Original Study

A New Genetic Risk Score to Predict the Outcome of Locally Advanced or Metastatic Breast Cancer Patients Treated With First-Line Exemestane: Results From a Prospective Study

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Abstract

Currently there are no reliable biomarkers to predict outcome of exemestane treatment. We designed a prospective study to investigate whether constitutive genetic background might affect response to therapy. In a population of 302 advanced breast cancer patients treated with exemestane we showed that a 5–polymorphism-based genetic score could be used to identify patients with different risks of progression and death.

Introduction: Approximately 50% of locally advanced or metastatic breast cancer (MBC) patients treated with first-line exemestane do not show objective response and currently there are no reliable biomarkers to predict the outcome of patients using this therapy. The constitutive genetic background might be responsible for differences in

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the outcome of exemestane-treated patients. We designed a prospective study to investigate the role of germ line polymorphisms as biomarkers of survival. **Patients and Methods:** Three hundred two locally advanced or MBC patients treated with first-line exemestane were genotyped for 74 germ line polymorphisms in 39 candidate genes involved in drug activity, hormone balance, DNA replication and repair, and cell signaling pathways. Associations with progression-free survival (PFS) and overall survival (OS) were tested with multivariate Cox regression. Bootstrap resampling was used as an internal assessment of results reproducibility. **Results:** Cytochrome P450 19A1-rs10046TC/CC, solute carrier organic anion transporter 1B1-rs4149056TT, adenosine triphosphate binding cassette subfamily G member 2-rs2046134GG, fibroblast growth factor receptor−4-rs351855TT, and X-ray repair cross complementing 3-rs861539TT were significantly associated with PFS and then combined into a risk score (0-1, 2, 3, or 4-6 risk points). Patients with the highest risk score (4-6 risk points) compared with ones with the lowest score (0-1 risk points) had a median PFS of 10 months versus 26.3 months (adjusted hazard ratio [AdjHR], 3.12 [95% confidence interval (CI), 2.18-4.48]; $P < .001$) and a median OS of 38.9 months versus 63.0 months (AdjHR, 2.41 [95% CI, 1.22-4.79], $P = .012$), respectively. **Conclusion:** In this study we defined a score including 5 polymorphisms to stratify patients for PFS and OS. This score, if validated, might be translated to personalize locally advanced or MBC patient treatment and management.

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Keywords: Advanced breast cancer, Aromatase inhibitor, Hormone therapy, Polymorphisms, Survival

Introduction

Despite that personalized medicine has assumed a crucial role in recent years, pharmacogenetic investigations, aiming at identifying predictive/prognostic biomarkers, usually represent only secondary aims in clinical trials. In this scenario, setting up prospective studies, designed ad hoc to investigate the effect of genetic variants in candidate genes and pathways, could be an effective strategy to produce reliable and clinically useful results.

Exemestane is a steroidal aromatase inhibitor (AI) for the treatment of postmenopausal patients affected by hormone receptor-positive (HR^+) breast cancer (BC). It is registered for the adjuvant and the advanced settings for the treatment of locally advanced or metastatic BC (MBC).[1] Exemestane has shown efficacy in several clinical trials,[2-4] but almost the 50% of patients, after an initial benefit, experience disease progression.[4-6] Unfortunately, there are still no biomarkers to predict the outcome of exemestane-treated patients.[7-9]

A great effort has been done in the past years to identify germ line variants as predictive and/or prognostic biomarkers in patients treated with AIs, including exemestane.[10-13] Polymorphisms in genes affecting either the activity or the expression of the drug target (aromatase, cytochrome P450 19A1 [*CYP19A1*]), or influencing drug/hormone activity (*CYP17A1*,[14,15] estrogen receptor 1 [*ESR1*],[10,16] *ESR2*,[10] PR/SET domain 2 [*PRDM2*][17]), metabolism (Catechol-O-methyltransferase [*COMT*],[14,15] *CYP1B1*,[15] uridine diphosphate [UDP] glucuronosyltransferase family 1 member A1 [*UGT1A1*],[18] *CYP3A4*,[19,20] *CYP3A5*[19]), or transport (adenosine triphosphate [ATP] binding cassette [*ABC*],[18] and solute carrier organic anion [*SLCO*] transporters[21]) have been investigated as potential predictive or prognostic biomarkers of efficacy.[22]

In this context, another mechanism of interest is the DNA repair pathway, that is deregulated in BC. The deficiency in DNA repair capacity is considered a hallmark of breast carcinogenesis.[23] A recent report on the outcome of patients treated with AIs has highlighted somatic mutations on genes involved in DNA replication, repair, cell cycle, and tumor protein p53 signaling pathways. These somatic mutations were associated with AI resistance.[24] However, the role of the germ line variants in these pathways has not been investigated.

To assess the clinical value of germ line polymorphisms as potential indicators of exemestane outcome, we performed a prospective multicenter study, specifically designed to investigate the role of germ line DNA variants to predict survival in patients treated with exemestane. This study was carried out on 302 HR^+ locally advanced or MBC patients treated with exemestane as first-line hormonal therapy. Seventy-four polymorphisms in 39 candidate genes involved in drug activity, hormone balance, DNA replication and repair, and cell signaling pathways were investigated to identify biomarkers of progression-free survival (PFS) and overall survival (OS).

Patients and Methods

Patients

This prospective study involved 23 Italian centers. All procedures were approved by the ethics committee of the sponsoring center, National Cancer Institute Centro di Riferimento Oncologico di Aviano (protocol number 1003/D; November 11, 2005), and investigations were performed in accordance with Declaration of Helsinki. HR^+ locally advanced or MBC postmenopausal patients treated with exemestane were enrolled from 2007 to 2012. Patients signed a written informed consent for the purpose of this research.

Eligibility criteria included: blood sample availability, measurable and nontarget lesions defined according to Response Evaluation Criteria In Solid Tumors version 1 (RECIST) criteria, Eastern Cooperative Oncology Group performance status 0 to 2, absolute neutrophil count $\geq$1500/μL, platelets $\geq$100,000/μL, hemoglobin $\geq$9.0 g/dL, and HER2-negative. Exclusion criteria were: previous exemestane treatment or other hormonal therapy in the advanced/metastatic setting, brain metastasis, serious infectious disease, serious functional alteration of visceral and metabolic

Table 1 Patient Clinical and Demographic Characteristics

Patient and Tumor Characteristic	Value	PFS HR, *P*	OS HR, *P*
Total, n	302		
Age, Years			
Median (range)	71 (35-93)		
<60, n (%)	58 (19.2)	Ref	Ref
61-70, n (%)	88 (29.1)	NS	NS
71-80, n (%)	108 (35.8)	0.69, .051	NS
>80, n (%)	48 (15.9)	**0.44, .001**	NS
Sex, n (%)			
Female	301 (99.6)		
Male	1 (0.4)		
Stage at Diagnosis, n (%)			
I-II	130 (43.0)	Ref	Ref
III-IV	170 (56.3)	**1.38, .015**	**2.04, .001**
Unknown	2 (0.7)		
Dominant Metastatic Site, n (%)			
Visceral	183 (60.6)		
Bone[a]	102 (33.8)[a]		
Soft tissue	17 (5.6)		
Liver Involvement			
No	257 (85.1)	Ref	Ref
Yes	45 (14.9)	**2.49, .001**	**1.83, .008**
Metastatic Sites at Recruitment, n (%)			
1	91 (30.1)		
2-3	157 (52.0)		
4-5	54 (17.9)		
ER/PgR Status			
ER^+/PgR^+	257 (85.1)		
ER^+/PgR^-	44 (14.6)		
ER^-/PgR^+	1 (0.3)		
ER Expression, n (%)			
0-50%	100 (33.1)		
51-75%	49 (16.2)		
76-100%	153 (50.7)		
PgR Expression, n (%)			
0-10%	87 (28.8)	Ref	Ref
11-100%	215 (71.2)	**0.58, .001**	**0.41, .001**
Surgery, n (%)			
No	60 (19.9)	Ref	Ref
Yes	242 (80.1)	NS	**0.44, .001**
Previous Chemotherapy, n (%)			
No	138 (45.7)	Ref	Ref
Yes	164 (54.3)	**1.56, .001**	NS
Neo- or adjuvant	124 (41.1)		
First-line[b]	40 (13.2)[b]		
Previous Hormone Therapy, n (%)			
No	140 (46.4)	Ref	Ref
Adjuvant tamoxifen	98 (32.5)	NS	NS
Adjuvant AI[c]	64 (21.1)[c]	**1.49, .018**	NS
PFS			
Progression, n (%)	238 (78.8)		
Median PFS (range)	15.4 (0.8-115.6)		
OS			
Deaths, n (%)	141 (46.7)		
Median OS (range)	26.8 (1.5-152.6)		

Significant results are shown in bold text.
Abbreviations: AI = aromatase inhibitor; ER = estrogen receptor; HR = hazard ratio; NS = not significant; OS = overall survival; PFS = progression-free survival; PgR = progesterone receptor; Ref = reference category.
[a]Ten of 302 patients had bone and soft tissue lesions.
[b]Seventeen patients also underwent neo- or adjuvant therapy.
[c]Thirty-one patients have also taken tamoxifen.

disease, radiotherapy or major surgery within 4 weeks from start of exemestane treatment, previous or concomitant neoplasm (excluding in situ cervical cancer), and inability to attend periodical clinical and/or radiological evaluations.

Clinical data were collected on case report forms. Details on primitive tumor, previous treatments, exemestane therapy, and follow-up information are reported in Table 1. Data were reviewed by an internal board and stored in a database. Hormone receptor status was assessed according to the 2010 American Society of Clinical Oncology guidelines.

Data Statement

The data set generated and analyzed during the current study is not publicly available because the biological material and clinical data were collected from patients only for the purposes of this study. However, the data set is available from the corresponding author upon reasonable request.

Efficacy Evaluation

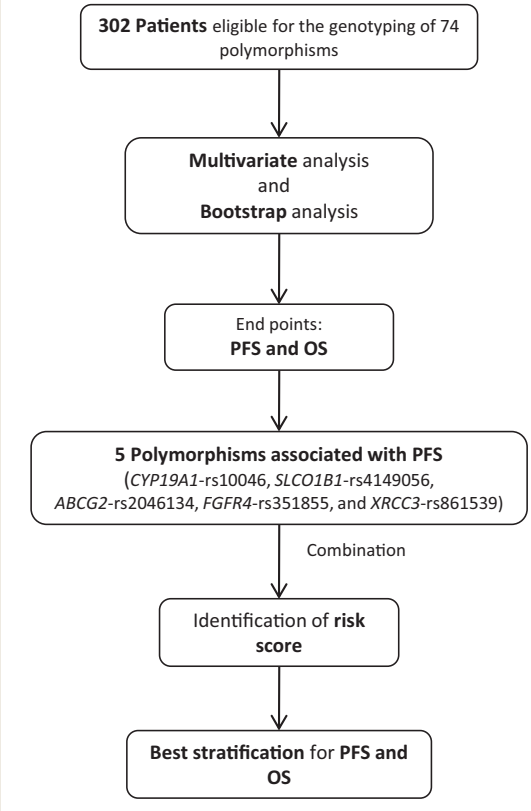
Patients were treated with 25 mg of daily oral exemestane. Efficacy evaluation occurred every 8 weeks for at least 24 weeks according to RECIST criteria. Treatment was continued until disease progression, unacceptable toxicity, death, or consent withdrawal.

Progression-free survival was defined as the time between treatment initiation and tumor progression or death from any cause. OS was defined as the time between treatment initiation and death from any cause. Patients lost at follow-up were censored at the time of their last follow-up.

Gene Variants and Genotyping

Genes and polymorphisms of interest were selected upon literature analysis on the basis of association with cancer, BC, and AIs

Figure 1 Study Flow Chart. Three Hundred Two Patients Were Enrolled in the Period From 2007 to 2012. Among Them, at Least 298 Were Genotyped for All of the 74 Polymorphisms Investigated. All of the Polymorphisms Were Tested for Association With Progression-Free Survival (PFS) and Overall Survival (OS). Cox Multivariate Regression and Bootstrap Analyses Highlighted 5 Polymorphisms as Significantly Associated With PFS. None of the Tested Polymorphisms Was Significantly Associated With OS. The Risk Score Obtained From the Combination of These 5 Polymorphisms Was Subsequently Tested for Association With PFS and OS, Showing a Better Stratification Ability Than the Single Polymorphisms for PFS and a Trend for Association With OS



outcome. Moreover, to assess the involvement of selected genes into pathways of interest, each gene was searched in the Gene database of the National Center for Biotechnology Information . Genes and polymorphisms investigated are listed in Supplemental Table 1 in the online version. Linkage disequilibrium between variants was assessed using the Genome Variation Server (http://gvs.gs.washington.edu/GVS150/). A 3-mL blood sample was obtained from each patient before the first administration of exemestane. Genomic DNA was automatically extracted with BioRobot EZ1 kit (Qiagen SPA, Milan, Italy).

Polymorphisms were genotyped with Automated Fragment Analysis on Genetic Analyzer ABI PRISM 3100 (Applied Biosystems, Foster City, CA), TaqMan Assays on a 7500 Real-Time PCR System (Applied Biosystems), PSQ96 MA Pyrosequencing (Biotage AB, Uppsala, Sweden), or a custom-designed Illumina GoldenGate Assay on a BeadXpress Reader (Illumina, San Diego, CA). Analyses were performed according to manufacturer's instructions including negative and positive controls. Twelve polymorphisms analyzed using the GoldenGate Assay were also analyzed using TaqMan (Applied Biosystems) or Pyrosequencing (Biotage AB) as control.

Statistical Analyses

Progression-free survival and OS were assessed using multivariate Cox regression; clinical variables were included in the model if associated with a P value $< .05$ at univariate analysis, with one of the outcomes considered (Table 1). Proportional hazards assumption was assessed using Schoenfeld residuals. All tests of statistical significance were 2-sided and medians were reported with their relative minimum and maximum range or 95% confidence interval (CI). Robust standard errors were calculated to take into account the possible lack of independence between patients from the same hospital.[25] Additive, dominant, and recessive genetic models were evaluated, and the most statistically significant model for each polymorphism was selected. Ninety-five percent CIs and P values were estimated using a bootstrap resampling technique with 1000 replications. A P value cutoff of .05 after bootstrapping was considered to be statistically significant. Unadjusted differences in PFS and OS according to genotypes were assessed using Kaplan–Meier estimates and the statistical significance using the log rank test. Genotypes individually associated with a higher risk of progression or not showing a protective effect were used to generate a genetic risk score. All the analyses were performed using STATA version 13 software (StataCorp LLC, Cary, NC).

Results

Patients and Clinical Outcome

Three hundred two patients with locally advanced or MBC treated with first-line exemestane were enrolled in this prospective pharmacogenetic study (Figure 1). All of the subjects were of Caucasian origin (self-reported). Patient median age was 71 (range, 35-93) years and the median follow-up was 35 (range, 2-153) months. The median treatment duration was 11.7 months (range, 0.7-84.8). Patients, tumor, and treatment characteristics are summarized in Table 1.

Genetic Analyses

Seventy-four polymorphisms in 39 genes involved in drug activity, hormone balance, DNA replication and repair, and cell signaling pathways were identified. The allele frequencies are reported in Supplemental Table 1 in the online version and were consistent with those previously reported (https://www.ncbi.nlm.nih.gov/snp). As a test for genotyping quality control, genotype data were obtained using 2 different techniques in 247 patients for *CYP19A1*-rs4646, *CYP3A5*-rs776746, *COMT*-rs4680, *ESR1*-rs2234693, *ESR2*-rs1256049, *ESR2*-rs4986938, *CYP17A1*-rs743572, *CYP19A1*-rs700519, *CYP19A1*-rs10046, *CYP3A4*-rs2740574, and *PRDM2*-rs2308040 variants. These analyses had a complete concordance rate (100%).

Table 2 Variants Significantly Associated With PFS

Gene	rsID	Variant	Model	AdjHR (95% CI)	*P*	95% CI Bootstrapped Value	*P* Bootstrapped Value
ABCG2	rs2046134	G>A	Dominant	0.62 (0.41-0.93)	.020	0.39-0.97	.038
CYP19A1	rs10046	T>C	Additive	1.15 (1.02-1.29)	.021	1.01-1.31	.038
SLCO1B1	rs4149056	T>C	Dominant	0.54 (0.41-0.71)	.000	0.36-0.80	.002
FGFR4	rs351855	C>T	Recessive	1.85 (1.15-2.99)	.011	1.03-3.34	.039
XRCC3	rs861539	C>T	Recessive	1.72 (1.15-2.57)	.008	1.02-2.90	.043

The results were adjusted for the clinical variables significantly associated with PFS and OS. 95% CI and *P* values were estimated using the bootstrap resampling method by drawing 1000 samples from the original data set.
Abbreviations: AdjHR = adjusted hazard ratio; OS = overall survival; PFS = progression-free survival; rsID = reference single-nucleotide polymorphism identification number.

Progression-Free Survival and OS Analyses

The following clinical variables were associated with either PFS and/or OS and were used as covariates for multivariate analyses: age, stage at diagnosis, progesterone receptor expression, liver involvement, surgery, chemotherapy, and adjuvant hormonal therapy (Table 1).

In multivariate analysis, 5 polymorphisms were significantly associated with PFS: *CYP19A1*-rs10046, solute carrier organic anion transporter 1B1 (*SLCO1B1*)-rs4149056, ATP binding cassette subfamily G member 2 (*ABCG2*)-rs2046134, fibroblast growth factor receptor−4 (*FGFR4*)-rs351855, and X-ray repair cross complementing 3 (*XRCC3*)-rs861539 (Table 2, Figure 2).

In particular, the variant C allele of *CYP19A1*-rs10046, on the aromatase gene, was significantly associated with an increased risk of progression (additive model, adjusted hazard ratio [AdjHR], 1.15; bootstrapped 95% confidence interval [95%CI_{bootsr}, 1.01-1.31; bootstrapped *P* value (p_{bootsr}) = .038). A reduced risk of progression was observed for *SLCO1B1*-rs4149056, and for *ABCG2*-rs2046134, on genes involved in steroid transport (*SLCO1B1*-rs4149056 dominant model, AdjHR = 0.54 [95%CI_{bootsr}, 0.36-0.80], p_{bootsr} = .002; *ABCG2*-rs2046134 dominant model, AdjHR = 0.62 [95%CI_{bootsr}, 0.39-0.97], p_{bootsr} = .038). The homozygous variant TT genotypes of *FGFR4*-rs351855 and of *XRCC3*-rs861539 were associated with a higher risk of progression compared with wild type or heterozygous patients (*FGFR4*-rs351855 recessive model, AdjHR = 1.85 [95%CI_{bootsr}, 1.03-3.34], p_{bootsr} = .039; *XRCC3*-rs861539 recessive model, AdjHR = 1.72 [95%CI_{bootsr}, 1.02-2.90], p_{bootsr} = .043).

To assess the role of the simultaneous presence of different risk alleles, 0, 1, or 2 points were assigned to the 6 genotypes previously described and a risk score was generated grouping the 5 polymorphisms associated with shorter PFS. A point was assigned to each genotype of the 5 polymorphisms according to its risk of progression, as shown in Table 3: additive model: 0 points if patients had 0 risk alleles, 1 point for 1 risk allele, 2 points for 2 risk alleles; dominant models: 0 points if patients had at least 1 protective allele, 1 point for 0 protective alleles; recessive models: 0 points if patients had 0 or 1 risk allele, and 1 point for 2 risk alleles. According to the genotype of each polymorphism, patients had a total score derived according to the sum of the assigned points, ranging from 0 to 6. Patients were then aggregated into 4 risk groups: 0 to 1 risk points (n = 26 patients), 2 risk points (n = 84 patients), 3 risk points (n = 111 patients), and 4 to 6 risk points (n = 75 patients).

Figure 3A and Table 4 show PFS in the 4 groups of patients stratified according to the risk score. As shown, patients in the 4 to 6 risk group performed worse than those in the 3, 2, and 0 to 1 groups (log rank *P* = .0002). Accordingly, the risk of progression increased with the number of risk points: patients in the 4 to 6 risk group and those in the 3 risk group had a significantly greater risk of progression than patients in the 0 to 1 risk group (AdjHR for the 4-6 risk group = 3.12 [95% CI, 2.18-4.48], *P* < .001; AdjHR for the 3 risk group = 2.01 [95% CI, 1.20-3.36], *P* = .008). Even if the difference was not statistically significant, the group of patients carrying 2 risk points had a greater risk of progression than patients with 0 to 1 risk points (AdjHR for the 2 risk group = 1.39 [95% CI, 0.88-2.20], *P* = .149).

Patients with the highest score (4-6 risk points) exhibited the lowest median PFS: 10.0 months (95% CI, 8.1-14.4 months), whereas patients with the lowest score (0-1 risk points) showed a median PFS of 26.3 months (95% CI, 15.9-39.0 months; *P* = .0002).

None of the germ line variants individually investigated was significantly associated with OS. Nonetheless, the risk score allowed a global stratification of patients according to OS with the same trend (*P* = .07) observed for PFS (Figure 3B). Intriguingly, by comparing only the groups with the highest risk score (4-6 points) versus the lowest risk score (0-1 points), the difference was significant in the univariate and in the multivariate models (Table 4).

Discussion

In the modern armamentarium of the treatment of HR^+ locally advanced or MBC, AIs still retain a crucial role. However, although a first stabilization of the tumor burden takes place in several locally advanced or MBC patients treated with AIs, disease progression almost always occurs, frequently after only a few months of treatment. Prognostic or predictive biomarkers for exemestane outcome are, hence, a clinical need.

Recently, somatic DNA variations have been described to affect AI response during neoadjuvant treatment, but the role of germ line variants in PFS and OS after first-line exemestane treatment remains to be elucidated. A prospective multicenter study was designed to identify germ line variants associated with PFS and OS in locally advanced or MBC patients treated with first-line exemestane. Polymorphic variants in genes involved in exemestane pharmacokinetics and pharmacodynamics, hormone balance, DNA repair, and cell signaling pathways were considered. Multivariate and bootstrap analyses highlighted 5 polymorphisms as significantly

Figure 2 Kaplan–Meier Estimates for Progression-Free Survival (PFS) According to 3 Polymorphisms in Hormone Balance Pathways: (A) *CYP19A1*-rs10046, Additive Model (add), (B) *SLCO1B1*-rs4149056, Dominant Model (dom), and (C) *ABCG2*-rs2046134, dom; and 2 Polymorphisms in DNA Repair and Cell Signaling Pathways: (D) *FGFR4*-rs351855, Recessive Model (rec), and (E) *XRCC3*-rs861539, rec. Adjusted Hazard Ratio (AdjHR) and *P* Values Were Determined Using a Multivariate Cox Regression and Bootstrap Analyses: *CYP19A1*-rs10046: AdjHR, 1.15 (95% CI Bootstrapped Value [bootsr], 1.01-1.31), $P_{bootsr} = .038$; *SLCO1B1*-rs4149056: AdjHR, 0.54 (95% CI_{bootsr}, 0.36-0.80), P_{bootsr}, .002; *ABCG2*-rs2046134: AdjHR, 0.62 [95% CI_{bootsr}, 0.39-0.97], $P_{bootsr} = .038$; *FGFR4*-rs351855: AdjHR, 1.85 (95% CI_{bootsr}, 1.03-3.34), $P_{bootsr} = .039$; *XRCC3*-rs861539: AdjHR, 1.72 (95% CI_{bootsr}, 1.02-2.90), $P_{bootsr} = .043$). "+" Indicates Censored

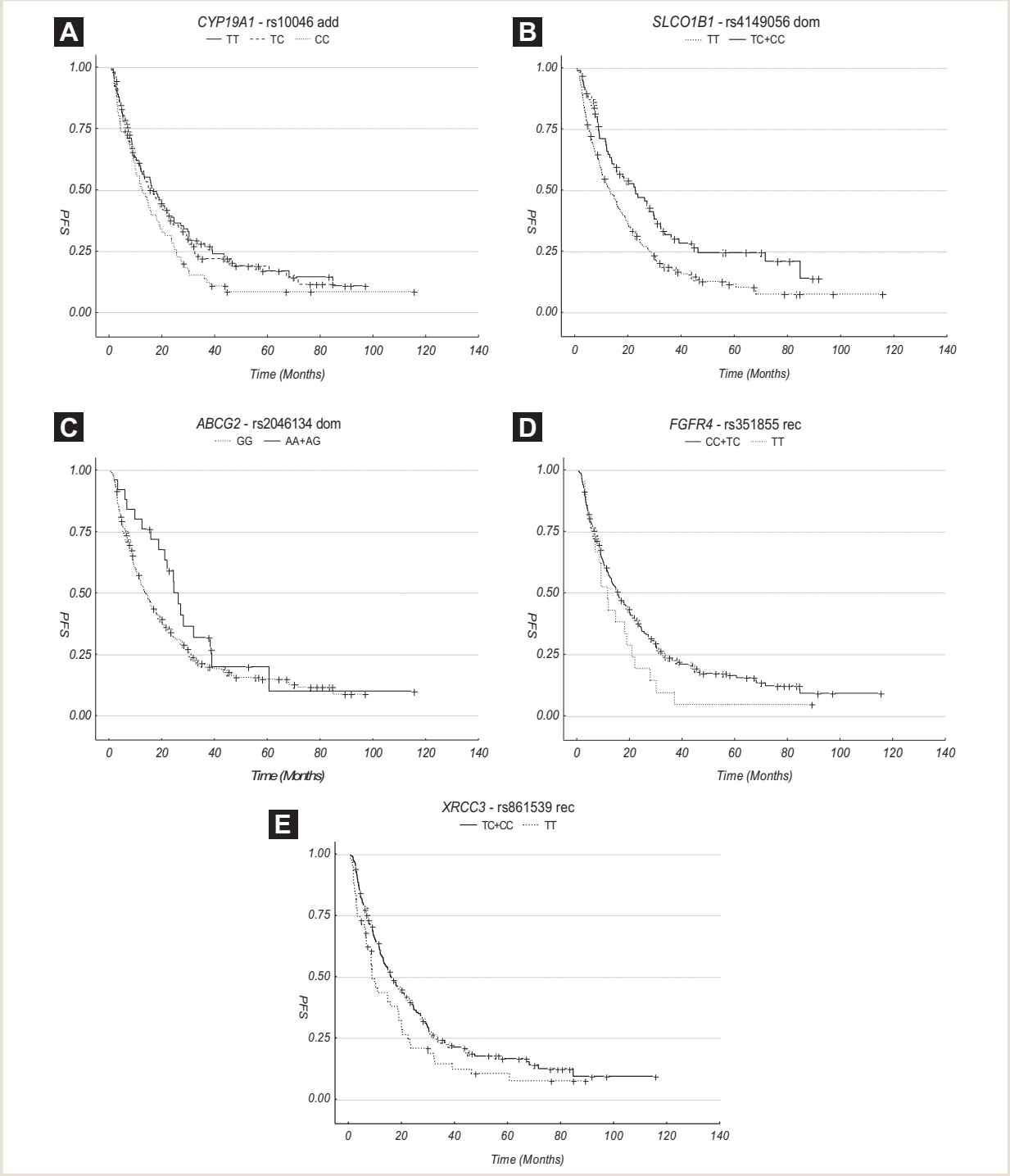


Table 3 Points Attributed to the Risk Genotypes Associated With PFS and OS

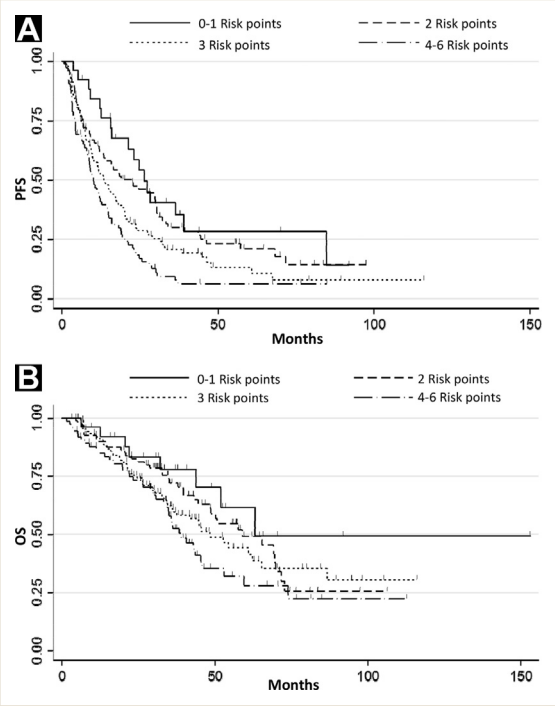
Polymorphism	Patient's Genotype	Type of Allele	Risk Points Attributed	Genetic Model
CYP19A1-rs10046	TT	C = risk allele	0	Additive
CYP19A1-rs10046	TC		1	
CYP19A1-rs10046	CC		2	
SLCO1B1-rs4149056	TC or CC	C = protective allele	0	Dominant
SLCO1B1-rs4149056	TT		1	
ABCG2-rs2046134	AA or AG	A = protective allele	0	Dominant
ABCG2-rs2046134	GG		1	
FGFR4-rs351855	TC or CC	T = risk allele	0	Recessive
FGFR4-rs351855	TT		1	
XRCC3-rs861539	TC or CC	T = risk allele	0	Recessive
XRCC3-rs861539	TT		1	

Abbreviations: OS = overall survival; PFS = progression-free survival.

associated with PFS: *CYP19A1*-rs10046, *XRCC3*-rs861539, *ABCG2*-rs2046134, *SLCO1B1*-rs4149056, and *FGFR4*-rs351855. The main findings reported in the literature regarding these polymorphisms are summarized in Supplemental Table 2 in the online version.

Cytochrome P450 19A1 codes for the aromatase enzyme, the target of exemestane. Aromatase catalyzes the conversion of C19 androgens into C18 estrogens, a critical step in estrogen biosynthesis, inhibited by exemestane. Extensive research has been done to investigate the role of *CYP19A1* polymorphisms in hormone therapy efficacy, but data produced so far are contradictory. A recent meta-analysis[26] described an association between the *CYP19A1*-rs4646 polymorphism and time to progression in AI-treated BC patients. The authors concluded that the effect of *CYP19A1* polymorphisms on clinical outcomes were most often detected in individual studies, underling the necessity of performing prospective validation studies. Our prospective study failed to confirm any association between rs4646 and PFS. The only *CYP19A1* polymorphism associated with PFS was the rs10046, a 3′ untranslated region variation, which has a low level of linkage disequilibrium with rs4646 (r^2 = 0.39 in the European 1000G phase 1 population). A recent work of Magnani et al[27] highlighted how AI-resistant patients show acquired *CYP19A1* amplification in their recurrent tumor. Amplification of this gene also occurred in vitro in AI-resistant models, showing a higher aromatase activity. In light of these results it seems that *CYP19A1* amplification at the tumor level might be more important than germ line variations in determining the response to exemestane.

Figure 3 Kaplan–Meier Curves for (A) Progression-Free Survival (PFS) and (B) Overall Survival (OS) According to the Genetic Risk Score. Patients Were Divided Into 4 Groups On the Basis of the Number of Risk Points. Kaplan–Meier Log Rank Survival Analysis (2-Sided) Was Used to Calculate *P* Values (PFS, *P* = .0002; OS, *P* = .07). "|" Indicates Censored



It is well known that genotoxic estrogen metabolites might cause DNA damage.[28] *XRCC3* is involved in the DNA synthesis and repair pathways. In our study, patients carrying the *XRCC3*-rs861539TT genotype (241 Met/Met) had an increased risk of progression compared with the TC and CC genotypes. The 241 Met/Met variant was associated with a decreased DNA repair capacity[29] and it has been considered a biomarker of survival in MBC patients treated with DNA-damaging chemotherapy.[30] Similarly, patients carrying the *XRCC3*-rs861539TT genotype might be unable to repair genotoxic estrogen metabolite damage or other genotoxic insults, allowing cell proliferation and cancer progression.[31]

Steroid transporters have a critical role in tumor response to hormone therapy in BC.[32] In our study we observed that polymorphisms in 2 genes (*ABCG2* and *SLCO1B1*) encoding for steroid transporters, were associated with an increased PFS. Patients carrying at least 1 variant allele of either *ABCG2*-rs2046134 (A) or *SLCO1B1*-rs4149056 (C-174Ala) had a significantly reduced risk of progression compared with the wild type alleles. *ABCG2* and *SLCO1B1* genes encode for BC resistance protein (BCRP) and

Table 4 Median PFS and OS According to the Risk Score: Univariate and Multivariate Analysis

Risk Points	Median Survival (95% CI), Months	Log Rank *P*	Univariate		Multivariate	
			HR (95% CI)	*P*	AdjHR (95% CI)	*P*
PFS						
0-1	26.3 (15.9-39.0)	**.0002**	Ref		Ref	
2	22.3 (13.2-30.3)		1.32 (0.98-1.79)	.07	1.40 (0.89-2.20)	.149
3	13.4 (9.7-17.9)		1.80 (1.21-2.66)	**.004**	2.00 (1.20-3.36)	**.008**
4-6	10.0 (8.1-14.4)		2.54 (1.90-3.39)	**<.001**	3.12 (2.18-4.48)	**<.001**
OS						
0-1	63.0 (43.9-not reached)	.07	Ref		Ref	
2	58.9 (48.5-69.8)		1.44 (0.79-2.63)	.233	1.34 (0.77-2.34)	.300
3	48.3 (36.5-65.1)		1.72 (0.85-3.47)	.131	1.77 (0.97-3.20)	.061
4-6	38.9 (34.3-45.8)		2.28 (1.61-4.50)	**.017**	2.41 (1.22-4.79)	**.012**

Significant results are shown in bold text.
Abbreviations: AdjHR = adjusted hazard ratio; HR = hazard ratio; OS = overall survival; PFS = progression-free survival; Ref = reference category.

organic anion-transporting polypeptide 1B1, respectively. These are transporters also involved in the detoxification process of xenobiotic and antineoplastic drugs.[33,34] Very recently the *SLCO1B1*-rs4149056 polymorphism has been associated with exemestane pharmacokinetics. In a study involving few healthy volunteers, women carrying at least 1 variant C allele showed a statistically significant higher area under the time/concentration curve for exemestane and its metabolite.[35] Moreover, *SLCO1B1*-rs4149056 is a predictor of statins and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors disposition. Intriguingly, in our study, patients carrying at least 1 C allele had a prolonged PFS, probably as a result of an increased drug exposure because of the effect of this polymorphism. Regarding BCRP, at present, no data have been reported on its effect on exemestane. However, it must be considered that exemestane and its metabolites share similar chemical structures of the steroidal derivatives that are BCRP substrates.

The FGFR is a transmembrane tyrosine kinase receptor involved in multiple biological processes, including cell proliferation, differentiation, and apoptosis. Previous reports indicate that endocrine resistance involves a cross-talk between growth factor pathways and estrogen signaling.[9,36] In BC, aberrant FGFR signaling, including the *FGFR4*-rs351855 polymorphism (Gly388Arg), has been involved in tumor progression and resistance to tamoxifen.[37] In vitro reports showed an increased motility of mammary cells with 388 Arg/Arg variant and also an increased extracellular matrix degradation.[37] According to this detrimental effect, we observed that patients carrying *FGFR4*-rs351855 homozygous variant genotype (TT-388 Arg/Arg) were at a significantly increased risk of progression compared with TC and CC genotypes.

The 6 risk genotypes significantly associated with a shorter PFS (*CYP19A1*-rs10046TC/CC, *SLCO1B1*-rs4149056TT, *ABCG2*-rs2046134GG, *FGFR4*-rs351855TT, and the *XRCC3*-rs861539TT) were combined into a genetic risk score, allowing the definition of 4 risk groups of patients. As a result, we obtained a better stratification of patients according to their PFS, with a substantial improvement in the predictive ability of the score compared with the individual polymorphisms. Patients carrying 2, 3, and 4 to 6 risk points had a 40%, 100%, and more than 200% increased risk of progression, respectively, than patients with 0 to 1 risk points. We observed an overall not statistically significant trend for the association of OS with the risk score. The fact that we have not taken into account treatments after progression, which can affect OS, might explain this lack of significance. However, when considering only the groups with the highest and lowest risk points (4-6 vs. 0-1), the difference in OS became statistically significant, indicating that this score might be helpful in OS prediction for at least these extreme groups of patients, allowing identification of the ones at the highest and the lowest risk of death.

Another limitation of this study is that an external validation cohort is missing, due to the difficulty of finding a prospective study with similar characteristics. However, the bootstrap analysis, consisting in the replication of the findings by drawing 1000 samples from the original data set, allowed an internal assessment of reproducibility. Moreover, this study could evaluate only the prognostic role of the germ line polymorphisms. The assessment of the predictive value of the biomarkers could not be established, because of the lack of a control arm with patients not treated with exemestane.

To the best of our knowledge, this is the largest prospective study specifically designed to evaluate pharmacogenetic biomarkers of PFS and OS in patients treated with exemestane. In addition, the long follow-up (median: almost 3 years) is another point of strength of the study.

Conclusion

Our findings show that germ line polymorphisms in hormone balance, drug activity, DNA replication and repair, as well as in signaling pathways are associated with PFS and OS of exemestane-treated patients. The joint effect of polymorphisms from multiple pathways included in a multifactorial genetic score might better define groups of patients with different prognoses. Replication studies, in external cohorts of patients, are nonetheless required to finally ascertain the clinical utility of these markers.

Clinical Practice Points

- Several studies investigated the role of genetic variations in genes involved in exemestane pharmacokinetics, pharmacodynamics, and hormone balance pathways. Even if several associations have been found with exemestane outcome, no consensus has been reached on polymorphisms to be translated into clinical practice as predictive or prognostic biomarkers.
- This study highlighted 5 polymorphisms on genes involved in AI pathways and also in DNA repair pathways. The combination of these 5 polymorphisms into a score allow better stratification of patients according to their PFS and OS.
- The use of this pharmacogenetic score might help, through a simple blood test, to identify patients who might require a different or more aggressive therapeutic approach because of their higher risk of progression and/or death.

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Disclosure

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Supplemental Data

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Supplemental Data

Supplemental Table 1 List of the Analyzed Polymorphisms

Pathway	Gene	rsID	Polymorphism	Patients, n	Variant Allele	%
Estrogen Synthesis	*CYP19A1*	rs10046	T>C	302	C	48
Estrogen Synthesis	*CYP19A1*	rs60271534	(TTTA) 7-13	302	L	50
Estrogen Synthesis	*CYP19A1*	rs4646	C>A	302	A	29
Estrogen Synthesis	*CYP19A1*	rs700519	C>T	302	T	3
Estrogen Synthesis	*CYP17A1*	rs743572	A>G	302	G	42
Estrogen Activity	*ESR1*	rs9340799	A>G	302	G	38
Estrogen Activity	*ESR1*	rs2234693	T>C	302	C	46
Estrogen Activity	*ESR2*	rs1256049	G>A	302	A	2
Estrogen Activity	*ESR2*	rs4986938	G>A	302	A	41
Estrogen Activity	*PRDM2*	rs2308040	D>I	302	I	37
Estrogen Metabolism	*COMT*	rs4680	A>G	302	A	50
Estrogen Metabolism	*CYP1B1*	rs1056836	C>G	302	G	44
Estrogen Metabolism	*UGT1A1*	rs8175347	(TA) 5-8	300	L	35
Estrogen Metabolism	*CYP2C9*	rs1799853	C>T	301	T	11
Exemestane Metabolism	*CYP3A4*	rs2740574	A>G	302	G	2
Exemestane Metabolism	*CYP3A5*	rs776746	G>A	302	A	5
Steroids Transport, MDR	*ABCB1*	rs10276036	T>C	299	C	43
Steroids Transport, MDR	AB*CB1*	rs2235013	A>G	300	G	48
Steroids Transport, MDR	*ABCB1*	rs2235015	G>T	299	T	19
Steroids Transport, MDR	*ABCB1*	rs2235033	C>T	299	T	48
Steroids Transport, MDR	*ABCB1*	rs3213619	T>C	300	C	4
Steroids Transport, MDR	*ABCB1*	rs3842	A>G	298	G	14
Steroids Transport, MDR	*ABCC1*	rs2074087	G>C	301	C	17
Steroids Transport, MDR	*ABCC1*	rs212088	C>T	300	T	16
Steroids Transport, MDR	*ABCC1*	rs2230671	G>A	302	A	23
Steroids Transport, MDR	*ABCC1*	rs35587	T>C	299	C	33
Steroids Transport, MDR	*ABCC1*	rs35588	A>G	302	G	31
Steroids Transport, MDR	*ABCC1*	rs35605	C>T	302	T	19
Steroids Transport, MDR	*ABCC1*	rs3765129	C>T	299	T	14
Steroids Transport, MDR	*ABCC1*	rs4148356	G>A	302	A	0
Steroids Transport, MDR	*ABCC1*	rs60782127	G>T	302	T	1
Biliary Transport, MDR	*ABCC2*	rs17216177	T>C	302	C	7
Biliary Transport, MDR	*ABCC2*	rs2002042	C>T	302	T	23
Biliary Transport, MDR	*ABCC2*	rs2273697	G>A	301	A	19
Biliary Transport, MDR	*ABCC2*	rs3740066	G>A	302	A	37
Biliary Transport, MDR	*ABCC2*	rs4148396	C>T	302	T	38
Biliary Transport, MDR	*ABCC2*	rs717620	G>A	302	A	18
Biliary Transport, MDR	*ABCC2*	rs8187710	G>A	302	A	7
Steroids Transport, BCRP	*ABCG2*	rs2046134	G>A	299	A	5
Steroids Transport, BCRP	*ABCG2*	rs2231142	C>A	302	A	9
Steroids Transport, BCRP	*ABCG2*	rs2622604	C>T	299	T	22
Steroids Transport, BCRP	*ABCG2*	rs3219191	D>I	299	I	45
Steroids Transport	*SLCO1B1*	rs4149056	T>C	300	C	16
Cell Cycle, DNA Repair	*ATM*	rs1801516	G>A	302	A	14
Cell Cycle, DNA Repair	*CDKN1A*	rs1801270	C>A	302	A	7
Cell Cycle, DNA Repair	*MDM4*	rs4245739	A>C	299	C	30
Cell Cycle, Signaling	*FGFR4*	rs351855	C>T	301	T	27
DNA Repair	*APEX1*	rs1130409	T>G	302	G	46
DNA Repair	*MSH6*	rs3136228	T>G	301	G	37
DNA Repair	*OGG1*	rs1052133	C>G	302	G	21

Supplemental Table 1 Continued

Pathway	Gene	rsID	Polymorphism	Patients, n	Variant Allele	%
DNA Repair	*ERCC5*	rs17655	C>G	302	G	22
DNA Repair	*XRCC1*	rs1799782	C>T	302	T	6
DNA Repair	*XRCC1*	rs25487	G>A	302	A	33
DNA Repair	*XRCC1*	rs25489	G>A	300	A	6
DNA Repair	*XRCC3*	rs1799794	A>G	302	G	22
DNA Repair	*XRCC3*	rs1799796	A>G	301	G	27
DNA Repair	*XRCC3*	rs861539	C>T	302	T	42
DNA Repair, NER	*ERCC1*	rs11615	T>C	302	C	40
DNA Repair, NER	*ERCC1*	rs3212986	G>T	302	T	27
DNA Repair, NER	*ERCC2*	rs13181	T>G	301	G	44
DNA Synthesis	*ATIC*	rs2372536	C>G	302	G	36
DNA Synthesis, Folate Cycle	*FOLR1*	rs2071010	G>A	302	A	6
DNA Synthesis, Folate Cycle	*FOLR1*	rs9282688	C>T	302	T	2
DNA Synthesis, Folate Cycle	*FPGS*	rs10106	A>G	302	G	37
DNA Synthesis, Folate Cycle	*GGH*	rs11545078	C>T	301	T	11
DNA Synthesis, Folate Cycle	*MTHFD1*	rs2236225	C>T	302	T	43
DNA Synthesis, Folate Cycle	*MTHFR*	rs1801131	A>C	302	C	32
DNA Synthesis, Folate Cycle	*MTR*	rs1805087	A>G	301	G	19
DNA Synthesis, Folate Cycle	*MTRR*	rs1801394	A>G	301	G	49
DNA Synthesis, Folate Cycle	*SHMT1*	rs2273029	C>T	302	T	25
DNA Synthesis, Folate Cycle	*TYMS*	rs16430	I>D	302	D	38
DNA Synthesis, Folate Cycle	*TYMS*	rs2790	A>G	302	G	25
DNA Synthesis, Folate Cycle	*TYMS*	rs699517	C>T	301	T	38
TP53 Signaling	*TP53*	rs1042522	G>C	302	C	29

List of the analyzed polymorphisms with the pathway they are involved in, the number of patients genotyped, and the frequencies of the variant alleles. Underlined, polymorphisms in linkage disequilibrium, according to r^2 threshold = 0.8. For the polymorphisms *CYP19A1*-rs60271534 and for *UGT1A1*-rs8175347 alleles were associated into 2 groups, long (L) and short (S) alleles: L $\geq$ 7 TTTA, S $<$ 7 TTTA repeats, and L = 7-8 TA, S = 5-6 TA repeats, respectively.

Abbreviations: BCRP = breast cancer resistance protein; D = deletion; I = insertion; MDR = multidrug resistance; NER = nucleotide excision repair; rsID = reference single-nucleotide polymorphism identification number; TP53 = tumor protein 53.

Supplemental Table 2 Role, Function, and Associations Found in the Literature for the Polymorphisms Significantly Associated With PFS

Gene	SNP (Amino Acid Change)	Location	Predicted or Functional Role	Cancer Type	Treatment	End Point	Patient n	Association With the Variant Allele or Gene	Reference
CYP19A1	rs10046	3′ UTR	↓ Estrogen levels	Healthy subjects	None	Estrogen levels	1975	↓ Estrogen levels	
				BC	Adjuvant LET	Estrogen levels	204	None	1
				BC	NR	DFS	Total 1257, premenopausal 439	↑ DFS (premenopausal)	2
				BC	TAM and/or LET	Bone AEs	4861	↑ Bone AE risk	3
				BC	NR	BC Risk	522 Cases/1221 controls	↑ BC risk	4
				BC	NR	BC Risk	20,098 (meta-analysis)	None	4
				BC	NR	BC Risk	1164 Cases/2111 controls	None	5
				BC	LET; ANA	TTP	67,272	None	6,7
				BC	Neoadjuvant LET	Response	95	None	8
				BC	ANA, LET, EXE	TTP, AEs	NR	None	9
				BC	AIs	OS	53	↑ OS with WT allele	10
SLCO1B1	rs4149056 (Val174Ala)	Exonic	↓ Transport activity	Healthy subjects	None	Activity	NR	↓ Transport activity	11
				BC cell line	NA	Activity	NA	↓ Transport activity	12
				BC	TAM	OS	296	↓ OS	13
FGFR4	rs351855 (Gly388Arg)	Exonic	↑ Tumor cell motility	BC cell line	NA	Cell motility	NA	↑ Tumor cell motility	14
				BC	None, CMF, and/or TAM	DFS	84	↓ DFS	14
				PC cell line	NA	Tumor invasion	NA	↑ ECM degradation	15
				BC transgenic mouse	NA	BC progression	NA	↑ Tumor progression	16
				Multiple, including BC	NA	OS	2537 (Pooled analysis)	↓ OS	10
				BC	Adjuvant CMF and/or TAM	DFS/OS	372	↓ DFS/OS	17
				BC	TAM	CB/PFS	285	↓ CB/DFS	18
ABCG2	rs2046134	Intronic	Transcription factor binding site (predicted)[a]	Tissue from healthy donors/GIST AI-resistant cells/ xenograft tumors	Imatinib LET, EXE	Functional effect AI resistance	44 + 60 + 28 Tissues/82 GIST patients	↑ Protein expression Involvement of *ABCG2* in AI resistance	ConSite[19-21,a]
XRCC3	rs861539 (Thr241Met)	Exonic	Possibly damaging (predicted)[b]	GIST	Imatinib	OS	81	↓ OS	Polyphen-2[22,b]
				BC	Anthracyclines	OS	150	↑ OS	23
				BC	NR	BC risk	70 Cases/70 controls	BC risk	24
				BC	NR	BC risk	19,575 cases/21, 125 controls	↑ BC risk	25

The literature analysis was focused on the identification of the role of the SNPs with cancer risk, toxicities, patients' prognosis, and cellular transformation. In case of no functional data available in literature, we referred to the results obtained with bioinformatic analysis exploiting ConSite (http://compbio.cs.queensu.ca/F-SNP) and PolyPhen (http://genetics.bwh.harvard.edu/ggi/pph2/e8dbeaa52a8642d83df5575a0830d51a00e71f38/5502954.html).

Abbreviations: AE = adverse event; AI = aromatase inhibitor; ANA = anastrozole; BC = breast cancer; CB = clinical benefit; CMF = cyclophosphamide/methotrexate/fluorouracil; DFS = disease free survival; ECM = extracellular matrix; EXE = exemestane; GIST = gastrointestinal stromal tumor; LET = letrozole; OS = overall survival; PC = prostate cancer; PFS = progression-free survival; SNP = single nucleotide polymorphism; TAM = tamoxifen; TTP = time to progression; UTR = untranslated region; WT = wild type.

[a]Predicted by ConSite.

[b]Predicted by PolyPhen-2.

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