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Review

Targeting of tamoxifen to enhance antitumour action for the treatment and prevention of breast cancer: The 'personalised' approach?

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ABSTRACT

Tamoxifen is a standard endocrine therapy for the treatment of steroid receptor positive breast cancer. Tamoxifen efficacy depends on the formation of clinically active metabolites 4-hydroxytamoxifen and endoxifen which have a greater affinity to the oestrogen receptor and ability to control cell proliferation as compared to the parent drug. The cytochrome P450 2D6 enzyme plays a key role in this biotransformation and lack of tamoxifen efficacy has been linked to low activity. There is now considerable mechanistic, pharmacologic and clinical pharmacogenetic evidence in support of the notion that CYP2D6 genetic variants and phenocopying effects through drug interaction by CYP2D6 inhibitors influence plasma concentrations of active tamoxifen metabolites and negatively impact tamoxifen outcome. These interrelations are particularly critical for patients with non-functional (poor metaboliser) and severely impaired (intermediate metaboliser) CYP2D6 variants, and, moreover, for patients in need of co-medication such as serotonin re-uptake inhibitors to control adverse effects such as hot flashes and other menopausal symptoms. Therefore, in the future, a personalised approach for an optimal tamoxifen benefit should consider a CYP2D6 genotype guided adjuvant endocrine treatment strategy and avoid non-adherence as well as strong CYP2D6 inhibitors such as co-medications.

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Review criteria

Apart from select historically relevant papers and review articles, literature was identified by searching the PubMed database for relevant publications written in English between December 2003 and April 2009. Search terms included 'tamoxifen, CYP2D6 metabolism', 'tamoxifen outcome, CYP2D6', and 'tamoxifen adherence' matched by 'pharmacogenetics' and/or 'hot flashes'.

1. Introduction

Tamoxifen, a non-steroidal antioestrogen[1] (Fig. 1), is used for the treatment of all stages of breast cancer[2–4] and in the US is available to reduce the incidence of breast cancer in both pre- and postmenopausal women at elevated risk.[5–7] It is important to remember that during early clinical studies tamoxifen did not show any improvement in efficacy over standard hormonal treatments (high dose oestrogen or androgen) for

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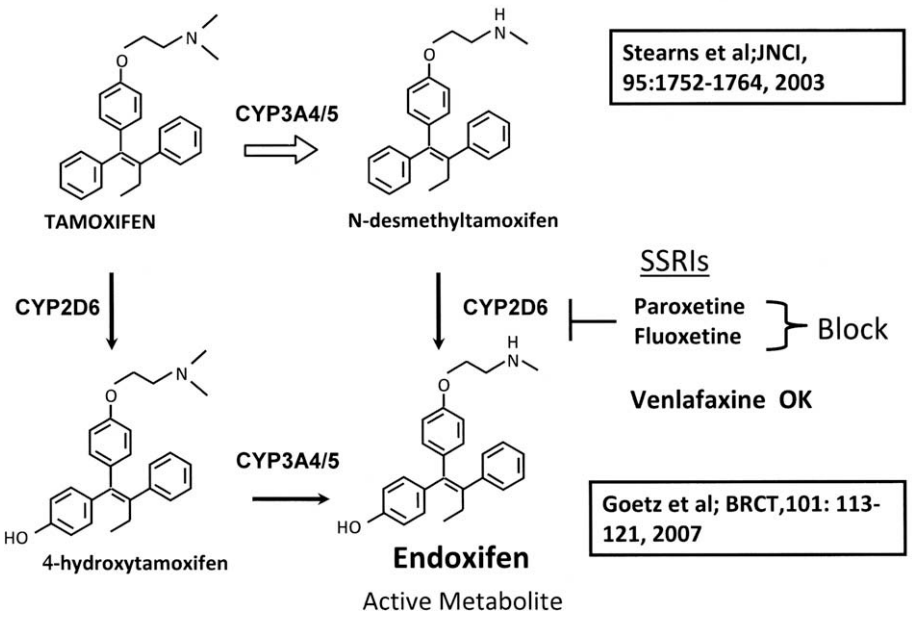

Fig. 1 – The principal metabolites of tamoxifen illustrating the route of metabolism for endoxifen via the CYP2D6 enzyme. An increase in the intensity of hot flashes and menopausal symptoms during tamoxifen therapy has prompted the widespread use of selective serotonin re-uptake inhibitors (SSRIs) to improve the quality of life. However, the SSRIs such as paroxetine and fluoxetine are also metabolised by the CYP2D6 enzyme as it can block the production of endoxifen. Venlafaxine has a low affinity for the CYP2D6 enzyme and is therefore recommended as an alternative to ameliorate menopausal symptoms.

metastatic breast cancer.[2,8] The only advantage of tamoxifen was a reduced incidence of side effects for those 30% of patients who responded for about 1 year. However, laboratory studies to target the tumour oestrogen receptor (ER)[9] employed long term adjuvant therapy[10] and considered the chemoprevention of breast cancer.[11,12] Tamoxifen was thus reinvented from an orphan drug to the 'gold standard' for the endocrine treatment of breast cancer between 1984 and 2004. The targeting of tamoxifen to block oestrogen stimulated breast tumour growth with long term (5 years) adjuvant tamoxifen therapy[13] resulted in a major improvement in patient survivorship and has contributed significantly to the reduction in national death rates from breast cancer.[14,15] The recent development of aromatase inhibitors as an effective treatment for breast cancer in postmenopausal patients[16] has improved disease-free survival and reduced the side effects of endometrial cancer and blood clots noted with tamoxifen.[17–20] However, aromatase inhibitors are not universally available in national health care systems worldwide because of significant financial constraints. Tamoxifen remains a cheap, life-saving, targeted therapy for both pre- and postmenopausal patients with breast cancer.

The application of the ER as a tumour target to treat breast cancer patients appropriately provided a valuable, but admittedly not perfect, test to increase the probability of tumour growth control during long term adjuvant treatment. Tamoxifen does not enhance either disease-free or overall survival in patients with ER negative tumours.[14,15]

At present, there are no universally accepted tumour markers to improve response rates for patients with ER positive tumours. However, emerging data on the pharmacogenomics of tamoxifen metabolism through the CYP2D6 enzyme and new knowledge of potential drug interactions with selective serotonin re-uptake inhibitors (SSRIs), to control hot flashes, provide valuable new information to aid in the selection of the appropriate long term endocrine treatment for breast cancer patients with ER positive disease.

The goal of this concise review is to describe the new understanding of the metabolic activation of tamoxifen to its putative active agent endoxifen[21–23] and consider the clinical significance of CYP2D6 polymorphisms together with phenocopying effects through drug interaction. We will summarise the actions necessary to improve the value of tamoxifen as a 'personalised targeted treatment for breast cancer'.

2. Clinical pharmacology of tamoxifen

2.1. *Tamoxifen efficacy*

Our evolving understanding of the relevance of tamoxifen metabolism for its pharmacology has recently been reviewed.[24] Nevertheless, the important pharmacological issues and conclusions will be restated to provide a scientific background for evaluating the role of the CYP2D6 enzyme and underlying genetics for the antitumour actions of tamoxifen.

Tamoxifen is a pro-drug that requires metabolic activation to 4-hydroxytamoxifen[25,26] and 4-hydroxy-N-desmethyltamoxifen (endoxifen) (Fig. 1) in order to exert its therapeutic effect.[3,4,22,23] 4-Hydroxylation of tamoxifen and its major metabolite N-desmethyltamoxifen increases the affinity for the ER,[26–28] and although both metabolites are equipotent with respect to ER binding and inhibition of 17beta-oestradiol induced cell proliferation, it is proposed that endoxifen is the

principal antioestrogenic metabolite for the antitumour activity observed in breast cancer patients treated at the 20 mg daily dose of tamoxifen.[29] Endoxifen was found at more than six-fold higher concentrations in the plasma of tamoxifen treated patients as compared to 4-hydroxytamoxifen. The metabolism of interest is illustrated in Fig. 1 and the principal metabolites of interest are 4-hydroxytamoxifen and endoxifen. Both metabolites induce similar changes on global gene expression patterns, i.e. the gene array analysis of the spectrum of genes activated or suppressed by 4-hydroxytamoxifen and endoxifen in MCF-7 breast cancer cells is almost the same.[30] There are 4062 total genes either up or down regulated by oestradiol but in the presence of oestradiol and 4-hydroxytamoxifen or endoxifen, 2444 and 2390 genes were affected, respectively. Both tamoxifen metabolites showed overlapping effects on 1365 oestradiol sensitive genes and there was reasonable confirmation with selected genes by RT-PCR. The overall conclusion was that 4-hydroxytamoxifen and endoxifen are almost identical.[30] Together with the ER binding profile and the antiproliferative action of 4-hydroxytamoxifen and endoxifen in MCF-7 cells being identical,[28] but the circulating levels of endoxifen in patients being higher than that of 4-hydroxytamoxifen,[23,29] based on the Law of Mass Action, endoxifen would be anticipated to be the principal metabolite blocking the binding of oestradiol at the tumour ER.

An intriguing aspect of the investigations of the molecular pharmacology of endoxifen is the recent report that the antioestrogen targets ERα for rapid destruction in breast cancer cells.[31] The implication is that the shape of the endoxifen ERα complex is perturbed significantly for rapid proteasomal degradation. Profound structural perturbations of the ER are noted with the pure antioestrogen ICI164384[32] and the SERM GW5638[33] with both compounds causing rapid destruction of ER. In contrast, the structure of endoxifen is almost identical to the related metabolite 4-hydroxytamoxifen (Fig. 1) that causes accumulation of the ER. The structure of the 4 hydroxyl tamoxifen ER complex has been resolved.[34] Perhaps crystallisation of the endoxifen ER complex would provide insight into the actions of endoxifen at the ER.

2.2. *Tamoxifen pharmacogenomics*

2.2.1. *The role of cytochrome P450 2D6*

Numerous drug metabolising enzymes, particularly of the cytrochrome P450 (CYP) iso-enzyme family, are involved in the metabolism of tamoxifen. Among these, CYP2D6 plays the dominant role in the conversion from the major, but clinically inactive, metabolite N-desmethyltamoxifen into the clinically active endoxifen (Fig. 1).[35] Together with CYP2B6, CYP2C9, CYP2C19 and CYP3A4, it is also involved in the formation of 4-hydroxytamoxifen. With CYP2D6 being at the heart of tamoxifen action, host factors, by virtue of the patients genetic makeup, must be taken into account, in addition to tumour characteristics such as ER status, in order to understand drug efficacy. This is owing to the fact that the CYP2D6 phenotype is variable and that this variability differs with respect to degree, underlying genetic variation and frequencies across ethnic groups. By way of clinical observation, the first CYP2D6 phenotypic variation (sparteine/debrisoquine polymorphism) distinct from an extensive metaboliser (EM) phenotype was identified more than 30 years ago and termed poor metaboliser (PM).[36,37] Since then, based on drug oxidation capacity, four different CYP2D6 phenotypes, namely EM, intermediate metaboliser (IM), PM, and ultrarapid metaboliser (UM), have been identified.[38–40] Their frequencies and global distributions have been investigated and extensively reviewed.[41] Although not all CYP2D6 phenotypic variations can be attributed to genetic variations, as of today, there are more than 100 known different alleles of the CYP2D6 (http://www.cypalleles.ki.se).

The PM phenotype is present in 7 to 10% of the European population with PM individuals carrying two non-functional (null) alleles leading to a loss of enzyme function. Of the numerous known null alleles the CYP2D6 *3, *4, and *5 alleles are prevalent in populations of European descent with *4 being present in 70–90% of all PMs. PM status, i.e. lack of catalytic function, can be deduced with greater than 99% certainty from the presence of two non-functional alleles and, therefore, can be accurately predicted from the patients genotype without the need to phenotype.[38,40,42,43] Ten to 15% of Europeans are IM, characterised by severely impaired CYP2D6 expression and function due to the presence of *9, *10, and *41 alleles.[39,44–46] IMs are genetically either homozygous for IM mutations or compound heterozygous for an IM allele in combination with one null allele.[45,47] The EM phenotype results from the presence of one or two alleles with normal expression level and catalytic function such as *1 and *2 and represents the most frequent CYP2D6 phenotype within the European population accounting for 60–70%. EMs can be separated into homozygous or heterozygous EMs depending on whether they carry two or one functional allele. Heterozygous EMs carrying one *1 or *2 allele in combination with an IM or PM allele have a somewhat impaired enzyme expression and function, a reason why they have been classified as 'intermediate metabolisers' assuming a gene dose effect. However, due to the substantial overlap both in enzyme content and activity between homozygous and heterozygous EMs, this is not correct and, therefore, the predictive value of the heterozygous EM genotype is rather poor. Importantly, the IM is a phenotype and genotype distinct from the heterozygous EM based on the presence of *9, *10, and *41 and/or non-functional alleles.[39,46] The UM phenotype is present in 10–15% of the European population and a gene duplication with up to 13 gene copies involving *1, *2, and *4 alleles has been identified as an underlying molecular mechanism.[48,49] Such an increase in enzyme activity can have profound consequences on the plasma concentrations of drug metabolites[50,51]; however, only 20–30% of the UM phenotype in the Caucasian population are accessible through genotyping and, therefore, the predictive value is rather low.[38,40,52]

While CYP2D6 tamoxifen pharmacogenomics for patients of European descent must primarily focus on PM and IM, but also include UM, the PMs play a minor role in individuals of non-European descent. Rather, within Asian populations, IMs are prevalent based on a much higher frequency of the *10 allele, i.e. 57% in Han Chinese[41] and, therefore, tamoxifen pharmacogenomics in Asia requires a focus on IM. Furthermore, North Eastern African populations would require a focus on gene duplication due to a much higher frequency

e.g. 29% in Ethiopia[53] and 21% in Saudi Arabia[54] as compared to 1–5% in populations of European descent.[41,43,55]

2.2.2. CYP2D6 genotype – tamoxifen outcome relationship

Within recent years an increasing awareness of the CYP2D6 phenotypes and underlying genotypes[29,56] sparked a number of international clinical studies to assess retrospectively the potential value of tamoxifen pharmacogenomics for the prediction of treatment outcome of (mainly) early breast cancer. The first evidence linking CYP2D6 variants with treatment response was obtained by Goetz et al.[57] from a US prospective randomised phase III trial of postmenopausal women with primary ER positive breast cancer (North Central Cancer Treatment Group Adjuvant Breast Cancer Trial 89-30-52) investigating the effect of adding the androgen fluoxymestrone, for 1 year, to the standard 5-year adjuvant tamoxifen (20 mg/day). Patients who had received 20 mg/daily adjuvant tamoxifen ($n = 223$ of 256 eligible; mainly of European descent) were genotyped for CYP2D6 variants *4 and *6. Their genomic DNA was obtained from paraffin-embedded tissue specimens. Of the 190 patients for whom CYP2D6 (*4) genotyping was possible, 137 (72.1%) had wt/wt, 40 (21.1%) wt/*4, and 13 (6.8%) *4/*4 genotype. The concordance rate between the genotype obtained from additional buccal cells in 17 patients and the corresponding tumour tissue was 100%. After a median follow-up of 11.4 years, the CYP2D6 *4/*4 genotype was associated with poor patient outcome. CYP2D6 *4/*4 was associated with worse relapse-free ($P = 0.023$) and disease-free survival ($P = 0.012$). The genotype did not have an impact on overall survival ($P = 0.169$). The authors confirmed their findings in an extended study of 256 patients.[58]

A robust association between CYP2D6 genotypes and treatment outcome has been obtained by Schroth et al.[59] from a non-randomised retrospective cohort of ER-positive postmenopausal breast cancer cases from Germany. The study included 206 breast cancer patients treated with adjuvant tamoxifen monotherapy (standard dose) and 280 patients without tamoxifen. The comprehensive genotyping approach using constitutional DNA derived from formalin-fixed paraffin-embedded normal breast tissues included the CYP2D6 variants *4, *5, *10, and *41 to cover the vast majority of PM and IM genotypes (e.g. 95% and 90%, respectively). The analyses were aimed at the investigation of approximately 15–25% of patients expected to be carriers of PM or IM alleles and genotypes. At a median follow-up of 71 months, carriers of CYP2D6 *4, *5 *10 and *41 alleles had significantly more breast cancer recurrences, shorter relapse-free time (hazard ratio (HR) = 2.24; 95% confidence interval (CI), 1.16–4.33; $P = 0.02$), and worse event-free survival (HR = 1.89; 95% CI, 1.10–3.25; $P = 0.02$) compared to carriers of functional alleles (Fig. 2). These associations were not observed in postmenopausal ER positive patients not treated with tamoxifen. This study also included other tamoxifen metabolising genes (i.e. CYP2C19, CYP2B6, CYP2C9, and CYP3A5) and variants. Interestingly, the CYP2C19*17 (UM) allele also had a favourable effect on tamoxifen treatment outcome in that patients with a homozygous *17 genotype had significantly less breast cancer recurrences, longer relapse-free time and better event-free survival (HR = 0.45; 95% CI, 0.21–0.92; $P = 0.03$) compared to non *17 carriers.[59] Altogether, this study suggested that genotyping for CYP2D6 *4, *5, *10 and *41 can identify patients who will derive little benefit from adjuvant tamoxifen. However, EM patients, accounting for approximately 50% of all patients, are likely to benefit from tamoxifen and this benefit will be at a maximum for those with a combination of CYP2D6 functional and CYP2C19 UM alleles. The latter applies to approximately one third of all patients pointing to the relevance of tamoxifen pharmacogenomics for a large fraction of patients receiving endocrine treatment.

Supportive evidence has been provided by studies from Korea,[60] China[61] and Japan.[62] As expected for Asian populations, the CYP2D6 *10 allele significantly contributed to the overall fraction of IM genotypes and observed effects in these patient cohorts. The Korean study by Lim et al.[60] included 202 patients with either primary or metastatic breast cancer treated with 20 mg/daily tamoxifen for more than 8 weeks. Genotype frequencies were 31.6% for wt/wt, 44% for wt/*10, and 24.2% for *10/*10. Patients with *10/*10 genotype ($n = 49$) had significantly lower steady-state plasma concentrations of endoxifen and 4-OH-tamoxifen than those with other

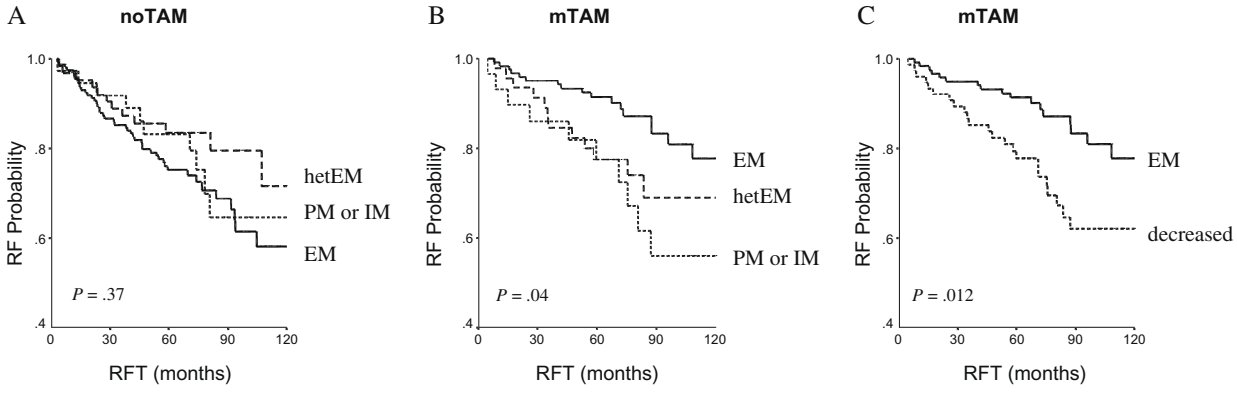

Fig. 2 – Kaplan–Meier estimates of relapse-free time (RFT) for CYP2D6 metaboliser phenotypes predicted from genotypes. (A) Patients not treated with tamoxifen (noTAM); (B) patients treated with adjuvant tamoxifen monotherapy (mTAM); (C) carriers of one or two impaired CYP2D6 alleles predictive for decreased enzyme activity were combined; EM, IM, PM, extensive, intermediate, poor metaboliser, hetEM, heterozygous extensive.[59]

genotypes ($n = 153$). In a small cohort of 21 patients with metastatic breast cancer and treated with tamoxifen, all six patients with progressive or stable disease lasting less than 24 weeks carried the *10/*10 genotype ($P = 0.0186$). The median time to progression for *CYP2D6*10/*10* patients was significantly shorter than that for all other genotypes (5.0 versus 21.8 months, $P = 0.0032$). The Chinese study by Xu et al.[61] investigated 152 patients with 20 mg/daily adjuvant tamoxifen for 5 years and a cohort of 141 patients not treated with tamoxifen. Overall genotype frequencies were 24% for *10 wt/wt (C/C), 28% for wt/*10 (C/T), and 48% for *10/*10 (T/T). At a median follow-up time of 63 months, carriers of the CYP2D6 *10/*10 genotype had a significantly worse disease-free survival (89% versus 96%, $P = 0.005$), an association that was not observed in the patient cohort not treated with tamoxifen. Moreover, among 37 patients taking tamoxifen for at least 4 weeks, 4-hydroxytamoxifen levels were significantly lower in CYP2D6*10 homozygous genotype carriers than in patients with homozygous CYP2D6 wt/wt genotype ($P = 0.04$). The Japanese study by Kiyotani et al.[62] investigated 67 patients treated with 20 mg/daily tamoxifen for 5 years with a median follow-up of 10 years. Frequencies were 29.9% for CYP2D6 *1/*1 (wt/wt), 34.3% for *1/*10 and 22.4% for *10/*10. Patients with a CYP2D6 *10/*10 genotype showed a significantly higher incidence of recurrence than those with a CYP2D6 *1/*1 genotype ($P = 0.0057$) or a combined patient group carrying at least one *1 allele ($P = 0.0031$ for trend). Although some of the sample sizes in the Asian studies were low, their findings of an implication of CYP2D6 genotypes predictive for tamoxifen outcome are in line with the studies from Europe[59] and the US.[57,58]

No favourable association of CYP2D6 genetics and tamoxifen outcome was reported in studies from the US, by Nowell et al. (162 patients with tamoxifen, 175 patients without tamoxifen), and Sweden, by Wegmann et al. (112 patients with 40 mg/daily tamoxifen, and mean follow-up of 10.7 years), respectively.[63,64] While Nowell et al. reported no association between CYP2D6 *4 and tamoxifen response or breast cancer prognosis,[63] Wegman et al. reported a decrease in the number of recurrences in tamoxifen treated patients who carried the CYP2D6 *4 variant (odds ratio (OR) = 0.28; 95% CI, 0.11–0.74; $P = 0.0089$).[64] Wegman et al. in addition investigated a cohort of 677 tamoxifen-treated postmenopausal patients, 238 of whom were randomised to 2 versus 5 years of treatment. Patients homozygous for CYP2D6 *4 had a significantly better disease-free survival compared to patients homozygous or heterozygous for the *1 allele ($P = 0.05$ and $P = 0.04$, respectively); however, this effect was not significant in multivariate Cox analysis ($P = 0.055$).[65]

So far, most available evidence in favour of a relationship between CYP2D6 variation and tamoxifen treatment outcome is derived from patients with mainly adjuvant tamoxifen treatment. However, preliminary evidence suggests that this relationship may also play a role in breast cancer chemoprevention as reported from the Italian tamoxifen trial including 5408 healthy hysterectomised women aged 35–70 years who were randomly assigned to receive 20 mg daily tamoxifen or placebo. In a nested case-control study including 46 women who developed breast cancer and 136 controls, the frequency of CYP2D6 *4/*4 genotype was significantly higher in women who developed breast cancer than in those who did not: all women (tamoxifen and placebo): 9% versus 1% $P = 0.015$); tamoxifen treated women: 15% versus 2% ($P = 0.04$).[66] Unexpectedly, hot flashes were reported for all three CYP2D6 *4/*4 allele carriers who developed breast cancer during tamoxifen treatment.

Finally, a small study of hereditary breast cancer patients being tumour suppressor mutation carriers of BRCA1 (47 patients) or BRCA2 (68 patients) and treated with tamoxifen suggested a relationship between CYP2D6 PM status and worse survival.[67] This relationship was observed for BRCA2 but not for BRCA1 carriers. Due to small numbers, as well as ER positive and ER negative patients being included in the analysis, further investigation will be needed to distinguish a pharmacogenetic effect from a poor prognosis effect.

2.3. *Effects of metabolite levels and drug interaction on tamoxifen efficacy and outcome*

It is clear that patients must complete a 5-year course of tamoxifen because 5 years of tamoxifen is superior to 1 or 2 years of adjuvant treatment. This principle is elegantly demonstrated in the overview analysis of clinical trials for premenopausal patients with ER positive breast cancer (Fig. 3).[15] Although, in general, rates of tamoxifen adherence are higher than those observed for other medications, discontinuation of adjuvant tamoxifen in older women with ER positive breast cancer has been evaluated. Randomised clinical trials of adjuvant therapy reported 5-year discontinuance rates of 23% and 40%[4,68], and the primary prevention trial reported a 5-year dis-

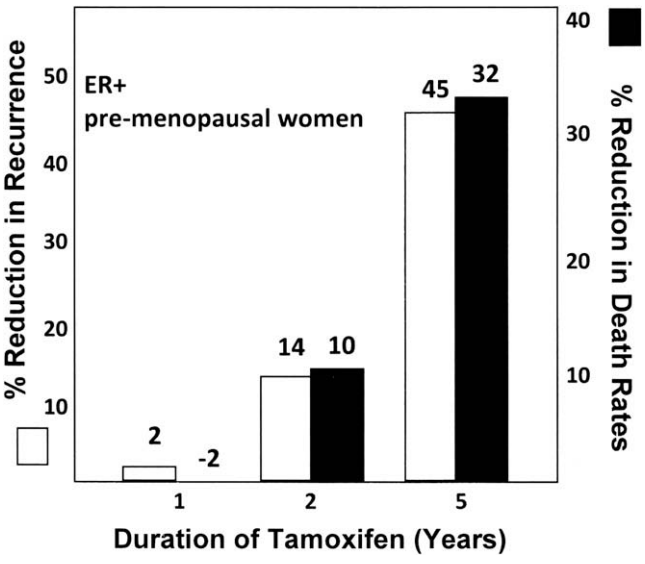

Fig. 3 – The influence of the durations of adjuvant tamoxifen therapy administered to premenopausal patients with oestrogen receptor (ER) positive (+) breast cancer.[15] The enhancement of a reduction of recurrences and a reduction of death rates between women taking only 1 year of adjuvant tamoxifen compared to 5 years serves to illustrate the benefits of the drug, the need for compliance, and the need to ensure that patients are neither poor metabolisers by virtue of aberrations of CYP2D6 or phenocopying by taking SSRIs to reduce menopausal symptoms.

continuance rate of 24%.[69] In clinical practice, discontinuation rates range from 15% to 50%.[70–74] Health-care data-based analyses revealed that as many as half of the patients stop their medication in the course of the 5-year adjuvant treatment with tamoxifen and as many as 15% and 22% of patients stop taking tamoxifen during the first year.[75–77]

The main obstacle to compliance is unacceptable side effects such as severe hot flashes and related menopausal symptoms.[70] However, there is accumulating evidence that hot flashes are an indicator of tamoxifen efficacy and, therefore, the patient's lack of compliance imposes an obstacle to successful treatment. This has recently been suggested by data from the Women's Healthy Eating and Living trial (WHEL)[78] which enrolled primary breast cancer patients ($n = 3088$ between 18 and 70 years of age) between 2 to 48 month after initial diagnosis to either dietary intervention ($n = 1537$) or observation ($n = 1551$) alone. At study entry, among the 864 women taking tamoxifen 78% reported hot flashes, and among those 69% also reported night sweats; only 4% reported night sweats without hot flashes, and 18% did not report either hot flashes or night sweats. Patients reporting hot flashes at baseline were less likely to develop recurrent breast cancer than those who did not report hot flashes (12.9% versus 21%, $P = 0.01$; 127 women had a confirmed breast cancer recurrence after 7.3 years follow-up). Moreover, hot flashes were more predictive of outcome than age, grade, hormone receptor status, or stage II cancer.[78] Goetz et al. showed that the incidence of hot flashes during adjuvant tamoxifen improved therapeutic outcomes and correlated with the CYP2D6 genotype.[57] In their study none of the patients with CYP2D6 *4/*4 genotype (0/13) reported moderate or severe hot flashes compared to 20% (36/177) in the groups of *4/wt and wt/wt patients ($P = 0.064$). Accordingly, hot flashes can be attributed to higher tamoxifen metabolite levels in patients with functional CYP2D6 and drug efficacy. These data which link the occurrence of hot flashes with CYP2D6 genotype and adjuvant tamoxifen outcome, clearly extend previous prospective cohort studies of adjuvant tamoxifen treatment that have already demonstrated that there is a wide inter-individual variability in the formation of tamoxifen metabolites and that steady-state endoxifen plasma concentrations during tamoxifen treatment are substantially reduced in women carrying CYP2D6 genetic variants.[23,29,56] Similar relationships have been reported in studies from Asia[60,61] and Europe.[79] Moreover, at the level of chemoprevention, higher levels of N-desmethyltamoxifen (i.e. endoxifen precursor, Fig. 1) have been reported for carriers of CYP2D6 variants after 1 year of tamoxifen, suggesting that the conversion into the clinically active endoxifen may be impaired.[80] In the light of these genotype–metabolite relationships it is of utmost importance that patients experiencing hot flashes sustain adjuvant tamoxifen despite the discomfort of adverse reactions.

To aid compliance, patients are routinely prescribed selective serotonin re-uptake inhibitors (SSRIs, Fig. 4) that reduce menopausal symptoms.[81–83] This, however, imposes a new challenge because it is known, that some SSRIs have a high affinity for the CYP2D6 enzyme[84,85] and, therefore, SSRIs can inhibit CYP2D6 activity and interfere with tamoxifen efficacy by blocking the conversion of tamoxifen to endoxifen. The relative inhibitory concentrations of SSRIs for the CYP2D6 enzyme product are noted in the legend of Fig. 4. While the effect of SSRIs on the plasma levels of endoxifen had been previously reported by Stearns et al.,[23] this endoxifen lowering effect has been subsequently linked to the patients' CYP2D6 genotype by Jin et al.[29] Plasma concentrations after 4 months of tamoxifen therapy were significantly lower in patients with a CYP2D6 homozygous variant (20 nM; 95% CI: 11.1–28.9 nM) or heterozygous genotype (43.1 nM, 95% CI: 33.3–52.9 nM) than those with homozygous wild type (78.0 nM; 95% CI: 65.1–90.1 nM) (both $P = 0.003$). In this study, 24 of the 78 patients took CYP2D6 inhibitors including paroxetine, fluoxetine, sertraline, citalopram, amiodarone and metoclopramide. Among patients who carried a homozygous wild type genotype, the mean plasma endoxifen concentration for patients using CYP2D6 inhibitors was 58% lower than that of patients not using SSRI co-medication (38.6 nM versus 91.4 nM, $P = 0.0025$), and in patients who were heterozygous

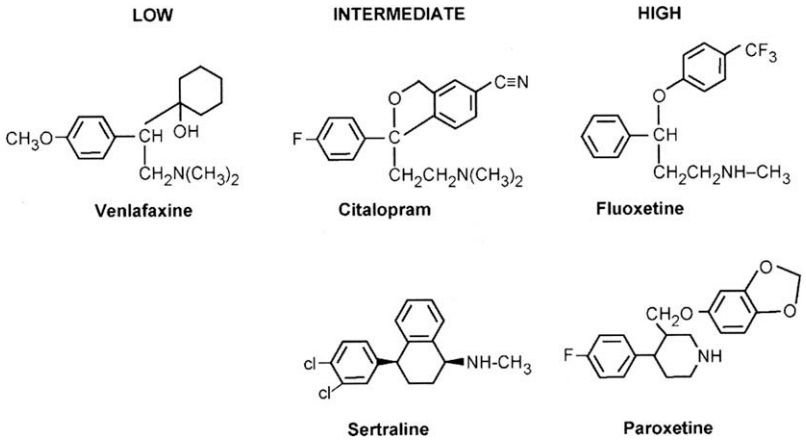

Fig. 4 – The selective serotonin re-uptake inhibitors (SSRIs) used to ameliorate hot flashes and menopausal symptoms during tamoxifen therapy. The SSRIs are CYP2D6 substrates and compete with N-desmethyltamoxifen for the CYP2D6 enzyme. Thy can be classified in high, intermediate, and low binding substrates for the CYP2D6 enzyme. The inhibitor constants for venlafaxine (low), citalopram (intermediate), and sertraline (intermediate), fluoxetine and paroxetine (high) are 33, 7, 1.5, 0.17 and 0.05, respectively.

for a non-functional CYP2D6 allele (wt/vt) this difference was 38% (31.0 nM versus 51.7, $P = 0.08$). Moreover, women taking the weak CYP2D6 inhibitor venlafaxine (a serotonine noradrenaline re-uptake inhibitor (SNRI)) had slightly reduced plasma endoxifen concentrations compared to women taking the potent CYP2D6 inhibitor paroxetine.[29] These findings suggest that both pharmacogenomic effects and pharmacological interactions of drugs at CYP2D6 have an influence on the metabolism of tamoxifen and, therefore, ultimately affect drug efficacy.

The extended investigations of Borges et al.[56] scrutinised the quantitative relationship between CYP2D6 variants, i.e. PM, IM and UM genotype, on endoxifen plasma concentrations in 158 patients at 4 months of 20 mg daily tamoxifen. They found that CYP2D6 genoptypes are highly associated with endoxifen plasma concentrations and account for their variability. While there were no significant differences in mean plasma concentrations of tamoxifen, N-desmethyltamoxifen and 4-hydroxytamoxifen between users and non-users of concomitant CYP2D6 inhibitors, the mean endoxifen plasma concentration was significantly lower in patients taking CYP2D6 inhibitors compared to that in patients who did not (39.6 ± 28.4 nmol/L versus 71.5 ± 41.2 nmol/L; $P < 0.01$).[56] When the authors divided the CYP2D6 inhibitors into potent (paroxetine, fluoxetine, $n = 19$) and weak (SSRI: sertraline and citalopram [$n = 14$] as well as celecoxib, diphenydramine and chlorpheniramine [$n = 13$]), they found a more pronounced decrease in mean endoxifen plasma concentrations with potent inhibitors than with weak inhibitors. Concomitant use of venlafaxine, which is considered the least potent inhibitor, did not show any significant effect. Taking into account CYP2D6 genotypes, the authors observed that the mean plasma endoxifen concentration was significantly lower in CYP2D6 EM patients who were taking potent CYP2D6 inhibitors compared to that in patients who were not (23.5 ± 9.5 nmol/L versus 84.1 ± 39.4 nmol/L, $P < 0.001$).[56] Thus, CYP2D6 genotype and concomitant potent CYP2D6 inhibitors are highly associated with plasma endoxifen concentrations and may substantially impact outcome during tamoxifen treatment by phenocopying effects i.e. converting an EM into a PM phenotype.

The phenocopying effect of SSRI with respect to their interplay with CYP2D6 genotype and effect on clinical outcome was explored by Goetz et al. in their recent follow-up of the NCCTG trial.[58] They investigated the role of CYP2D6 inhibitors in 256 patients that had been randomised to the tamoxifen alone arm. Patients with CYP2D6 wt/wt genotype who did not take CYP2D6 inhibitors were classified as EM ($n = 115$), whereas patients with either one or two *4 alleles or those taking a CYP2D6 inhibitor were classified as IM or PM ($n = 65$), depending on the strength of the inhibitor. Following these assignments, patients with decreased metabolism had shorter time to breast recurrence ($P = 0.015$), relapse-free ($P = 0.007$), disease-free ($P = 0.009$), and overall survival ($P = 0.082$) compared to those with extensive CYP2D6 metabolism.[58] The authors concluded that CYP2D6 metabolism, as measured by genetic variation and enzyme inhibition, is an independent predictor of breast cancer outcome in postmenopausal primary breast cancer patients receiving adjuvant tamoxifen. Accordingly, outcome during tamoxifen treatment may be influenced by its pharmacogenetics as well as co-prescription of drugs interfering with the CYP2D6 mediated tamoxifen metabolism.

3. Conclusion

In summary, we can conclude that endoxifen is formed by the CYP2D6 enzyme[21–23,28,35] and it is therefore anticipated that aberrant genotypes and other medicines that are metabolised by the same enzyme impair the actions of tamoxifen in patients.[29] We addressed the veracity of the hypothesis from the current literature to explore the possibility of targeting tamoxifen to improve women's health. There is now strong evidence that hot flashes are indicators of tamoxifen efficacy and that tamoxifen efficacy and outcome depend on the drug's metabolism which is subject to CYP2D6 genotype and pharmaco-interations. Data from numerous international studies[29,56–62] yielded consistent results in linking active tamoxifen metabolite plasma concentrations with genetically determined CYP2D6 metaboliser status, interference with strong CYP2D6 inhibitors, as well as clinical outcome. Few conflicting data[63–65] may be explained by variations in patient inclusion criteria into respective studies (e.g. variations in tamoxifen doses, length of treatment, additional chemotherapy regimens, lack of consistent ER testing). Importantly, most authors agree that genetic CYP2D6 variants, as well as CYP2D6 inhibition by prescribed co-medications such as antidepressants, may decrease tamoxifen metabolism, and thus negatively impact tamoxifen efficacy and treatment outcome.

There are a number of potential clinical consequences from these emerging data. First of all, strict compliance with tamoxifen treatment is critical for efficacy and outcome and, therefore, deviations from the prescribed course of adjuvant tamoxifen must be avoided even when side effects occur. Second, potent SSRIs such as paroxetine or fluoxetine should not be used for the relief of hot flashes in breast cancer patients receiving tamoxifen. Even though SSRIs are one of the few evidence-based therapy options for menopausal vasomotor symptoms,[86] available data indicate that they may compromise tamoxifen efficacy due to their interference with CYP2D6 dependent tamoxifen metabolism. Yet, this interference depends on the strength of the CYP2D6 inhibitor.[84,85] If treatment of hot flashes is indicated, a SSRI such as citalopram or escitalopram or a SNRI such as venlafaxine should be used because these substances showed no significant inhibition of CYP2D6.[29] Third, the CYP2D6 genotype/phenotype-treatment outcome relationship points to the possible benefit of upfront CYP2D6 genotyping prior to the initiation of endocrine treatment. A comprehensive robust, standardised, and quality controlled CYP2D6 genotyping test will need to analyse all relevant genetic variants that may affect tamoxifen metabolism which should include common PM alleles (*3, *4 and *5) and IM alleles depending on the individual's ethnic origin.[57–62] Of note, *41 is the most frequent IM allele in Europeans, *17 is the principal IM allele in Africans, and *10 dominates in Asians (in addition *9 should also be considered).[41] Other areas of interest with respect to clinical application are the measurement of endoxifen plasma levels as a surrogate of CYP2D6 phenotype and a possible dose increase of

tamoxifen to overcome impaired CYP2D6 metabolism; however, the latter option requires further investigation before definite conclusions can be made.

Given alternative treatment options, i.e. tamoxifen versus aromatase inhibitors (AI), and considering the available scientific and clinical evidence, an individualised approach for endocrine treatment of postmenopausal breast cancer patients is desirable. One might speculate that tamoxifen alone may be adequate for CYP2D6 EM/EM (wt/wt) carriers whereas postmenopausal patients with variant CYP2D6 alleles may fare better with upfront AI therapy. However, currently, a formal recommendation on the integration of CYP2D6 genotypes in treatment decisions must await their validation in statistically powered and/or prospective clinical trials. While these may be under way it will be interesting to see whether the small difference in the outcome benefit of AI as compared to tamoxifen recently reported from landmark trials BIG 1–98[87] and ATAC[17,88] can be attributed to the lack of CYP2D6 genotype stratification. This possibility should be considered particularly in the light of insights from a biomathematical modelling exercise of the estimated benefit of adjuvant tamoxifen according to CYP2D6 gene status. Using the BIG 1–98 information on recurrence probabilities and assuming that AI metabolism was CYP2D6 independent, it has been suggested that the benefit of 5 years of adjuvant tamoxifen may even exceed that of upfront AI treatment in postmenopausal CYP2D6 wt/wt patients.[89] In the meantime, the International Tamoxifen Pharmacogenetics Consortium (http://www.pharmgkb.org/views/project.jsp?pId=63) is making an effort towards pooled analysis of available data to further strengthen our understanding of the relationship between CYP2D6 metabolism status and tamoxifen efficacy.

Finally, the personalised approach in targeting tamoxifen seems feasible and should await timely translation into clinical practise. Indeed, the CYP2D6 genotype might be one of the first predictors of therapeutic response in cancer care. Because this approach is genome-based by utilising CYP2D6 genotyping for the prediction of a patient's metaboliser phenotype, ethical issues need to be sufficiently addressed. In the light of acceptable alternatives, an informed choice about adjuvant endocrine treatment and, most importantly, avoidance of a therapy that might potentially lack efficacy must be prime interests. It will therefore be important to make patients and their care takers aware of these issues and also to initiate discussions with regulatory authorities.

Conflict of interest statement

None declared.

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