A pharmacogenetic interaction analysis of bevacizumab with paclitaxel in advanced breast cancer patients

Luigi Coltelli1,2,16, Giacomo Allegrini1,2,16, Paola Orlandi3,16, Chiara Finale1,2, Andrea Fontana4, Luna Chiara Masini1-2, Marco Scalese5, Giada Arrighi1,2, Maria Teresa Barletta1,2, Ermelinda De Maio1,2, Marta Banchi3, Elisabetta Fini3, Patrizia Guidi4, Giada Frenzilli3, Sara Donati6, Simona Giovannelli7, Lucia Tanganelli2, Barbara Salvadori8, Lorenzo Livi9, Icra Meattini9, Ilaria Pazzagli9, Marco Di Lieto9, Mirco Pistelli10, Virginia Casadei11, Antonella Ferro12, Samanta Cupini1,2, Francesca Orlandi1,2, Damiana Francesca1,13, Giulia Lorenzini4, Leonardo Barellini1,14, Alfredo Falcone4,15, Alessandro Cosimi1 and Guido Bocci1,16

To investigate pharmacogenetic interactions among VEGF-A, VEGFR-2, IL-8, HIF-1α, EPAS-1, and TSP-1 SNPs and their role on progression-free survival (PFS) in metastatic breast cancer (MBC) patients treated with bevacizumab plus first-line paclitaxel or with paclitaxel alone. Analyses were performed on germline DNA, and SNPs were investigated by real-time PCR technique. The multifactor dimensionality reduction (MDR) methodology was applied to investigate the interaction between SNPs. The present study was an explorative, ambidirectional cohort study: 307 patients from 11 Oncology Units were evaluated retrospectively from 2009 to 2016, then followed prospectively (NCT01935102). Two hundred and fifteen patients were treated with paclitaxel and bevacizumab, whereas 92 patients with paclitaxel alone. In the bevacizumab plus paclitaxel group, the MDR software provided two pharmacogenetic interaction profiles consisting of the combination between specific VEGF-A rs833061 and VEGFR-2 rs1870377 genotypes. Median PFS for favorable genetic profile was 16.8 vs. the 10.6 months of unfavorable genetic profile (p = 0.0011). Cox proportional hazards model showed an adjusted hazard ratio of 0.64 (95% CI, 0.5–0.9; p = 0.004). Median OS for the favorable genetic profile was 39.6 vs. 28 months of unfavorable genetic profile (p = 0.0103). Cox proportional hazards model revealed an adjusted hazard ratio of 0.71 (95% CI, 0.5–1.01; p = 0.058). In the 92 patients treated with paclitaxel alone, the results showed no effect of the favorable genetic profile, as compared to the unfavorable genetic profile, either on the PFS (p = 0.509) and on the OS (p = 0.732). The pharmacogenetic statistical interaction between VEGF-A rs833061 and VEGFR-2 rs1870377 genotypes may identify a population of bevacizumab-treated patients with a better PFS.

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INTRODUCTION

The treatment of metastatic breast cancer (MBC) patients with hormone-receptors positive (HR+) and human epidermal receptor 2 negative (HER2−) tumors is dramatically changed over the years. In this setting, cyclin-dependent kinase 4/6 inhibitors (CDK4/6i), such as palbociclib, ribociclib and abemaciclib, in combination with aromatase inhibitors or fulvestrant represent today the first and later lines of therapy1. However, chemotherapy-based treatment is still a therapeutic choice when hormone resistance occurs, in triple-negative tumor or in case of visceral crisis2,3. In this scenario, the humanized monoclonal antibody bevacizumab, in combination with paclitaxel, is a treatment option compared to chemotherapy alone4. Although a significant improvement in progression-free survival (PFS) was observed from three comparative studies, the US Food and Drug Administration (FDA), but not the European Medicines Agency (EMA), revoked the initial approval of bevacizumab for the first-line treatment of MBC patients because of the lack of benefit in terms of overall survival (OS). However, it has been theorized that when a long survival post first-line progression is expected after a first-line chemotherapy, such as in breast cancer, the lack of an apparent benefit in OS could not mean a lack of improvement in OS for the first line of treatment5-8.

Different strategies have been investigated to find possible predictive biomarkers and select those patients with the best chance of response to bevacizumab. Indeed, the PFS improvement due to bevacizumab was identical for magnitude in all subgroups of patients with different clinical and pathological characteristics9, and therefore new selective biomarkers should be needed to identify those patients who can have a major advantage in terms of outcome. Despite many attempts have been done, no validated biomarkers are currently available in the clinical practice and the prospective MERIDIAN trial failed to demonstrate a possible role of VEGF-A baseline in predicting the response to bevacizumab in breast cancer patients10-15.
Germline and somatic polymorphisms of genes involved in the angiogenic pathways have also been widely investigated in this research area to predict bevacizumab outcome, with contrasting results\(^\text{12,16-18}\). Due to the retrospective nature of these studies and to their inconclusive results, the role of single nucleotide polymorphisms (SNPs) as predictive markers remains to define\(^\text{19}\). Therefore, the current approach of correlating the bevacizumab response to a single SNP may be replaced by a genetic analysis of the interaction between SNPs, defined as non-linear interaction or epistasis. Moore and colleagues have established and validated a drug response \(^\text{20}\). To test this hypothesis, we conducted a retrospective study on 113 MBC patients to assess the ability of MDR methodology to identify a favorable pharmacogenetic profile associated to PFS in patients treated with bevacizumab, combined with first-line paclitaxel. The MDR analysis provided two pharmacogenetic interaction profiles consisting of the combination between specific \(VEGFR-2\) rs11133360 and \(IL-8\) rs4073 genotypes. The median PFS was 14.1 months (95% CI, 11.4–16.8) and 10.2 months (95% CI, 8.8–11.5) for the favorable and the unfavorable genetic profile, respectively (HR = 0.44; 95% CI, 0.29–0.66; \(p < 0.0001\))\(^\text{21}\).

Based on these encouraging results, our study was planned to evaluate the effects of the combination of paclitaxel with bevacizumab on patients harboring other different genetic profiles, exploring the possibility to predict the best favorable profile in terms of PFS, our primary endpoint. The second step was to test if the eventual seen PFS advantage could be maintained also in terms of OS (our secondary endpoint) in these patients even after the end of the administration of bevacizumab combined therapy. The analysis was extended to a group of patients treated without bevacizumab, during the same period of time, with the purpose of having a control group.

RESULTS

Patients

Two-hundred and fifteen patients treated with bevacizumab in combination with paclitaxel and 92 patients treated with first-line chemotherapy without bevacizumab, entered the present MDR analysis. In the bevacizumab plus paclitaxel group, the median number of cycles administered were 7 (range 4–18) and maintenance with bevacizumab alone was continued in 152 patients (70.7%).

All the 215 patients were evaluated for the response. 21 (10%) and 126 (58%) experienced a complete and a partial response, respectively; 52 patients (24%) reported a stable disease (SD) and in 16 patients (8%) a progression was observed. None of the analyzed polymorphisms was associated with the response rate (data not shown).

When the present analysis was performed, 215 out of 215 patients (100%) progressed and 170 out of 215 patients (79%) died from the metastatic disease. No patients died of cancer-unrelated causes. The median PFS and median OS were 11.8 months (95% CI, 10.9–12.7 months) and 30.7 months (95% CI, 26–35.5 months), respectively. Data were censored after 70 months.

The main characteristics of the patients are reported in Table 1. While the differences observed between groups may be due to the retrospective nature of the study, the characteristics between favorable and unfavorable group were superimposable.

### Table 1. Characteristics of patients at baseline treated with paclitaxel (PTX) plus bevacizumab (BEV) or paclitaxel alone.

| Factor                        | PTX + BEV | % PTX + BEV | PTX | % PTX | \(p\)-value |
|-------------------------------|-----------|-------------|-----|-------|-------------|
| Adjuvant CHT                  | No        | 76          | 35.3| 42    | 45.7        | 0.089       |
|                               | Yes       | 139         | 64.7| 50    | 54.3        |             |
| Adjuvant CHT with taxanes     | No        | 157         | 73.4| 71    | 77.2        | 0.483       |
|                               | Yes       | 57          | 26.6| 21    | 22.8        |             |
| Adjuvant HT                   | No        | 84          | 39.4| 35    | 38          | 0.819       |
|                               | Yes       | 129         | 60.6| 57    | 62          |             |
| DFI \(\geq 12\) months        | No        | 57          | 26.5| 31    | 33.7        | 0.202       |
|                               | Yes       | 158         | 73.5| 61    | 66.3        |             |
| \(\geq 3\) Pathological sites | No        | 145         | 67.8| 74    | 80.4        | 0.024       |
|                               | Yes       | 69          | 32.2| 18    | 19.6        |             |
| Visceral disease              | No        | 68          | 31.6| 38    | 41.3        | 0.102       |
|                               | Yes       | 147         | 68.4| 54    | 58.7        |             |
| HR positive                   | No        | 37          | 17.5| 9     | 9.8         | 0.086       |
|                               | Yes       | 175         | 82.5| 83    | 90.2        |             |
| Age > or <65 years            | >         | 171         | 79.5| 48    | 52.2        | 0.0001      |
|                               | <         | 44          | 20.5| 44    | 47.8        |             |

\(CHT\) chemotherapy, \(DFI\) disease-free interval, \(HR\) hormone receptor, \(HT\) hormonal therapy.
The MDR analysis revealed a genetic interaction profile, consisting of the combination between specific VEGF-A rs833061 and VEGFR-2 rs1870377 genotypes, significantly associated with PFS and OS benefit. Particularly, two pharmacogenetic profiles were identified in patients, as reported in Table 5. The first one was associated with a greater PFS and OS benefit, whereas the second one with a lower PFS and OS after paclitaxel plus bevacizumab treatment. The characteristics at baseline of patients treated with paclitaxel alone or paclitaxel + bevacizumab harboring the pharmacogenetic favorable and unfavorable profile are reported in Tables 6 and 7, respectively.

The median PFS for the favorable genetic profile was 16.8 months (95% CI, 13.1–20.5 months) vs. the 10.6 months of the unfavorable genetic profile (95% CI, 9.4–11.7 months; \( p = 0.0011 \), log-rank test; Fig. 1). The Cox proportional hazards model, which was performed to assess the adjusted hazard ratio for the PFS of the favorable genetic profile, showed a value of 0.64 (95% CI, 0.5–0.9; \( p = 0.004 \); Table 8). Furthermore, a formal test of interaction confirmed the predictive nature of the favorable profile in the bevacizumab + paclitaxel group as reported in Supplementary Table 1. Remarkably, the patients included in the favorable genetic profile also had the best probability of OS benefit, and the difference was significant as compared to the OS of the unfavorable genetic profile (Fig. 2). The median OS for the favorable genetic profile was 39.6 months (95% CI, 30.2–40.1 months) vs. the 28 months of the unfavorable genetic profile (95% CI, 24–32 months; \( p = 0.0103 \), log-rank test; Fig. 2). The Cox proportional hazards model, including the same significant parameters described in Table 8, revealed an adjusted hazard ratio for the OS of the favorable genetic profile of 0.71 (95% CI, 0.5–1.01; \( p = 0.058 \)), at the limit of significance. Of note, the probability of an estimated 1-year survival rate was 90.9% (95% CI, 90.4–91.4) in the favorable genetic profile and 80.5% (95% CI, 80.2–80.8) in the unfavorable genetic profile; the estimated 2-year survival was 75.7% (95% CI, 75.2–76.2) and 57% (95% CI, 56–57.4), respectively. The estimated 3-year survival rate was 56.1% (95% CI, 56.1–57.1) in the favorable genetic profile and 38.9% (95% CI, 38.4–39.3) in the unfavorable. The observed objective responses were 69.7% in the favorable genetic profile as compared with 69.1% in the unfavorable genetic profile.

Also, the 92 MBC patients treated with a first-line chemotherapy including paclitaxel without bevacizumab were investigated to test the impact of the two genetic profiles in both PFS and OS. The results revealed no effect of the favorable genetic profile, as compared to the unfavorable genetic profile, either on the PFS (\( p = 0.509 \), log-rank test; Supplementary Fig. 1a) or on the OS (\( p = 0.732 \), log-rank test; Supplementary Fig. 1b).
and novel targeted therapies. The CDK4/6 inhibitor palbociclib, ribociclib, and fulvestrant have dramatically changed the treatment of this setting of patients.

Despite these treatments’ advances, the chemotherapy maintains its key role because almost all metastatic patients with HR+ and HER2− disease develop resistance over time to endocrine therapy. As well, chemotherapy represents the first choice of treatment in triple negative, BRCA wild type and PD-L1 negative disease.

In this scenario, bevacizumab in combination with paclitaxel can still represent an option but the lack of any advantage in terms of OS, led to a slow decline in its use in the clinical practice during last years. Moreover, a recent meta-analysis has investigated, in head-to-head comparisons, in metastatic breast cancer with HR+ and HER2− disease, in first and later lines of treatment, is represented by combinations of hormone and novel targeted therapies. The CDK4/6i palbociclib, ribociclib, and abemaciclib in combination with aromatase inhibitors or fulvestrant have dramatically changed the treatment of this setting of patients1.

### DISCUSSION

The current standard therapy of patients suffering of metastatic breast cancer with HR+ and HER2− disease, in first and later lines of treatment, is represented by combinations of hormone and novel targeted therapies. The CDK4/6i palbociclib, ribociclib, and abemaciclib in combination with aromatase inhibitors or fulvestrant have dramatically changed the treatment of this setting of patients1.

Despite these treatments’ advances, the chemotherapy maintains its key role because almost all metastatic patients with HR+ and HER2− disease develop resistance over time to endocrine therapy. As well, chemotherapy represents the first choice of treatment in triple negative, BRCA wild type and PD-L1 negative disease.

In this scenario, bevacizumab in combination with paclitaxel can still represent an option but the lack of any advantage in terms of OS, led to a slow decline in its use in the clinical practice during last years. Moreover, a recent meta-analysis has investigated, in head-to-head comparison, the role of endocrine treatment versus chemotherapy in postmenopausal setting with HR+ and HER2− metastatic disease.
highlighting that bevacizumab in combination with paclitaxel was the only regimen that was significantly better than paclocilb plus letrozole in terms of response rate. Thus, the identification of pharmacodynamic biomarkers could better select patients with the best chance of bevacizumab response and clarify the role of this antiangiogenic antibody in the management of MBC patients.

The multifactor dimensionality reduction (MDR) methodology has been previously used to identify genetic polymorphisms interactions profiles able in predicting drug response in metastatic colorectal cancer patients. In the study published by Pander and colleagues, an interaction between VEGF+405G>C and TMYMS-TSER polymorphisms, instead of an individual polymorphism, seemed to predict the CAPOX-B (capectabine, oxaliplatin, and bevacizumab combination) response in terms of PFS, suggesting a paradigm shift from SNPs to a more complex interaction gene analysis able to predict response to antitumor agents.

The current study was planned to evaluate the effects of the combination of paclitaxel with bevacizumab versus paclitaxel alone on MBC patients harboring different genetic profiles, exploring the possibility to predict the best favorable profile in terms of PFS. The second step was to test if the eventual seen PFS advantage could be maintained also in terms of OS in these patients. The previous study on VEGFR-2 and IL-8 genetic interaction analysis, the favorable profile in terms of PFS was not predictive of OS benefit. In the present study, the seen advantage in PFS was indeed confirmed in OS (an adjusted hazard ratio of 0.71) but with a p = 0.058, a value very close to a statistically significance, but not significant. However, the reported data, although statistically negative, seem to suggest that the favorable profile in terms of PFS may probably be maintained also in terms of OS and undoubtedly merits further investigations in a validation prospective study. Indeed, evaluation and confirmation of these findings in an independent cohort is critical because of the exploratory nature of our ambidirectional trial.

The analyses conducted with the MDR methodology in this unselected population of MBC patients revealed more than a genetic interaction profile, consisting of the combination between specific genotypes, but, due to nature of the MDR methodology, we investigated the genetic profile with a benefit in terms of both PFS and OS. The analysis conducted revealed a genetic interaction profile, consisting of the combination between specific genotypes of VEGF rs833061 and VEGFR-2 rs1870377. Particularly, two genetic profiles were identified in patients, as reported in Table 5. The first one was associated with a greater both PFS and OS benefit compared to the second one. However, this model considered all the candidates and allows for any and all combination of SNPs to correlate with outcome. Thus, there are other significant or borderline permutations. Indeed, we have also included, as an example in the supplementary data (Supplementary Table 2 and Supplementary Fig. 2), another interesting genetic profile with a significant advantage in term of PFS but without any advantage in OS (not even a tendency).

Therefore, in our study we demonstrated, through the MDR methodology, a statistical interaction between VEGF-A and VEGFR-2 gene SNPs that potentially relates to bevacizumab efficacy on both PFS and OS. The two genes, and, consequently, the two proteins belong to the same signaling pathway, and it has been clearly demonstrated that VEGF-A stimulates VEGFR-2 phosphorylation and tumor angiogenesis. Based on these premises, it is conceivable to hypothesize that, in patients carrying the favorable genetic profile, the tumor angiogenesis is successfully inhibited in the presence of bevacizumab. The pharmacological inhibition of the angiogenic process by bevacizumab could be effective because of the physiological (not increased) production of VEGF-A due to the presence of VEGF-A rs833061 CC genotype or C allele. Indeed, for this SNP VEGF-A rs833061 C>T it has been described an increased promoter activity due to the T allele that may explain an eventual resistance to the treatment. Moreover, the VEGF-A rs1870377 is a nonsynonymous SNP substituting glycine with histidine (Q472H) located in the extracellular ligand binding region of the receptor, potentially impacting VEGFR-2 degradation. The VEGFR-2 rs1870377 TT genotype or T allele present in the favorable profile synthesize a receptor not modified in its structure, suggesting that it is not abnormally activated or degraded. Therefore, it might be plausible that the genetic background characterized by a physiological activation of the VEGF-A pathway may be responsible, in part, for the positive effect of bevacizumab maintenance therapy in these MBC patients. In contrast, in patients with an unfavorable genetic profile, the microenvironment conditions due to the different genotype combinations may result in an increase of the VEGF-A production and/or the presence of an

Table 7. Characteristics of patients treated with paclitaxel plus bevacizumab with pharmacogenetic favorable (fav) and unfavorable (unfav) profile at baseline.

|                      | fav (N) | % N    | unfav (N) | % N   | p-value |
|----------------------|---------|--------|-----------|-------|---------|
| Adjuvant CHT         | No      | 24     | 36.4      | 52    | 34.9    | 0.836   |
|                      | Yes     | 42     | 63.6      | 97    | 65.1    |         |
| Adjuvant CHT with taxanes | No | 49     | 74.2      | 108   | 73.0    | 0.846   |
|                      | Yes     | 17     | 25.8      | 40    | 27.0    |         |
| Adjuvant HT          | No      | 29     | 44.6      | 55    | 37.2    | 0.305   |
|                      | Yes     | 36     | 55.4      | 93    | 62.8    |         |
| DFI ≥ 12 months      | No      | 20     | 30.3      | 37    | 24.8    | 0.402   |
|                      | Yes     | 46     | 69.7      | 112   | 75.2    |         |
| ≥3 Pathological sites| No      | 49     | 74.2      | 96    | 64.9    | 0.175   |
|                      | Yes     | 17     | 25.8      | 52    | 35.1    |         |
| Visceral disease     | No      | 22     | 33.3      | 46    | 30.9    | 0.720   |
|                      | Yes     | 44     | 66.7      | 103   | 69.1    |         |
| HR positive          | No      | 10     | 15.2      | 27    | 18.5    | 0.553   |
|                      | Yes     | 56     | 84.8      | 119   | 81.5    |         |
| Age > or <65 years   | >       | 10     | 84.8      | 34    | 22.8    | 0.199   |
|                      | <       | 56     | 15.2      | 115   | 77.2    |         |

CHT chemotherapy, DFI disease-free interval, HR hormone receptor, HT hormonal therapy.
altered VEGFR-2 on tumor endothelial cells which may be capable to proliferate, migrate or survive because the VEGF action is not completely blocked by bevacizumab.

The absence of any advantage in terms of efficacy in the patients treated with chemotherapy without bevacizumab could suggest a possible predictive role of the favorable genetic profile for bevacizumab response, but the exploratory nature of this ambidirectional study may limit this hypothesis. However, the main findings of our analyses support the conclusion that a genetic profile may identify a group of patients with longer PFS and OS, predicting the response to bevacizumab in combination with paclitaxel.

The MDR approach is a major reason for differences between our trial and other studies on bevacizumab biomarkers such as E210016. There are additional aspects between the E2100 US patients and the Italian population of our study that may account for different results. First of all, Italian patients were of Caucasian

| Table 8. Multivariable Cox regression model, including significant variables at the univariate analysis in patients treated with paclitaxel and bevacizumab. |
| --- |
| Progression-free survival (N = 215) |
| Characteristics | HR | 95% CI | \( p \) |
| Hormone Receptor Negative | 1 | | |
| Positive | 0.48 | 0.33–0.70 | <0.0001 |
| Sites involvement | | |
| <3 | 1 | | |
| \( \geq 3 \) | 1.51 | 1.11–2.04 | 0.008 |
| Favorable genetic profile No | 0.64 | 0.47–0.86 | 0.004 |
| Yes | 1 | | |
| HR hazard ratio, CI confidence interval |

Fig. 1 Progression-free survival (PFS) curves in patients treated with paclitaxel and bevacizumab calculated by the Kaplan–Meier method, according to the favorable (blue line) and unfavorable (green line) genetic profiles, with the adjusted hazard ratio (HR). CI confidence interval.
origin and no patients of African origin were represented. Secondly, although the frequencies of our studied VEGF-A and VEGFR-2 SNPs were superimposable with the ones of the Caucasian patients published in the article by Schneider and colleagues, there was an exception regarding the VEGFR-2 889A/G (rs2071559). In that case, the frequency of the minor allele A in our population was 0.49 whereas in the E2100 study was 0.09.

New pharmacogenetic favorable biomarkers of bevacizumab-combined therapies could be retrieved from a genetic analysis of the interaction among SNPs rather than from the examination of a single SNP of a single gene. Surely, a multigene-risk biomarkers may be more beneficial from a comprehensive agnostic approach using genome-wide association studies (GWAS) rather than a candidate gene approach as the one that we have used in our study. However, some challenges have been faced when scientists tried the scaling of MDR to big data, as the one from GWAS, such as the necessity to filter the data prior to MDR analysis, also using biological knowledge through tools such as BioFilter. Moreover, our work can definitively be strengthened by the biological characterization of the VEGF expression in the pre-treatment tissue.

Indeed, since rs833061 is located in the promoter region of VEGF-A, the difference in expression levels of VEGF-A in tumors of patients harboring the favorable vs. unfavorable profile could be an important strategy to confirm our statistical findings.

In conclusion, the MDR methodology could be successfully used as witnessed by the experience in this unselected MBC patients where the investigation of an interaction between VEGF-A rs833061 and VEGFR-2 rs1870377 gene polymorphisms resulted in the identification of a genetic profile associated with a longer PFS.

METHODS

Study population

This is an explorative, ambidirectional cohort study, meaning that eligible patients were enrolled and evaluated retrospectively from January 2009 until September 2016 and then followed prospectively. The oncology units, all located in the north or center of Italy, were selected based on their clinical experience in the use of the combination of paclitaxel and bevacizumab as first-line therapy in histologically confirmed HER-2-negative MBC patients. Two-hundred fifteen patients from 11 Italian
Pharmacogenetic study. Ninety-two MBC patients treated with a schedule and criteria for the prospective follow-up. The protocol was approved by the ethical committee of Azienda Ospedaliero-Universitaria Pisana (CESM-AOUP 3077/2010; clinicaltrials.gov identifier NCT01935102) for Pisa, Livorno, Lucca, Massa Carrara, Versilia, and Pontedera Hospitals, and by the ethic committees of all participating centers.

Genotyping analyses

Blood samples (3 ml) were collected in EDTA tubes and stored at −80 °C. Genes and polymorphisms, involved in the angiogenesis pathways, were selected for the present analyses based on our previous study21. In the Table 9, the selected polymorphisms are reported. Germline DNA extraction was performed using QiAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). Allelic discrimination of genes was performed using an ABI PRISM 7900 SDS (Applied Biosystems, Carlsbad, CA, USA) and with validated TaqMan® SNP genotyping assays (Table 9; Applied Biosystems). PCR reactions were carried out according to the manufacturer’s protocol. Genotyping was not performed until an adequate number of events (>80% on study population) was reported in terms of PFS. All the samples were analyzed twice to replicate the obtained genotype.

Statistical analysis

The investigators responsible for data analysis were blinded to which samples were from patients treated with paclitaxel alone and paclitaxel plus bevacizumab.

The aim of the present study was to identify a favorable genetic profile in terms of PFS in MBC patients treated with bevacizumab in association with paclitaxel. The corresponding OS in these patients remained a secondary endpoint as well as response rate. All polymorphisms were analysed for deviation from the Hardy–Weinberg Equilibrium (HWE) by means of comparison between observed allelic distributions with those expected from the HWE by on χ² test (see Supplementary Tables 3 and 4). Any association between gene polymorphisms and response rate was analysed by the two-sided Fisher’s exact test. The association between each individual polymorphism and the most relevant clinical-pathological characteristics with PFS and OS was tested using a Cox proportional hazards model. In these analyses we used Bonferroni’s correction and the p value <0.00357 (0.05/14 SNPs = 0.00357) was accepted as statistically significant. The multifactor dimensionality reduction (MDR) methodology was applied (MDR software version 2.0 beta 6 on http://sourceforge.net/projects/mdr/, last access January 2021) to investigate the interaction between gene polymorphisms and to identify favorable genetic profiles associated with the greater PFS in this population of patients. MDR was developed as a non-parametric and genetic model-free data mining strategy for identifying combinations of SNPs that are predictive of a discrete clinical endpoint. MDR approach is a constructive induction algorithm that creates a new attribute by pooling genotypes from multiple SNPs16,18. The difference both in PFS and OS between favorable genetic profiles and the unfavorable genetic profiles were assessed with the log-rank test and the Kaplan–Meier method to evaluate survival curves. A Cox proportional hazards model, with the possible genetic profiles and the clinical and pathological patient’s characteristics individually related with both the PFS and OS, was used to calculate the adjusted hazards ratio (HR) and the 95% confidence interval (95% CI). The Kaplan–Meier and Cox proportional hazards analyses were performed using the SPSS version 17.0 (SPSS, Chicago, IL). For the genotype combination we used a statistical correction. Indeed, the p value for the statistical significance was obtained using 1000-fold permutation testing (software available on https://sourceforge.net/projects/mdr/files/mdrpt/), and the significance was set for values less than 0.05.

As an explorative study in nature, no estimation of power and sample size was performed because of the absence of previous published data regarding the specific investigated genetic profiles and the administered combination treatment. No data were excluded from the analysis.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.
