SNP of Aromatase Predict Long-term Survival and Aromatase Inhibitor Toxicity in Patients with Early Breast Cancer: A Biomarker Analysis of the GIM4 and GIM5 Trials

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ABSTRACT

Purpose: In estrogen receptor–positive (ER^+) breast cancer, single-nucleotide polymorphisms (SNP) in the aromatase gene might affect aromatase inhibitors (AI) metabolism and efficacy. Here, we assessed the impact of SNP on prognosis and toxicity of patients receiving adjuvant letrozole.

Experimental Design: We enrolled 886 postmenopausal patients in the study. They were treated with letrozole for 2 to 5 years after taking tamoxifen for 2 to 6 years, continuing until they completed 5 to 10 years of therapy. Germline DNA was genotyped for SNP rs4646, rs10046, rs749292, and rs727479. Log-rank test and Cox model were used for disease-free survival (DFS) and overall survival (OS). Cumulative incidence (CI) of breast cancer metastasis was assessed through competing risk analysis, with contralateral breast cancer, second malignancies and non-breast cancer death as competing events. CI of skeletal and cardiovascular events were assessed using DFS events as competing events. Subdistribution HR (sHR) with 95% confidence intervals were calculated through Fine-Gray method.

Results: No SNP was associated with DFS. Variants rs10046 [sHR 2.03, (1.04–2.94)], rs749292 [sHR 2.11, (1.12–3.94)], and rs727479 [sHR 2.62, (1.17–5.83)] were associated with breast cancer metastasis. Three groups were identified on the basis of the number of these variants (0, 1, >1). Variant-based groups were associated with breast cancer metastasis (10-year CI 2.5%, 7.6%, 10.7%, $P = 0.035$) and OS (10-year estimates 96.5%, 93.0%, 89.6%, $P = 0.030$). Co-occurrence of rs10046 and rs749292 was negatively associated with 10-year CI of skeletal events (3.2% vs. 10%, $P = 0.033$). A similar association emerged between rs727479 and cardiovascular events (0.3% vs. 2.1%, $P = 0.026$).

Conclusions: SNP of aromatase gene predict risk of metastasis and AI-related toxicity in ER^+ early breast cancer, opening an opportunity for better treatment individualization.

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Translational Relevance

Patients with estrogen receptor–positive early breast cancer face a continued risk of recurrence for more than 20 years after diagnosis. While extended endocrine treatment with aromatase inhibitors beyond 5 years can lower this risk, it can also lead to clinically significant toxicities such as bone fractures and cardiovascular events. This study identified specific germline SNP within the gene responsible for encoding the aromatase enzyme. These SNP have been linked to a higher cumulative incidence of metastasis in breast cancer and an increased risk of death. However, they are also associated with a lower cumulative incidence of bone fractures and fewer adverse cardiovascular outcomes 10 years after diagnosis. The findings suggest that tailoring endocrine treatment extension based on aromatase SNP can optimize treatment benefits while minimizing risks. This research opens an avenue to better personalize treatment decision-making and survivorship care in breast cancer survivors.

Introduction

Estrogen receptor–positive (ER^+) early breast cancer is characterized by a substantial risk of late metastasis, with 10% to 40% of recurrences occurring 5 to 20 years from diagnosis (1). Extended endocrine therapy with aromatase inhibitors (AI) beyond 5 years reduces the risk of these late events by 20% to 30% (2–4), but such benefit comes at the price of increased incidence of skeletal and cardiovascular (CV) events (5–8).

SNP in the gene encoding for the aromatase enzyme (*CYP19A1*) may affect aromatase activity and circulating estradiol levels (9, 10), potentially leading to increased risk of ER^+ breast cancer recurrence (11, 12) and decreased AI efficacy (11, 13, 14) and toxicity (15–17).

On the basis of these premises, we conducted a biomarker analysis to assess the impact of SNP of *CYP19A1* on the long-term outcomes of 886 postmenopausal patients treated with adjuvant letrozole within the GIM4 and GIM5 trials.

Materials and Methods

Participants and procedures

This study includes two prospective cohorts of postmenopausal women with ER^+ early breast cancer who remained free of recurrence for 2 to 6 years from breast surgery and were treated with 2 to 5 years of adjuvant letrozole.

The GIM5 trial (EudraCT number: 2005-001213-18) was a multicenter noncomparative study, which assessed the correlation between SNP of *CYP19A1* and the efficacy and safety of 5 years of extended adjuvant letrozole in patients already treated with 4 to 6 years of adjuvant tamoxifen (18). To be eligible, patients had to be postmenopausal and have one of the following high-risk criteria: axillary nodal involvement (N+), high tumor grade (G3), tumor size >50 mm or 20 to 50 mm, and G≥2.

The GIM4 trial randomized 2,056 postmenopausal patients with ER^+ operable breast cancer who already received 2 to 3 years of adjuvant tamoxifen to either 2 to 3 years or 5 years of letrozole (19). The study protocol was amended in August, 2005, to assess germline aromatase SNP and evaluate their association with efficacy and tolerability of adjuvant letrozole. As a result, 591 out of 2,056 enrolled patients were evaluated for SNP. Both studies defined postmenopausal status with one of the following: ≥55 years and cessation of menses, <55 years and cessation of menses for at least 1 year, <55 years with spontaneous menses within the past 1 year but gonadotropin or estradiol concentrations within the postmenopausal range, or previous bilateral oophorectomy.

Both trials were open-label with no blinding. In both trials, patients were followed once every 6 months for 5 years after study entry and every 12 months thereafter. Bisphosphonates, calcium, and vitamin D were allowed as per local guidelines.

The study was approved by the ethics committees of participating institutions and according to the Declaration of Helsinki. Written informed consent was obtained from all patients before study entry.

Blood collection, DNA extraction, and genotyping assays

Genomic DNA was extracted from peripheral blood using QIAamp DNA blood Midi Kit (Qiagen) and genotyped for SNP of aromatase rs749292 and rs727479, located respectively in exonic and intronic regions of *CYP19A1*, and rs4646 and rs10046, both located in the 3′ untranslated region (3′-UTR) of *CYP19A1*. Polymorphisms in 3′-UTR were genotyped with the hexaprimer amplification refractory mutation system PCR (H-ARMS-PCR; refs. 18, 20); rs749292 and rs727479 were genotyped by standard ARMS-PCR.

Outcomes

Disease-free survival (DFS) was the prespecified primary endpoint. Overall survival (OS) and safety were secondary endpoints. DFS events included local or distant recurrence, invasive breast cancer, second primary malignancy, and death from any cause. Following the publication of MA 17.R (21) and NSABP B-42 (7) trials showing an increased contribution of contralateral breast tumors and second malignancies to DFS events at longer follow-up, a competing risk model for cumulative incidence of metastasis was included, to better account for competing causes of morbidity and death. Distant recurrence and death with breast cancer were defined as outcomes of interest, while contralateral invasive breast tumors, second primary malignancy, and death without breast cancer were considered competing events.

To investigate the association between SNP of aromatase and AI-related adverse events, cumulative incidence of skeletal and CV events were also analyzed as safety endpoints within a competing risk model. Outcomes of interest were bone fractures for skeletal events, venous thrombosis, embolism, stroke, angina, or myocardial infarction for CV events. Competing events for both endpoints were defined on the basis of the established criteria outlined by Rabaglio and colleagues (22). As per this definition, breast cancer recurrence (local or distant), invasive breast cancer, second primary malignancy, and death were considered as competing events. The adoption of this comprehensive definition stems from the potential associations of breast cancer recurrence and second malignancies with bone metastases and modifications in oncologic therapies, thereby exerting a substantial influence on the overall risk of adverse events, expecially bone fractures. To further validate our findings, an additional analysis considering death as the only competing event was performed. OS events were defined as death from any cause as per standard definition. As prespecified in the study protocol, follow-up and all survival endpoints were calculated from the time of enrollment in either the GIM4 or the GIM5 study, which corresponds to the time of assignment to letrozole for both studies.

Statistical analysis

Deviation from Hardy–Weinberg equilibrium was evaluated by a χ^2 with one degree of freedom with the SNPassoc R package. Haplotypes

and the deviation between observed and expected frequencies were evaluated by χ^2 of 3 × 3 table through the haplo.stats R package (23). The association with outcomes of interest was assessed for each SNP under the additive, dominant, and recessive genetic model. SNP which showed significant associations with outcomes were combined together, and the effect of these composite genotypes was also explored.

Log-rank test and Cox models were applied to assess the association of SNP with DFS and OS and to adjust for covariates. Cumulative incidence functions in a competing risk model were used to estimate cumulative incidences of metastasis, skeletal events, and CV events. The Fine and Gray model was used to estimate the corresponding subdistribution HR (sHR) and adjust for covariates (24). For survival endpoints, the following covariates were included in the Cox and Fine-Gray models: tumor stage (pT1 vs. pT2 vs. pT3–4), nodal status (node-positive vs. node-negative), age at diagnosis (as continuous variable), (neo)adjuvant chemotherapy receipt (no vs. yes), and study cohort (GIM5 vs. GIM4). Smoking history, age at diagnosis, body mass index (BMI), previous bisphosphonate the use, and preexisting CV risk factors (i.e., uncontrolled hypertension or dyslipidemia, history of stroke, angina or cardiomyopathy) were the covariates included in the models for safety endpoints, with age and BMI categorized according to published algorithms for the risk assessment of bone fractures and CV events in patients with breast cancer (25, 26).

Data availability

Data from this study have been deposited in the ClinVar public archive under the accession numbers SCV004037380, SCV004037381, and SCV004037379. Patient-level, pseudonymized clinical and SNP data will be made available upon reasonable request through a data transfer agreement (DTA). Requests should be directed to lucia.delmastro@hsanmartino.it. A DTA template can be found in the Supplementary Materials and Methods.

Results

Study population

From August 1, 2005 to May 19, 2010, 591 patients from the GIM4 trial and 295 patients from the GIM5 trial were genotyped, for a total of 886 patients (Supplementary Fig. S1). Patients in the SNP cohorts were representative of the main GIM4 and GIM5 populations (Supplementary Tables S1–S3). Compared with patients in the GIM4-SNP cohort, those in the GIM5-SNP cohort had higher nodal stage ($P < 0.001$), higher tumor stage ($P < 0.001$), and were treated with tamoxifen and letrozole for more years (**Table 1**). The median time between the initial diagnosis of breast cancer and study enrollment was 2.7 years. Because most patients began adjuvant treatment before the approval of adjuvant trastuzumab in Italy (2006), only one patient out of 34 with known HER2-positive status received adjuvant trastuzumab.

Alleles and genotypes frequencies of SNP of aromatase are listed in **Table 2**. No deviations from Hardy–Weinberg equilibrium were observed. The T alleles of rs10046, rs749292, and rs727479 were in positive linkage disequilibrium and co-occurred in 39% of patients (Supplementary Fig. S2a and S2b). Notably, patient characteristics were homogenous across SNP (Supplementary Tables S4–S7).

Survival outcomes

At a median follow-up of 12 years from study enrollment, 159 DFS events were reported, of which 75 (47%) were distant recurrences or breast cancer deaths. No SNP was associated with DFS, neither in the overall population (Supplementary Table S8), nor when the association was assessed separately in the GIM5 study and in the short (2–3 years) and long (5 years) letrozole arm of the GIM4 trial (Supplementary Table S9). In the overall population, an increased cumulative incidence of breast cancer metastasis and death with breast cancer was observed in patients homozygous for the minor T allele in rs10046 (sHR 2.03; 95% CI, 1.04–2.94) and in rs749292 (sHR 2.11; 95% CI, 1.12–3.94) and for those harboring the common T allele in rs727479, either in homozygosis or heterozygosis (sHR 2.62; 95% CI, 1.17–5.83). These high-risk variants were also associated with worse OS, with HR of 2.12 (95% CI, 1.14–3.9,3), 1.83 (95% CI, 1.03–3.26), and 2.62 (95% CI, 1.17–5.83), respectively (Supplementary Table S8).

On the basis of these results, patients were stratified in three groups according to the number of high-risk variants (no high-risk SNP, one high-risk SNP, >1 high-risk SNP; Supplementary Fig. S3). Overall, 133 patients had no high-risk SNP (15%), 530 had at least one high-risk SNP (60%), and 223 had >1 high-risk SNP (25%). Importantly, all clinical-pathologic features were homogenously distributed across SNP-based prognostic groups (Supplementary Table S10). These groups identified patients with significant differences in their cumulative incidence of metastasis and breast cancer death, with 10-year estimates of 2.5%, 7.6%, and 10.7%, respectively ($P = 0.035$; **Fig. 1A**). Conversely, the incidence of competing events (i.e., contralateral breast tumors, second primary malignancy, and death without breast cancer) was similar across groups (10-year estimates of 4.8%, 5.8%, and 6.1%, respectively, $P = 0.953$). In a multivariable competing risk model including age, tumor size, nodal status, chemotherapy receipt and study cohort, the intermediate- and high-risk groups remained independent predictors of breast cancer metastasis and breast cancer death, with an sHR of 2.55 (95% CI, 1.00–6.45) and 3.48 (95% CI, 1.33–9.13), respectively (**Table 3**). Notably, while no association emerged between SNP-based groups and DFS, the relative contribution of breast cancer distant recurrence to DFS outcomes increased progressively in patients with zero (27%), one (46%), and >1 high-risk SNP (59%). SNP-based groups were also significantly associated with OS, with 10-year survival estimates of 96.5%, 93.0%, and 89.6%, respectively ($P = 0.030$; **Fig. 1B**). The association remained significant after adjustment for age, tumor size, nodal status, chemotherapy receipt, and study cohort (**Table 4**).

Impact of tamoxifen adjuvant treatment on SNP prognostic effect

Patients in the GIM4 and GIM5 cohorts were treated with different durations of adjuvant tamoxifen before starting adjuvant letrozole. Because the mechanism of action of tamoxifen is independent from aromatase inhibition (27), we explored whether longer exposure to previous tamoxifen could reduce the impact of SNP on distant recurrence through a carryover effect. When the interaction between tamoxifen duration and SNP-based group was fitted in a competing risk model for breast cancer metastasis and breast cancer death, no significant interaction was observed ($P_{interaction} = 0.364$). Moreover, when the SNP-based groups were assessed separately in the GIM4 and GIM5 trial, their prognostic effects appeared to be similar in the two cohorts, both in terms of DFS, OS, and cumulative incidence of breast cancer metastasis and breast cancer death (Supplementary Table S11; Supplementary Fig. S4).

Adverse events

In the overall population, skeletal and CV events occurred in 79 and 19 patients, respectively. When breast cancer recurrence, invasive breast cancer, second primary malignancy, and death were considered

Table 1. Baseline patients' characteristics in the GIM4-SNP cohort, GIM5-SNP cohort, and in the combined SNP cohort. *P* values represent the comparisons between the GIM4-SNP cohort (including both the short and long arm) and GIM5-SNP cohort.

	GIM4 SNP cohort *N* = 591 (%)	GIM5 SNP cohort *N* = 295 (%)	Total SNP cohort *N* = 886 (%)	*P* value
Age (median, IQR)	60 (54–67)	61 (55–69)	60 (54–67)	0.03
Ever smoker	123 (20.8)	48 (16.3)	171 (19.3)	0.128
BMI (kg/m^2)				
<24	198 (33.5)	89 (30.2)	287 (32.4)	0.220
≥24	373 (63.1)	205 (69.5)	578 (65.2)	
Missing	20 (3.4)	1 (0.3)	21 (2.4)	
Tumor size				
pT1	407 (68.9)	147 (49.8)	554 (62.5)	
pT2	149 (25.2)	114 (38.6)	263 (29.7)	<0.001
pT3–4	24 (4.1)	26 (8.8)	50 (5.6)	
Missing	11 (2.0)	8 (2.7)	19 (2.1)	
Nodal status				
pN0	357 (60.4)	78 (26.4)	435 (49.1)	
pN1–3	223 (37.7)	217 (73.6)	440 (49.7)	<0.001
Missing	11 (1.9)	0 (0)	11 (1.2)	
Tumor grade				
G1	118 (20.0)	35 (11.9)	150 (16.9)	
G2	307 (51.9)	159 (54.0)	466 (52.6)	<0.001
G3	121 (20.5)	88 (29.8)	209 (23.6)	
Missing	45 (7.6)	13 (4.4)	58 (6.5)	
HER2 status[a]				
Negative	378 (64.0)	279 (94.6)	657 (74.2)	
Positive	18 (3.0)	16 (5.4)	34 (3.8)	<0.001
Unknown	195 (33.0)	0 (0)	195 (22.0)	
(Neo)adjuvant CT				
No	273 (46.2)	31 (10.5)	304 (34.3)	
Yes	316 (53.5)	264 (89.5)	580 (65.5)	<0.001
Missing	2 (0.3)	0 (0)	2 (<1)	
Tamoxifen, y (median, IQR)	2.4 (2.1–2.6)	5.1 (4.9–5.3)	2.7 (2.2–4.9)	<0.001
Letrozole, y (median, IQR)	3.0 (2.1–5-0)	4.7 (1.4–5.0)	3.1 (2.3–5.0	0.060

[a]HER2-positive tumors were defined by a finding of at least 10% of tumor cells with HER2 protein expression assessed by an IHC assay or by positivity of an *in situ* hybridization assay.

as competing events, the T/T genotype in rs10046 (sHR 0.49; 95% CI, 0.25–0.97; $P = 0.034$) and in rs749292 (0.34; 95% CI, 0.14–0.85, $P = 0.020$) was associated with a decreased cumulative incidence of skeletal events (Supplementary Table S12). Patients with double homozygosis of the T allele in rs10046 and rs749292 showed a significantly lower incidence of skeletal events at 10 years (3.2%) compared with patients with no homozygosis (9.1%) and those with homozygosis only in one SNP (10.5%, $P = 0.033$; **Fig. 2A**). The double T/T homozygosis in rs10046 and rs749292 remained a significant predictor of lower incidence of skeletal events in a multivariable competing risk model including age (≤65 vs. >65 years), BMI (≥24 vs. <24), smoking habit (no vs. yes), and previous use of byphosphonates (sdHR 0.30; 95% CI, 0.10–0.88; $P = 0.028$; Supplementary Table S13). Similarly, the T/T genotype in rs727479 was associated with a decreased cumulative incidence of CV events (sHR 0.23; 95% CI, 0.05–0.99; $P = 0.048$; Supplementary Table S14). Only one CV event was observed in patients with T/T homozygosis in rs727479, while the 10-year cumulative incidence was 3.1% for other genotypes ($P = 0.026$; **Fig. 2B**). The association appeared to be mantained when it was fitted in a multivariable competing risk model including age (≤65 vs. >65 years), BMI (≥ 30 vs. <30), smoking habit, and preexisting CV risk factors (sHR 0.23; 95% CI, 0.05–1.02; $P = 0.053$; Supplementary Table S14). The association of these SNP genotypes with skeletal and CV events was mantained when the competing risk analysis was repeated considering death as the only competing event (Supplementary Table S15).

Table 2. Minor allele frequency and genotype of the four SNP of *CYP19A1*.

SNP	No. assessed	MAF	Genotype, *n* (%) Common variant	Heterozigous	Homozigous	HWE *P* value
rs4646 (G>T)	886	0.28	451 (50.9)	373 (42.1)	62 (7.0)	0.212
rs10046 (C>T)	886	0.48	229 (25.8)	450 (50.8)	207 (23.4)	0.639
rs749292 (C>T)	886	0.42	300 (33.8)	435 (49.1)	151 (17.0)	0.782
rs727479 (T>G)	886	0.40	311 (35.1)	442 (49.8)	133 (15.0)	0.262

Abbreviations: HWE, Hardy-Weiberg equilibrium; MAF, minor allele frequency.

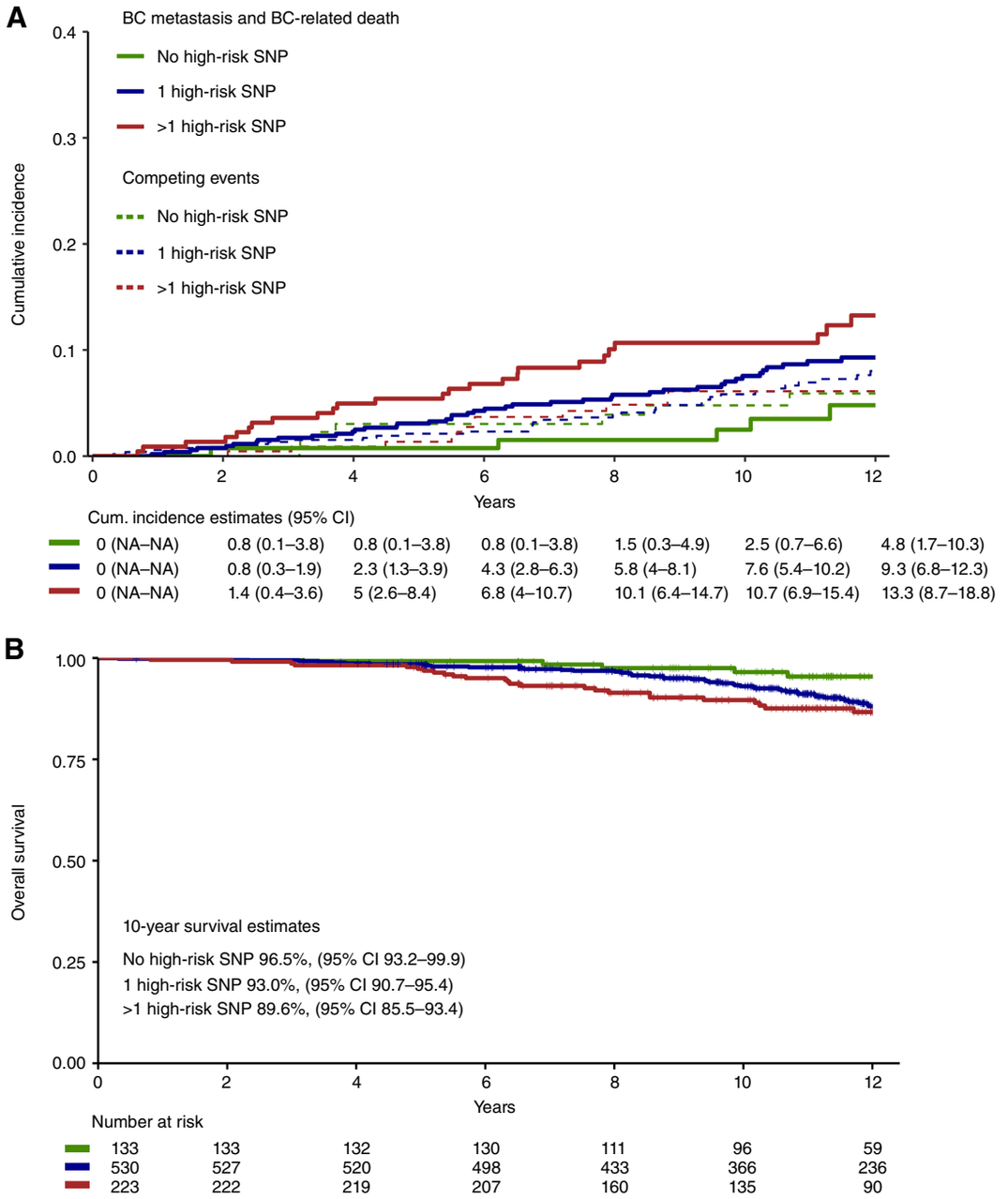

Figure 1.

Association of SNP of aromatase with survival outcomes. **A,** Cumulative incidence of breast cancer metastasis or breast cancer–related death and competing events across SNP-based groups; **B,** Kaplan–Meier curves for OS according to SNP-based groups. In **A**, solid lines represent incidence of the events of interest, and dashed lines represent the incidence of competing event from the cumulative incidence function.

Discussion

Our biomarker analysis of the GIM4 and GIM5 trials including women with ER^+ early breast cancer receiving adjuvant endocrine therapy with AI demonstrated that SNP of the aromatase are independent predictors of survival and AI-related adverse events. Through competing risk analysis, SNP identified three groups of patients with large differences in their risk of breast cancer metastasis and breast cancer-related deaths over 12 years of follow-up. Although such differences were not reflected in DFS overall, the relative contribution of distant recurrences to DFS outcomes increased progressively in patients with zero (27%), one (46%), and >1 (59%) high-risk SNP, leading to meaningful differences in OS. Intriguingly, SNP associated with the risk of breast cancer metastasis had a protective effect on the incidence of skeletal and CV events, indicating that women at higher risk of distant recurrence were also less likely to experience major AI-related toxicities over time.

Table 3. Multivariable competing risk model for breast cancer metastasis and breast cancer death versus competing events according to SNP-based prognostic groups and patients' baseline characteristics.

	BC metastasis and BC death $N = 878$, events $= 73$		Competing events $N = 878$, events $= 58$	
Variable	**sHR (95% CI)**	***P* value**	**sHR (95% CI)**	***P* value**
SNP-based groups				
0 high-risk SNP	1		1	
1 high-risk SNP	2.55 (1.00–6.45)	0.048	1.18 (0.55–2.55)	0.671
>1 high-risk SNP	3.48 (1.33–9.13)	0.011	1.06 (0.44–2.58)	0.890
Tumor size				
pT1	1		1	
pT2	1.90 (1.13–3.20)	0.016	1.29 (0.71–2.34)	0.402
pT3–4	3.56 (1.79–7.10)	<0.001	1.28 (0.44–3.73)	0.652
Nodal status				
pN0	1		1	
pN+	3.15 (1.62–6.13)	<0.001	1.16 (0.62–2.17)	0.647
(Neo)adjuvant chemotherapy				
No	1		1	
Yes	1.22 (0.52–2.84)	0.652	1.16 (0.62–2.17)	0.990
Age at diagnosis	0.99 (0.97–1.03)	0.940	1.03 (1.00–1.07)	0.037
Study cohort				
GIM4	1		1	
GIM5	0.95 (0.58–1.56)	0.835	0.37 (0.18–0.78)	0.009

Abbreviation: BC, breast cancer.

The lack of association between SNP and DFS is likely due to the increased contribution of contralateral breast tumors and non-breast cancer events in defining DFS over time, a trend which has already been observed in adjuvant studies with long-term follow-up (28). In our study, distant recurrences accounted for approximately half (47%) of DFS events, a percentage consistent with clinical trials on extended adjuvant endocrine therapy, where distant recurrences represented 33% to 47% of DFS outcomes (7, 29). These findings underscore the need of distinguishing between types of DFS events at long-term follow-up to improve patient prognostication and better inform treatment decisions. In fact, only patients for whom risk of distant recurrences remains the primary DFS event may derive a true survival benefit from prolonging adjuvant endocrine therapy.

Table 4. The association of SNP-based groups with OS on the basis of a multivariable Cox model.

	Overall survival $N = 878$, events $= 91$	
Variable	**HR (95% CI)**	***P* value**
SNP-based groups		
0 high-risk SNP	1	
1 high-risk SNP	2.42 (1.04–5.70)	0.040
>1 high-risk SNP	3.00 (1.24–7.32)	0.015
Tumor size		
pT1	1	
pT2	1.59 (0.99–2.59)	0.057
pT3–4	2.92 (1.55–5.51)	<0.001
Nodal status		
pN0	1	
pN+	2.16 (1.24–3.76)	0.007
(Neo)adjuvant chemotherapy		
No	1	
Yes	0.87 (0.45–1.66)	0.667
Age at diagnosis	1.06 (1.03–1.09)	<0.001
Study cohort		
GIM4	1	
GIM5	0.78 (0.46–1.28)	0.325

From this standpoint, deciding whether to extend endocrine therapy in patients who already completed 5 years of treatment remains a challenging clinical decision. Despite consistent improvements in DFS (2, 6, 19), treatment extension with AI for an additional 2 to 5 years failed to significantly improve OS in most randomized trials (2, 4). In exchange for this uncertain survival benefit, longer exposure to AI has been associated with a well-documented increase in clinically relevant toxicities, including skeletal (5, 8, 19) and CV (7, 8, 30, 31) events. In this clinical context, SNP of aromatase identified a group of patients for whom distant recurrence continued to be the main DFS event and the leading cause of death at longer follow-up, opening an opportunity for better treatment individualization. In women with >1 high-risk SNP, the 10-year incidence of metastasis (10.7%) was almost double the combined incidence of contralateral breast tumors, second malignancies, and non-breast cancer deaths (6%), making these patients likely to derive an OS benefit from extended endocrine treatment. On the other extreme, breast cancer metastasis exhibited a 10-year incidence of just 2.5% in patients without high-risk SNP. Because in this case breast cancer metastasis contributed less to OS than competing events, extended endocrine therapy is much less likely to improve OS in this population.

In assessing the indication to extended endocrine treatment, the prediction of toxicity is also becoming increasingly relevant (32). In our study, SNP associated with increased risk of breast cancer metastasis exhibited a protective effect on the incidence of skeletal and CV events. Such inverse impact aligns with previous data, linking SNP to significant changes in aromatase activity and circulating estrogen levels, which, in turn, may affect both efficacy and toxicity of the adjuvant endocrine therapy (11, 18). A substudy of the TEAM trial showed that SNP supposedly linked to decreased aromatase activity were associated with early musculoskeletal and vasomotor symptoms

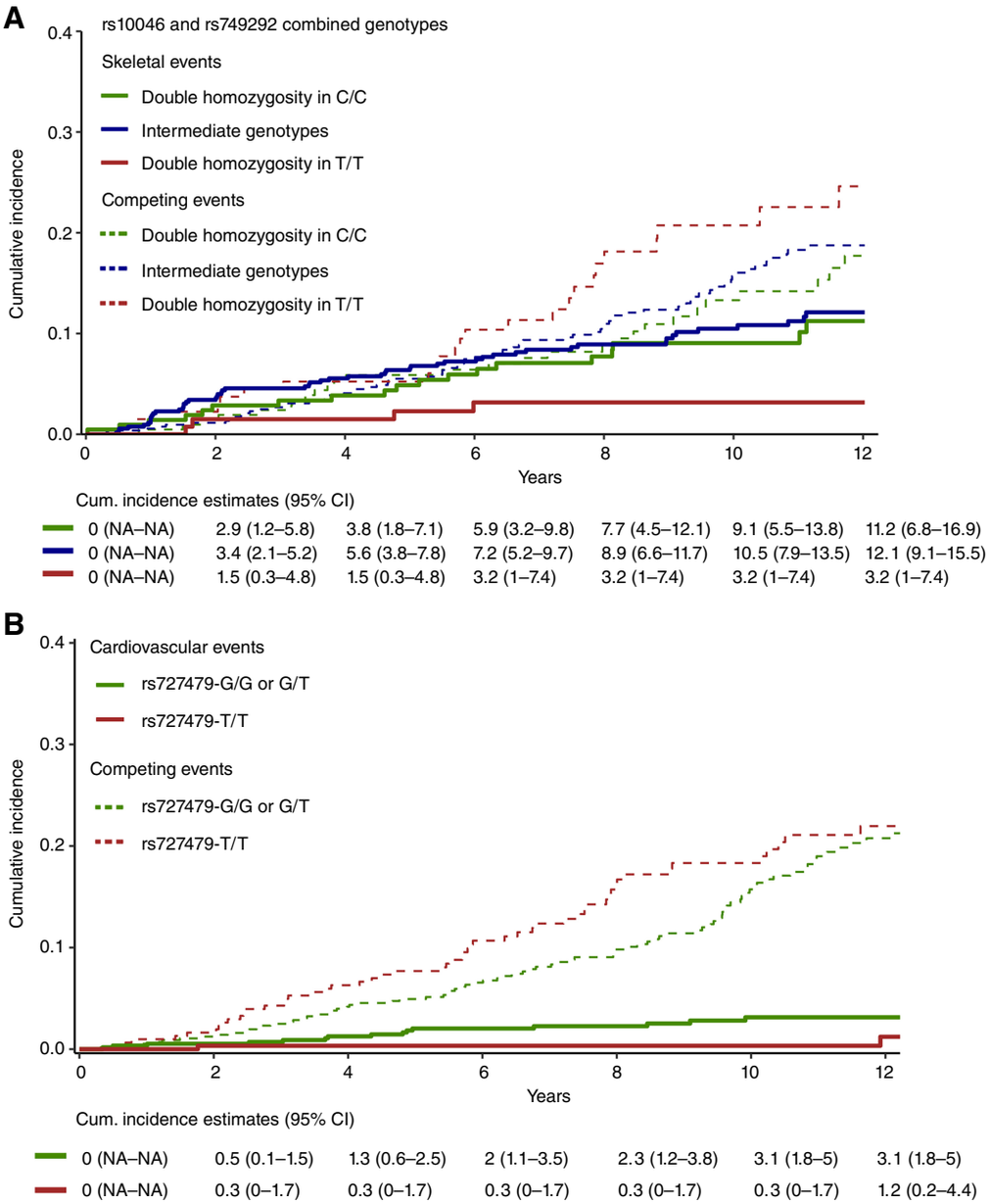

Figure 2.

Association of SNP of aromatase with letrozole-related toxicities. **A,** Cumulative incidence of skeletal events and competing events according to SNP rs10046 and rs749292 combined genotypes. **B,** Cumulative incidence of cardiovascular events and competing events according to SNP rs727479. Solid lines represent the incidence of the events of interest, and dashed lines represent the incidence of competing events from the cumulative incidence function.

in postmenopausal patients receiving exemestane (33). In another study, these SNP appeared to accelerate the negative effect of adjuvant AI on bone mineral density (34). On the other hand, SNP linked to increased aromatase activity may lead to a decrease in the efficacy of endocrine treatments, resulting in less side effects but also in worse prognosis. In the TEXT trial, patients with the T allele in rs10046 showed less vasomotor symptoms with ovarian suppression plus exemestane or tamoxifen, suggesting that women carrying this SNP may have less estrogen inhibition under endocrine therapy (15). Another study on 125 postmenopausal patients confirmed that rs10046 is associated with higher circulating estradiol and estrone levels, leading to a higher risk of breast cancer recurrence (12).

From a clinical standpoint, this Janus-faced effect offers the opportunity to pursue a more balanced assessment of individual benefits and risks of adjuvant endocrine treatment. Furthermore, it could present an avenue to better personalize survivorship care based on the individual risk of late adverse events. Bone fractures related to prolonged AI use have an established detrimental effect on patients' morbidity and quality of life (5), and while bone-modifying agents may counteract such effect, their use is associated

with additional costs and toxicity (35). Including SNP in the clinical evaluation of the risk of fractures could help selecting patients who may safely receive extended AI and be spared the side effects of bone-modifying agents. Among women with >1 high-risk SNP, those homozygous for the T allele in rs10046 and rs749292 had a 10-year incidence of skeletal events three times lower than that of breast cancer metastasis (3.2% vs. 10.7%). Therefore, these patients would be ideal candidates for endocrine treatment escalation and bone-targeted treatment de-escalation. The overall incidence of CV events was lower than expected in our study (31), probably as a result of limited enrollment of patients with CV risk factors. Nevertheless, SNP revealed significant differences in CV outcomes between patients. This finding is in line with previous evidence from *post hoc* analyses (8, 36), population-based studies (30, 31, 37), and results from the NSABP B-42 trial (7) indicating that longer AI exposure could lead to increased incidence of adverse CV events. In a real-world setting, SNP of aromatase might help identifying patients at higher risk of experiencing these adverse events with extended AI treatment and who may benefit from specific survivorship interventions.

This biomarker analysis presents constraints to be adressed in future studies. At the time of study enrollment, patients in the GIM5 trial had been free from recurrence for a significantly longer time compared with patients in the GIM4 trial (i.e., 4–6 years vs. 2–3 years). To address potential biases arising from such difference, all the results were adjusted for the study cohort in the multivariable analyses. Second, all patients in our study were treated with 2 to 6 years of adjuvant tamoxifen before starting letrozole. Although we did not observe any impact of longer tamoxifen exposure on SNP prognostic effect, it could not be ruled out that such an effect could be even greater on patients who are started on AI upfront. Future studies should investigate the magnitude of SNP effect in this population. Third, our study is characterized by heterogeneity in terms of timepoint and duration of extended treatment with AI, which precluded us to rule out whether SNP are predictive of extended AI benefit. The SNP associated with a higher risk of breast cancer metastasis are also expected to diminish the estrogen-deprivation activity of AI. Therefore, the question remains whether the detrimental effect of high-risk SNP on survival could be counteracted with extended AI. Moreover, because these SNP affect the tolerability of AI, their impact on treatment adherence and the potential consequences on patients' outcomes are also worthy to be explored in future studies. With respect to skeletal events, our study lacks longitudinal records on the prescription of bisphosphonates and denosumab. To establish the clinical use of SNP, future validation studies should investigate their role in bone toxicity prediction considering different durations of AI treatment and different patterns of bone-targeted agent administration. Similarly, the clinical utility of SNP in predicting CV toxicity should be further investigated in the context of patients' preexisting CV risk factors. Finally, our study population was entirely composed of white women. Because there are significant differences in linkage disequilibrium across ethnic groups (38), our study should be replicated in diverse populations.

In conclusion, this is the first study specifically designed to investigate the impact of SNP of aromatase on the long-term survival and toxicity outcomes of patients with ER^+ early breast cancer. Compared with previous reports, our study has a larger sample size, longer follow-up, and more clinically relevant endpoints to evaluate the long-term safety of adjuvant endocrine treatment. By providing complementary information on prognosis and AI-related toxicities at long-term follow-up, SNP of aromatase could present an avenue to individualize both adjuvant endocrine treatment and survivorship interventions based on one, ready-to-use, circulating biomarker.

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Authors' Contributions

B. Conte: Conceptualization, data curation, formal analysis, writing–original draft. **L. Boni:** Supervision. **G. Bisagni:** Project administration. **A. Durando:** Investigation. **G. Sanna:** Investigation. **S. Gori:** Investigation. **O. Garrone:** Investigation. **S. Tamberi:** Investigation. **S. De Placido:** Investigation. **F. Schettini:** Formal analysis. **A. Pazzola:** Investigation. **R. Ponzone:** Investigation. **F. Montemurro:** Investigation. **G. Lunardi:** Investigation, methodology. **R. Notaro:** Investigation, methodology. **M. De Angioletti:** Investigation. **A. Turletti:** Investigation. **M. Mansutti:** Investigation. **F. Puglisi:** Investigation. **A. Frassoldati:** Investigation. **M. Porpiglia:** Investigation. **A. Fabi:** Investigation. **D. Generali:** Investigation. **G. Scognamiglio:** Investigation. **M. Rossi:** Investigation. **F. Brasó-Maristany:** Investigation. **A. Prat:** Writing–review and editing. **B. Cardinali:** Methodology. **P. Piccioli:** Investigation, Methodology. **M. Serra:** Investigation. **S. Lastraioli:** Methodology. **C. Bighin:** Investigation. **F. Poggio:** Investigation. **M. Lambertini:** Writing–review and editing. **L. Del Mastro:** Conceptualization, data curation, project administration, writing–review and editing.

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