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ORIGINAL ARTICLE

Impact of ABCB1 and CYP2D6 polymorphisms on tamoxifen treatment outcomes and adverse events in breast cancer patients

Sona Argalacsova[1,2], Ondrej Slanar[1,3], Hana Bakhouche[1], Lubos Pertuzelka[2]

[1]Institute of Pharmacology, [2]Department of Oncology, [3]Institute of Clinical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University and General Teaching Hospital, Prague, Czech Republic

Summary

Purpose: *This study was designed to evaluate the effect of CYP2D6 and ABCB1 polymorphisms and co-medication on the outcomes and adverse events (AEs) of tamoxifen therapy.*

Methods: *In total, 258 women (187 postmenopausal and 71 premenopausal) with hormone positive breast carcinoma were retrospectively evaluated. CYP2D6 polymorphisms were evaluated with AmpliChip (Roche), and polymorphisms of ATP-binding cassettes B1 (P-glycoprotein) (ABCB1) rs2032582 and rs1045642 with restriction fragment length polymorphisms polymerase chain reaction (RFLP-PCR).*

Results: *CYP2D6 polymorphisms or co-medication affecting CYP2D6 activity demonstrated no statistically significant effect on the efficacy of tamoxifen therapy or AE incidence. There was only a trend towards shortening the time to event (TTE) in CYP2D6-poor metabolisers. ABCB1 polymorphism rs2032582 was not associated with clinical outcomes, while a trend towards an increase in TTE, in variant allele carriers, was noted. The ABCB1 polymorphism rs1045642 demonstrated statistical significance, albeit only in premenopausal patients, i.e. the effect of two variant alleles on the TTE extension was demonstrated only in the premenopausal group (p=0.0012, HR 0.69; 95% CI 0.21-2.31), and statistical significance (p=0.0106) only for gynaecological/vasomotor AEs (p=0.0221, HR=1.0588), with no evidence of any influence on the incidence and onset of venous complications (i.e. deep venous thrombosis or pulmonary embolism).*

Conclusions: *Although no conclusive statistical association between the examined polymorphisms and the outcome or incidence of AEs in tamoxifen therapy was found, the impact of ABCB1 polymorphisms warrants further research. The importance of finding predictive pharmacogenomic biomarkers is a major challenge for individualization and pharmaco-economic rationalization of therapy. The latest international guidelines support this notion.*

Key words: *adverse events, breast cancer, CYP2D6, P-glycoprotein, polymorphism, tamoxifen*

Introduction

Five years of adjuvant therapy with tamoxifen, the gold standard of hormonal therapy for about 40 years, can reduce the annual risk of breast cancer relapse by 39% [1,2]. In 2008, the higher efficacy of aromatase inhibitors (AI) was demonstrated in the adjuvant treatment of postmenopausal women, but the analysis of studies indicating AI benefits over tamoxifen showed clearly that differences in the relapse rate (RR) between tamoxifen and AI were fewer than 5% [3-6]. The question arises, therefore, whether it is possible to identify a group of postmenopausal women, for whom primary tamoxifen therapy has the same efficacy as AI as well as its economic aspects.

The anti-oestrogenic effects of tamoxifen are mostly mediated by its active metabolites of 4-OH-TAM and endoxifen, with 100 times higher affinity to oestrogen receptors (ER) and 30-100 times higher

Correspondence to: Sona Argalacsova, MD. Institute of Pharmacology, 1st Faculty of Medicine, Charles University and General Teaching Hospital, Albertov 4 street, 128 00 Prague 2, Czech Republic.
Tel: + 42 0224968104, E-mail: argi@centrum.cz; ondrej.slanar@lf1.cuni.cz
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ability to stop proliferation [7-9]. Tamoxifen's rate of conversion into its active moieties is mostly dependent on CYP2D6 activity, which can be influenced by genetics as well as iatrogenic factors, i.e. CYP2D6 inhibitors such as SSRI antidepressants [10,11]. The most common variant alleles of *CYP2D6* in European populations is the *CYP2D6*4* allele (allelic frequency 12-21%); in Asian populations it is *CYP2D6*10* (allelic frequency >50%), and *CYP2D6*17* (allelic frequency 20-35%) in Afro-American populations [12-14]. The European population consists of 5-10% poor metabolisers (PM) and 40% intermediate metabolisers (IM) [15-18]. There is evidence that extensive metabolisers (EM) with two fully active CYP2D6 alleles in postmenopausal patients, compared to patients treated with AI, have the same or an even better 5-year disease-free survival (DFS) rate [19]. Other, mostly retrospective, studies suggest that patients with variant alleles (vt) treated with tamoxifen have a higher disease recurrence rate (RR) risk, shorter DFS and worse median overall survival (mOS) [4,10,20-25]. In contrast, there is a roughly comparable number of 'negative studies' that do not demonstrate the benefits of *CYP2D6* polymorphism testing [26-29].

The uncertain impact of the anticipated decrease in CYP2D6's effectiveness in relation to tamoxifen therapy has led to a search for other candidate polymorphisms. P-glycoprotein (P-gp, ABCB1) is a human membrane efflux transporter, responsible for the active transport of drugs and xenobiotics [30]. It has been shown that overexpression of P-gp, e.g. in association with chemotherapy induction, leads to significantly shorter DFS in breast cancer [19,31,32].

In 2012, Teh et al. reported that influence of mutual combination of several polymorphisms on the effect of tamoxifen therapy may be statistically significant even if influence of individual polymorphisms shows no significance. For example, the combination of IM for CYP2D6 and wild-type (wt) homozygotes for *ABCB1* SNP *rs1045642* is associated with significantly shorter relapse-free survival (RFS) [19]. The results of our previously published work in 71 premenopausal patients correlate with Teh's conclusions, in which clear and statistically significant TTE prolongation in the carriers of vt alleles (*T3435T/C*) for *rs1045642*, without any significance attached to the influence of CYP2D6, was found [33].

The role of polymorphisms and their influence on the occurrence of gynaecological and vasomotor AEs associated with tamoxifen therapy are barely mentioned in the literature. These issues also deserve further research, because any possibility of predicting AEs that would lead to the discontinuation of therapy is yet another option for therapy individualisation.

Several authors have attempted to systemise and streamline existing data. One meta-analysis of 12 studies (n=4,973 patients) confirmed a significant reduction in DFS (HR 1.25, p=0.009), but only if specific inclusion criteria were followed in the selection of patients [25]. Another meta-analysis showed a non-zero but still minimal effect of polymorphisms in *CYP2D6* on the efficacy of tamoxifen therapy, with a stronger association of *CYP2D6*10* alleles than *CYP2D6*4*, and also a minor effect of CYP2D6 inhibitors. Their conclusion saw some merit in searching for links between multiple polymorphisms involved in the metabolism and transport of tamoxifen, and it seems that any resolution in the future will reside in the "whole genome approach" [34,35]. With regard to the controversial results, attempts at 'genotype-guided hormonal therapy' have not led to implementation into clinical practice, and the routine testing of *CYP2D6* and other polymorphisms, at present at least, is not recommended anywhere in the world. In 2015, new indications appeared that the results of BIG-98 and ATAC studies were burdened with a large deviation away from the Hardy-Weinberg equilibrium, probably because of an improper *CYP2D6* genotyping methodology for paraffin-embedded tumor samples, which leads to loss of heterozygosity and a false increase in homozygotes [36-38]. This fact is slowly being reflected in the latest guidelines of professional societies (ITCP and ASCO), which, contrary to previous negative attitudes on the benefits of *CYP2D6* genotyping, now cautiously admit its possible benefits in practice and call for further development of an accurate algorithm [39-41]. The positive results of our previous premenopausal study lead us to confirm our results in a complex sample of premenopausal and postmenopausal women and also demonstrate the clinical validity of the studied polymorphisms in terms of most common AEs, with the intention of maximising their predictive value in routine clinical practice.

Methods

This project was approved by the Ethics Committee of the General Teaching Hospital in Prague. All patients entering the research were briefed thoroughly about the purpose of the project and expressed their free will to participate in the study by signing an informed consent form.

Clinical characteristics of patients and follow-up

The study included patients regardless of menopausal status and with sufficient immunohistochemical oestrogen receptor (ER) positivity (ER at least 10%),

with a primarily localised or a locoregionally advanced stage of the disease, who were treated at the Oncology Department, 1st Medical Faculty of Charles University, in Prague between 1985 and 2011. Another inclusion criterion for patient selection was the initiation of hormone treatment with tamoxifen. A patient database was created and the following information was recorded during the research: demographic data, clinical data, histological and immunohistochemical tumor parameters, the extent of disease and therapy courses and the outcomes of tamoxifen therapy, as well as data on co-medication that could iatrogenically alter CYP2D6 activity (mostly used inhibitors: fluoxetine, paroxetine, citalopram and escitalopram; mostly used inducers: metoprolol and atenolol). The therapy was not influenced by the result of polymorphism testing.

Sample preparation and genotyping

DNA was isolated from the blood samples (K_2EDTA) using standardised method based on the QIAamp DNA Blood Mini Kit (Qiagen Ltd., Hilden, Germany). In the subsequent detection of *CYP2D6* polymorphisms, the AmpliChip (Roche Molecular Diagnostics, Alameda, USA) microchip was used. The procedure detected the alleles *CYP2D6*3, *4, *5, *6, *7, *8, *9, *10, *11, *14, *15, *17, *19, *202, *25, *26, *29, *30, *31, *35, *36, *40* and **41* as well as gene duplication or multiplication. If the listed alleles were excluded, the case was determined as a 'wt' allele *CYP2D6*1*. Genotyping of the monitored *ABCB1* polymorphisms (*rs2032582* and *rs1045642*) was carried out through the previously described and validated RFLP-PCR method [33,42].

Statistics

The primary analysis aimed to determine the TTE (time period from the start of tamoxifen up to any relapse or disease progression, or until the appearance AEs). The data of patients without disease relapse were censored on the date of the last visit in the monitored period with lasting total remission of disease. The distribution of alleles was compared with the Hardy-Weinberg equilibrium. The dependence of categorical data was studied using Pearson's x^2 test. Statistical significance was tested using the nonparametric Kruskal-Wallis test or one-way ANOVA. The primary analysis of TTE was performed using the Mantel-Cox test. The probability of an 'event' was evaluated and illustrated using the Kaplan-Meier method with log-rank test, and the subsequent multivariate testing of predictive power of the monitored parameters was carried out using the usual Cox proportional regression analysis. The statistical significance threshold was set at $p<0.05$.

Results

Of the 258 evaluated patients 187 were postmenopausal and 71 premenopausal. Basic demographic, therapeutic and histological data are presented in Table 1. The disease relapsed in 11 of the 71 premenopausal patients (15.5%), with an average TTE of 56.5 months. In the postmeno-

Table 1. Demographic, histological and clinical characteristics of patients

Characteristics	*Premenopausal n=71*	*Postmenopausal n=187*
Age[1], years	44 (26-52)	57 (43-80)
Follow-up length[2]	56 (8-198)	91 (6-244)
Length of TMX therapy[3], months	43 (8-84)	60 (6-121)
T stage, n (%)		
T1	43 (60.6)	115 (61.5)
T2	19 (26.8)	39 (20.8)
T3	3 (4.2)	6 (3.2)
T4	1 (1.4)	16 (8.6)
Tx	2 (2.8)	2 (1)
Tis	3 (4.2)	9 (4.9)
N stage, n (%)		
N0-mic	45 (63.4)	133 (71.1)
N1	21 (29.6)	39 (29.6)
N2-3	2 (2.8)	6 (3.3)
Nx	3 (4.2)	9 (5.0)
Histology, n (%)		
IDC	52 (73.2)	139 (74.3)
ILC	10 (14.1)	28 (15.0)
Ductolobular mixed (ILC+IDC)	4 (5.6)	13 (6.9)
Other histological types	5 (7.4)	7 (3.8)
Grade, n (%)		
G1	15 (21.1)	23 (12.3)
G2	24 (33.8)	67 (35.8)
G3	11 (15.5)	35 (18.8)
Gx	21 (29.6)	62 (33.1)
PR status		
PR positive (> 10%), n (%)	62 (87.3)	126 (67.4)
PR negative	7 (9.9)	40 (21.4)
PR unknown	2 (2.8)	21 (11.2)
HER-2 status, n (%)		
Negative	46 (64.8)	107 (57.2)
FISH positive	6 (8.5)	31 (16.6)
Unknown	19 (26.7)	49 (26.2)
Chemotherapy, n (%)		
Adjuvant	27 (38.0)	52 (27.8)
Neoadjuvant	9 (12.7)	18 (9.6)
Combined- neoadjuvant and adjuvant	4 (5.6)	4 (2.1)
Without chemotherapy	31 (43.7)	113 (60.5)
Adjuvant radiotherapy, n (%)		
Yes	48 (67.6)	92 (49.2)
No	20 (28.2)	95 (50.8)
Unknown	3 (4.2)	0
Surgery, n (%)		
Mastectomy	29 (40.8)	109 (58.3)
Segmentectomy/ Tumorectomy/ Lumpectomy	42 (59.2)	78 (41.7)

[1]Mean age at diagnosis±SD (median);
[2]Mean length of follow-up±SD (median);
[3]Mean length of tamoxifen therapy±SD (median)
SD: standard deviation

pausal cohort, 53 (31%) patients failed tamoxifen therapy, with an average TTE of 65.9 months (p=0.03).

The frequencies of studied polymorphisms and co-medication usage in all subsets is shown in Table 2. Co-medication was not significantly associated with the outcome of tamoxifen therapy. There was only a trend towards TTE shortening by co-medication of CYP2D6 inhibitors. *CYP2D6* polymorphisms did not show any statistically significant effect on the results of tamoxifen therapy (premenopausal: p=0.1955, x^2=4.6959; postmenopausal: p=0.2854, x^2=2.5076), whilst Cox multivariate analysis only showed a trend towards statistical significance of PM polymorphism (p=0.0899, 95% CI=0.08875-5.5506). In the *ABCB1* polymorphism *rs2032582*, there was an assumption that the presence of a variant allele would lead to decreased P-gp function and consequent lower conversion and elimination of tamoxifen and its active metabolites, with prolonged exposure and higher efficacy of tamoxifen therapy as a result. In the premenopausal cohort, we found a trend toward a significant effect on TTE (p=0.1006), while the subset of postmenopausal women showed a clear statistical association of *rs2032585* and TTE (p=0.0357) (Figure 1). The results were consistent with our assumption – a statistically significant prolongation of TTE for GT and TT carriers (p=0.0187, x^2=13.5544) – albeit only for T2 and higher stages. In Cox multivariate analysis for the entire cohort and the two subsets, we tested the predictive power of all the observed genetic covariates and co-medication, and found no statistically significant or important correlation in premenopausal and in the whole subset, while the postmenopausal cohort showed a significant shortening of TTE only in the minor group of AA carriers in *rs2032585* (p=0.0130, 95% Cl=1.8249-149.2781), a trend toward significant prolongation of TTE in the most common vt-homozygotes TT and heterozygous GA (p=0.0891, 95% CI=0.1049-1167), and shorter TTE in users of CYP2D6 inhibitors with no statistical significance (p=0.1802, 95% CI=0.7916-3.5277) (Figure 2). In contrast to these results, no significant association was confirmed in the postmenopausal or the whole subset (p=0.6591 and p=0.1882, respectively).

AEs were noted in 81 (31%) patients: 56 patients had gynaecological AEs (endometrial hyperplasia or cancer and/or vasomotor hot flushes); 24 patients had venous complications (deep venous thrombosis or pulmonary embolism) and one patient had both types of complications. An overview of the demographics and distribution of individual AEs is shown in Table 3. The average time taken to develop gynaecological AEs (TTE) was 21 (range 6-78) months and venous complica-

Table 2. Frequency of the *CYP2D6* and *ABCB1* polymorphisms and co-medication usage in patient groups

		Postmenopausal		*Premenopausal*		*All patients*	
		No progression/ relapse n (%)	*Disease progression/ relapse n (%)*	*No progression/ relapse n (%)*	*Disease progression/ relapse n (%)*	*No progression/ relapse n (%)*	*Disease progression/ relapse n (%)*
CYP2D6[1]	UM	4 (2)	0	1 (1)	0	5 (2)	0
	EM	89 (48)	27 (51)	36 (48)	4 (36)	125 (48)	31 (48)
	IM	80 (42)	22 (41)	32 (42)	6 (55)	112 (43)	28 (43)
	PM	14 (8)	4 (8)	7 (9)	1 (9)	21 (9)	5 (9)
rs2032582[2]	GG (wt)	46 (26)	13 (24)	27 (36)	6 (55)	73 (29)	13 (23)
	GT	91 (53)	30 (57)	35 (46)	5 (45)	126 (50)	35 (60)
	GA	3 (2)	2 (4)	2 (3)	0	5 (2)	2 (3)
	TT	29 (16)	5 (9)	12 (16)	0	41 (17)	5 (9)
	AA, TA (VT)	5 (3)	3 (6)	0	0	5 (2)	3 (6)
rs1045642[3]	C3435C (WT)	55 (31)	14 (26)	18 (24)	5 (45)	73 (29)	19 (30)
	C3435T	83 (48)	26 (49)	44 (58)	5 (45)	127 (51)	31 (48)
	T3435T (VT)	37 (21)	13 (25)	14 (18)	1 (10)	51 (20)	14 (22)
Co-medication usage	inducer	25 (13)	10 (19)	5 (7)	1 (9)		
	inhibitor	27 (14)	9 (17)	12 (17)	3 (27)		

[1]Polymorphisms CYP2D6
UM: Ultra-rapid metabolisers, EM: extensive metabolisers, IM: intermediate metabolisers, PM: poor metabolisers,
[2]Polymorphism of ABCB1 (P-glycoprotein) SNP rs2032582
WT: wild-type homozygous (GG), Variant heterozygous (GT, GA, TA), VT: Variant heterozygous (major TT, minor AA).
[3]Polymorphism of ABCB1 (P-glycoprotein) SNP rs1045642
WT: wild-type homozygous (C3435C), Variant heterozygous (C3435T), VT: Variant heterozygous (T3435T).

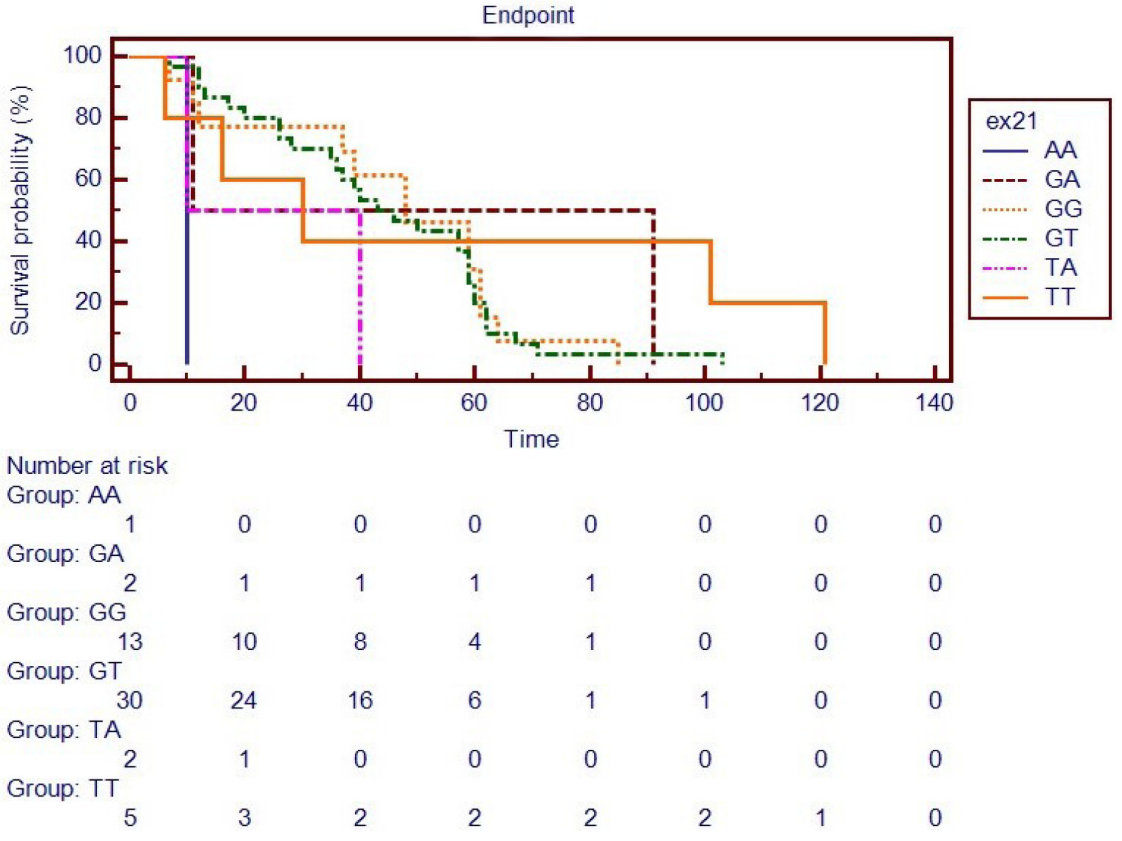

Figure 1. Kaplan-Meier analysis of TTE (months) in postmenopausal patients in relation to rs2032585 ABCB1 polymorphism (p=0.0357).

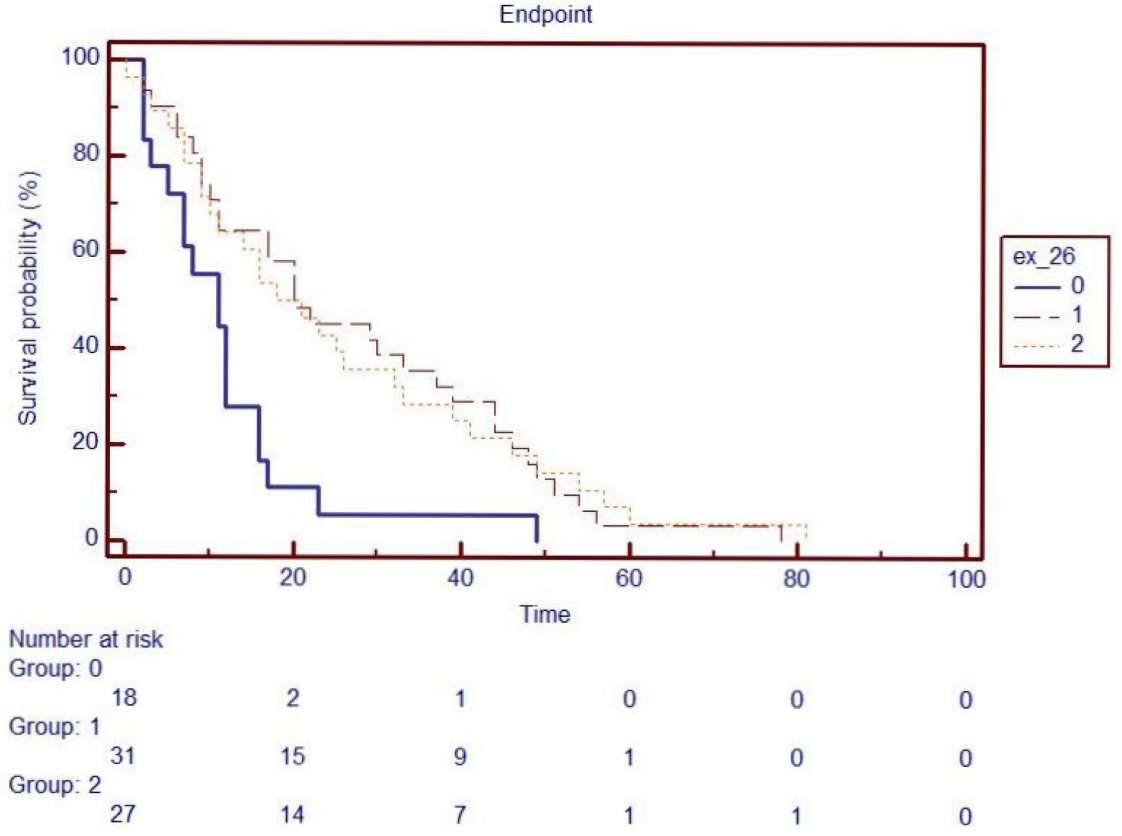

Figure 2. Kaplan-Meier analysis of TTE (months) for non-selected AEs in relation to rs1045642 of ABCB1 polymorphisms (p=0.0106). exon26 = rs1045642 of ABCB1, group 0 = C3435C – wt-homozygote, group 1 = C3435T – heterozygote, group 2 = T3435T – vt-homozygote

Table 3. Demographic parameters and association between analysed polymorphisms or co-medication in patient groups with and without adverse events. Data presented as mean ± SD or n (%)

	All patients	*No AE*	*Gynaecological AE*	*Venous AE*	*Both Gynecological and venous AE*
Age (years)	53±27	53.5±23.5	59±21	65±15	58
Follow up (Months)	125±119	126.5±117.5	107±91	98±58	60
TMX Treatment duration (Months)	46±38	48.5±39.5	42±36	34±26	16
CYP2D6 phenotype					
EM, n(%)	125 (47)	87 (47)	29 (53)	9 (29)	0
TTE AE (Months)			40±38	42±39	
IM, n(%)	112 (42)	76 (41)	22 (40)	13 (61)	1
TTE AE (Months)			26±23	31±29	16
PM, n(%)	23 (9)	19 (10)	3 (5)	1 (5)	0
TTE AE (Months)			25.5±23.5	44	
UM, n(%)	5 (2)	2 (1)	2 (2)	1 (5)	0
TTE AE (Months)			8±3	46	
rs2032582					
wt/wt, n(%)	73 (29)	45 (26)	20 (38)	8 (33.3)	0
TTE AE (Months)			26.5±25.5	29.5±21.5	
wt/v, n(%)	136 (54)	107 (62)	21 (40)	8 (33.3)	0
TTE AE (Months)			40.5±37.5	24±22	
v/v, n(%)	41 (17)	20 (12)	12 (22)	8 (33.3)	1
TTE AE (Months)			32±25	42±39	16
rs1045642					
Wt/wt, n(%)	73 (29)	55 (32)	13 (26)	5 (21)	0
TTE AE (Months)			25±24	15.5±7.5	
wt/v, n(%)	127 (51)	96 (55)	21 (43)	10 (42)	0
TTE AE (Months)			40±38	26.5±24.5	
v/v, n(%)	51 (20)	22 (13)	18 (31)	9 (37)	1
TTE AE (Months)			32±25	41.5±39.5	16
Co-medication					
Without Co-medication, n(%)	226 (87)	160 (89)	42 (76)	21 (88)	1
Inhibitor users, n(%)	24 (9)	17 (11)	7 (12)	0	0
TTE AE (Months)			26±15		
Inducer users, n(%)	10 (4)	0	7 (12)	3 (12)	0
TTE AE (Months)			13±10	22.5±10.5	

UM – ultrarapid metabolizers
EM- extensive metabolizers
IM- intermediate metabolizers
PM- poor metabolizers
TTE AE – Mean time to appearance of adverse event

tion 25 (range 8-60) months. The initial regression analysis confirmed the independence of AEs from the expression of ER in the tumour (p=0.782, F-ratio=0.07724) and age at diagnosis (p=0.102, F-ratio=2.7303). A summary of individual gene polymorphisms for the entire reference group is provided in Table 4. Our analysis didn't confirm any of the above assumptions, and there was no statistically significant effect of CYP2D6 polymorphisms or co-medication on the incidence of AEs during tamoxifen therapy. The initial statistical evaluation of the association between individual polymorphisms and co-medication, and the time to appearance of AEs (TTE) only demonstrated a statistically significant reduction of TTE among the wt carriers of *ABCB1 rs1045642* (x^2=9.0960, p=0.0106), as illustrated in Figure 2. Furthermore, there was noticeable statistical trend toward a significant effect of *ABCB1* polymorphism *rs2032582* and co-medication on the development of AEs (p=0.1300, x^2=4.0804 and p=0.1714, x^2=3.5272, respectively). Cox multivariate analysis of the influence of all studied *ABCB1* polymorphisms on the development of AEs demonstrated again a statistically significant reduction in TTE in carriers of the *ABCB1 rs1045642* (C3435C) wt allele (p=0.0135, 95% CI 1.1906-4.4054) and a statistically significant influence of the variant polymorphism

Table 4. Association between analysed polymorphisms or co-medication and adverse events

	All patients	*Without AE*	*Gynaecol AE*	*Venous AE*	*Both AE*
CYP2D6					
EM, n (%)	125 (47)	87 (47)	29 (53)	9 (29)	0
Average AE appearance, mos (range)			23 (2-78)	24 (3-81)	0
IM, n (%)	112 (42)	76 (41)	22 (40)	13 (61)	1
Average AE appearance, mos (range)			18 (3-49)	25 (2-60)	16
PM, n (%)	23 (9)	19 (10)	3 (5)	1 (5)	0
Average AE appearance, mos (range)			18 (2-49)	44	0
UM, n (%)	5 (2)	2 (1)	2 (2)	1 (5)	0
Average AE appearance, mos (range)			8 (5-11)	46	0
ABCB1 rs2032582					
Wt-homozygous (GG), n (%)	73 (29)	45 (26)	20 (38)	8 (33.3)	0
Average AE appearance, mos (range)			15 (1-52)	25 (8-51)	0
Heterozygous (GT/A), n (%)	136 (54)	107 (62)	21 (40)	8 (33.3)	0
Average AE appearance, mos (range)			24 (3-78)	21 (2-46)	0
vt-homozygous (TT/AA), n (%)	41 (17)	20 (12)	12 (22)	8 (33.3)	1
Average AE appearance, mos (range)			28 (7-57)	32 (3-81)	16
ABCB1 rs1045642					
C3435C, n (%)	73 (29)	55 (32)	13 (26)	5 (21)	0
Average AE appearance, mos (range)			11 (1-49)	14 (8-23)	0
C3435T, n (%)	127 (51)	96 (55)	21 (43)	10 (42)	0
Average AE appearance, mos (range)			26 (2-78)	28 (2-51)	0
T3435T, n (%)	51 (20)	22 (13)	18 (31)	9 (37)	1
Average AE appearance, mos (range)			24 (7-57)	32 (2-81)	16
Co-medication	51 (20)				
Without	226 (87)	160 (89)	42 (76)	21 (88)	1
with, n (%)					0
Inhibitors	24 (9)	17 (11)	7 (12)	0	0
Average AE appearance, mos (range)			23 (11-41)	0	0
Inducers, n (%)	10 (4)	0	7 (12)	3 (12)	0
Average AE appearance, mos (range)			9 (3-23)	23 (12-33)	0

UM – ultrarapid metabolizers
EM- extensive metabolizers
IM- intermediate metabolizers
PM- poor metabolizers

of *rs2032582 TT*. A more detailed analysis of the impact of *ABCB1 rs1045642* polymorphism on the development of individual types of AEs only confirmed a statistical significance in gynaecological AEs (p=0.0221, HR=1.0588), with no proven effect on the risk for venous complications.

Discussion

The advantages of this study primarily include the fact that the identification of polymorphisms was carried out in blood samples, so there is no distortion of genotype distribution due to loss of heterozygosity, as seen in many patient groups in earlier studies [37,38]. This is also indicated by the relatively balanced distribution of demographics and the representation of individual polymorphisms in the two subgroups, which correlates with a similar proportion in other publications, and even with respect to the determination of *ABCB1* polymorphisms, it is a rather large cohort [4,10,15-29,32]. The cohort of postmenopausal patients was 2.5-fold larger, because the discussion on the benefit of 'genotype-guided tamoxifen therapy' is currently ongoing in postmenopausal women. Another advantage is the systematic analysis of AEs during treatment with regard to all studied polymorphisms. A definite disadvantage is the retrospective, monocentric nature of the study, though the same can be stated for the majority of published studies on this topic. Another disadvantage is the inclusion of hot flushes as a vasomotor AE in the group of gynaecological AEs. We considered hot flushes as an effect associated with hormonal changes, and the main intention was to distinguish venous AEs from the other examples.

In our follow-up, *CYP2D6* polymorphisms and relevant co-medication did not show any statistically significant effect on AE appearance during tamoxifen therapy. Cox multivariate analyses repeatedly demonstrated only a tendency of poor metabolizers (PM) for TTE shortening, and even this was usually in conjunction with other *ABCB1* polymorphisms. Thus, our study supports the results of a series of 'negative studies' or the conclusions of meta-analyses that show a non-zero but very minor effect in CYP2D6 PM phenotype as well as a very minor effect of CYP2D6 inhibitors used with tamoxifen [26-29,34]. Statistically significant effects of variant alleles of *ABCB1* polymorphism *rs1045642* on the prolongation of TTE were only demonstrated in the premenopausal group in our previous publication [33], but they were not confirmed in the postmenopausal or the whole cohort herein. The question as to why the polymorphism is only significant in premenopausal patients remains open. The first consideration was the stimulation of P-gp via adjuvant chemotherapy, but our results did not demonstrate separate significance for stages T1 and T2, and, moreover, where one might expect adjuvant chemotherapy prior to hormonal therapy in T2 and higher stages, especially in premenopausal patients; therefore, this reasoning certainly does not explain our results. The analysis of the whole cohort didn't show certain tendency of polymorphisms toward statistical significance (p=0.1882). The study published by Teh et al. confirms the reduction in DFS in wt (*rs1045642*) and a further shortening in combination with the PM phenotype of CYP2D6 (from 48 to 12 months) [19]. We suggest, however, that statistical significance in the premenopausal cohort was caused rather by chance due to the low number of patients, though there could be a partial impact of this polymorphism on the effectiveness of tamoxifen therapy. The same can be considered for the *ABCB1* polymorphism *rs2032582* results, where, on the contrary, we only found a trend toward a significant effect on TTE in the postmenopausal cohort. However, the specific analysis of particular stages only found significance in T2 and higher stages, while Cox regression analysis only confirmed the statistical significance in minor AA polymorphism (p=0.0130), thereby demonstrating no clear trend toward significant prolongation of TTE in the most common variant homozygotes and shorter TTE in CYP2D6 inhibitors' co-medication (p=0.1802). Kyiotani et al. found no significant effect of our studied *ABCB1* polymorphisms, though significance was demonstrated for another *rs3740065 ABCC2* polymorphism [10]. A certain, albeit unclear, tendency toward statistical significance of multiple examined polymorphisms indicates the greater role of a 'complex genotype' with several polymorphisms included rather than the effect of one polymorphism, which supports the findings of the studies published by Kyiotani et al. and The et al. [10,19].

There were 81 patients (33%) with non-selected AEs, 23% gynaecological-vasomotor AEs and 10% venous complications. Our analysis did not show a statistically significant effect of *CYP2D6* polymorphisms or co-medication on the development of AEs, thereby confirming the findings of previously published studies [43-45]. Evaluation of the association of individual polymorphisms and co-medication, and the time to the development of AEs, demonstrated a statistically significant relationship but only in *ABCB1* polymorphism *rs1045642* (p=0.0106), and only for gynaecological and vasomotor AEs (p=0.0221, HR=1.0588). These results support the conclusion of Teh et al., who demonstrated a reduction in the expression of P-gp in variant homozygous patients (TT), with an expected reduction in the elimination of tamoxifen and its active metabolites, thereby prolonging its effect [19]. The reason for statistical significance solely in gynaecological and vasomotor AEs can only be speculated, so further studies are needed to delineate whether it is caused by the differential expression of P-gp in the endometrium and the venous wall, or whether this disparity is a result of a deviation in one of the studied AE groups.

In conclusion, our results support the 'negative studies', as we found no conclusive statistical dependence between the examined polymorphisms and the outcome and incidence of AEs in tamoxifen therapy. However, the implied statistical trends and associations in ABCB1 polymorphism *rs1045642* may lead to the conclusion that 'genotype-guided tamoxifen therapy' is not just a 'dead end', as it seemed in recent years, but could be a real sign of new predictors and an increase in the effectiveness of hormone therapy for breast cancer, as confirmed by recent statements made by professional societies [39-41,43].

Abbreviations

4-OH-TAM - 4-hydroxy-tamoxifen, ABCs – ATP-binding cassettes, ABCB1 – ATP-binding cassettes B1 (P-glycoprotein), AEs – Adverse events, AI – Inhibitors of Aromatase, ASCO – The Committee of the American Society of Clinical Oncology, DFS – Disease free survival, EM – Extensive metabolisers: patients with two fully functional (wild-type) alleles, ER – Oestrogen receptor / Oestrogen Receptor, IM – Intermediate metabolisers: patients with one deficient and one wild type allele, ITPC – International Tamoxifen Pharmacogenetics Consortium, OS – Overall survival, p – P-value, P-gp – P-glycoprotein:

a human membrane efflux transporter belonging to the family of ABCs, PM – Poor metabolisers: patients with two deficient (variant/) alleles, PR – Progesterone receptor, RFLP-PCR – Restriction fragment length polymorphisms Polymerase Chain reaction, RFS – Relapse free survival, RR – Recurrence Rate, SNPs – Single-nucleotide polymorphisms, SSRIs – Selective serotonin reuptake inhibitors, antidepressant (e.g. paroxetiin, fluoxetine), T – Tumour size, TTE – Time to Event: a) Time from when tamoxifen therapy starts until relapse or progression of the disease. b) Time to the appearance of adverse events, UM – Ultra-rapid metabolisers: patients with more than two functional alleles, wt – Wild-type allele – fully functional allele, wt-homozygous – consists of two fully function alleles, vt – variant-type-homozygous: consists of two variant (deficient) alleles.

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Conflict of interests

The authors declare no confict of interests.

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