Single-nucleotide polymorphisms associated with outcome in metastatic renal cell carcinoma treated with sunitinib

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Background: There are no validated markers that predict response in metastatic renal cell cancer (RCC) patients treated with sunitinib. We aim to study the impact of single-nucleotide polymorphisms (SNPs) that have recently been proposed as predictors of outcome to anti-VEGF-targeted therapy in metastatic RCC in an independent cohort of patients.

Methods: We genotyped 16 key SNPs in 10 genes involved in sunitinib pharmacokinetics, pharmacodynamics and VEGF-independent angiogenesis in patients with metastatic clear-cell RCC treated with sunitinib as the first-line targeted therapy. Association between SNPs, progression-free survival (PFS) and overall survival (OS) were studied by multivariate Cox regression using relevant clinical factors associated with PFS and OS as covariates.

Results: In a series of 88 patients, both PFS and OS were associated significantly with SNP rs1128503 in ABCB1 (P = 0.027 and P = 0.025), rs4073054 in NR1/3 (P = 0.025 and P = 0.035) and rs307821 in VEGFR3 (P = 0.032 and P = 0.011). Progression-free survival alone was associated with rs2981582 in FGFR2 (P = 0.031) and rs2276707 in NR1/2 (P = 0.047), whereas OS alone was associated with rs2307424 in NR1/3 (P = 0.048) and rs307826 in VEGFR3 (P = 0.013).

Conclusion: Our results confirm former communications regarding the association between SNPs in ABCB1, NR1/2, NR1/3 and VEGFR3 and sunitinib outcome in clear-cell RCC. Prospective validation of these SNPs is now required.

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Inactivation of the von Hippel–Lindau (VHL) tumour-suppressor gene is the most frequent molecular alteration in clear-cell renal cell cancer (RCC). Inactivated VHL leads to elevated protein levels of hypoxia-induced factor-α that upregulates vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) expression. Targeted therapies directed against some of these proteins have significantly improved the perspectives of patients with metastatic RCC. Sunitinib malate is an orally administered tyrosine kinase receptor inhibitor (TKI) that targets VEGF and PDGF receptors, KIT, FLT-3, colony stimulating factor-1 receptor and RET. In a randomised controlled trial, sunitinib significantly prolonged progression-free survival (PFS; 11 vs 5 months, \( P < 0.001 \)) as compared with interferon-α (Motzer et al., 2007, 2009). Median overall survival (OS) was 26.4 and 21.8 months, respectively (\( P = 0.051 \)). Sunitinib is a standard treatment option in clear-cell RCC, but other anti-VEGFR and anti-PDGF-targeted TKIs such as sorafenib, pazopanib and axitinib are also used in different stages of the disease.

Although 50% of RCC patients receiving sunitinib experience an objective response and 43% achieve disease stabilisation, 7% will experience progressive disease (PD) at first evaluation, probably because of intrinsic resistance or other factors (Motzer et al., 2009). Moreover, even patients with an initial clinical benefit will finally progress because of 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. On the other hand, the identification of biomarkers for acquired resistance could provide novel directions to develop therapies that block these resistance pathways. Although different mechanisms of resistance have been proposed (Rini and Atkins, 2009), reliable biomarkers predictive of sunitinib sensitivity or primary/secondary resistance are still lacking.

Several clinical and biochemical markers for PFS and OS are available for sunitinib-treated patients (Heng et al., 2009; Patil et al., 2011). 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 presence of bone metastases, time between nephrectomy and start of systemic therapy, baseline serum LDH level, baseline haemoglobin, baseline calcium and baseline ECOG. The last five criteria are part of the Memorial Sloan Kettering Cancer Centre (MSKCC) score that categorises patients into a favourable-, intermediate- and poor-prognosis group (Motzer et al., 2004). These established clinical and biochemical markers are indicators of the general condition of the patient and the extension or stage of the disease. They do not take into account sunitinib pharmacokinetics (absorption, metabolisation) or pharmacodynamics (interaction of sunitinib with its molecular targets). Recently, a meta-analysis of pharmacokinetic data from 443 patients treated with sunitinib showed that higher plasma levels of sunitinib and its active metabolite SU12662 were associated with prolonged TTP and OS (Houk et al., 2010). Factors influencing the concentration of sunitinib in plasma are dose and schedule of the drug and patient compliance, but importantly, also the concentration of efflux pumps and metabolising enzymes. Moreover, sunitinib efficacy can be influenced by the expression level and variants of the molecular targets of the drug.

Recently, a number of studies have proposed that genetic variability in genes involved in sunitinib pharmacokinetics and pharmacodynamics alter the efficacy of sunitinib (van der Veldt et al., 2010; Garcia-Donas et al., 2011) or pazopanib (Xu et al., 2011a,b) in metastatic RCC. As each of these studies investigated a different set of single-nucleotide polymorphisms (SNPs), these findings need to be validated independently. The aim of the present study is therefore to replicate association of these SNPs to sunitinib outcome by assessing an independent cohort of patients with metastatic clear-cell RCC treated with first-line sunitinib.

### MATERIALS AND METHODS

For this retrospective study, germline DNA samples were collected in the CIT-rein kidney tumour bank and in patients treated at the University Hospitals Leuven. The French-Belgian multicentric CIT-rein kidney tumour bank contains more than 250 frozen kidney tumour samples collected at 20 academic hospitals. We selected the samples of patients with pathologically confirmed clear-cell RCC treated in first line with sunitinib and for whom frozen normal kidney tissue was available. Eligible patients could have received cytokines as systemic treatment for kidney tumours before starting sunitinib as a monotherapy, but they could not have received any other TKI or mTOR (mammalian target of rapamycin) inhibitor before starting sunitinib. To make sure that the effect of sunitinib was accurately measured, patients had to take sunitinib during at least one complete cycle of 28 days and had to reach at least the first evaluation by CT scan. In the whole CIT-rein kidney tumour bank, 79 frozen normal kidney samples corresponded to these selection criteria. In order to extend the series, we added nine patients visiting the University Hospitals Leuven and complying to the same inclusion criteria. As no frozen normal kidney tissue was available for these patients, peripheral blood was sampled during out day clinic from July 2011 till December 2011.

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 biologic material from patients who had already died and for whom a general positive advice for the utilisation of remaining tissue was foreseen by the institutional board.

All the patients were treated in routine clinical practice. Drug schedule, dose-reduction policy and timing of radiological assessments were left to the discretion of the attending doctors in accordance with current local practice guidelines. All the patients started their sunitinib therapy at the standard sunitinib dose of 50 mg day\(^{-1}\), 4 weeks on and 2 weeks off. The patient characteristics considered relevant for PFS and OS analysis were the five risk factors according to the MSKCC prognostic criteria and additional factors such as baseline neutrophil count, baseline platelet count, the presence or absence of liver metastases, the presence or absence of a component of sarcomatoid differentiation and the presence or absence of bone metastases. The latter two parameters were associated to outcome on sunitinib in recent publications (Golshayan et al., 2009; Beuselinck et al., 2011; Patil et al., 2011).

The SNPs previously associated with TKI efficacy in RCC were selected from the literature (Table 1). These SNPs are located in genes affecting sunitinib pharmacokinetics (i.e., genes involved in sunitinib absorption, such as ABCB1, or metabolism, such as CYP3A5, NRI/2 and NRI/3), sunitinib pharmacodynamics (i.e., genes involved in PDGF- and VEGF-dependent angiogenesis such as HIF1A, PDGFRα, VEGFR2 and VEGFR3) or VEGF-independent alternative pro-angiogenic pathways (FGFR2 and IL8). DNA was isolated at INSERM U674 in Paris, France, from fresh frozen normal kidney tissue sampled in the nephrectomy specimen 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 at the Vesalius Research Center in Leuven with the Qiaquick extraction kit (Qiagen) and final DNA concentration quantified with Nanodrop (Nanodrop, Wilmington, DE, USA). High-throughput SNP genotyping was performed at the Vesalius Research Center in Leuven, Belgium, using the Sequenom MassArray platform.
SNPs and outcome on sunitinib in renal cell cancer

The first day on sunitinib and the date of death or last date of follow-up. Objective response was assessed by the treating doctors and classified as complete response (CR), partial response (PR), stable disease (SD), or PD. Timing for assessments was dictated by individual institution policy.

All patient characteristics were tested in an univariate analysis and classified as complete response (CR), partial response (PR), stable disease (SD), or PD. Timing for assessments was dictated by individual institution policy.

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Table 2. Patient characteristics at diagnosis and at the start of sunitinib treatment and baseline clinical and biochemical parameters associated with PFS and OS

|                                | Total  | Median PFS (months) | P-value, HR (95% CI) | 95% CI of median PFS |
|--------------------------------|--------|---------------------|----------------------|----------------------|
| **At initial diagnosis**       |        |                     |                      |                      |
| Male                           | 68%    | (60/88)             | —                    | —                    |
| **Ethnic origin**              |        |                     |                      |                      |
| Caucasian                      | 94%    | (83/88)             | —                    | —                    |
| Unknown                        | 6%     | (5/88)              | —                    | —                    |
| M1 (synchronous metastases)   | 55%    | (46/84)             | —                    | —                    |
| **Fuhrman**                    |        |                     |                      |                      |
| Grade 1–3                      | 68%    | (58/85)             | —                    | —                    |
| Grade 4                        | 32%    | (27/85)             | —                    | —                    |
| **Sarcomatoid dedifferentiation** |   |                     |                      |                      |
| Present                        | 9%     | (8/88)              | 4                    | 0.09                 |
| Absent                         | 91%    | (80/88)             | 18                   | 0.37 (0.12–1.18)     |
| **At the start of sunitinib**  |        |                     |                      |                      |
| ECOG PS                        |        |                     |                      |                      |
| >0                             | 49%    | (43/88)             | 15                   | 0.08                 |
| 0                              | 51%    | (45/88)             | 21                   | 0.63 (0.37–1.06)     |
| Neutrophils                    |        |                     |                      |                      |
| >4500 per mm$^3$               | 40%    | (34/85)             | 9.5                  | 0.13                 |
| <4500 per mm$^3$               | 60%    | (51/85)             | 19                   | 0.65 (0.38–1.14)     |
| Platelets                      |        |                     |                      |                      |
| >400,000 per mm$^3$            | 15%    | (13/88)             | 11                   | 0.25                 |
| <400,000 per mm$^3$            | 85%    | (75/88)             | 18                   | —                    |
| Haemoglobin                    |        |                     |                      |                      |
| Low (<11.5 g dl$^{-1}$ (women) or <13 g dl$^{-1}$ (men)) | 42%    | (37/88)             | 14                   | 0.98                 |
| Normal                         | 58%    | (51/88)             | 18                   | —                    |
| LDH                            |        |                     |                      |                      |
| >1.5 ULN                       | 10%    | (8/84)              | 10.5                 | 0.09                 |
| ≤1.5 ULN                       | 90%    | (76/84)             | 18.0                 | 0.40 (0.14–1.16)     |
| Corrected calcium              |        |                     |                      |                      |
| >10 mg dl$^{-1}$               | 7%     | (6/84)              | 22                   | 0.9                  |
| ≤10 mg dl$^{-1}$               | 93%    | (78/84)             | 15                   | —                    |
| Time from nephrectomy to systemic treatment |   |                     |                      |                      |
| <12 months                     | 66%    | (58/88)             | 18                   | 0.30                 |
| >12 months                     | 34%    | (30/88)             | 15                   | —                    |
| Immunotherapy before sunitinib | 28%    | (24/87)             | —                    | —                    |
| Site of metastasis             |        |                     |                      |                      |
| Lung                           | 84%    | (74/88)             | —                    | —                    |
| Liver metastases               | 18%    | (16/88)             | 15                   | 0.59                 |
| No liver metastases            | 82%    | (72/88)             | 18                   | —                    |
| Bone metastases                | 35%    | (31/88)             | 15                   | 0.5                  |
| No bone metastases             | 65%    | (57/88)             | 18                   | —                    |
| Brain                          | 6%     | (5/88)              | —                    | —                    |
| MSKCC prognosis                |        |                     |                      |                      |
| Favourable                     | 15%    | (13/85)             | Not reached          | 0.21                 |
| Intermediate                   | 56%    | (48/85)             | 15                   | 8–Not reached        |
| Poor                           | 28%    | (24/85)             | 15                   | 11–21                |
|                                |        |                     |                      | 4–25                 |
Table 2. Continued

| At initial diagnosis | Total | Median OS (months) | P-value, HR (95% CI) | 95% CI of median OS |
|----------------------|-------|--------------------|----------------------|---------------------|
| Male                 | 68% (60/88) | — | — | — |
| Ethnic origin        |       |                    |                      |                     |
| Caucasian            | 94% (83/88) | — | — | — |
| Unknown              | 6% (5/88) | — | — | — |
| M1 (synchronous metastases) | 55% (46/84) | — | — | — |
| Fuhrman              |       |                    |                      |                     |
| Grade 1–3            | 68% (58/85) | — | — | — |
| Grade 4              | 32% (27/85) | — | — | — |
| Sarcomatoid dedifferentiation |       |                    |                      |                     |
| Present              | 9% (8/88) | 16.5 | 0.19 | 5–Not reached |
| Absent               | 91% (80/88) | 30 | 0.45 (0.13–1.50) | 23–42 |
| At the start of sunitinib |       |                    |                      |                     |
| ECOG PS              |       |                    |                      |                     |
| >0                   | 49% (43/88) | 23 | 0.08 | 17–34 |
| 0                    | 51% (45/88) | 35 | 0.60 (0.34–1.06) | 23–Not reached |
| Neutrophils          |       |                    |                      |                     |
| >4.500 per mm$^3$    | 40% (34/85) | 22 | 0.39 | — |
| <4.500 per mm$^3$    | 60% (51/85) | 34 | — | — |
| Platelets            |       |                    |                      |                     |
| >400.000 per mm$^3$  | 15% (13/88) | 27 | 0.45 | — |
| <400.000 per mm$^3$  | 85% (75/88) | 29 | — | — |
| Haemoglobin          |       |                    |                      |                     |
| Low (<11.5 g dl$^{-1}$ (women) or <13 g dl$^{-1}$ (men)) | 42% (37/88) | 27 | 0.42 | — |
| Normal               | 58% (51/88) | 34 | — | — |
| LDH                  |       |                    |                      |                     |
| >1.5 ULN             | 10% (8/84) | 24.5 | 0.19 | 19–34 |
| £1.5 ULN             | 90% (76/84) | 34 | 0.51 (0.19–1.38) | 23–45 |
| Corrected calcium    |       |                    |                      |                     |
| >10 mg dl$^{-1}$     | 7% (6/84) | 42 | 0.98 | — |
| £10 mg dl$^{-1}$     | 93% (78/84) | 29 | — | — |
| Time from nephrectomy to systemic treatment |       |                    |                      |                     |
| <12 months           | 66% (58/88) | 27 | 0.13 | 22–35 |
| >12 months           | 34% (30/88) | Not reached | 1.50 (0.88–2.85) | 19–Not reached |
| Immunotherapy before sunitinib | 28% (24/87) | — | — | — |
| Site of metastasis   |       |                    |                      |                     |
| Lung                 | 84% (74/88) | — | — | — |
| Liver metastases     | 18% (16/88) | 22 | 0.60 | — |
| No liver metastases  | 82% (72/88) | 29 | — | — |
| Bone metastases      | 35% (31/88) | 22 | 0.06 | 11–34 |
| No bone metastases   | 65% (57/88) | 35 | 0.54 (0.29–1.03) | 24–Not reached |
| Brain                | 6% (5/88) | — | — | — |
| MSKCC prognosis      |       |                    |                      |                     |
| Favourable           | 15% (13/85) | Not reached | 0.0097 | Not reached–not reached |
| Intermediate         | 56% (48/85) | 24 | — | — |
| Poor                 | 28% (24/87) | 27 | — | — |

Abbreviations: PFS = progression-free survival; OS = overall survival; ECOG PS = Eastern Cooperative Oncology Group Performance Status; LDH = lactate dehydrogenase; ULN = upper limit of normal; MSKCC = Memorial Sloan Kettering Cancer Center; HR = hazard ratio; 95% CI = 95% confidence interval. In the univariate analysis, median PFS and median OS were estimated by Kaplan-Meier and $P$-values were derived from a log-rank test. The impact of the presence of lung metastases and previous immunotherapy was not assessed as these parameters have not been strongly linked to PFS or OS.
Of the 11 clinical parameters assessed in the univariate analysis (for 88 patients), there were 13 missing values (1.3%). For the multivariate analysis, 82 patients with complete data could be included. Statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) and XLSTAT software (Addinsoft, Paris, France).

RESULTS

We enrolled 88 patients who started sunitinib between November 2005 and July 2011 and closed the follow-up database in April 2012. Table 2 shows the clinical characteristics of enroled patients. Mean age at diagnosis was 59 years (range 38–84). The majority of patients (>94%) were of Caucasian origin. According to the MSKCC prognostic criteria, 15% of patients were categorised into the favourable risk group, and 56% had intermediate and 28% poor risk.

At the time of analysis, 57 (64.8%) patients had reached progression and 48 (54.5%) had died. The median follow-up was 15.0 months (95% CI 11.0–23.0 months) and the median OS was 29.0 months (range 4.0–51.0 months) after the start of sunitinib. The median PFS was 46.0 months (range 1.0–73.0 months; 95% confidence interval (CI) 36.9–51.0 months).

Table 3. Genotype and allele distribution of selected SNPs

| Gene          | RS ID     | Polymorphism | Location or functional consequence | n   | Wild-type/variant, n (%) | Variant/variant, n (%) | Observed minor allele frequency (%) | Minor allele frequency in dbSNP (%) |
|---------------|-----------|--------------|------------------------------------|-----|-------------------------|------------------------|------------------------------------|-----------------------------------|
| ABCB1         | rs1045642 | 3435C>T      |                                    | 87  | 25 (29)                 | 43 (49)                | 19 (22)                            | 46.6                              |
|               | rs1128503 | 1236C>T      |                                    | 88  | 38 (43)                 | 35 (40)                | 15 (17)                            | 36.9                              |
|               | rs2032582 | 2677G>T or G>A |                                    | 80  | 32 (40)                 | 36 (45)                | 12 (15)                            | 37.5                              |
| CYP3A5        | rs776746  | 6986G>A      |                                    | 75  | 69 (92)                 | 6 (8)                  | 0 (0)                              | 4.0                               |
| NR1/2         | rs3814055 | 25385C>T     |                                    | 82  | 32 (39)                 | 35 (43)                | 15 (18)                            | 39.6                              |
|               | rs2276707 | 8055C>T      |                                    | 83  | 57 (69)                 | 21 (25)                | 5 (6)                              | 18.7                              |
| NR1/3         | rs2307424 | 5719C>T      |                                    | 88  | 45 (51)                 | 32 (36)                | 11 (12.5)                          | 30.7                              |
|               | rs2307418 | 7738A>C      |                                    | 86  | 61 (71)                 | 22 (26)                | 3 (3)                              | 16.3                              |
|               | rs4073054 | 7837T>G      |                                    | 87  | 40 (46)                 | 35 (40)                | 12 (14)                            | 33.9                              |
| Sunitinib pharmacokinetics
| HIF1A         | rs11549467 | 1790G>A      |                                    | 84  | 83 (99)                 | 1 (1)                  | 0 (0)                              | 0.6                               |
| PDGFRα        | rs35597368 | 1580T>C      |                                    | 88  | 69 (78)                 | 18 (20)                | 1 (1)                              | 11.3                              |
| VEGFR2        | rs1870377 | 1718T>A      |                                    | 81  | 46 (57)                 | 28 (35)                | 7 (9)                              | 25.9                              |
| VEGFR3        | rs307826  | 3971G>T      |                                    | 88  | 64 (73)                 | 23 (26)                | 1 (1)                              | 14.2                              |
| Alternative VEGF-independent proangiogenic pathways
| FGFR2         | rs2981582  | 906C>T       |                                    | 87  | 23 (26)                 | 52 (60)                | 12 (14%)                           | 43.6                              |
|               | rs4073    | 251T>A       | 5’ near gene                     | 79  | 25 (31)                 | 42 (53)                | 12 (15%)                           | 41.8                              |
| IL8           | rs2981582  | 906C>T       |                                    | 87  | 23 (26)                 | 52 (60)                | 12 (14%)                           | 43.6                              |
|               | rs4073    | 251T>A       | 5’ near gene                     | 79  | 25 (31)                 | 42 (53)                | 12 (15%)                           | 41.8                              |

Abbreviations: SNP = single-nucleotide polymorphism; VEGF = vascular endothelial growth factor; UTR = untranslated region; dbSNP = SNP database.
Table 4. Univariate and multivariate analyses: association between SNPs and outcome

| Gene (a) SNP ID | Polymorphism | No. of pts | Median PFS (months) | P-value (UV) | P-value (MV) | HR | 95% CI of HR | 95% CI of median PFS (months) |
|----------------|--------------|------------|---------------------|--------------|--------------|----|--------------|-------------------------------|
| ABCB1 rs1045642 3435C>T | CC | 25 | 14 | 0.67 | NA | NA | NA | NA |
| | CT | 43 | 15 | 0.031 | 0.027 | 0.464 | 0.234–0.918 | 11–25 |
| | TT | 19 | 18 | 0.45 | NA | NA | NA | NA |
| ABCB1 rs1128503 1236C>T | CT + CC | 73 | 19 | 0.0078 | 0.047 | 2.978 | 1.012–8.761 | 12–25 |
| | TT | 15 | 8 | 0.26 | NA | NA | NA | NA |
| ABCB1 rs2032582 2677G>T or G>A | GG | 32 | 14 | 0.18 | 0.155 | 1.513 | 0.856–2.675 | 11–38 |
| | GT/GA | 36 | 19 | 0.04 | 0.15 | 1.864 | 1.082–3.210 | 12–38 |
| | TT/TA | 12 | 15 | 0.813–2.870 | 11–25 |
| ABCB1 TCG copy | Present | 16 | 15 | 0.68 | NA | NA | NA | NA |
| | Absent | 64 | 19 | NA | NA | NA | NA | NA |
| CYP3A5 rs776746 6986G>A | GG | 69 | 18 | 0.36 | NA | NA | NA | NA |
| | AG | 6 | 21 | NA | NA | NA | NA | NA |
| NR1/2 s3814055 25385C>T | CC + CT | 67 | 18 | 0.047 | 0.025 | 1.864 | 1.082–3.210 | 12–38 |
| | TT | 15 | 19 | NA | NA | NA | NA | NA |
| NR1/2 rs2276707 8055C>T | CC + CT | 78 | 18 | 0.0078 | 0.047 | 2.978 | 1.012–8.761 | 12–25 |
| | TT | 5 | 7 | 0.26 | NA | NA | NA | NA |
| NR1/3 rs2037424 5719C>T | CC | 45 | 20 | 0.18 | 0.155 | 1.513 | 0.856–2.675 | 11–38 |
| | CT + TT | 43 | 15 | NA | NA | NA | NA | NA |
| NR1/3 rs2307418 7738A>C | AA | 61 | 14 | 0.45 | NA | NA | NA | NA |
| | AC + CC | 27 | 28 | NA | NA | NA | NA | NA |
| NR1/3 rs4073054 7837T>G | TT | 40 | 12 | 0.04 | 0.025 | 1.864 | 1.082–3.210 | 12–38 |
| | TG + GG | 47 | 21 | NA | NA | NA | NA | NA |
| NR1/3 CAT copy | Present | 51 | 15 | 0.67 | NA | NA | NA | NA |
| | Absent | 36 | 15 | NA | NA | NA | NA | NA |
| FGFR2 rs2981582 906C>T | TT | 12 | 7.5 | 0.012 | 0.031 | 2.669 | 1.094–6.511 | 5–11 |
| | CC | 23 | 14 | NA | NA | NA | NA | NA |
| IL8 rs4073 251T>A | TT | 25 | 8 | 0.22 | NA | NA | NA | NA |
| | AA | 12 | 21 | NA | NA | NA | NA | NA |
| PDGFRA rs35597368 1580T>C | TT | 69 | 19 | 0.088 | 0.188 | 1.528 | 0.813–2.870 | 11–25 |
| | TC + CC | 19 | 14 | NA | NA | NA | NA | NA |
| VEGFR2 rs1870377 1718T>A | TT | 48 | 15 | 0.76 | NA | NA | NA | NA |
| | TA + AA | 40 | 19 | NA | NA | NA | NA | NA |
| VEGFR3 (b) rs3078213971G>T | GT + TT | 24 | 10 | 0.077 | 0.032 | 1.981 | 1.060–3.702 | 7–21 |
| | GG | 64 | 18 | NA | NA | NA | NA | NA |
| VEGFR3 rs307826 1480A>G | AG + GG | 23 | 10 | 0.022 | 0.051 | 1.800 | 0.996–3.250 | 6–19 |
| | AA | 65 | 19 | NA | NA | NA | NA | NA |

| Gene (a) SNP ID | Polymorphism | No. of pts | Median OS (months) | P-value (UV) | P-value (MV) | HR | 95% CI of HR | 95% CI of median OS (months) |
|----------------|--------------|------------|---------------------|--------------|--------------|----|--------------|-------------------------------|
| ABCB1 rs1045642 3435C>T | CC | 25 | 45 | 0.37 | NA | NA | NA | NA |
| | CT | 43 | 27 | NA | NA | NA | NA | NA |
| | TT | 19 | 24 | NA | NA | NA | NA | NA |
| ABCB1 rs1128503 1236C>T | CT + CC | 73 | 34 | 0.055 | 0.025 | 0.415 | 0.193–0.894 | 23–45 |
| | TT | 15 | 21 | NA | NA | NA | NA | NA |
In this retrospective study, we aim to observe the impact of SNPs that have recently been proposed as predictors of outcome to antiangiogenic therapy in metastatic RCC in an independent cohort of patients. We observed significant associations between SNPs in genes involved in sunitinib pharmacokinetiks (ABCB1, NR1/2, and NR1/3), sunitinib pharmacodynamics (VEGFR2 and VEGFR3) and VEGF-independent pro-angiogenic pathways (FGFR2) and the therapeutic outcome of sunitinib in metastatic clear-cell RCC patients. For each of these associated SNPs, we observed similar

**Table 4. Continued**

| Gene (a) SNP ID | Polymorphism | No. of pts | Median PFS (months) | P-value (UV) | P-value (MV) | HR | 95% CI of HR | 95% CI of median PFS (months) |
|----------------|--------------|------------|---------------------|-------------|-------------|----|--------------|------------------------------|
| ABCB1 rs2032582 | GG           | 32         | 33                  | 0.49        | NA          | NA | NA           | NA                           |
| 2677 G>T or G>A | GT/GA        | 36         | 34                  |             | NA          | NA | NA           | NA                           |
| TT/TG          | 12           | 24         |                     |             |             | NA | NA           | NA                           |
| ABCB1 TCG copy | Present      | 16         | 26                  | 0.74        | NA          | NA | NA           | NA                           |
| Absent         | 64           | 34         |                     |             | NA          | NA | NA           | NA                           |
| CYP3A5 rs776746 | GG           | 69         | 30                  | 0.92        | NA          | NA | NA           | NA                           |
| 6986G>A        | AG           | 6          | NR                  |             |             | NA | NA           | NA                           |
| NR1/2 rs3814055 | CC + CT      | 67         | 30                  | 0.46        | NA          | NA | NA           | NA                           |
| TCS585>T       | 15           | 31         |                     |             |             | NA | NA           | NA                           |
| NR1/2 rs2276707 | CC + CT      | 78         | 31                  | 0.092       | 0.080       | 2.828 | 0.884–9.044 | 24–45                       |
| 8055C>T        | TT           | 5          | 12                  |             |             | 0.057 | 0.048       | 1.913                      |
| NR1/2 rs2307424 | CC           | 45         | 42                  |             | 0.057       | 0.048 | 1.913       | 25–Not reached              |
| 5719C>T        | CT + TT      | 43         | 23                  |             |             | 1.913 | 1.006–3.636 | 16–34                      |
| NR1/3 rs2307418 | AA           | 61         | 30                  | 0.86        | NA          | NA | NA           | NA                           |
| 7738A>C        | AC + CC      | 27         | 27                  |             | NA          | NA | NA           | NA                           |
| NR1/3 rs4073054 | TT           | 40         | 22                  | 0.03        | 0.035       | 1.927 | 1.046–3.549 | 14–34                      |
| 78377T>G       | TG + GG      | 47         | 35                  |             |             | 1.927 | 1.046–3.549 | 14–34                      |
| NR1/3 CAT copy | Present      | 51         | 28                  | 0.58        | NA          | NA | NA           | NA                           |
| Absent         | 36           | 30         |                     |             | NA          | NA | NA           | NA                           |
| FGFR2 rs2981582 | TT           | 12         | 23                  | 0.97        | NA          | NA | NA           | NA                           |
| 906C>T        | CC           | 36         | 30                  |             |             | NA | NA           | NA                           |
| ILB rs40735258 | TA           | 25         | 23                  | 0.68        | NA          | NA | NA           | NA                           |
| 2511T>A       | AA           | 12         | 31                  |             |             | NA | NA           | NA                           |
| PDGFRA rs35597368 | TT          | 69         | 35                  | 0.025       | 0.302       | 1.440 | 0.721–2.875 | 24–Not reached              |
| 1580T>C       | TC + CC      | 19         | 23                  |             |             | 1.440 | 0.721–2.875 | 24–Not reached              |
| VEGFR2 rs1870377 | TT           | 48         | 24                  | 0.63        | NA          | NA | NA           | NA                           |
| 1718T>A       | TA + AA      | 40         | 30                  |             | NA          | NA | NA           | NA                           |
| VEGFR3 (b) rs307821 | GT + TT   | 24         | 34                  | 0.056       | 0.011       | 2.265 | 1.202–4.268 | 11–42                      |
| 3971G>T       | GG           | 64         | 29                  |             |             | 2.265 | 1.202–4.268 | 11–42                      |
| VEGFR3 rs307826 | AG + GG      | 23         | 22                  | 0.0058      | 0.013       | 2.223 | 1.187–4.163 | 11–34                      |
| 1480A>G       | AA           | 65         | 31                  |             |             | 2.223 | 1.187–4.163 | 11–34                      |

**Abbreviations:** SNP = single nucleotide polymorphism; pts = patients; PFS = progression free survival; OS = overall survival; UV = univariate analysis; MV = multivariate analysis; NA = not applicable; HR = hazard ratio; 95% CI = 95% confidence interval. In the univariate analysis, P-values were calculated by a log rank test. In the multivariate analysis, P-values were calculated by Cox proportional hazards. Whenever possible, variants were combined as it was done in the original publications: this was the case for FGFR2 (IU), NR1/2, VEGFR2 and VEGFR3. For ABCB1 and NR1/3, the original publication only reported haplotypes. The haplotypes were tested against PFS and OS in our series and no association with PFS and OS could be shown. Therefore, we checked for each SNP the three subgroups and analysed the PFS and OS curves. For ABCB1, when analysing TT vs CC in rs1045642 or GG vs GT/GA vs TT/TG in rs2032582, the three curves were overlapping for PFS and OS. Only in ABCB1 rs11389303, when analysing TT vs TC vs CC variants, the CC and CT results were overlapping for PFS and OS and clearly different from the TT results, allowing us to group the results of the CT and CC variants. Concerning NR1/3, for SNP rs2307424, the PFS and OS curves of the CT and TT variants were overlapping, but the curves of the CC variant were clearly distinct. For SNP rs2307418, there were only two CC variant patients: they were grouped with the AC variant patients and tested against the AA variant patients. For SNP rs4073054, the PFS and OS curves of the TG and GG variants were overlapping, but the curves of the TT variant were clearly distinct. This distribution allowed us to test the impact of the CC variant in rs2307424, the AA variant in rs2307418 and the TT variant in rs4073054 vs the combination of the other variants. In case of CYP3A5, there were no AA variants in our series. For PDGFRA, there was only one CC variant: this patient was grouped with the TC variant. The HR for survival for patients with the GT or TT variants in rs307821 in VEGFR3 vs patients with the GG variant was 2.268, favouring longer survival in patients with the GG genotype. Nevertheless, because of a crossing of the curves, the median OS was longer in the GT and TT variants (see curves).

**DISCUSSION**

In this retrospective study, we aim to observe the impact of SNPs that have recently been proposed as predictors of outcome to antiangiogenic therapy in metastatic RCC in an independent cohort of patients. We observed significant associations between SNPs in genes involved in sunitinib pharmacokinetics (ABCB1, NR1/2 and NR1/3), sunitinib pharmacodynamics (VEGFR2 and VEGFR3) and VEGF-independent pro-angiogenic pathways (FGFR2) and the therapeutic outcome of sunitinib in metastatic clear-cell RCC patients. For each of these associated SNPs, we observed similar
SNPs and outcome on sunitinib in renal cell cancer

The expression of cytochrome P450 CYP3A4, thought to be the key enzyme for the hepatic biotransformation of sunitinib, is regulated by the ligand-activated nuclear receptors NR1I2 (pregnane X receptor) and NR1I3 (constitutive androstane receptor). We observed a significant association between the TT genotype in rs2276707 8055C>T in NR1I2 and a shorter PFS. Data on the impact of rs2981582 in FGFR2 on outcome on TKIs are only available in patients treated with pazopanib. In a series of 380 RCC patients, the TT variant was associated with inferior OS compared with the CC genotype (median OS 21.4 vs 28.0 months, P=0.002) (Xu et al, 2011b).

The expression of cytochrome P450 CYP3A4, thought to be the key enzyme for the hepatic biotransformation of sunitinib, is regulated by the ligand-activated nuclear receptors NR1I2 (pregnane X receptor) and NR1I3 (constitutive androstane receptor). We observed a significant association between the TT genotype in rs2276707 8055C>T in NR1I2 and a shorter PFS and OS. van der Veldt et al (2010) also found a significant difference in PFS between patients with the TT genotype (28 vs 20 months, P=0.009) (Xu et al, 2011b).

rs2032582). Functional studies have shown that the haplotype of these three SNPs (rs1046542, rs1128503 and rs2032582) is a silent mutation and alters the function of the efflux transporter including its substrate specificity. We observed a significant association between the TT variant in rs1128503 1236C>T and shorter PFS and OS. In 89 RCC patients treated with sunitinib, Garcia-Donas et al (2011) observed an association, although not significant, between rs1128503 and PFS (HR 1.42, P=0.089) and OS (HR 1.75, P=0.055), favoring the patients with a C-allele. In 129 RCC patients treated with sunitinib, van der Veldt et al (2010) observed that the presence of a TCG haplotype (rs1045642, rs1128503 and rs2032582) in ABCB1 (and thus the presence of the C-variant in rs1128503) was associated with prolonged PFS (P=0.033) and a tendency for prolonged OS (P=0.078). In 241 patients treated with pazopanib, the wild-type CC variant of rs1128503 was associated with improved OS compared with the wild-type TT genotype (28 vs 20 months, P=0.009) (Xu et al, 2011b).

Figure 1. (A and B) Kaplan–Meier curves for PFS and OS for SNP rs1128503 in ABCB1. The P-values are indicated for the univariate (UV) and multivariate (MV) analyses.

As sunitinib was used as a monotherapy and as a first-line treatment, our results were not confounded by concomitant or previous therapies and we could detect significant associations in a series involving only a limited number of patients.

The expression of cytochrome P450 CYP3A4, thought to be the key enzyme for the hepatic biotransformation of sunitinib, is regulated by the ligand-activated nuclear receptors NR1I2 (pregnane X receptor) and NR1I3 (constitutive androstane receptor). We observed a significant association between the TT genotype in rs2276707 8055C>T in NR1I2 and a shorter PFS and OS. van der Veldt et al (2010) also found a significant difference in PFS between patients with the CC/CT genotype and patients with the TT genotype, 10.8 vs 6.7 months (P=0.025), but they could not confirm these results on multivariate analysis. Concerning
NR1/3, we observed a significant association between the TT variant in SNP rs4073054 and shorter PFS and OS. Prolonged PFS (13.3 vs 8.0 months, \( P = 0.017 \)) was found in 136 patients with absence of a CAT copy in the NR1/3 haplotype (rs2307424, rs2307418 and rs4073054; \( P = 0.021 \)) (van der Veldt \textit{et al}, 2010). This corresponds with our results, as rs4073054 concerns the T in the NR1/3 between the CC genotype in rs 2307424 in the CAT haplotype. We also observed a significant association between the AG or GG variants in rs4073054 and shorter PFS and OS. The T in rs35597368 vs G and shorter OS. In a series of 89 RCC patients treated with sunitinib, TTP for the GT variant of rs35597368). van der Veldt \textit{et al} (2010) observed on univariate analysis a better OS in patients with a GCGT haplotype in both alleles (GCGT–GCGT), and thus in patients with a TT variant of the SNP, whereas patients with a GCG–other or other–other haplotype had a poorer median OS: 24.2 vs 14.8 months (\( P = 0.002 \) on univariate analysis but 0.108 on multivariate analysis). We could not confirm the association with PFS and OS on multivariate analysis. The functional impact of this SNP is presently unknown.

The \textit{VEGFR3} signalling is involved in embryonic angiogenesis, adult lymphangiogenesis and tumoural angiogenesis (Partanen \textit{et al}, 1999; Valtola \textit{et al}, 1999) and is one of the main targets of sunitinib. We observed a significant association between the GT or TT variant in rs307821 3971G \( \rightarrow \) T in \textit{VEGFR3} and shorter PFS and OS. Note that because of a crossing of the curves, the median OS was longer in the GT and TT variants than in the GG variants of rs307821. Nevertheless, the HR for survival for patients with the GT or TT variants in rs307821 in \textit{VEGFR3} vs patients with the GG variant was 2.265 (95% CI 1.202–4.238). The crossing of the curves is probably because of the limited number of patients in our series. We also observed a significant association between the AG or GG variant in rs307826 1480A \( \rightarrow \) G and shorter OS. In a series of 89 RCC patients treated with sunitinib, TTP for the GT variant of rs307821 of was 6.7 months vs 13.7 months for patients with the GG genotype (\( P = 0.00085 \)) and TTP for the GA variant of
rs307826 was 3.6 months vs 13.7 months for patients with the AA genotype \((P = 0.00049)\). There was no significant association with OS (Garcia-Donas et al., 2011). In 228 patients treated with pazopanib, OS was 26 months in the AA variant vs 23 months in the AG variant \((P = 0.04)\) of rs307826 but, surprisingly, these authors did not find any association between the SNP and PFS (Xu et al., 2011a,b). This matches the observation of van der Veldt et al. (2010), who reported no significant effect of rs307826 on PFS after the trial \(11.0\) and OS 26.0 months; Motzer et al., 2007). This difference is likely because of the patient selection in our series: all the patients had to complete at least one cycle of sunitinib and had to reach at least the first evaluation by CT scan.

Our study has several potential limitations. (1) It was a retrospective analysis of patients treated in several centres without a central protocol dictating schedule and dose modifications or timing of radiological assessments. (2) 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. (3) We failed to genotype SNP rs1126647 in IL-8 because of technical reasons. (4) In case of polymorphisms may be less frequent. (5) We confirmed several associations between polymorphisms in genes linked to pharmacokinetics and pharmacodynamics of sunitinib and therapeutic outcome of patients receiving sunitinib for metastatic RCC. These associations had previously been described in other series of patients treated with sunitinib or pazopanib.

The impact of SNPs in pathways linked to pharmacokinetics and pharmacodynamics of sunitinib shows that besides acquired genetic characteristics of tumour cells, patient’s germline genetic variation may also affect the efficacy of anticancer therapy.

We confirmed several associations between polymorphisms in pathways linked to pharmacokinetics and pharmacodynamics of sunitinib and therapeutic outcome of patients receiving sunitinib for metastatic RCC. These associations had previously been described in other series of patients treated with sunitinib or pazopanib.
Time (months)

0 6 12 18 24 30 36 42 48

Subjects at risk (AA) 65 54 37 27 18 15

Subjects at risk (AG+GG) 23 16 9 8 5 3 1

P = 0.022 (UV) and 0.051 (MV)

Figure 7. (A and B) Kaplan–Meier curves for PFS and OS for SNP rs307826 in VEGFR3. The P-values are indicated for the univariate (UV) and multivariate (MV) analyses.

Table 5. Distribution of SNP genotypes in patients exhibiting progressive disease and partial response as the best response

| Gene (a)                  | SNP ID     | In patients with PD as their best response (n = 10) | In patients with SD, PR or CR as their best response (n = 78) | P-value by Fisher’s exact | Adjusted P-value by logistic regression | Odds ratio (95% CI) |
|---------------------------|------------|---------------------------------------------------|-----------------------------------------------------------------|--------------------------|----------------------------------------|-------------------|
| Genes involved in pharmacokinetics |            |                                                   |                                                                  |                          |                                        |                   |
| ABCB1                     | rs1045642  | CC 2/10 (20%)                                     | CC 23/77 (30%)                                                  | NS                       | —                                      | —                 |
|                          | rs1128503  | TT 3/10 (30%)                                     | TT 12/78 (15%)                                                  | NS                       | —                                      | —                 |
|                          | rs2032582  | TT or TA 2/8 (25%)                                | TT or TA 10/72 (14%)                                            | NS                       | —                                      | —                 |
|                          | TCG copy   | Not present 7/7 (100%)                            | Not present 54/70 (77%)                                         | NS                       | —                                      | —                 |
|                          | rs776746   | GG 8/8 (100%)                                     | GG 61/67 (91%)                                                  | NS                       | —                                      | —                 |
|                          | rs3814065  | TT 3/8 (38%)                                      | TT 12/74 (16%)                                                  | NS                       | —                                      | —                 |
|                          | rs2276707  | TT 2/8 (25%)                                      | TT 3/5 (4%)                                                    | NS                       | —                                      | —                 |
|                          | rs2307424  | CC 6/10 (60%)                                     | CC 36/78 (46%)                                                 | NS                       | —                                      | —                 |
|                          | rs2307418  | AA 8/10 (80%)                                     | AA 49/78 (63%)                                                 | NS                       | —                                      | —                 |
|                          | rs4073054  | TT 7/10 (70%)                                     | TT 31/78 (40%)                                                 | NS                       | —                                      | —                 |
|                          | CAT copy   | Present 8/10 (80%)                                | Present 40/77 (52%)                                             | 0.09                     | NS                                     | —                 |
| Genes involved in pharmacodynamics |            |                                                   |                                                                  |                          |                                        |                   |
| PDGFRA                    | rs35597368 | TT 8/10 (80%)                                     | TT 61/78 (78%)                                                 | NS                       | —                                      | —                 |
| VEGFR2                    | rs1870377  | TT 7/10 (70%)                                     | TT 41/78 (53%)                                                 | NS                       | —                                      | —                 |
| VEGFR3                    | rs307821   | GT + TT 5/10 (50%)                                | GT + TT 18/78 (23%)                                            | 0.07                     | 0.05                                   | 5.763 (0.986–33.693) |
|                          | rs307826   | GA + GG 6/10 (60%)                                | GA + GG 17/78 (22%)                                            | 0.01                     | 0.02                                   | 7.011 (1.372–42.209) |
| Genes in alternative proangiogenic factors |            |                                                   |                                                                  |                          |                                        |                   |
| FGFR2                     | rs2981582  | TT 2/10 (20%)                                     | TT 10/77 (13%)                                                 | NS                       | —                                      | —                 |
| IL8                       | rs4073     | AA 1/9 (11%)                                      | AA 11/70 (16%)                                                 | NS                       | —                                      | —                 |

Abbreviations: PR = partial response; PD = progressive disease; CR = complete response; SNP = single-nucleotide polymorphism; 95% CI = 95% confidence interval; SD = stable disease; NS = non-significant. The logistic regression analysis was adjusted for the presence of sarcomatoid dedifferentiation, the MSKCC score and baseline neutrophils. Variants were combined as follows: ABCB1: a TCG copy was linked to better outcome in van der Veldt et al (2010). Therefore, we analysed the impact of CC in rs1045642, TT in rs1128503 and TT (or TA) in rs2032582; CYP3A5: the TT variant was linked to poor outcome in van der Veldt et al (2010); NR1/2: rs3814055 and rs2276707: the TT variant was linked to poor outcome in van der Veldt et al (2010); NR1/3: a CAT copy was linked to poor outcome in van der Veldt et al (2010). Therefore, we analysed the impact of CC in rs2307424, AA in rs2307418 and TT in rs4073054; FGFR2: the TT variant was linked to poor outcome in Costa et al (2010). Therefore, we analysed the impact of CC in rs307824; AA in rs270419 and TT in rs407304; VEGFR3: the TT variant was linked to poor outcome in Garcia-Donas et al (2011); VEGFR3 rs307826: the GA/GG variant was linked to poor outcome in Garcia-Donas et al (2011).
Moreover, germline DNA is inherited, fixed and relatively insensitive to time and environmental factors, which makes it more reliable than nucleotide and protein biomarkers linked to the tumour.

If the impact of these and other SNPs on outcome on sunitinib could be validated prospectively in independent series, scoring systems based on the combination of several unfavourable or favourable SNPs could be elaborated. When combining these SNPs with clinical and biochemical parameters associated with outcome, we will probably be able to predict more precisely the chance of response to sunitinib and identify primary resistant patients in order to direct them towards other therapies, avoiding unnecessary side effects and costs. Similarly, we will be able to predict more accurately disease progression, which is the time point of secondary resistance to sunitinib. Polymorphisms could also help us to identify those patients whose ideal starting dose of sunitinib could be higher than the usual 50 mg daily, for instance, patients with genotypes and haplotypes leading to lower sunitinib plasma levels.

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CONFLICT OF INTEREST

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