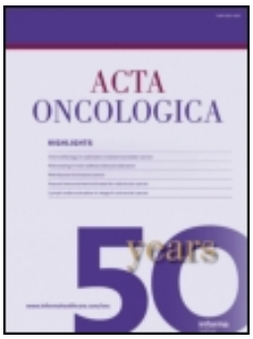

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ORIGINAL ARTICLE

VEGFR1 single nucleotide polymorphisms associated with outcome in patients with metastatic renal cell carcinoma treated with sunitinib – a multicentric retrospective analysis

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Abstract

Background. There are no validated markers that predict outcome in metastatic renal cell cancer (mRCC) patients treated with sunitinib. Recently, single nucleotide polymorphism (SNP) rs9582036 in VEGFR1 has been proposed as a predictor of progression-free survival (PFS) and overall survival (OS) to bevacizumab in patients with pancreatic cancer and rs7993418 in VEGFR1 as predictor for PFS in mRCC-patients treated with bevacizumab. Here, we aim to study the impact of these SNPs in mRCC patients treated with sunitinib. *Methods.* We included patients with mRCC treated in 15 institutions in France and Belgium. Patients received sunitinib as first-line targeted therapy. We assessed response, time-to-tumor progression (TTP), OS, and clinical and biochemical parameters associated with outcome. We genotyped rs9582036 and rs7993418 as well as three other surrounding SNPs in VEGFR1: rs9554320, rs9554316 and rs9513070. Association between SNPs and treatment outcome were studied by univariate analysis and by multivariate Cox regression using relevant clinical factors associated with TTP and OS as covariates. *Findings*. Ninety-one patients were included. We found that mRCC patients with the CC-variant in rs9582036 in VEGFR1 have a poorer response rate (RR) (0% vs. 46%, $p = 0.028$), a poorer PFS (10 vs. 18 months, $p = 0.033$ on univariate and 0.06 on multivariate analysis) and a poorer OS (14 vs. 31 months, $p = 0.019$ on univariate and 0.008 on multivariate analysis) compared to patients with the AC- and AA-genotypes. mRCC patients with the AA-variant in rs9554320 in VEGFR1 have a poorer PFS (12 vs. 21 months, $p = 0.0066$ on univariate and 0.005 on multivariate analysis) and a poorer OS (22 vs. 34 months, $p = 0.019$ on univariate

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and 0.067 on multivariate analysis) compared to patients with the AC- and CC-genotypes. *Interpretation.* mRCC patients with the CC-genotype in VEGFR1 SNP rs9582036 have a poorer response rate, PFS and OS when treated with sunitinib. These findings are in agreement with the association of rs9582036 and outcome observed in bevacizumab treated pancreatic cancer patients. Prospective validation of this SNP is warranted.

Clear cell RCC is characterized by an inactivated von Hippel-Lindau (*VHL*) tumor suppressor gene. Inactivation of the *VHL*-gene leads to elevated protein levels of hypoxia-induced factor-α which upregulates vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) expression. Targeted therapies directed against some of these proteins have significantly improved progression-free survival (PFS) of patients with metastatic RCC (mRCC) as compared to historical treatment options. Sunitinib malate is an orally administered tyrosine kinase receptor inhibitor (TKI) that targets VEGF-receptor (VEGFR) -1, -2 and -3, PDGF-receptor (PDGFR)-α and -β, KIT, FLT-3, colony stimulating factor-1 receptor, and RET. In a randomized controlled trial sunitinib significantly prolonged the PFS (11 vs. 5 months, $p < 0.001$) as compared to interferon-α [1,2]. Median OS was 26.4 and 21.8 months ($p = 0.051$), respectively. Sunitinib is currently a standard treatment in RCC, but other anti-VEGFR and anti-PDGFR-targeted TKIs like sorafenib, pazopanib and axitinib are also used in different stages of the disease.

Although roughly 50% of RCC patients receiving sunitinib experience an objective response and 43% achieve disease stabilization, 7% will experience progressive disease (PD) at first evaluation due to intrinsic resistance or due to other factors [1]. Moreover, all patients with clinical benefit will ultimately relapse due to acquired resistance or for other reasons. The identification of biomarkers able to predict intrinsic resistance could avoid unnecessary costs and side effects, guiding alternative treatment decisions for these patients. On the other hand, the identification of biomarkers for acquired resistance could provide novel directions to develop therapies that block resistance pathways. Although different mechanisms of resistance have been proposed [3], reliable biomarkers predictive of sunitinib sensitivity or primary/secondary resistance are still lacking.

Several clinical and biochemical markers linked to PFS and OS are available for sunitinib treated patients [4,5]. For PFS, these are baseline serum lactate dehydrogenase (LDH) level, the presence of two or more metastatic sites, no prior nephrectomy, Eastern Cooperative Oncology Group Performance Status (ECOG PS), and baseline platelet count. For OS, factors include the presence of bone metastases, time between nephrectomy and start of systemic therapy, baseline serum LDH level, baseline hemoglobin, baseline calcium and baseline ECOG PS. The last five criteria are part of the Memorial Sloan-Kettering Cancer Center (MSKCC) score, which categorize patients into a favorable, intermediate, and poor prognosis group [6]. These established clinical and biochemical markers are indicators of the general condition of the patient and the extension or stage of the disease.

Recently, a number of studies have proposed that genetic variability in genes involved in sunitinib pharmacokinetics and pharmacodynamics alter the efficacy of sunitinib [7–9] or pazopanib [10] in mRCC linking SNPs in *ABCB1, CYP3A5*, *NR1/2, NR1/3, HIF1A, PDGFRA, VEGFR2, VEGFR3, FGFR2,* and *IL8* to sunitinib efficacy. Other studies have shown links between polymorphisms in genes involved in VEGF-dependent-angiogenesis and outcome in patients treated with bevacizumab [11]. Bevacizumab, a humanized monoclonal antibody that binds to VEGF, was the first anti-VEGF-specific drug to be approved for clinical use. When used together with standard therapies, bevacizumab is effective in several cancers such as metastatic colorectal [12], non-small cell lung [13], breast cancer [14], RCC [15], and in recurrent glioblastoma [16].

Lambrechts et al. recently showed the predictive impact of two polymorphisms in *VEGFR1*, a receptor involved in VEGF-dependent tumoral angiogenesis, on outcome in pancreatic cancer and RCC treated with bevacizumab. In 77 patients with pancreatic carcinoma included in the AVITA trial (randomizing patients in arm A with gemcitabine, erlotinib, and bevacizumab and arm B with gemcitabine, erlotinib, and placebo) and treated with bevacizumab, patients with the CC-variant of rs9582036 319A > C had a worse PFS (3.4 months) compared to patients with the AA-variant (7.2 months) ($p = 0.0091$) and the AC-variant (4.5 months) ($p = 0.0002$) and a worse OS (4.7 months) compared to AA-carriers (10.2 months) ($p = 0.018$) and AC-carriers (5.9 months) ($p = 0.0015$). As no effects were seen in placebo-treated patients, the authors concluded that this SNP has a predictive value [17]. Fine-mapping experiments of the *VEGFR1* locus identified rs7993418, a synonymous SNP affecting tyrosine 1213 in the *VEGFR1* tyrosine-kinase domain, as the functional variant underlying the association. This SNP causes a shift in codon usage, leading to increased *VEGFR1* expression and downstream *VEGFR1* signaling. In 59 patients with mRCC treated with bevacizumab in the AVOREN trial (randomizing patients in arm A with

bevacizumab plus interferon versus arm B with interferon alone), Lambrechts et al. found that rs7993418 correlated significantly with PFS, which was 17.5 months in the TT-variants, 10.9 months in the TC-variants and 14.5 months in the CC-variants ($p = 0.033$). Again, no effect was observed in placebo treated patients.

Hansen et al. observed an impact of SNP rs9582036 in *VEGFR1* on RR in 218 metastatic colorectal cancer patients treated with bevacizumab plus chemotherapy: patients with the CC-genotype had 36% of objective response, meanwhile those with CA- and CC-genotype had respectively, 40% and 56% of objective response ($p = 0.048$) [18].

The aim of the present study is to replicate the association of these SNPs observed with bevacizumab therapy in pancreatic carcinoma, RCC and colorectal carcinoma in a retrospective cohort of RCC patients treated with sunitinib.

Patients and methods

For this retrospective study, germ-line DNA samples were selected from the 'CIT-rein' RCC tumor bank. This French-Belgian multicentric RCC tumor bank contains more than 250 frozen primary kidney tumor samples. We selected the samples of patients treated with sunitinib as first-line anti-VEGF-targeted therapy and of whom frozen normal kidney tissue was available. For 11 additional patients, of whom no frozen normal kidney tissue was available, peripheral blood was used. The protocol was approved by the medical ethics review boards of all participating institutions, and signed consent was obtained from all patients. In some cases, we used frozen biological material originating from patients who had already died and for whom approval for the utilization of remaining tissues was foreseen by the institutional review board.

Eligible patients could have received cytokines as systemic treatment for kidney tumors before starting sunitinib as a monotherapy, but they could not have received any other TKI or mammalian target of rapamycine (mTOR) inhibitor before starting sunitinib. Most of the patients were treated in routine clinical practice, some were included in clinical trials. All patients had progressive disease when sunitinib was started. Drug schedules, dose-reduction policy, and the timing of radiological assessments were left to the discretion of the attending doctors in accordance with current local practice guidelines. Patient characteristics considered relevant for TTP and OS analysis were the five risk factors according to MSKCC prognostic criteria and additional factors such as baseline neutrophil and platelet count, and the presence of liver or bone metastases. All patients had to reach at least the first response evaluation, which was usually foreseen after two treatment cycles of sunitinib.

DNA was isolated from fresh frozen normal kidney tissue sampled during nephrectomy using the Qiaquick extraction kit (Qiagen, Valencia, CA, USA) and quantified by fluorometry (Fluoroskan Thermo Labsystems, Cergy-Pontoise, France). DNA was isolated from peripheral blood with the Qiagen DNA kit (Qiagen, Valencia, CA, USA) and final DNA concentration quantified with Nanodrop (Nanodrop, Wilmington, USA). High-throughput SNP genotyping was performed using the Sequenom MassArray platform (Sequenom, San Diego, CA, USA) [19]. Genotyping analysis was performed by investigators blinded for the clinical data.

We genotyped rs9582036 and four surrounding tagging SNPs: rs9554316, rs9513070, rs9554320 and rs7993418, the latter the underlying variant causing a higher expression of *VEGFR1*. These SNPs were selected in the study of Lambrechts et al. [17].

We defined TTP as the time between the first day of sunitinib treatment and the date of radiological disease progression. In most cases, RECIST was used. Patients who had not progressed at database closure were censored at last follow-up. OS was defined as the time between the first day of sunitinib treatment and the date of death or last date of follow-up. TTP was chosen above PFS because one patient died from a surgical intervention after eight months of sunitinib. Objective response was assessed by treating doctors and classified as complete response, partial response (PR), stable disease (SD), or PD. Patients with bone metastases only (two patients) were excluded for the TTP analysis, as bone lesions are not target lesions for RECIST. Timing for assessments was dictated by individual institution policy.

All patient characteristics were tested in an univariate analysis against TTP and OS using Kaplan-Meier statistics and in the multivariate model with Cox-regression analysis. Fisher exact test was used to correlate SNPs with RR. Variables with a $p \leq 0.2$ on univariate analysis were selected as covariates for multivariate Cox-regression analysis. The MSKCC score was not used in the multivariate model, as the individual factors of the score were used if significant on univariate analysis. Results with a p-value of <0.05 were considered as significant in the multivariate analysis. As our approach was the validation of previously published results, although with another class of anti-angiogenics, no correction for multiple testing was made. Statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, La Jolla, UCLA) and XLSTAT software (Addinsoft, Paris, France).

Results

We included 91 patients who started sunitinib between November 2005 and January 2012 and closed the follow-up database in December 2012. Table I shows the clinical characteristics of included patients. The majority of patients were of Caucasian origin. According to MSKCC prognostic criteria, 18% of patients were categorized into the favorable, 55% in the intermediate and 27% into the poor-risk group.

At the time of analysis, 62 (68%) patients had reached progression and 58 (64%) had died. The median follow-up was 50 months (range 1–75) after the start of sunitinib. Median TTP was 15.0 months and median OS 30.0 months in the total group. The difference with outcome on sunitinib in the pivotal trial (PFS 11.0 and OS 26.0 months) is likely due to the patient selection in our series: all the patients had to reach at least the first evaluation by CT scan after approximately 10 weeks of treatment. Moreover, all but one patient underwent nephrectomy. Best response assessment was available in 85 patients (response assessment was poorly defined in the medical records of six patients, but they had a clinical benefit of at least five months, although it was unclear whether the best response was either PR or SD). Six of 84 (7%) patients had a complete response, 36/84 (37%) patients a PR, 38/84 (45%) a SD and 10/90 (11%) PD as best response.

Table I. Patient characteristics at diagnosis and at the start of sunitinib treatment.

At initial diagnosis	Total
Number of patients	91
Mean age (years)	59
Male	68% (62/91)
Ethnic origin	
Caucasian	93% (85/91)
Indian or unknown	7% (6/91)
Fuhrman	
Grade 1–3	48% (44/91)
Grade 4	52% (47/91)
At the start of sunitinib	
ECOG PS >0	47% (43/91)
Neutrophils $>4.500/mm^3$	38% (35/91)
Platelets $>400.000/mm^3$	14% (13/91)
Hemoglobin <11.5 g/dl (women) or <13 g/dl (men)	42% (38/91)
LDH >1.5x ULN	9% (8/91)
Corrected calcium >10 mg/dl	8% (7/87)
Time nephrectomy to systemic treatment <12 months	66% (60/91)
Immunotherapy before sunitinib	27% (24/90)
Site of metastasis	
Lung	84% (76/91)
Liver	18% (16/91)
Bone	36% (33/91)
Brain	5% (5/91)
Mean number of metastases	2.35
MSKCC prognosis	
Favorable	18% (16/89)
Intermediate	55% (49/89)
Poor	27% (24/89)

ECOG PS, Eastern cooperative oncology group performance status; LDH, lactate dehydrogenase; MSKCC, Memorial Sloan-Kettering Cancer Center.

Table II reports the allele frequencies of the genotyped polymorphisms in our series. Overall, the SNPs were successfully genotyped with success rates $\geq 91\%$ for each SNP and an overall average success rate of 95%.

We detected a minor allele frequency of 31.3% for the C-allele in rs9582036, corresponding to the minor allele frequency reported in the dbSNP (dbSNP build 136) database or 1000 Genomes Project (31.7%). Table II also reports the linkage disequilibrium (r^2 test) between the different SNPs showing a complete correlation between rs7993418 and rs9554316, but not between the other polymorphisms.

Table III shows the clinical and biochemical parameters associated with TTP and OS. In the multivariate analysis for TTP, four parameters exhibited a $p \leq 0.2$ (baseline ECOG PS, baseline LDH activity, baseline neutrophil count, and the presence of bone metastases [20]) and were considered as covariates in the multivariate analysis for TTP. For OS, baseline ECOG PS, the laps of time between nephrectomy and start of systemic therapy and the presence of bone metastases were taken into account.

Table IV and Figures 1–4 show the results of univariate and multivariate Cox regression analysis for the SNPs in *VEGFR1* for TTP and OS. Patients homozygous for the rs9582036 C-allele in *VEGFR1* have a poorer PFS (10 vs. 18 months) ($p = 0.033$ on univariate and 0.06 on multivariate analysis) and OS (14 vs. 31 months) ($p = 0.019$ on univariate and 0.008 on multivariate analysis) compared to patients with the AC- and AA-genotypes, while the curves for AC- and AA-genotypes were overlapping both for PFS and OS. In the CC-carriers, there were no PRs, meanwhile in the AC- and AA-carriers, there were 46% of PRs ($p = 0.028$; Fisher exact test). Patients homozygous for the rs7993418 C-allele had a poorer PFS and OS compared to TC- and TT-carriers, but this difference was not statistically significant. As rs7993418 was in perfect linkage disequilibrium with rs9554316 (see Table II), the same was observed for rs9554316 TT-carriers vs. GT- and GG-carriers. Patients with the AA-variant in rs9554320 had a poorer PFS (12 vs. 21 months) ($p = 0.0066$ on univariate and 0.005 on multivariate analysis) and OS (22 vs. 34 months) ($p = 0.019$ on univariate and 0.067 on multivariate analysis) compared to CA-and CC-carriers. Finally, no impact of rs9513070 on PFS or OS could be observed.

Table II. Genotype and allele distribution of the genotyped SNPS in VEGFR1 and corelation of polymorphisms (r^2).

rs ID	Polymorphism	Location	Wildtype/ Wildtype (%)	Wildtype/ Variant (%)	Variant/ Variant (%)	Observed minor allele frequency
rs9582036	A>C	Intron	38/91 (42%)	46/91 (51%)	7/91 (8%)	31.3%
rs7993418	T>C	Exon 28	44/86 (51%)	38/86 (44%)	4/86 (5%)	26.7%
rs9513070	A>G	Intron	20/83 (24%)	46/83 (55%)	17/83 (20%)	42.2%
rs9554316	G>T	Intron	42/84 (50%)	38/84 (45%)	4/84 (5%)	27.4%
rs9554320	C>A	Intron	25/86 (29%)	40/86 (47%)	21/86 (24%)	47.7%

r^2	rs7993418	rs9513070	rs9554316	rs9554320	rs9582036
rs7993418	–	0.127	1.000	0.364	0.645
rs9513070	0.127	–	0.130	0.060	0.122
rs9554316	1.000	0.130	–	0.362	0.660
rs9554320	0.364	0.060	0.362	–	0.509
rs9582036	0.645	0.122	0.660	0.509	–

Discussion

We observed a significant association between SNP rs9582036 in *VEGFR1,* one of the molecular targets of sunitinib involved in tumoral VEGF-dependent angiogenesis, and outcome (PFS, OS and RR) in mRCC patients treated with sunitinib. As sunitinib was used as single agent in our series, our results were not confounded by concomitant chemotherapy and we could obtain significant links in a series involving only a limited number of patients.

Table III. Baseline clinical and biochemical parameters associated with TTP and OS.

	Median TTP (months)	p*	Median OS (months)	p*
Neutrophils >4.500/mm³ (n=35)	9	0.07	22	0.41
Neutrophils ≤4.500/mm³ (n=53)	19		34	
Platelets >400.000/mm³ (n=13)	11	0.29	27	0.21
Platelets ≤400.000/mm³ (n=78)	18		31	
ECOG PS>0 (n=43)	15	0.035	24	0.12
ECOG PS 0 (n=48)	21		35	
LDH>1.5ULN (n=8)	10.5	0.10	24.5	0.26
LDH≤1.5ULN (n=80)	18		31	
Hb low [<11.5 g/dl (women) or <13 g/dl (men)] (n=38)	14	0.71	25	0.46
Hb normal (n=53)	18		34	
Corrected Ca>10 mg/dl (n=7)	23	0.82	31	0.67
Corrected Ca≤10 mg/dl (n=80)	15		30	
Time from nephrectomy (or initial diagnosis) to systemic treatment<12 mo (n=60)	16	0.34	27	0.14
Time from nephrectomy (or initial diagnosis) to systemic treatment>12 mo (n=31)	15		55	
Liver metastases (n=16)	14.5	0.48	22	0.35
No liver metastases (n=75)	16		31	
Bone metastases (n=33)	13	0.08	22	0.05
No bone metastases (n=58)	20		34	
Clear cell histology (n=87)	16	0.31	30	0.30
Papillary histology (n=4)	12		16.5	
MSKCC Good (n=16)	NA	NA	59	0.053
MSKCC Intermediate (n=49)	NA		25	
MSKCC Poor (n=24)	NA		28	

ECOG PS, Eastern cooperative oncology group performance status; LDH, lactate dehydrogenase; MSKCC, Memorial Sloan-Kettering Cancer Center; NA, not applicable; NR, not reached; OS, overall survival; TTP, time-to-tumor progression; ULN, upper limit of normal.

*The p-value was calculated by log-rank test.

Note: The parameters that were considered for the multivariate analysis for TTP were baseline neutrophil count, baseline LDH and baseline ECOG PS as well as the presence of bone metastasis. The parameters that were considered for the multivariate analysis for OS were baseline ECOG PS, the laps of time between nephrectomy to systemic treatment as well as the presence of bone metastasis.

Table IV. Univariate and multivariate analysis: association between SNPS and outcome.

rs ID	Polymorphism	Number of patients	TTP (months)	p-value (univariate)* p-value (multivariate)**	HR (95% CI of HR)	OS (months)	p-value (univariate)* p-value (multivariate)**	HR (95% CI of HR)
rs9582036	AA + AC	84	18	0.033	0.2545	31	0.019	0.2493
A > C	CC	7	10	0.06	(0.07214–0.8978)	14	0.008	(0.07778–0.7992)
	AA	38	18	0.12	NA	30	0.065	NA
	AC	46	19	NA	NA	34	NA	NA
	CC	7	10			14		
rs7993418	TT + TC	82	18	0.32	NA	31	0.35	NA
T > C	CC	4	14	NA	NA	23.5	NA	NA
	TT	44	20	0.38	NA	35	0.43	NA
	TC	38	15	NA	NA	30	NA	NA
	CC	4	14			23.5		
rs9554316	GG + GT	80	18	0.32	NA	31	0.36	NA
G > T	TT	4	14	NA	NA	23.5	NA	NA
	GG	42	20	0.40	NA	35	0.45	NA
	GT	38	15	NA	NA	30	NA	NA
	TT	4	14			23.5		
rs9554320	CC + CA	70	21	0.0066	2.713	34	0.019	2.286
C > A	AA	21	12	0.005	(1.321–5.575)	22	0.067	(1.147–4.555)
	CC	25	22	0.025	ND	NR	0.0076	ND
	CA	40	21	ND	ND	30	ND	ND
	AA	21	12			22		
rs9513070	AA	20	15	0.22	NA	24	0.35	NA
A > G	AG	46	23	NA	NA	42	NA	NA
	GG	17	15			22		

CI, confidence interval; HR, hazard ratio; OS, overall survival; NA, not applicable; NR, not reached; ND, not done; TTP, time-to-tumor progression.

*The p-value for the univariate analysis was calculated by log-rank test; ** The p-value for the multivariate analysis was the result of a Cox proportional hazards model.

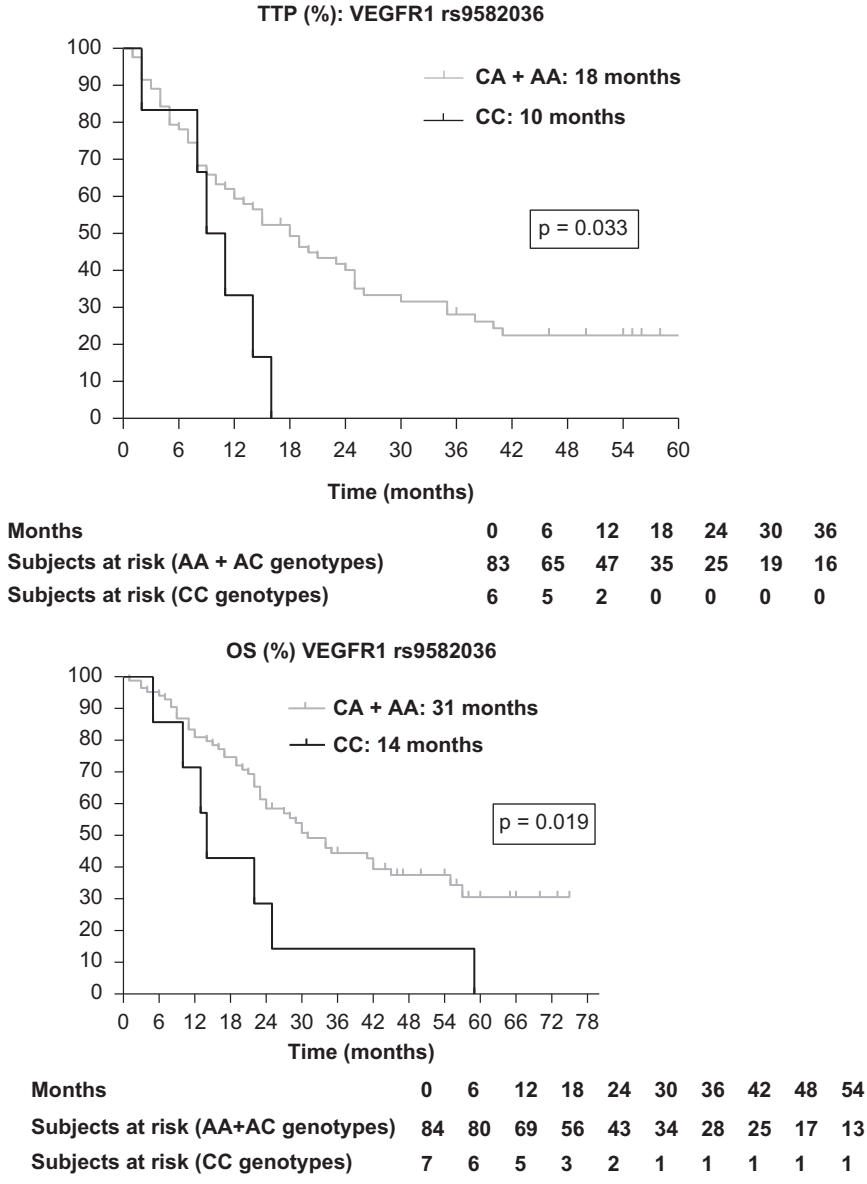
Note: VEGFR1 rs9582036: when analyzing AA versus AC versus CC, the TTP and OS curves for AA and AC are completely overlapping, allowing us to analyze AA + AC against CC. rs7993418: all the CC-variants were also rs9582036 CC-variants. rs9554316: all the TT-variants were also rs9582036 CC-variants. rs9554320: all the rs9582036 CC-variants were rs9554320 AA-variants. For rs9513070 it was not possible to group variants, because the three curves were overlapping.

Concerning rs9582036, two reports in literature, in patients with pancreatic cancer or colorectal cancer, treated with bevacizumab and chemotherapy, are in accordance with our findings.

In AVITA [21], a multicenter, randomized phase III trial, patients with metastatic pancreatic adenocarcinoma were randomly assigned to receive gemcitabine and erlotinib plus either bevacizumab or placebo. Lambrechts et al. investigated DNA of 154 AVITA-patients, of whom 77 received bevacizumab and 77 placebo. SNP rs9582036 319A > C in *VEGFR1* was significantly associated with OS in the bevacizumab group: median OS was 4.7 months in CC-carriers, 5.9 months in AC-carriers, and 10.2 months in AA-carriers. After adjustment for neutrophil count, C-reactive protein level, and tumor location, association of rs9582036 with OS showed a per-allele HR of 1.9 (95% CI 1.27–2.92) and a p-value of 0.002. SNP rs9582036 was also associated with PFS in bevacizumab-treated patients: AC- and CC-carriers of this SNP exhibited HRs for PFS of 2.0 (95% CI 1.19–3.36; p = 0.0091) and 4.72 (95% CI 2.08–10.68; p = 0.0002) relative to AA-carriers. As no effects were seen in placebo-treated patients and a significant genotype by treatment interaction (p = 0.041) was recorded, the authors concluded that the *VEGFR1* locus containing this SNP serves as a predictive marker for bevacizumab treatment outcome in AVITA [17].

Hansen observed in 218 metastatic colorectal cancer patients treated with bevacizumab plus chemotherapy a statistically significant difference in RR: 36% of patients with the CC-genotype had objective responses, while those with CA- and AA-genotype had 40% and 56% of objective responses, respectively (p = 0.048) [18].

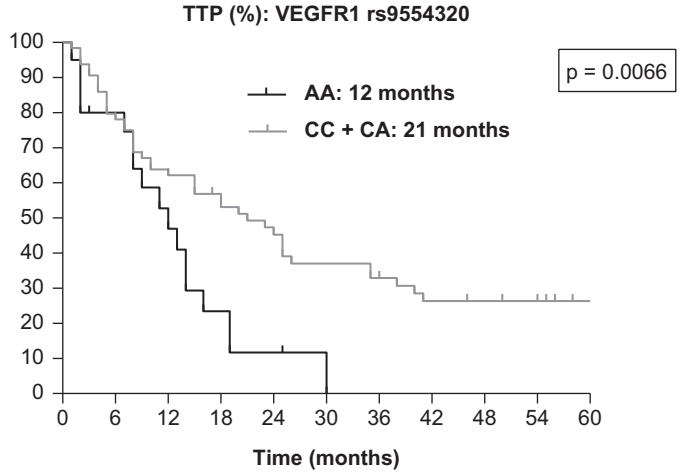
The rs7993418 SNP was identified by Lambrechts et al. as the functional variant underlying the association between rs9582036 and outcome. In particular, this SNP causes a shift in codon usage, leading to increased *VEGFR1* expression and downstream *VEGFR1* signaling. In 59 patients with mRCC treated with bevacizumab in the AVOREN trial [15] (randomizing patients in arm A bevacizumab plus interferon vs. arm B interferon alone), Lambrechts et al. found that rs7993418 correlated significantly with



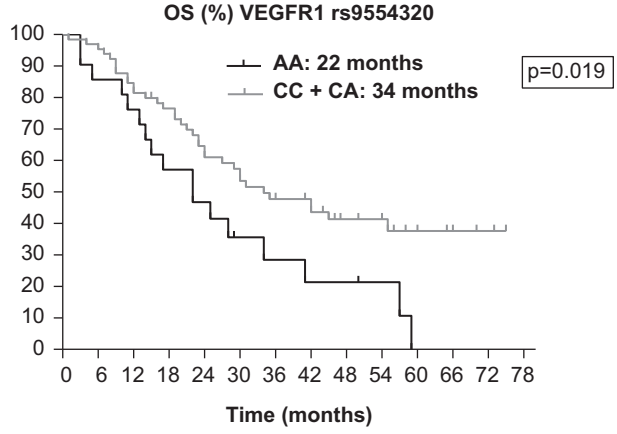
Figures 1 and 2. Kaplan-Meier curves of the significant associations between SNP rs9582036 and time-to-tumor progression (TTP) and overall survival (OS) in our series. P-values are the p-values from the univariate analysis.

PFS, which was 17.5 months in the TT-variants, 10.9 months in the TC-variants and 14.5 months in the CC-variants ($p = 0.033$), while no effect was observed in 51 placebo treated patients. No correlation with OS was noted, but the authors explained this by the low number of death events in their patient series and frequent post-protocol treatments with the anti-angiogenic TKIs sorafenib and sunitinib, which might affect association of *VEGFR1* SNPs with bevacizumab treatment outcome. Although in our series, we did notice a shorter PFS and OS in patients with the CC-variant, these results were not significant. This can probably been explained by the fact that the minor allele variant in rs7993418 is less frequent than the minor allele variant in rs9582036. As a consequence, we only had four patients with the CC-genotype in our series.

Concerning the other surrounding polymorphisms in *VEGFR1* (rs9554316, rs9513070, and rs9554320), Lambrechts et al. found them to be correlated with OS in the bevacizumab group of AVITA, but they did not pass the p-value threshold adjusted for multiple testing ($p = 0.00042$ for rs9554316 favoring the GG- and GT-genotypes, $p = 0.0081$ for rs9513070 favoring the AA- and AG-genotypes, and $p = 0.0097$ for rs9554320 favoring the CC- and CA-genotypes). In AVOREN, due to high linkage disequilibrium with rs7993418 in this patient series, variants rs9554316 (favoring the GG- and GT-genotypes) and rs9513070 (favoring the AA- and AG-genotypes) also correlated significantly with PFS in the bevacizumab group. The other two SNPs in the *VEGFR1* locus, rs9582036



Months	0	6	12	18	24	30	36	42
Subjects at risk (CC + CA genotypes)	64	50	39	30	23	18	16	12
Subjects at risk(AA genotypes)	20	15	9	4	2	1	0	0



Months	0	6	12	18	24	30	36	42	48	54
Subjects at risk (CC + CA genotypes)	65	63	54	45	36	30	25	23	15	12
Subjects at risk(AA genotypes)	21	18	16	11	9	5	4	3	3	2

Figures 3 and 4. Kaplan-Meier curves of the significant associations between SNP rs9554320 and time-to-tumor progression (TTP) and overall survival (OS) in our series. P-values are the p-values from the univariate analysis.

and rs9554320, were in lower linkage disequilibrium with rs7993418 and were not significantly associated with PFS. In our series, the only significant findings were the association between rs9554320 and PFS and OS, favoring patients with the AC- and CC-genotype, the latter confirming the data of Lambrechts et al. [17].

Our study has several potential limitations. First of all, it was a retrospective analysis of patients treated in several centers without a central protocol dictating the treatment schedule, dose modifications or timing of radiological assessments. Secondly, because our patients were mainly white, the relevance of these polymorphisms needs to be assessed in other ethnic groups, in whom the described polymorphisms may be less frequent. Finally, the major handicap of this study is that due to the lack of a control group treated with placebo, we cannot affirm that rs9582036 has a predictive value. Nevertheless, given the impact both on PFS and OS and given the fact that in bevacizumab treated patients this SNP is a predictive marker, it is probable that SNP rs9582036 is also of predictive value in RCC patients treated with sunitinib.

The emerging evidence of the impact of SNPs in pathways linked to pharmacokinetics and pharmacodynamics of sunitinib shows that besides acquired genetic characteristics of tumor cells, patient's germline genetic variation may also affect the effectiveness of anticancer therapy. Germline DNA has significant advantages over other nucleotide and protein biomarkers in that it is inherited, fixed, and relatively insensitive to time and environmental factors.

If the impact of these and other SNPs on outcome on sunitinib could be confirmed prospectively

in independent series, scoring systems based on the combination of several unfavorable or favorable SNPs could be elaborated. When combining these SNPs with clinical and biochemical parameters linked to outcome, we will probably be able to predict more precisely the chance of response on sunitinib and identify primary resistant patients in order to orient them towards other therapies avoiding unnecessary side effects and costs. We will probably also be capable of predicting more accurately PFS and thus the moment of secondary resistance.

Contributors

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Benoit Beuselinck, Diether Lambrechts, Robert Paridaens, Jean-Jacques Patard, Jessica Zucman-Rossi, and Stéphane Oudard designed the study. Benoit Beuselinck, Alexandra Karadimou, Gabrielle Couchy, and Joost Berkers were responsible for DNA-extraction. Bart Claes and Diether Lambrechts were responsible for SNP-genotyping. Virginie Verkarre, Vincent Molinié, Nathalie Rioux-Leclercq, and Evelyne Lerut were involved in pathology review. Benoit Beuselinck, Pascal Wolter, Florence Joly, Thierry Lebret, Gwénaelle Gravis, Gaël Deplanque, Aurélien Descazeaud, Nathalie Rioux-Leclercq, Jean-Jacques Patard, Corine Teghom, Stéphane Oudard, Arnaud Méjean, Hendrik Van Poppel, Vincent Molinié, and Reza Elaidi collaborated in data collection and provided study materials or recruited patients. All authors critically reviewed the manuscript and approved the final version.

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