Reported mean concentration in steady state as CYP2D6 normal metabolizer	Half-life	Time to steady-state	Metabo-lism	Excre-tion	Refs.
Tamoxifen[a]		4-7 hours	±40[a] ng/ml	122 ng/ml	7 days	4 weeks	Hepatic	Feces	48
Endoxifen	**Endoxifen as metab-olite of tamoxifen citrate**	N.A.	N.A	10.830-15.982 ng/ml	N.A	N.A.	Hepatic	Not repor-ted	30,82
	Endoxifen as z-endox-ifen hydro-chloride	2-4 hours	64.8[b]-635[c] ng/ml	146[b]-1950[c] ng/ml	49[1]-68.1[4] hours	7 days	Hepatic	Not repor-ted	23

[a]with 20 mg of tamoxifen; [b] with 20 mg of endoxifen;[c] with 160 mg of endoxifen [d] with 100 mg of endoxifen; N. A.: not applicable; Refs.: references

Other minor enzymes in tamoxifen metabolism
Besides the enzymes involved in the main transformations of tamoxifen into NDM-tamoxifen and 4-hydroxy-tamoxifen, other drug-metabolizing enzymes are also involved in the conversion of tamoxifen into active and inactive metabolites of tamoxifen (**Figure 2.1** and **Figure 2.2**).

In **Table 2.2**, an overview with all the minor enzymes in tamoxifen metabolism is listed. Genetic variants in the gene encoding the UGT1A4 enzyme, which catalyses the transformation from tamoxifen into tamoxifen-N-glucuronide, include *UGT1A4_48_Val* and *UGT1A4_48_Leu.* To date, only the variant *UGT1A4_48_Val* has been associated with higher tamoxifen-N-glucuronide levels [50-52]. Other phase I drug-metabolizing enzymes, FMO1 and FMO3, are also involved in tamoxifen metabolism. In this case, both enzymes transform tamoxifen into tamoxifen-N-oxide. Interestingly, the enzyme CYP3A4 enzyme can transform tamoxifen-N-oxide into tamoxifen *in vivo*, and it has been postulated that tamoxifen-N-oxide might behave as repository for tamoxifen, since the tamoxifen-N-oxide/tamoxifen ratio is reduced when higher tamoxifen dosages are used[53-55] (**Figure 2.2**).
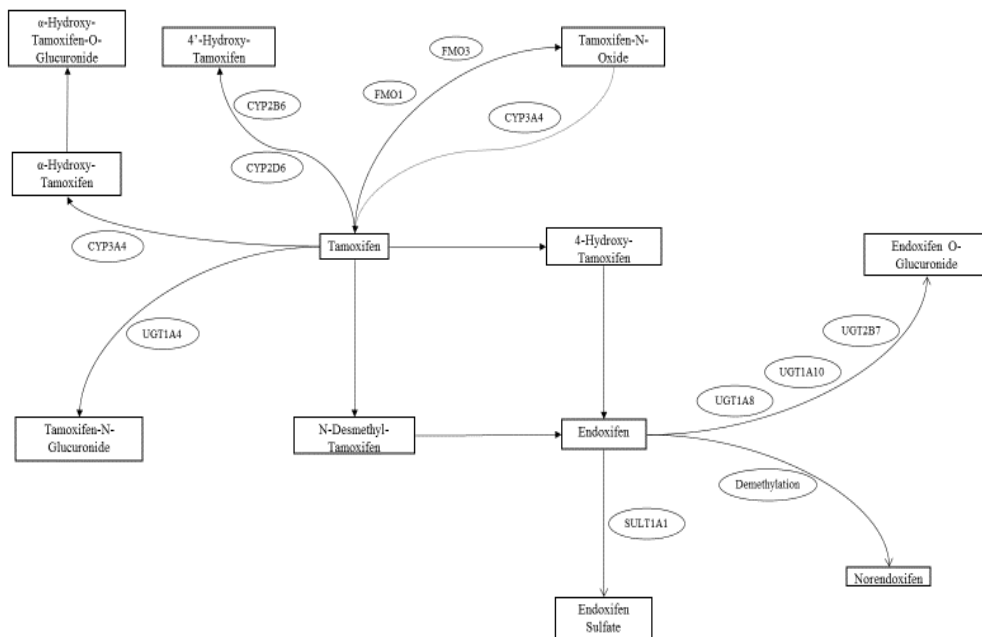
Figure 2.2. Minor enzymes of tamoxifen and endoxifen metabolism

Table 2.2. Minor enzymes involved in tamoxifen metabolism.

Gene	Conversion	Comparison	Impact on levels	Refs.
UGT1A4	Tamoxifen to Tamoxifen-N-glucuronide	*UGT1A4*_48_ Val versus UGT1A4 _48_Leu	Higher Tamoxifen-N-glucuronide levels in UGT1A4_48_Val variant	49-51
CYP3A4 and CYP3A5	Tamoxifen to α-Hydroxy-Tamoxifen		CYP3A4 has a higher implication in the formation of α-Hydroxy-Tamoxifen compared to CYP3A5. α-Hydroxy-Tamoxifen has been postulated to be involved in the adduct formation, which could play a role in the development of endometrial cancer.	59,60
CYP2D6 and CYP2B6	Tamoxifen to 4'-hydroxy-tamoxifen		Tamoxifen is metabolized by CYP2D6 and CYP2B6 to 4'-hydroxy-tamoxifen, which might activate a non-apoptotic cytotoxic effect in the endometrium.	56-58
FMO1 and FMO3	Tamoxifen to Tamoxifen-N-Oxide		Tamoxifen can be metabolized by FMO1 and FMO3 to Tamoxifen-N-Oxide and it can be metabolized back to Tamoxifen by hemoglobin and NADPH-P450 oxidoreductase. Tamoxifen-N-oxide could behave as repository for Tamoxifen: Tamoxifen-N-Oxide/Tamoxifen ratio is reduced when higher tamoxifen dosages are taken.	52-54 52-54
CYP3A4	Tamoxifen-N-Oxide to Tamoxifen			52-54

FMO1: flavin monooxygenases 1; FMO3: flavin monooxygenases 3; SULT1A1: Sulfotransferases 1A1   UGT: UDP-glucuronosyltransferases;

Other subproducts of tamoxifen metabolism are 4'-hydroxy-tamoxifen and α-hydroxy-tamoxifen. Both metabolites have been associated with the development of endometrial cancer[56], different enzymes are found in these processes. While CYP2D6 and CYP2B6 are involved in the transformation of tamoxifen into 4'-hydroxy-tamoxifen[57-59], CYP3A4 and CYP3A5 enzymes catalyse the conversion of tamoxifen into α-hydroxy-tamoxifen[60,61] (**Figure 2.2**).

Other minor enzymes in endoxifen metabolism

In contrast to tamoxifen, a few endoxifen elimination and inactivation enzymes have been investigated and associated with clinical survival outcomes and tamoxifen active metabolites concentrations and metabolic ratios. In the same manner, **Table 2.3** summarizes all the minor enzymes in endoxifen metabolism.

Sulfotransferases (SULTs), UDP-glucuronosyltransferases (UGTs) and demethylases are the most significant enzymes participating in endoxifen elimination and inactivation (**Figure 2.2**). Within all the SULTs enzymes, SUL1A1 enzyme is responsible for the inactivation of endoxifen into endoxifen sulfate. Genetic polymorphisms in the SULT1A1 enzyme have been analysed for their association with different survival outcomes and drug concentrations. Nowell and colleagues reported a poorer overall survival for *SULT1A1*2/*2* carriers, compared to *SULT1A1*1/*1* or *SULT1A1*1/*2* individuals[62]. Later on, the same research group described again another statically significant poorer overall outcomes in *SULT1A1*2/*2* and *UGT2B15*2* patients[63]. On the contrary, Wegman and colleagues found a better outcome for *SULT1A1*1/*1*[64]. However, none of these results were confirmed by other authors [65,66]. Interestingly, SULT1A1 copy number of variation, has also been evaluated in these studies, but no significant differences in terms of survival were found. [65,66]. Similarly, no statistically significant differences in endoxifen concentration levels were observed between when comparing patients carrying *SULT1A1*1/*1*, *SULT1A1*1/*2*, *SULT1A1*2/*2* and SULT1A1 copy number of variation [67-69].

UGTs enzymes are also implicated in endoxifen metabolism, but only a few studies have examined their role in the conversion into endoxifen-O-glucuronide (**Figure 2.2**). In an *in vitro* study, genetic polymorphisms in UGT1A10 were studied, but no significant differences in endoxifen concentration levels were observed [70]. Another relevant variant allele is the non-functional allele *UGT1A8*3*, since it was suggested that oral bioavailability might be altered[71]. Mizuma and colleagues studied the variant allele *UGT1A8*3* in raloxifene-treated patients, and concluded that *UGT1A8*3* carriers had higher oral bioavailability, which could be explained by the no functionality of glucuronidation of this variant, mainly at intestinal level. However, these results have not been observed in tamoxifen-treated patients. In this case, when the non-functional variant *UGT1A8*3* and the active allele *UGT1A8*1* were compared, no differences in endoxifen levels [70] or in survival outcomes [72] were found. In line with these observations, *UGT2B7*2* and its decreased activity were suggested to have an impact on endoxifen

concentration levels, but no significant differences in concentrations[50,51,70,73,74] or in clinical outcome [40,72] have been found (**Table 2.3**; **Figure 2.2**).

Another interesting transformation is the conversion of endoxifen into norendoxifen via N-de-methylation. Lim and colleagues described that *CYP2C19*2* carriers were associated with lower norendoxifen concentration levels[27].

Non-genetic factors affecting tamoxifen and endoxifen metabolism

All of the non-genetic factors are described and presented in **Table 2.4.**

Table 2.3. Minor enzymes involved in endoxifen metabolism.

Gene	Conversion	Comparison	Impact on levels	Impact on survival outcome	Refs.
UGT1A8	Endoxifen to Endoxifen O-glucuronide	*1/*1 versus *1/*3 or *3/*3	No differences in endoxifen levels (*in vitro*)		69
				No differences in survival	71
UGT1A10	Endoxifen to Endoxifen O-glucuronide	*1a versus *2a	No differences in endoxifen levels (*in vitro*)		69
UGT2B7	Endoxifen to Endoxifen O-glucuronide	*1 versus *2	No differences in endoxifen levels		49,50, 69,72, 133
		*1/*1 versus *1/*2 or *2/*2		No differences in survival	71,134
SULT1A1	Endoxifen to Endoxifen sulfate	*1/*1 versus *1/*2 versus *2/*2 and copy number variation	No differences in endoxifen levels		66-68
		*1/*1 or *1/*2 versus *2/*2		*2/*2: worst OS	61
				*SULT1A1*2/*2* and *UGT2B15*2:* worst OS	62
		*1/*1 versus *1/*2 and *2/*2		*1/*1:better survival	63
		*1/*1 versus *1/*2 or *2/*2 and/or copy number variation		No differences in survival	64,65
N-de-methy-lation	Endoxifen to Norendoxifen	*CYP2C19*2*	Lower Norendoxifen levels		27

Age
Among other non-genetic factors that could potentially influence metabolism, age has

been described to affect both tamoxifen and endoxifen metabolism. Lien and colleagues reported significant higher endoxifen, tamoxifen NDM-tamoxifen concentration levels among patients in the oldest patient's group (> 69 years old)[86] compared to younger patients. In the same way, Wu [78] and Peyrade [79] observed significantly higher concentrations of the four metabolites in older patients. In contrast, Antunes *et al.* found an inverse correlation between endoxifen and 4-hydroxy-tamoxifen concentration and age, whilst tamoxifen and NDM-tamoxifen concentrations were significantly higher among older patients (**Table 2.4**) [77]. Yet, these differences in the literature might be explained by natural physiological changes in humans due to the aging process, such as menopause[87], lower metabolic hepatic enzyme activity[88], due to poly-pharmacy[89] or co-morbidities [90].

Body mass index (BMI)
Different studies have demonstrated an inverse correlation between BMI and serum concentrations of tamoxifen and its metabolites [49,78,81,91]. A simple explanation for this association is given by the larger volume of distribution in individuals with higher BMI, consequently leading to lower drug concentration levels. Despite of this association, there are no general recommendations of dose adjustments for tamoxifen in the clinical practice[5,81], whereas despite of the discrepancies in the current evidence, some clinicians tend to prescribe daily 40 mg of tamoxifen in patients with a BMI classified as overweight (25-30 kg/m^2)or obese (>30 kg/m^2) (**Table 2.4**) [92].

Food, circadian rhythm and seasonal variation
Other non-genetic factors influencing tamoxifen and endoxifen metabolism are food, moment of drug intake and seasonal variation. In the case of the effect of food on the pharmacokinetics, no clinically relevant effects have been described. Generally, tamoxifen is recommended to be administrated with or without food[93].

In contrast to food, the time of drug intake influences tamoxifen and endoxifen metabolism. Binkhorst and colleagues reported 15 % and 3 % higher endoxifen and tamoxifen systemic exposure (AUC$_{0-24}$) when tamoxifen was taken in the morning, whilst more balanced concentrations were found in the evening schedule [83]. Regardless of these differences, the authors concluded this phenomenon would probably not be clinically important for tamoxifen efficacy.

Interestingly, seasonal variation may also influence tamoxifen and endoxifen pharmacokinetics. Teft *et al.* observed that during the winter season, significantly lower endoxifen concentrations were found than were detected during the other seasons[82]. In addition, authors also found an association between lower vitamin D levels and lower endoxifen concentrations. In a study by Antunes and colleagues, 116 breast cancer patients who were receiving adjuvant tamoxifen were analysed[77]. In this research, comparable results to Teft and colleagues were found[82], showing significant higher endoxifen concentration levels and vitamin D during the summer time, whilst a

trend towards also lower tamoxifen concentrations was observed (**Table 2.4**)[77]. Yet, in both studies only a hypothetically relationship between vitamin D and endoxifen concentration levels are described, however, which of both elements is the cause and which the consequence, remains unclear. Still, no general recommendations for vitamin D supplements are given aimed at achieving higher anti-estrogenic exposure to tamoxifen or endoxifen.

Table 2.4. No genetic factors affecting tamoxifen and endoxifen concentrations. [1]Study as tamoxifen metabolite

Factor	Comparison	Tamoxifen concentration	Endoxifen concentration	Clinical outcome	Refs.
Age	Young (30-40) versus old (>65 year-old) women	↑	↑[1]	Higher concentrations of both metabolites with higher age	13,75-77
Gender	Male versus female	?	↓[1]	Lower endoxifen concentrations in male patients	91
BMI	Higher BMI versus lower BMI (normal)	↓	↓[1]	Lower endoxifen concentrations compared to patients with normal BMI (<24.9)	75,77, 82,84
Season	Winter and other seasons	↓	↓[1]	Lower endoxifen concentration levels during winter	77,88
Intake moment	Morning versus night	↑	↑[1]	15 % and 3 % higher endoxifen and tamoxifen systemic exposure after morning intake	87
Smoking	Smokers versus non-smokers	-	-	Higher tamoxifen-related nausea, headache and depression in smokers compared to non-smokers	96
Total Cholesterol	Total cholesterol before and after at least 1 cycle of treatment	-	-	Lower total cholesterol	23,135
LDL cholesterol	LDL cholesterol before and after at least 1 cycle of treatment	-	-	Lower LDL cholesterol	23,135
HDL cholesterol	HDL cholesterol before and after at least 1 cycle of treatment	-	-	Lower HDL cholesterol	23,135
Triglycerides	Triglycerides before and after at least 1 cycle of treatment	-	-	Lower triglycerides	23,135

Gender

The majority of breast cancer new cases are female individuals, but around 0.5-1 % of the newly diagnosed patients are male subjects[1,94]. In these cases, the first choice of treatment as adjuvant endocrine therapy is tamoxifen, whilst aromatase inhibitors are not recommended, as they appear to be inferior compared to tamoxifen in this clinical setting[95]. To date, only one study performed by Lenehan and colleagues, has demonstrated that male patients reached significantly lower endoxifen concentration levels compared to female patients (**Table 2.4**)[80]. In general, female and male patients differ in response to drug treatments, and these variances are mainly explained by body differences[96]. Normally, men tend to have higher BMI, total body water and plasma volume compared to women, which would lead to lower mean drug concentrations. However, it remains unknown if this difference is clinically relevant for the clinical efficacy of tamoxifen and endoxifen.

Smoking

Cigarette smoking has been associated with higher breast cancer risk[97,98], but to the best of our knowledge, no studies have been performed on the influence of smoking on tamoxifen and endoxifen pharmacokinetics. In one study, Persson and colleagues[99] described that older patients (> 50 years) who smoked during endocrine therapy with aromatase inhibitors did have higher probability of breast cancer events (HR: 2.97; 95 %CI: 1.44-6.13), distant metastasis (HR: 4.19; 95% CI:1.81-9.72) and death (HR: 3.52; 95 % CI: 1.59-7.81), whereas in the group treated with tamoxifen no association between tamoxifen efficacy and actively smoking during endocrine therapy was found (**Table 2.4**). However, Zhan and colleagues reported that smoking patients treated with tamoxifen presented higher probability of side effects like nausea, headaches and depression in comparison with non-smokers[84]. Although there is no clear evidence that tamoxifen efficacy is influenced by actively smoking, in daily practice all patients are recommended to quit smoking.

Triglycerides and cholesterol

In addition to its anti-estrogenic effect, tamoxifen use has been described to be beneficial in lowering the risk of cardiovascular diseases, since tamoxifen influences lipid metabolism by decreasing LDL- and HDL-cholesterol, and total cholesterol and triglycerides[100,101]. A study performed by Clarke and colleagues, found a significant lowering effect of cholesterol and triglycerides in tamoxifen-treated male patients[102]. These results were also observed by Shewmon *et al.* in healthy post-menopausal women[103]. Goetz and colleagues observed these same lowering effects on cholesterol and triglycerides in endoxifen-treated patients (**Table 2.4**)[23]. These observations suggest a clinically relevant cardiovascular risk reduction in both endoxifen and tamoxifen users. Yet, no clinical guidelines recommend the use of tamoxifen or endoxifen in order to lower cholesterol or triglycerides.

Tamoxifen, endoxifen and CYP2D6-inhibitors

Drug-drug interactions are an important point of discussion, since interactions could have an important role on clinical efficacy and occurrence or worsening of adverse events. Over the last decade, few drug-drug interactions have been as contentious as the tamoxifen and selective serotonin reuptake inhibitors (SSRIs) or selective serotonin and norepinephrine reuptake inhibitors (SNRIs) interactions. Since around 10-25% of female breast cancer patients suffer from depression [104,105], these patients might require antidepressant treatment with SSRIs or SNRIs. In addition, these drugs are frequently prescribed for treating hot-flashes due to tamoxifen use, because in those cases estrogen or progesterone combinations are not an option to be used. Both specific SSRIs and SNRIs are recognized as CYP2D6-inhibitors [106], and since CYP2D6 is the rate-limiting enzyme in tamoxifen activation into endoxifen, many studies have focused on this drug-drug interaction, with a wide range of controversial results.

Steams *et al.* observed an important decrease from a mean 12.4 ng/ml to 5.5 ng/ml of endoxifen concentrations among women who were treated with paroxetine [25], and consecutive studies showed significant lower concentrations of endoxifen among patients who were using potent CYP2D6-inhibitors, as paroxetine or fluoxetine [67,82,107]. Over the last ten years, there seems to be a change in trend co-prescribing tamoxifen and an SSRI. In a study in the U.S.A., it was observed that there was a significant reduction in the co-prescription of both drugs (tamoxifen and SSRI) (from 34 % during the period between 2004 and 2006 compared 15 % in 2010)[108]. Likewise, a Belgian and a Dutch study demonstrated a dropping in the co-prescription of strong CYP2D6-inhibitors and tamoxifen, whilst a preference for a weak CYP2D6-inhibitor was observed [109,110]. To analyse the effect of switching from a strong to a weak CYP2D6-inhibitor, Binkhorst and colleagues, analysed endoxifen concentration levels, before and after this switch. Interestingly, an improvement to higher endoxifen levels was reported after a switch from a strong inhibitor to escitalopram, a weak CYP2D6-inhibitor[111].

In theory, tamoxifen efficacy may be affected by the concomitant use of tamoxifen and CYP2D6-inhibitors, due to a decrease in endoxifen exposure. However, a large discrepancy in studies investigating the effect of SSRIs on breast cancer survival outcomes using tamoxifen has been published [25,67,82,107,112]. This high variance in the literature may be explained by the CYP2D6-inhibitor analysed, the source of information and the lack of information on *CYP2D6* genotype and compliance and the relative time in which patients had concomitantly used the CYP2D6 inhibitor. Still, one recent study with 16887 patients concluded that no increased risk among the women who were using antidepressants and tamoxifen was observed[113]. However, current guidelines with recommendations for endocrine therapy advise to avoid the co-prescription of CYP2D6-inhibitors in tamoxifen users due to the importance of drug-drug interactions[7,37,114].

Adherence to tamoxifen therapy

Tamoxifen treatment adherence is an important problem in daily clinical practice. In the literature, reported tamoxifen adherence vary widely from 41 % to 88 %[115-118]. However, tamoxifen discontinuation is principally seen after the first year of treatment[118]. In a recent prospective study analyzing tamoxifen adherence after the first year of endocrine treatment by quantifying tamoxifen concentrations, around 18.2 % of the enrolled patients were classified as poor or no adherence[119]. In the same manner, barely 50 % of patients achieve to finish the suggested period of five years[117,118]. In a recent review analyzing this importance of endocrine therapy, Chlebowski and colleagues highlighted the necessity of good treatment adherence (defined as > 80% use) in order to reach lower recurrence outcomes[118,120]. In this case, Chigwin and colleagues recently investigated the association of adherence to endocrine therapy and disease-free survival in the Breast Internation Group (BIG) 1-98 clinical trial. Authors reported worsened clinical outcome among the groups with poorer adherence to treatment (HR: 1.61; 95 % CI 1.08-2.38; p-value: 0.02). Interestingly, sequential therapies (either switching from tamoxifen to letrozole or from letrozole to tamoxifen) were associated with higher percentages of non-adherence (20.8 % and 20.3 % for the switch from tamoxifen to letrozole and from letrozole to tamoxifen, respectively) compared with the monotherapies of letrozole (17.6 %) and tamoxifen (16.9 %). In most of the cases, side effects were the principal cause for poorer adherence. Also, other reported factors in the literature associated with lower or non-adherence are[118]: medication cost, lack of network support, older age, absence or inadequate of doctor-patient relationship, among others. Consequently, treatment adherence is an important difficult, and therefore, strategies for detecting patients who could potentially discontinue endocrine therapy are extremely required.

Endoxifen: Towards individualizing tamoxifen treatment?

Active metabolites and mechanism of action

Almost thirty years ago, endoxifen was characterized for the first time by Lien and colleagues [24,121]. In contrast to tamoxifen, endoxifen has a higher affinity for estrogen receptor, whilst it is also categorized as a selective estrogen receptor modulator[122]. Initially, 4-hydroxy-tamoxifen was believed to be the principal active metabolite of tamoxifen, since it was found to be 30 to 100-times more potent as anti-estrogenic compared to tamoxifen[24]. In an effort to find a potential therapeutic alternatives, 4-hydroxy-tamoxifen was examined as a therapeutic drug, however, due to its unfavourable pharmacokinetics, it failed[123].

Both endoxifen and 4-hydroxy-tamoxifen are chemically related molecules, with comparable anti-estrogenic effect[25], although endoxifen reaches 5 to 10-fold larger concentrations[25]. Additionally, *in vitro* studies have suggested that the mechanism of action of endoxifen might differ from 4-hydroxy-tamoxifen[124,125]. Initially, Wu *et al.* described that endoxifen targets the estrogen-receptor α by blocking its transcriptional

activity and inhibiting estrogen breast cancer cell proliferation, only when high concentrations of endoxifen were used, in contrast to tamoxifen, NDM-tamoxifen and 4-hydroxy-tamoxifen[125]. Later, Hawse and colleagues compared the capacity of endoxifen, 4-hydroxy-tamoxifen and ICI (a pure antiestrogen and estrogen receptor down regulator)[124] to target estrogen receptor α for DNA binding in order to correctly identify differences in the gen expression profiles of MCF7 cells exposed to different concentrations of these drugs in the absence and presence of estrogen. Authors observed differences in gene expression profiles of MCF7 cells when high endoxifen concentrations were added, while when low concentrations were used, expression profiles barely varied. Based on these studies, the mechanism of action of endoxifen appears to be concentration dependent

Approaches for predicting endoxifen concentrations

Since endoxifen is considered the most relevant metabolite of tamoxifen metabolism, many different strategies have been investigated in order to predict the exposure to this metabolite. The majority of these approaches have mainly focused on using different variables in order to improve the explained inter-patient variability of endoxifen concentrations. *CYP2D6* polymorphisms are the main contributors to this inter-variability, yet they still only explain around 39-42.3% of the variability in concentrations of endoxifen[30,126]. For this reason, *CYP2D6* genotyping in the current form might be a too simplistic strategy for personalizing tamoxifen treatment. Another analysed approach is the use of ^{13}C-dextromethorphan breath test for *CYP2D6* phenotyping. This strategy allows to slightly improve the predictability of endoxifen concentrations to 47.5%[127]. In the same way, Teft and colleagues also developed an algorithm, including demographic data, use of SSRIs, *CYP2D6* genotypes and *CYP3A4*22*, among others, in order to predict endoxifen concentration levels[82].

Both phenotyping approaches and the algorithm of Teft and colleagues are strategies for predict endoxifen concentrations. Still, these models only minimally improve the prediction of endoxifen concentrations, which is considered an important limitation. Consequently, other strategies are still being awaited.

Endoxifen concentrations and tamoxifen efficacy

Due to the current limitations of *CYP2D6* genotyping, it has been hypothesized that monitoring endoxifen concentrations might be a better way for predicting tamoxifen efficacy. Based on this theory, endoxifen rather than *CYP2D6* genotyping may be closer to the pharmacological effect, and consequently may be a better approach for personalizing tamoxifen efficacy[22]. Also, a special consideration regarding endoxifen (Z-endoxifen), since it is the active isomer with the anti-estrogenic activity[30]. In this case, it is important to properly quantify Z-endoxifen, separately from E-endoxifen or other hydroxylated metabolites e.g. 4'hydroxy-desmethyltamoxifen[30], in order to adequately obtain the concentration of the active metabolite (Z-endoxifen), and avoiding too high concentrations from a mix of all metabolites, which may lead to misinterpretations in

further analysis.

Madlensky and colleagues were the first to study the relationship between endoxifen concentrations and clinical outcome[49]. In this analysis, a threshold of 5.97 ng/ml for endoxifen concentrations was associated with a 26 % lower chance of relapse (Adjusted HR: 0.76, 95 % Confidence Interval: 0.55-1.00). In this case, 1370 patients were divided in five groups (quintiles) according to their concentrations of endoxifen, and the lowest quintile had a higher chance of relapse, compare to the other groups. However, this endoxifen threshold concentration of 5.97 ng/ml has been highly commented, since the event rate across the other groups or quintiles could be seen as comparable

Another comparable limit-value for endoxifen concentration of 5.2 ng/ml was proposed for tamoxifen efficacy by Saladores and colleagues, but only in a cohort of 548 pre-menopausal women[19]. Following this approach, Helland and colleagues suggested an even lower concentration of 3.3 ng/ml as a threshold for improved clinical outcomes[128]. Interestingly, Helland used concentrations of 4-hydroxy-tamoxifen in his approach in order to also identify this low threshold concentration value of endoxifen

Despite of the relevance of these findings of the aforementioned studies, none of them were specifically designed for investigating the association between endoxifen concentration and clinical outcome. In addition, these studies were performed in retrospective cohorts. Consequently, the general utility of these outcomes in the clinical practice are still awaiting for validation in studies with larger populations and specifically designed for this purpose. Of note, no statistical difference in relapse-free survival was observed between groups with endoxifen concentration levels below and above the 5.97 ng/ml threshold in a recent study using a cohort of 667 Caucasian women receiving adjuvant tamoxifen[129]. In the same line, no association between endoxifen concentration and better clinical outcome, defined as progression free survival, clinical benefit and objective response rate, was found in the neoadjuvant and metastatic setting in a study population of 297 patients[130].

In contrast, Love *et al.* proposed that there might exist a range of endoxifen concentrations for tamoxifen efficacy, instead of a minimal threshold concentration[131]. In an exploratory analysis in a nested case-control including 48 patients, surprisingly high endoxifen concentrations (> 70 ng/ml) were related with higher risk of recurrence. Additionally, they also observed a trend towards lower endoxifen concentrations (under 20 ng/ml) in the patients with relapse, but no clear threshold of efficacy was defined. In the same manner, a recent analysis by Groenland *et al.* found no differences in clinically relevant toxicities among patients with high endoxifen concentration levels (>25 ng/ml)[132].

Expert commentary

For more than 40 years, tamoxifen has been a very successful and key element of the endocrine therapy of breast cancer. Yet, important variation in clinical response is still observed. Based on the current literature, predicting tamoxifen efficacy is still in its infancy. Initially, *CYP2D6* genotyping seemed the best option, since a poorer clinical outcome might be expected in poor and probably also in intermediate CYP2D6 metabolizers, who are at least partly unable to metabolize tamoxifen to its active metabolite endoxifen. However, since *CYP2D6* polymorphisms can only explain the interpatient variability of endoxifen co, ncentrations to a limited extent, there is as yet not enough evidence to generally recommend *CYP2D6* genotyping as a predictor for tamoxifen efficacy.

In a different approach, therapeutic drug monitoring (TDM) of endoxifen concentrations, might appear to have a potential role in individualizing tamoxifen treatment. However, there is no general agreement on the required endoxifen concentrations to be reached in order to predict tamoxifen efficacy. To the best of our knowledge, no study has specifically investigated how high or low should be the concentrations of endoxifen in order to predict tamoxifen efficacy. A potential explanation why it is difficult to properly study this association between endoxifen concentrations and clinical outcome might be due the mechanism of action of tamoxifen and its active metabolites. All of them block intracellularly the estrogen receptor, and therefore concentration levels in blood of tamoxifen, or any of its active metabolites, might not be representative of the block of estrogen receptor. Interestingly, Lash and colleagues reported that active metabolites of tamoxifen occupied between 99.63 % and 99.99 % estrogen receptor[130]. In theory, patients treated with adjuvant tamoxifen, normally receive the usual daily dose of tamoxifen is 20 mg. If concentrations of endoxifen are measured once steady-state is reached, normally after 2-3 months of treatment, and these endoxifen concentrations are lower than any of the above mentioned threshold for endoxifen concentration (5.97 ng/ml, 5.2 ng/ml or 3.3 ng/ml), applying a higher dose of tamoxifen will not always assure that the expected endoxifen concentrations would be reached, especially in *CYP2D6* poor metabolizer patients. Indeed, applying an increased daily dose of tamoxifen in IM patients but not in PM individuals, resulted in endoxifen concentrations comparable to EM patients[131,132]. Yet, the long-term consequences of these higher doses are still unknown.

In order to adequately investigate the putative role of TDM of endoxifen concentrations in order to predict tamoxifen efficacy, a large study with a substantial number of patients and a very long follow-up would be required. In addition, since also other metabolites e.g. 4-hydroy-tramoxifen exert antiestrogenic activity, monitoring serum endoxifen concentration levels as a predictor for tamoxifen efficacy, might not be currently the best approach. Another limitation for such a study might be related to the applied tamoxifen regimens and the study populations. Tamoxifen monotherapy

is mainly recommended for pre-menopausal women for 5 years, while for post-menopausal patients shorter tamoxifen regimens of 2-3 years is used[5,7]. Consequently, the ideal study population for such a study might not be possible to obtain, since in the daily clinical practice many diverse tamoxifen regimens and study populations might be difficult to investigate.

Although the concept of using TDM based on endoxifen concentrations as a manner for individualizing tamoxifen therapy is highly tempting, there is not enough evidence at present for using such an approach in routine care. Another relevant point is the complexity of tamoxifen metabolism and the high number of described active metabolites, which also might be important for predicting tamoxifen efficacy. Endoxifen is considered the most crucial active metabolite of tamoxifen metabolite, but it has a comparable anti-estrogenic activity to 4-hydroxy-tamoxifen. At the same time, other active metabolites e.g. norendoxifen could also potentially affect tamoxifen efficacy, since it has dual activity. As a consequence, the current approaches in which only a few elements of tamoxifen metabolism, such as *CYP2D6* phenotypes, are used, might not be the best manner to predict tamoxifen efficacy. Since many other enzymes and tamoxifen active metabolites are involved, together with non-genetic determinants of response, a more complex analysis including all these key elements could be required in order to improve the prediction of tamoxifen efficacy and safety.

Five-year view

Since tamoxifen has such a complex metabolism, in which many enzymes and metabolites are involved, it is to be expected that prediction of tamoxifen efficacy in individual patients relies on more genes than the *CYP2D6* gene alone. In addition, other non-genetic factors that may also alter tamoxifen pharmacokinetics and pharmacodynamics need to be considered. Therefore, models incorporating both genetic and non-genetic determinants of response may help to further improve the prediction of individual tamoxifen response.

Regarding the potential role of z-endoxifen hydrochloride, it is important to remark, that at present, z-endoxifen is mainly being investigated in the metastatic setting. In our opinion, z-endoxifen hydrochloride in the metastatic scenario may become an alternative therapy. However, more drug development research is needed before the role of z-endoxifen hydrochloride in breast cancer treatments becomes clear.

References

1. Torre LA, Siegel RL, Ward EM, et al: Global Cancer Incidence and Mortality Rates and Trends--An Update. Cancer Epidemiol Biomarkers Prev 25:16-27, 2016
2. World Health Organization: Breast cancer,
3. Huang B, Warner M, Gustafsson JA: Estrogen receptors in breast carcinogenesis and endocrine therapy. Mol Cell Endocrinol 418 Pt 3:240-4, 2015
4. Klein DJ, Thorn CF, Desta Z, et al: PharmGKB summary: tamoxifen pathway, pharmacokinetics. Pharmacogenet. Genomics 23:643-647, 2013
5. Burstein HJ, Temin S, Anderson H, et al: Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: american society of clinical oncology clinical practice guideline focused update. J Clin Oncol 32:2255-69, 2014
6. Rugo HS, Rumble RB, Macrae E, et al: Endocrine Therapy for Hormone Receptor-Positive Metastatic Breast Cancer: American Society of Clinical Oncology Guideline. J Clin Oncol 34:3069-103, 2016
7. Senkus E, Kyriakides S, Ohno S, et al: Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 26 Suppl 5:v8-30, 2015
8. Early Breast Cancer Trialists' Collaborative G: Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687-717, 2005
9. Early Breast Cancer Trialists' Collaborative G: Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 386:1341-1352, 2015
10. Early Breast Cancer Trialists' Collaborative G, Davies C, Godwin J, et al: Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 378:771-84, 2011
11. Cuzick J, Sestak I, Baum M, et al: Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 11:1135-41, 2010
12. Thurlimann B, Robertson JF, Nabholtz JM, et al: Efficacy of tamoxifen following anastrozole ('Arimidex') compared with anastrozole following tamoxifen as first-line treatment for advanced breast cancer in postmenopausal women. Eur J Cancer 39:2310-7, 2003
13. Lien EA, Soiland H, Lundgren S, et al: Serum concentrations of tamoxifen and its metabolites increase with age during steady-state treatment. Breast Cancer Res Treat 141:243-248, 2013
14. Lenehan JG, Teft WA, Kim RB: Comparison of endoxifen levels between male and female breast cancer patients treated with tamoxifen. Journal of Clinical Oncology Conference, 2016
15. Brauch H, Murdter TE, Eichelbaum M, et al: Pharmacogenomics of tamoxifen therapy. Clin Chem 55:1770-82, 2009
16. Goetz MP, Kamal A, Ames MM: Tamoxifen pharmacogenomics: the role of CYP2D6 as a predictor of drug response. Clin Pharmacol Ther 83:160-6, 2008
17. Brauch H, Schwab M: Prediction of tamoxifen outcome by genetic variation of CYP2D6 in

post-menopausal women with early breast cancer. Br J Clin Pharmacol 77:695-703, 2014

18. Knox SK, Ingle JN, Suman VJ, et al: Cytochrome P450 2D6 status predicts breast cancer relapse in women receiving adjuvant tamoxifen (Tam). Journal of Clinical Oncology 24:4S-4S, 2006

19. Saladores P, Murdter T, Eccles D, et al: Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics J 15:84-94, 2015

20. Schroth W, Goetz MP, Hamann U, et al: Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. JAMA 302:1429-1436, 2009

21. Sanchez-Spitman A, Dezentje V, Swen J, et al: Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. J Clin Oncol:JCO1800307, 2019

22. de Vries Schultink AHM, Huitema ADR, Beijnen JH: Therapeutic Drug Monitoring of endoxifen as an alternative for CYP2D6 genotyping in individualizing tamoxifen therapy. Breast 42:38-40, 2018

23. Goetz MP, Suman VJ, Reid JM, et al: First-in-Human Phase I Study of the Tamoxifen Metabolite Z-Endoxifen in Women With Endocrine-Refractory Metastatic Breast Cancer. J Clin Oncol 35:3391-3400, 2017

24. Lien EA, Solheim E, Lea OA, et al: Distribution of 4-hydroxy-N-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. Cancer Res 49:2175-83, 1989

25. Stearns V, Johnson MD, Rae JM, et al: Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst 95:1758-64, 2003

26. Lu WJ, Xu C, Pei Z, et al: The tamoxifen metabolite norendoxifen is a potent and selective inhibitor of aromatase (CYP19) and a potential lead compound for novel therapeutic agents. Breast Cancer Res Treat 133:99-109, 2012

27. Lim JS, Sutiman N, Muerdter TE, et al: Association of CYP2C19*2 and associated haplotypes with lower norendoxifen concentrations in tamoxifen-treated Asian breast cancer patients. Br J Clin Pharmacol 81:1142-52, 2016

28. Lv W, Liu J, Lu D, et al: Synthesis of mixed (E, Z)-, (E)-, and (Z)-norendoxifen with dual aromatase inhibitory and estrogen receptor modulatory activities. Journal of Medicinal Chemistry 56:4611-4618, 2013

29. Ma J, Chu Z, Lu JBL, et al: The Cytochrome P450 Enzyme Responsible for the Production of (Z)-Norendoxifen in vitro. Chem Biodivers, 2017

30. Murdter TE, Schroth W, Bacchus-Gerybadze L, et al: Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. Clin Pharmacol Ther 89:708-717, 2011

31. Brauch H, Schroth W, Goetz MP, et al: Tamoxifen use in postmenopausal breast cancer: CYP2D6 matters. J Clin Oncol 31:176-180, 2013

32. Schroth W, Winter S, Murdter T, et al: Improved Prediction of Endoxifen Metabolism by CYP2D6 Genotype in Breast Cancer Patients Treated with Tamoxifen. Front Pharmacol

8:582, 2017

33. Pharmacogene Variation Consortium: *CYP2D6* allele nomenclature. PharmVar, 2015

34. Sachse C, Brockmoller J, Bauer S, et al: Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 60:284-95, 1997

35. Bradford LD: CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics 3:229-43, 2002

36. Masimirembwa CM, Hasler JA: Genetic polymorphism of drug metabolising enzymes in African populations: implications for the use of neuroleptics and antidepressants. Brain Res Bull 44:561-71, 1997

37. Goetz MP, Sangkuhl K, Guchelaar HJ, et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and Tamoxifen Therapy. Clin Pharmacol Ther 103:770-777, 2018

38. Goetz MP, Rae JM, Suman VJ, et al: Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. J Clin Oncol 23:9312-8, 2005

39. Province MA, Goetz MP, Brauch H, et al: CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. Clin Pharmacol Ther 95:216-227, 2014

40. Rae JM, Drury S, Hayes DF, et al: CYP2D6 and UGT2B7 genotype and risk of recurrence in tamoxifen-treated breast cancer patients. J Natl Cancer Inst 104:452-60, 2012

41. Regan MM, Leyland-Jones B, Bouzyk M, et al: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. J Natl Cancer Inst 104:441-51, 2012

42. Dezentje VO, van Schaik RH, Vletter-Bogaartz JM, et al: CYP2D6 genotype in relation to tamoxifen efficacy in a Dutch cohort of the tamoxifen exemestane adjuvant multinational (TEAM) trial. Breast Cancer Res Treat 140:363-373, 2013

43. Stanton V, Jr.: Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. J Natl Cancer Inst 104:1265-6; author reply 1266-8, 2012

44. Pharoah PD, Abraham J, Caldas C: Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial and Re: CYP2D6 and UGT2B7 genotype and risk of recurrence in tamoxifen-treated breast cancer patients. J Natl Cancer Inst 104:1263-4; author reply 1266-8, 2012

45. Nakamura Y, Ratain MJ, Cox NJ, et al: Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. J Natl Cancer Inst 104:1264; author reply 1266-8, 2012

46. Goetz MP, Sun JX, Suman VJ, et al: Loss of heterozygosity at the CYP2D6 locus in breast cancer: implications for germline pharmacogenetic studies. J Natl Cancer Inst 107, 2014

47. Sanchez-Spitman A, Dezentje V, Swen J, et al: Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. J Clin Oncol 37:636-646, 2019

48. Administration FDa: Nolvadex (Tamoxifen Citrate) Tablets, 2004

49. Madlensky L, Natarajan L, Tchu S, et al: Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. Clin Pharmacol Ther 89:718-25, 2011

50. Murdter TE, Schroth W, Bacchus-Gerybadze L, et al: Activity levels of tamoxifen metabolites

at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. Clin Pharmacol Ther 89:708-17, 2011

51. Romero-Lorca A, Novillo A, Gaibar M, et al: Impacts of the Glucuronidase Genotypes UGT1A4, UGT2B7, UGT2B15 and UGT2B17 on Tamoxifen Metabolism in Breast Cancer Patients. PLoS One 10:e0132269, 2015

52. Sun D, Chen G, Dellinger RW, et al: Characterization of tamoxifen and 4-hydroxytamoxifen glucuronidation by human UGT1A4 variants. Breast Cancer Res 8:R50, 2006

53. Parte P, Kupfer D: Oxidation of tamoxifen by human flavin-containing monooxygenase (FMO) 1 and FMO3 to tamoxifen-N-oxide and its novel reduction back to tamoxifen by human cytochromes P450 and hemoglobin. Drug Metab Dispos 33:1446-52, 2005

54. Krueger SK, Vandyke JE, Williams DE, et al: The role of flavin-containing monooxygenase (FMO) in the metabolism of tamoxifen and other tertiary amines. Drug Metab Rev 38:139-47, 2006

55. Gjerde J, Gandini S, Guerrieri-Gonzaga A, et al: Tissue distribution of 4-hydroxy-N-desmethyltamoxifen and tamoxifen-N-oxide. Breast Cancer Res Treat 134:693-700, 2012

56. Kim SY, Suzuki N, Laxmi YR, et al: Genotoxic mechanism of tamoxifen in developing endometrial cancer. Drug Metab Rev 36:199-218, 2004

57. Dahmane E, Mercier T, Zanolari B, et al: An ultra performance liquid chromatography-tandem MS assay for tamoxifen metabolites profiling in plasma: first evidence of 4'-hydroxylated metabolites in breast cancer patients. J Chromatogr B Analyt Technol Biomed Life Sci 878:3402-14, 2010

58. Crewe HK, Notley LM, Wunsch RM, et al: Metabolism of tamoxifen by recombinant human cytochrome P450 enzymes: formation of the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen. Drug Metab Dispos 30:869-74, 2002

59. Cuevas ME, Lindeman TE: In vitro cytotoxicity of 4'-OH-tamoxifen and estradiol in human endometrial adenocarcinoma cells HEC-1A and HEC-1B. Oncol Rep 33:464-70, 2015

60. Mugundu GM, Sallans L, Guo Y, et al: Assessment of the impact of CYP3A polymorphisms on the formation of alpha-hydroxytamoxifen and N-desmethyltamoxifen in human liver microsomes. Drug Metab Dispos 40:389-96, 2012

61. Notley LM, Crewe KH, Taylor PJ, et al: Characterization of the human cytochrome P450 forms involved in metabolism of tamoxifen to its alpha-hydroxy and alpha,4-dihydroxy derivatives. Chem Res Toxicol 18:1611-8, 2005

62. Nowell S, Sweeney C, Winters M, et al: Association between sulfotransferase 1A1 genotype and survival of breast cancer patients receiving tamoxifen therapy. J Natl Cancer Inst 94:1635-40, 2002

63. Nowell SA, Ahn J, Rae JM, et al: Association of genetic variation in tamoxifen-metabolizing enzymes with overall survival and recurrence of disease in breast cancer patients. Breast Cancer Res Treat 91:249-58, 2005

64. Wegman P, Vainikka L, Stal O, et al: Genotype of metabolic enzymes and the benefit of tamoxifen in postmenopausal breast cancer patients. Breast Cancer Res 7:R284-90, 2005

65. Moyer AM, Suman VJ, Weinshilboum RM, et al: SULT1A1, CYP2C19 and disease-free survival in early breast cancer patients receiving tamoxifen. Pharmacogenomics 12:1535-

2

1543, 2011

66. Wegman P, Elingarami S, Carstensen J, et al: Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT2B15 and tamoxifen response in postmenopausal patients with breast cancer. Breast Cancer Res 9:R7, 2007

67. Jin Y, Desta Z, Stearns V, et al: CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 97:30-9, 2005

68. Gjerde J, Hauglid M, Breilid H, et al: Effects of CYP2D6 and SULT1A1 genotypes including SULT1A1 gene copy number on tamoxifen metabolism. Ann Oncol 19:56-61, 2008

69. Fernandez-Santander A, Gaibar M, Novillo A, et al: Relationship between genotypes Sult1a2 and Cyp2d6 and tamoxifen metabolism in breast cancer patients. PLoS One 8:e70183, 2013

70. Blevins-Primeau AS, Sun D, Chen G, et al: Functional significance of UDP-glucuronosyltransferase variants in the metabolism of active tamoxifen metabolites. Cancer Res 69:1892-900, 2009

71. Mizuma T: Intestinal glucuronidation metabolism may have a greater impact on oral bioavailability than hepatic glucuronidation metabolism in humans: a study with raloxifene, substrate for UGT1A1, 1A8, 1A9, and 1A10. Int J Pharm 378:140-1, 2009

72. Ahern TP, Christensen M, Cronin-Fenton DP, et al: Functional polymorphisms in UDP-glucuronosyl transferases and recurrence in tamoxifen-treated breast cancer survivors. Cancer Epidemiol Biomarkers Prev 20:1937-1943, 2011

73. Areepium N, Panomvana D, Rungwanonchai P, et al: Effects of CYP2D6 and UGT2B7 polymorphisms on pharmacokinetics of tamoxifen in Thai breast cancer patients. Breast Cancer (Dove Med Press) 5:73-8, 2013

74. Sutiman N, Lim JSL, Muerdter TE, et al: Pharmacogenetics of UGT1A4, UGT2B7 and UGT2B15 and Their Influence on Tamoxifen Disposition in Asian Breast Cancer Patients. Clin Pharmacokinet 55:1239-1250, 2016

75. Sutiman N, Lim JS, Muerdter TE, et al: Pharmacogenetics of UGT1A4, UGT2B7 and UGT2B15 and Their Influence on Tamoxifen Disposition in Asian Breast Cancer Patients. Clin Pharmacokinet 55:1239-1250, 2016

76. Rae JM, Drury S, Hayes DF, et al: CYP2D6 and UGT2B7 genotype and risk of recurrence in tamoxifen-treated breast cancer patients. J Natl Cancer Inst 104:452-460, 2012

77. Antunes MV, Timm TA, de O, V, et al: Influence of CYP2D6 and CYP3A4 Phenotypes, Drug Interactions, and Vitamin D Status on Tamoxifen Biotransformation. Ther Drug Monit 37:733-744, 2015

78. Wu AH, Pike MC, Williams LD, et al: Tamoxifen, soy, and lifestyle factors in Asian American women with breast cancer. J Clin Oncol 25:3024-30, 2007

79. Peyrade F, Frenay M, Etienne MC, et al: Age-related difference in tamoxifen disposition. Clin Pharmacol Ther 59:401-10, 1996

80. Lenehan JG, Teft WA, Kim RB: Comparison of endoxifen levels between male and female breast cancer patients treated with tamoxifen. Journal of Clinical Oncology 34, 2016

81. Sendur MA, Aksoy S, Ozdemir NY, et al: Effect of body mass index on the efficacy of adjuvant tamoxifen in premenopausal patients with hormone receptor-positive breast cancer. J BUON 21:27-34, 2016

82. Teft WA, Gong IY, Dingle B, et al: CYP3A4 and seasonal variation in vitamin D status in addition to CYP2D6 contribute to therapeutic endoxifen level during tamoxifen therapy. Breast Cancer Res Treat 139:95-105, 2013

83. Binkhorst L, Kloth JSL, de Wit AS, et al: Circadian variation in tamoxifen pharmacokinetics in mice and breast cancer patients. Breast Cancer Res Treat 152:119-128, 2015

84. Zhan M, Flaws JA, Gallicchio L, et al: Profiles of tamoxifen-related side effects by race and smoking status in women with breast cancer. Cancer Detect Prev 31:384-90, 2007

85. Sahebkar A, Serban MC, Penson P, et al: The Effects of Tamoxifen on Plasma Lipoprotein(a) Concentrations: Systematic Review and Meta-Analysis. Drugs 77:1187-1197, 2017

86. Lien EA, Soiland H, Lundgren S, et al: Serum concentrations of tamoxifen and its metabolites increase with age during steady-state treatment. Breast Cancer Res Treat 141:243-8, 2013

87. Davis SR, Lambrinoudaki I, Lumsden M, et al: Menopause. Nat Rev Dis Primers 1:15004, 2015

88. Tan JL, Eastment JG, Poudel A, et al: Age-Related Changes in Hepatic Function: An Update on Implications for Drug Therapy. Drugs Aging 32:999-1008, 2015

89. Maher RL, Hanlon J, Hajjar ER: Clinical consequences of polypharmacy in elderly. Expert Opin Drug Saf 13:57-65, 2014

90. Piccirillo JF, Vlahiotis A, Barrett LB, et al: The changing prevalence of comorbidity across the age spectrum. Crit Rev Oncol Hematol 67:124-32, 2008

91. Antunes MV, de Oliveira V, Raymundo S, et al: CYP3A4*22 is related to increased plasma levels of 4-hydroxytamoxifen and partially compensates for reduced CYP2D6 activation of tamoxifen. Pharmacogenomics 16:601-617, 2015

92. Goodwin PJ, Pritchard KI: Obesity and hormone therapy in breast cancer: an unfinished puzzle. J Clin Oncol 28:3405-7, 2010

93. Singh BN, Malhotra BK: Effects of food on the clinical pharmacokinetics of anticancer agents: underlying mechanisms and implications for oral chemotherapy. Clin Pharmacokinet 43:1127-56, 2004

94. Siegel RL, Miller KD, Fedewa SA, et al: Colorectal cancer statistics, 2017. CA Cancer J Clin 67:177-193, 2017

95. Eggemann H, Ignatov A, Smith BJ, et al: Adjuvant therapy with tamoxifen compared to aromatase inhibitors for 257 male breast cancer patients. Breast Cancer Res Treat 137:465-70, 2013

96. Soldin OP, Mattison DR: Sex differences in pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 48:143-57, 2009

97. Catsburg C, Kirsh VA, Soskolne CL, et al: Active cigarette smoking and the risk of breast cancer: a cohort study. Cancer Epidemiol 38:376-81, 2014

98. Catsburg C, Miller AB, Rohan TE: Active cigarette smoking and risk of breast cancer. Int J Cancer 136:2204-9, 2015

99. Persson M, Simonsson M, Markkula A, et al: Impacts of smoking on endocrine treatment response in a prospective breast cancer cohort. Br J Cancer 115:382-90, 2016

100. Imperato F, Marziani R, Perniola G, et al: [Effects of tamoxifen and estrogen replacement therapy on lipid metabolism and some other cardiovascular risk factors. A prospective study in hysterectomised women]. Minerva Ginecol 55:87-93, 2003

101. Nordenskjold B, Rosell J, Rutqvist LE, et al: Coronary heart disease mortality after 5 years of adjuvant tamoxifen therapy: results from a randomized trial. J Natl Cancer Inst 97:1609-10, 2005

102. Clarke SC, Schofield PM, Grace AA, et al: Tamoxifen effects on endothelial function and cardiovascular risk factors in men with advanced atherosclerosis. Circulation 103:1497-502, 2001

103. Shewmon DA, Stock JL, Rosen CJ, et al: Tamoxifen and estrogen lower circulating lipoprotein(a) concentrations in healthy postmenopausal women. Arterioscler Thromb 14:1586-93, 1994

104. Zainal NZ, Nik-Jaafar NR, Baharudin A, et al: Prevalence of depression in breast cancer survivors: a systematic review of observational studies. Asian Pac J Cancer Prev 14:2649-56, 2013

105. Pirl WF: Evidence report on the occurrence, assessment, and treatment of depression in cancer patients. J Natl Cancer Inst Monogr:32-9, 2004

106. Jeppesen U, Gram LF, Vistisen K, et al: Dose-dependent inhibition of CYP1A2, CYP2C19 and CYP2D6 by citalopram, fluoxetine, fluvoxamine and paroxetine. Eur J Clin Pharmacol 51:73-8, 1996

107. Borges S, Desta Z, Li L, et al: Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. Clin Pharmacol Ther 80:61-74, 2006

108. Dusetzina SB, Alexander GC, Freedman RA, et al: Trends in co-prescribing of antidepressants and tamoxifen among women with breast cancer, 2004-2010. Breast Cancer Res Treat 137:285-96, 2013

109. Dieudonne AS, De Nys K, Casteels M, et al: How often did Belgian physicians co-prescribe tamoxifen with strong CYP2D6 inhibitors over the last 6 years? Acta Clin Belg 69:47-52, 2014

110. Binkhorst L, Mathijssen RH, van Herk-Sukel MP, et al: Unjustified prescribing of CYP2D6 inhibiting SSRIs in women treated with tamoxifen. Breast Cancer Res Treat 139:923-9, 2013

111. Binkhorst L, Bannink M, de Bruijn P, et al: Augmentation of Endoxifen Exposure in Tamoxifen-Treated Women Following SSRI Switch. Clin Pharmacokinet 55:249-55, 2016

112. Dezentje VO, van Blijderveen NJ, Gelderblom H, et al: Effect of concomitant CYP2D6 inhibitor use and tamoxifen adherence on breast cancer recurrence in early-stage breast cancer. J Clin Oncol 28:2423-9, 2010

113. Haque R, Shi J, Schottinger JE, et al: Tamoxifen and Antidepressant Drug Interaction in a Cohort of 16,887 Breast Cancer Survivors. J Natl Cancer Inst 108, 2016

114. Burstein HJ, Lacchetti C, Anderson H, et al: Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: ASCO Clinical Practice Guideline Focused Update. J Clin Oncol:JCO1801160, 2018

115. Pagani O, Gelber S, Colleoni M, et al: Impact of SERM adherence on treatment effect: International Breast Cancer Study Group Trials 13-93 and 14-93. Breast Cancer Res Treat 142:455-9, 2013

116. Wigertz A, Ahlgren J, Holmqvist M, et al: Adherence and discontinuation of adjuvant hormonal therapy in breast cancer patients: a population-based study. Breast Cancer Res

Treat 133:367-73, 2012

117. Lash TL, Fox MP, Westrup JL, et al: Adherence to tamoxifen over the five-year course. Breast Cancer Res Treat 99:215-20, 2006

118. Chlebowski RT, Kim J, Haque R: Adherence to endocrine therapy in breast cancer adjuvant and prevention settings. Cancer Prev Res (Phila) 7:378-87, 2014

119. Pistilli B, Soulie P, Vanlemmens L, et al: 185O_PRSerum assessment of non-adherence to adjuvant endocrine therapy (ET) among premenopausal patients in the prospective multicenter CANTO cohort. Annals of Oncology 29, 2018

120. Chirgwin JH, Giobbie-Hurder A, Coates AS, et al: Treatment Adherence and Its Impact on Disease-Free Survival in the Breast International Group 1-98 Trial of Tamoxifen and Letrozole, Alone and in Sequence. J Clin Oncol 34:2452-9, 2016

121. Lien EA, Solheim E, Kvinnsland S, et al: Identification of 4-hydroxy-N-desmethyltamoxifen as a metabolite of tamoxifen in human bile. Cancer Res 48:2304-8, 1988

122. Ahmad A, Ali SM, Ahmad MU, et al: Orally administered endoxifen is a new therapeutic agent for breast cancer. Breast Cancer Res Treat 122:579-584, 2010

123. Jordan VC, Allen KE: Evaluation of the antitumour activity of the non-steroidal antioestrogen monohydroxytamoxifen in the DMBA-induced rat mammary carcinoma model. Eur J Cancer 16:239-51, 1980

124. Hawse JR, Subramaniam M, Cicek M, et al: Endoxifen's molecular mechanisms of action are concentration dependent and different than that of other anti-estrogens. PLoS One 8:e54613, 2013

125. Wu X, Hawse JR, Subramaniam M, et al: The tamoxifen metabolite, endoxifen, is a potent antiestrogen that targets estrogen receptor alpha for degradation in breast cancer cells. Cancer Res 69:1722-1727, 2009

126. Jin Y, Desta Z, Stearns V, et al: CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 97:30-39, 2005

127. Opdam FL, Dezentje VO, den HJ, et al: The use of the 13C-dextromethorphan breath test for phenotyping CYP2D6 in breast cancer patients using tamoxifen: association with CYP2D6 genotype and serum endoxifen levels. Cancer Chemother Pharmacol 71:593-601, 2013

128. Helland T, Henne N, Bifulco E, et al: Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Res 19:125, 2017

129. Sanchez-Spitman AB, Dezentjé VO, Swen JJ, et al: A prospective study on the effect of endoxifen concentration and CYP2D6 phenotypes on clinical outcome in early stage breast cancer patients receiving adjuvant tamoxifen. Journal of Clinical Oncology 36:523-523, 2018

130. Neven P, Jongen L, Lintermans A, et al: Tamoxifen Metabolism and Efficacy in Breast Cancer: A Prospective Multicenter Trial. Clin Cancer Res 24:2312-2318, 2018

131. Love RR, Desta Z, Flockhart D, et al: CYP2D6 genotypes, endoxifen levels, and disease recurrence in 224 Filipino and Vietnamese women receiving adjuvant tamoxifen for operable breast cancer. Springerplus 2:52, 2013

132. Groenland SL, Sanchez-Spitman AB, Moes DJAR, et al: 258PIncidence of clinically significant toxicities in patients with high endoxifen concentrations. Annals of