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Prospective analysis of the impact of *VEGF-A* gene polymorphisms on the pharmacodynamics of bevacizumab-based therapy in metastatic breast cancer patients

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Functional polymorphisms on the *VEGF-A* gene, known to be linked to cancer risk or to VEGF-A plasma concentrations, have been identified. So far, limited knowledge has been published on the relationships between toxicity/efficacy of bevacizumab-based therapy and *VEGF-A* polymorphisms (tumoral DNA). We therefore prospectively tested the impact of these five gene polymorphisms (blood DNA) on the pharmacodynamics of bevacizumab-based treatment administered in metastatic breast cancer patients.

WHAT THIS STUDY ADDS

- Present data obtained from a prospective study suggest a role for *VEGF-A* 936C > T polymorphism as a potential predictor of time to progression in breast cancer patients receiving bevacizumab-containing therapy. Also, the *VEGF-A* –634G > C polymorphism was linked to bevacizumab-related toxicity.

AIMS

To test prospectively the impact of *VEGF-A* gene polymorphisms on the pharmacodynamics of bevacizumab-chemotherapy in breast cancer patients.

METHODS

As part of the single-arm MO19391 trial, 137 women with locally recurrent or metastatic breast cancer receiving first-line bevacizumab-containing therapy were analysed. Patients received bevacizumab associated (76%) or not (24%) with taxane-based chemotherapy. Clinical evaluation included clinical response, time to progression (TTP) and a toxicity score corresponding to the sum of each maximum observed toxicity grade (hypertension, haemorrhage, arterial and venous thrombo-embolism). Functional *VEGF-A* polymorphisms at position –2578 C > A, –1498 T > C, –1154 G > A, –634 G > C and 936 C > T were analysed by PCR-RFLP (blood DNA).

RESULTS

Overall response rate (complete response (CR) + partial response (PR)) was 61%. Median TTP was 11 months. None of the *VEGF-A* polymorphisms was significantly linked to clinical response. Analysis of the 936C > T polymorphism revealed that the 96 patients homozygous for the 936C allele exhibited a marked tendency for a shorter TTP (median 9.7 months) than the 32 patients bearing the 936T allele (median 11.5 months, $P = 0.022$) of which 30 were CT and two were homozygous TT. Other polymorphisms did not influence TTP. *VEGF-A* –634 G > C was significantly related to the toxicity score with 39%, 49% and 81% of patients with score >1 in GG, GC and CC patients, respectively ($P = 0.01$).

CONCLUSIONS

The role for *VEGF-A* 936C > T polymorphism as a potential marker of TTP in breast cancer patients receiving bevacizumab-containing therapy concords with the known impact of *VEGF-A* 936C > T polymorphism on VEGF-A expression.

Introduction

Vascular endothelial growth factor A (VEGF-A) is a key angiogenic factor which promotes endothelial cell growth and tumour neovascularization. VEGF-A expression is a marker of invasiveness and tumour progression in various cancers, including breast cancer [1, 2]. Bevacizumab is a recombinant humanized monoclonal antibody that binds to VEGF-A and blocks VEGF binding to its receptors [3]. In a randomized phase III trial, the addition of bevacizumab to paclitaxel, administered as first-line treatment for metastatic breast cancer, significantly improved response rate and progression-free survival, along with minimal impact on toxicity [4].

In breast cancer, as well as in other cancer localizations, most studies have failed to identify biomarkers for predicting efficacy and/or toxicity of angiogenic agents, including bevacizumab [5–7]. One possible easy-to-perform approach for predicting inter-patient variability of drug effects is to study germinal gene polymorphisms potentially linked to drug pharmacodynamics and/or pharmacokinetics. Among genes potentially linked to inter-patient variability of bevacizumab pharmacodynamics is the *VEGF-A* gene which is highly polymorphic, with multiple common single nucleotide polymorphisms (SNPs) in the promoter, 5'untranslated and 3' untranslated regions [8]. Among them, five functional polymorphisms at positions –2578 C > A, –1498 T > C, –1154 G > A, –634 G > C and 936 C > T (Figure 1) have been associated with serum VEGF-A [9, 10] or cancer risk [11–13], including breast cancer risk or aggressiveness [10, 14, 15]. In a recent study conducted in patients receiving or not bevacizumab, it has been suggested that four out of these five polymorphisms may influence bevacizumab pharmacodynamics [16].

The aim of this prospective study was to test the impact of these five major functional *VEGF-A* gene polymorphisms on the efficacy of bevacizumab chemotherapy and on bevacizumab-related toxicity, in breast cancer patients treated as part of the large observational international MO 19391 (ATHENA) trial.

Methods

Patients and treatment

The single-arm, multinational, safety study MO19391 (ATHENA) EUDRACT # 2006-002529-21 was conducted in the context of general oncology practice in 2251 patients receiving bevacizumab in combination with taxane-based chemotherapy, for HER2 negative, locally recurrent or metastatic breast cancer. The study started in September 2006 and ended in August 2009. A companion study was conducted in 27 French centres to evaluate *VEGF-A* polymorphisms as potential predictors of treatment efficacy and safety. A total of 137 women were enrolled in this pharmacogenetic study, carried out with ethics committee approval. All patients gave informed consent. Patients received bevacizumab (10 mg kg^{-1} every 2 weeks or 15 mg kg^{-1} every 3 weeks), combined or not with a taxane-based standard chemotherapy, until disease progression, unacceptable toxicity or withdrawal. Adverse events related to bevacizumab were assessed according to the NCI CTCAE v3.0 criteria. Clinical response was assessed according to modified RECIST v1.0 criteria.

Pharmacogenetic analyses

VEGF-A gene polymorphisms were analysed by PCR-RFLP on DNA extracted from a 9 ml blood sample taken at baseline (Paxgene Blood DNA kit, Prenalytics), as previously described [8]. The positions of the analysed genotypes, given relative to the initiation of translation (Figure 1), were the following: –2578 C > A (promoter region), –1498 T > C (promoter region), –1154 G > A (promoter region), –634 G > C (5'UTR) and 936 C > T (3'UTR).

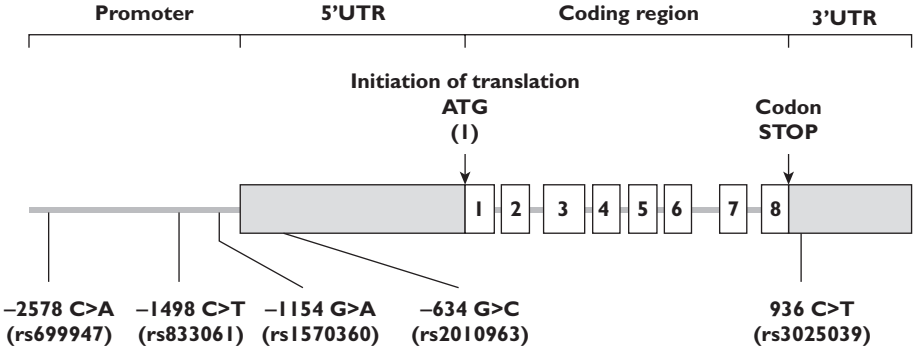

Figure 1

VEGF-A gene and analysed polymorphisms. UTR untranslated region

Statistics

Possible relationships between patient characteristics (age below *vs.* above the median value, Eastern Cooperative Oncology Groug (ECOG) performance status (PS) status 0 *vs.* 1–2, oestrogen receptor (ER) status, progesterone receptor (PR) status, number of metastatic lesions, associated chemotherapy taxane-containing regimen *vs.* non-taxane regimen *vs.* switched therapy) and clinical end-points were tested by means of chi-square or Fischer exact test. Time to progression (TTP) and overall survival (OS) were computed from the start of bevacizumab therapy. For OS, death due to any cause was considered. TTP and OS were analysed using the Kaplan-Meier method and the influence of the different polymorphisms was evaluated using a log-rank test. Survival curves for each polymorphism were also performed. Tumour response rate was analysed according to each polymorphism using a chi-square or Fischer exact test. To assess the possible relationships between *VEGF-A* gene polymorphisms and bevacizumab-related toxicities, we computed a toxicity score corresponding to the sum of each maximum observed toxicity grade in order to minimize the number of statistical tests. Thus, we did not report *P* values for each toxicity pattern but compared the genotype distribution of the five analysed polymorphisms between the group of patients having developed a toxicity score $\leq$ the median value and the group of patients exhibiting a toxicity score > the median value, by means of the Fischer exact test. Due to the number of analysed genotypes (five comparisons for each end-point), we applied the Bonferroni correction [17]: for a global risk α at 0.05, the *P* value to be applied in order to achieve a conclusive significant difference was thus 0.01. The above statistics were performed using SAS® 9.1.3. software. In addition, r^2 values of linkage disequilibria between the five analysed *VEGF-A* gene polymorphisms, along with *P* values (Fischer's Exact test), were computed using the SHEsis software platform dedicated to genetic analyses (http:analysis.bio-x.cn) [18].

Results

Patient population and genotypes

Baseline characteristics of the 137 enrolled patients are shown in Table 1. Median duration of bevacizumab treatment was 7 months. The genotype distribution of the five analysed *VEGF-A* polymorphisms agreed with those predicted by the Hardy–Weinberg equilibrium. Strong linkage disequilibria were observed between *VEGF-A* gene polymorphisms located in the promoter and in the 5'UTR region (Table 2).

Impact of gene polymorphisms on treatment efficacy

Clinical response, assessable in 127 patients, showed 10 complete responses (CR), 67 partial responses (PR), 41 stable diseases (SD) and nine patients with progressive disease (PD), accounting for an overall response rate (CR + PR) of 60.6%. None of the analysed *VEGF-A* polymorphisms was significantly linked to clinical response (Table 3).

Median follow-up was 16 months. All patients (n = 137) were assessable for TTP and OS. At time of analysis, 82 patients had developed progressive disease (60%) and 26 patients had died (19%), all from breast cancer. Median TTP was 11 months. Interestingly, analysis of the *VEGF-A* 936C > T polymorphism revealed that patients bearing the 936T allele (936CT, 30 patients and 936TT, two patients) exhibited a marked trend towards a longer TTP as compared with patients homozygous for the 936C allele (n = 96, Log Rank P = 0.022, Table 3, Figure 2). Median TTP was 11.5 months (95% CI 10.2, 25.8) in 936CT or 936TT patients *vs.* 9.7 months (95% CI 7.8, 12) in 936 CC patients. Other polymorphisms did not influence TTP (Table 3).

The influence of the *VEGF-A* gene polymorphisms on OS did not reveal any significant relationship, but was limited by the few number of events (n = 26).

Table 1

Patient characteristics (n = 137)

Age (years)	
Median	56
Range	24–79
Ethnicity	
Caucasian	136 (99.3%)
Other	1 (0.7%)
ECOG performance status	
0	87 (63.5%)
1	45 (32.8%)
2	5 (3.6%)
Hormonal receptor status (primary disease)	
Oestrogen receptor (positive/negative)	99/36 (72.3/26.3%)
Progesterone receptor (positive/negative)	81/52 (59.1/38%)
Number of metastatic lesions	
≤3	39 (29.1%)
>3	95 (70.9%)
Not documented	3
Associated chemotherapy	
Paclitaxel	32 (24.2%)
Docetaxel	55 (42%)
Navelbine	3 (2.3%)
Taxane combination	12 (9.2%)
Non-taxane combination	3 (2.3%)
Switched chemotherapy	26 (19.8%)
Not documented	6
Number of cycles with bevacizumab	
Median	12
Range	3–36
3 cycles	6 (4.6%)
4 to 6 cycles	26 (19.8%)
7 to 9 cycles	21 (16%)
>9 cycles	78 (59.5%)
Not documented	6

Table 2

Linkage disequilibria between *VEGF-A* gene polymorphisms (r^2 and *p* computed on SHEsis software)

		–2578C > A			–1498T > C			–1154G > A			–634G > C		
		CC	CA	AA	TT	TC	CC	GG	GA	AA	GG	GC	CC
–1498T > C	TT	39	4	0									
	TC	3	55	2									
	CC	0	1	2									
		r^2 = 0.816											
		P < 0.00001											
–1154G > A	GG	41	16	3	41	17	2						
	GA	1	44	11	2	44	11						
	AA	0	0	17	0	0	17						
		r^2 = 0.572			r^2 = 0.566								
		P < 0.00001			P < 0.00001								
–634G > C	GG	7	20	29	6	20	30	14	25	17			
	GC	20	40	2	23	40	0	31	32	0			
	CC	15	0	0	14	1	0	15	0	0			
		r^2 = 0.381			r^2 = 0.415			r^2 = 0.272					
		P < 0.00001			P < 0.00001			P < 0.00001					
936C > T	CC	34	41	24	33	44	23	47	39	14	45	43	12
	CT	8	17	6	10	15	6	13	15	3	10	18	3
	TT	0	2	1	0	2	1	0	3	0	1	2	0
		r^2 = 0.007			r^2 = 0.001			r^2 = 0.002			r^2 = 0.004		
		P = 0.17			P = 0.63			P = 0.48			P = 0.29		

Table 3

Links between *VEGF-A* genotypes and treatment efficacy*

Genotype	CR + PR	SD + PD	*P*†	Median TTP (month)	*P*‡
–2578					
CC	25 (64.1%)	14 (35.9%)		9.0	
CA	29 (51.8%)	27 (48.2%)	0.20	10.7	0.71
AA	20 (71.4%)	8 (28.6%)		11.5	
–1498					
TT	27 (64.3%)	15 (35.7%)		8.9	
TC	29 (50.9%)	28 (49.1%)	0.14	11.1	0.54
CC	19 (73.1%)	7 (26.9%)		11.5	
–1154					
GG	34 (60.7%)	22 (39.3%)		8.9	
GA	31 (58.5%)	22 (41.5%)	0.87	12.0	0.36
AA	10 (66.7%)	5 (33.3%)		10.4	
–634					
GG	34 (66.7%)	17 (33.3%)		11.1	
GC	33 (55.9%)	26 (44.1%)	0.54	10.7	0.46
CC	9 (60%)	6 (40%)		6.9	
936					
CC	53 (57%)	40 (43%)		9.7	
CT + TT	22 (71%)	9 (29%)	0.21	11.5	0.022

*127 and 137 patients were assessable for clinical response and TTP, respectively. Due to missing genotypes (poor quality DNA), relationships between clinical endpoints and genotypes were performed on lower figures, depending on the analysed genotype. †Fischer Exact Test, ‡Log Rank Test. CR complete response, PR partial response, SD stable disease, TPP time to progression.

None of the patient characteristics was associated with clinical response, TTP, or OS.

Impact of gene polymorphisms on bevacizumab-related toxicity

The analysed bevacizumab-related toxicities were hypertension, haemorrhage, arterial and venous thrombo-embolism. Globally, these toxicities were mild (Table 4). The toxicity score ranged between 0 and 6 (mean 1.8, median 1): 70 patients had a score 0–1 and 67 patients had a score 2 to 6. The toxicity score was not related to any of the patient characteristics. Table 5 shows that the *VEGF-A* –634 G > C polymorphism was significantly related to the toxicity score, with 39%, 49% and 81% of patients with score >1 in GG, GC and CC patients, respectively (P = 0.01). Close examination of each toxicity pattern included in this score revealed that patients with the –634C allele were prone to develop hypertension and thrombo-embolism.

Discussion

Elevated tumoral expression of VEGF-A, usually referred to as VEGF, is a very strong marker of cancer progression in node-negative breast carcinoma not receiving any adjuvant treatment [1, 2], and a strong predictor of poor efficacy of tamoxifen and chemotherapy in metastatic breast cancer patients [19]. This may suggest that breast tumours overexpressing VEGF-A may be good candidates for anti-VEGF target therapies, even though such a link has not been demonstrated so far [20]. Among the numerous

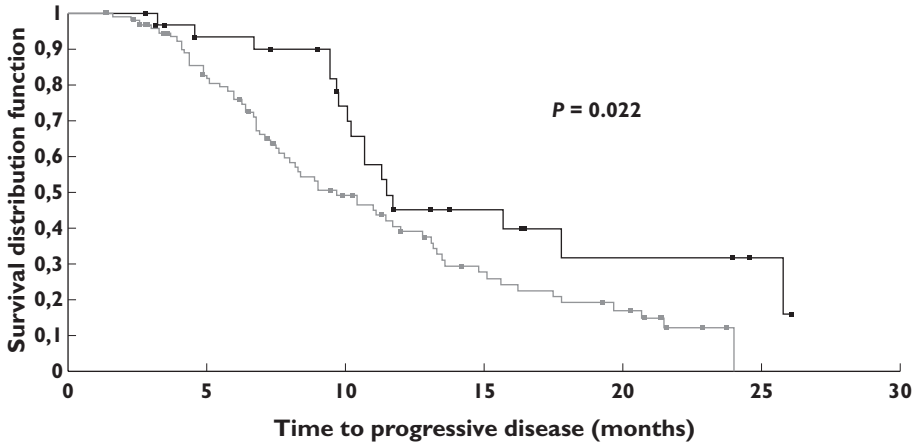

Figure 2

Time to progression (TTP) according to *VEGF*- 936C > T polymorphism. Median TTP was 9.7 months in 936CC patients *vs.* 11.5 months in patients bearing the 936T allele (Log Rank, $P = 0.022$). CC ($n = 96$) : 65 events (——); CT ($n = 30$) + TT ($n = 2$) : 17 events (——)

Table 4

Description of the bevacizumab-related toxicity*

	Grade 0 *n* (%)	Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)
Hypertension	81 (59.1%)	23 (16.8%)	20 (14.6%)	13 (9.5%)	0
Haemorrhage	37 (27.0%)	85 (62.0%)	15 (10.9%)	0	0
Arterial thrombo-embolism	135 (98.5%)	0	1 (0.7%)	0	1 (0.7%)
Venous thrombo-embolism	128 (93.4%)	1 (0.7%)	4 (2.9%)	3 (2.2%)	1 (0.7%)

*Maximum observed toxicity grade (NCI-CTCAE criteria).

studies aimed at evaluating the predictive value of VEGF-A expression on bevacizumab efficacy, one study conducted on 56 advanced breast cancer patients reported that low baseline circulating VEGF was significantly linked to longer TTP [21]. Interpretation of biomarker studies such as VEGF-A showed the difficulty to discriminate between its predictive value and its prognostic value. In this regard, the randomized trial by Dowlati *et al*. [22] conducted on 163 non-small-cell-lung cancer patients receiving carboplatin-paclitaxel ± bevacizumab is very informative. Patients with high baseline VEGF plasma concentrations had an increased probability of response with the addition of bevacizumab (33% *vs.* 7.7% with and without bevacizumab, respectively).

VEGF-A expression is controlled by complex mechanisms involving VEGF-A mRNA splicing that generates multiple VEGF-A isoforms including a pro-angiogenic isoform family and an anti-angiogenic isoform family [23] and various controls at the gene level itself, particularly at the 5′ and 3′ untranslated regions that contain key regulatory elements. Importantly, functional gene polymorphisms have been described in these regulatory regions, as well as in the promoter region (Figure 1). Since analysis of germinal *VEGF-A* polymorphisms from a blood sample is much more reliable and easy than analysis of tumoral or plasma VEGF-A (*ex vivo* release of VEGF has been reported in blood samples), the aim of the present study was to analyse prospectively the possible relationships between the five major functional germinal *VEGF-A* polymorphisms and pharmacodynamics of first-line bevacizumab-based therapy in metastatic breast cancer patients included in the single-arm safety MO19391 trial. This pharmacogenetic companion study was conducted on 137 women, whose characteristics were not significantly different from that of the entire cohort of patients (data not shown). Patient characteristics were not related to any of the analysed clinical end-points, thus justifying the univariate analyses performed to test the possible relationships between genotypes and toxicity score, TTP or OS.

The presently analysed pharmacodynamic–pharmacogenetic relationships were specific to bevacizumab for the toxicity since we strictly considered the bevacizumab-related toxicity, i.e. hypertension, haemorrhage and thrombo-embolism (Table 4) [24]. Patients bearing the –634 C allele presented a significant higher

Table 5

Links between *VEGF-A* genotypes and bevacizumab-related toxicity*

Polymorphism	*n*	% of patients with grade 1-2-3-4 hypertension	% of patients with grade 1-2-3-4 haemorrhage	% of patients with grade 1-2-3-4 arterial thromboembolism	% of patients with grade 1-2-3-4 venous thromboembolism	% of patients with toxicity score > 1
–2578C > A						
CC	42	45.2%	66.7%	4.8%	4.8%	57.1%
CA	60	41.7%	71.7%	0%	6.7%	48.3%, *P* = 0.18
AA	31	29.0%	83.9%	0%	6.5%	35.5%
–1498T > C						
TT	44	43.2%	65.9%	4.5%	4.5%	52.3%
TC	61	44.3%	72.1%	0%	8.2%	50.8%, *P* = 0.39
CC	30	26.7%	83.3%	0%	6.7%	36.7%
–1154G > A						
GG	60	41.7%	66.7%	3.3%	5.0%	53.3%
GA	57	40.4%	77.2%	0%	10.5%	47.4%, *P* = 0.43
AA	17	29.4%	82.4%	0%	0%	35.3%
–634G > C						
GG	56	30.4%	82.1%	0%	5.4%	39.3%
GC	63	44.4%	68.3%	1.6%	6.3%	49.2%, *P* = 0.012
CC	16	56.3%	62.5%	6.3%	12.5%	81.3%
936C > T						
CC	100	41.0%	75.0%	1.0%	9.0%	49.0%
CT	31	35.5%	67.7%	3.2%	0%	48.4%, *P* = 1.00
TT	3	33.3%	66.7%	0%	0%	33.3%

**P* values were not computed for each toxicity pattern in order to minimize the number of statistical tests. Fischer Exact tests were performed for the toxicity score.

risk to develop toxicity (Table 5). This result contrasts with data from Schneider *et al*. [16] focused on hypertension, who reported on 177 advanced breast cancer patients receiving bevacizumab-paclitaxel that *VEGF-A* –634CC genotype was associated with less grade 3–4 hypertension. This polymorphism, located in the 5′ untranslated region, has been shown to affect the protein translation efficiency and the –634C allele has been associated with lower VEGF-A expression [25]. However, the basis of the possible link between VEGF-A expression and bevacizumab-related systemic toxicity is clearly unknown.

The main objective of the study was to assess the influence of *VEGF-A* polymorphisms on the efficacy of a bevacizumab-based first-line therapy in advanced breast cancer patients. The majority of patients received concomitant taxane therapy. No relationship was observed between clinical response and *VEGF-A* genotypes. In contrast, patients bearing the *VEGF-A* 936T genotype presented a marked tendency towards a longer TTP than homozygous *VEGF-A* 936CC patients (Figure 2). The 936C > T polymorphism is located in a region of the gene corresponding to the 3′untranslated region of the VEGF mRNA and this mRNA domain is highly implicated in VEGF mRNA stability [26]. Three studies have reported that the *VEGF-A* 936T allele was significantly associated with decreased VEGF-A plasma concentrations [10, 27, 28]. Accordingly, in a series of 49 head and neck patients, our group reported that tumours harboring the *VEGF-A* 936T allele exhibited lower VEGF-A concentrations at the tumoral level [8]. To our knowledge, the opposite pattern concerning the impact of *VEGF-A* 936C > T genotype on VEGF-A expression has never been reported in the literature. It can thus be considered that the *VEGF-A* 936T allele likely governs VEGF-A expression. Thus, the longer TTP presently observed in patients bearing the 936T allele perfectly concords with the observations of Burstein *et al*. [21] showing a longer TTP in metastatic breast cancer patients with low plasma VEGF. In both studies, patients received bevacizumab-based therapy. It is however not clear-cut whether these observations reflect a prognostic or a predictive value. One can hypothesize that the 936T allele provides an advantage to bevacizumab-treated patients by two complementary mechanisms: a lower target level (at the systemic and tumoral levels), along with a less pro-angiogenic tumour environment reducing the risk of tumour growth.

Schneider *et al*. [16] investigated the same *VEGF-A* genotypes as ours, in tumour blocks of metastatic breast cancer patients receiving first-line paclitaxel or paclitaxel-bevacizumab therapy. They found that the *VEGF-A* –1154A and –2578A alleles were significantly associated with longer overall survival (but neither TTP nor responsiveness) only in patients included in the paclitaxel-bevacizumab arm. This may suggest a bevacizumab-dependent predictive value of *VEGF-A* –1154 and –2578 genotypes. The differences between the results of Schneider *et al*. and ours stand on the clinical end points, the nature of the *VEGF-A* genotypes and the nature of the

analysed tissues. In our study, no linkage disequilibria were observed between 936C > T, on the one hand, and −2578C > A or −1154G > A on the other (Table 2). As opposed to 936C > T genotype for which the functional impact on VEGF-A expression has been consistently observed [8, 10, 27, 28], the influence of −2578C > A and −1154G > A genotypes on expression and/or cancer risk is controversial [13]. Schneider *et al*. [16] analysed the influence of −2578C > A and −1154G > A genotypes on tumoral VEGF-A expression and did not observe significant relationships.

In conclusion, present data point out the potential role of *VEGF-A* 936C > T functional polymorphism for predicting disease progression in metastatic breast cancer patients receiving bevacizumab-based therapy. Further studies aimed at improving the knowledge of the impact of the numerous *VEGF-A* polymorphisms on outcome of bevacizumab-treated patients are warranted.

Competing Interests

J-YP, ME and GM have received funds for research by Roche. XP received an honorarium from Roche SA. CV has been reimbursed by Roche and other pharmaceutical companies for attending several conferences. MP is a Roche employee. There are no other competing interests to declare.

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