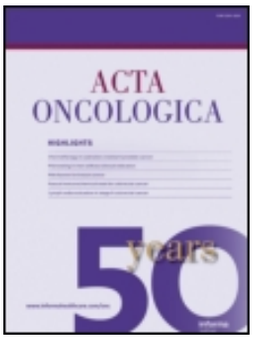

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ORIGINAL ARTICLE

Efflux pump ABCB1 single nucleotide polymorphisms and dose reductions in patients with metastatic renal cell carcinoma treated with sunitinib

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ABSTRACT

There is growing evidence that sunitinib plasma levels have an impact on treatment outcome in patients with metastatic renal cell carcinoma (mRCC). We studied the impact of single nucleotide polymorphisms (SNPs) in genes involved in sunitinib pharmacokinetics, and additionally, sunitinib pharmacodynamics on dose reductions of the tyrosine kinase inhibitor.

Methods. We retrospectively analyzed germ-line DNA retrieved from mRCC patients receiving sunitinib as first-line therapy. We genotyped 11 key SNPs, respectively, in *ABCB1, NR1/2, NR1/3* and *CYP3A5,* involved in sunitinib pharmacokinetics as well as *VEGFR1* and *VEGFR3*, which have been suggested as regulators of sunitinib pharmacodynamics. Association between these SNPs and time-to-dose-reduction (TTDR) was studied by Cox regression.

Results. We identified 96 patients who were treated with sunitinib and from whom germ-line DNA and data on dose reductions were available. We observed an increased TTDR in patients carrying the TT-genotype in *ABCB1* rs1125803 compared to patients with CC- or CT-genotypes (19 vs. 7 cycles; $p = 0.031$ on univariate analysis and $p = 0.012$ on multivariate analysis) and an increased TTDR in patients carrying the TT/TA-variant in *ABCB1* rs2032582 compared to patients with the GG- or GT/GA-variant (19 vs. 7 cycles; $p = 0.046$ on univariate analysis and $p = 0.024$ on multivariate analysis).

Conclusion. mRCC patients carrying the rs1128503 TT-variant or the TT/TA-variant in rs2032582 in *ABCB1*, which encodes for an efflux pump, do require less dose reductions due to adverse events compared to patients with the wild type or heterozygote variants in these genes.

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Inactivation of the von Hippel-Lindau (*VHL*) tumor suppressor gene is the most frequent molecular alteration in clear cell renal cell carcinoma (RCC). Inactivated *VHL* leads to elevated protein levels of hypoxia-induced factor-α which upregulates the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) pro-angiogenic signaling pathways. Targeted therapies directed against the VEGF- and PDGF-receptor have significantly improved the outcome of patients with metastatic renal cell carcinoma (mRCC). 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 randomized controlled trial sunitinib significantly prolonged progression-free survival (PFS) (11 vs. 5 months, $p < 0.001$) as compared to interferon alpha [1]. Median overall survival (OS) was 26.4 and 21.8 months, respectively ($p = 0.051$) [2]. Sunitinib is a current standard treatment option in mRCC, but other anti-VEGFR and anti-PDGFR-targeted TKIs like sorafenib, pazopanib and axitinib are also used in certain clinical settings.

Although 50% of RCC patients receiving sunitinib experience an objective response and 43% achieve disease stabilization, 7% will experience progressive disease (PD) at first evaluation probably due to intrinsic resistance or due to other factors [2]. Moreover, even patients with an initial clinical benefit will finally progress due to acquired resistance or for other reasons. Although different mechanisms of primary and secondary resistance have been proposed, reliable biomarkers predictive of sunitinib sensitivity or primary/secondary resistance are still lacking [3].

Sunitinib plasma levels are not dosed in clinical routine, even though it is known that sunitinib plasma levels might impact efficacy of sunitinib treatment in mRCC. A population pharmacokinetic analysis of sunitinib and its primary active metabolite, SU12662, found that the pharmacokinetics of both compounds were significantly influenced by several covariates including gender, age, and weight; however, the magnitude of the predicted changes in exposure minimized the necessity for dose adjustments [4]. 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 time-to-tumor-progression (TTP) and OS [5]. Other studies have shown that the occurrence of adverse events, in particular hypertension, is possibly linked to improved treatment outcome [6]. Finally, several studies in RCC have shown associations between polymorphisms in genes linked to sunitinib pharmacokinetics and outcome on sunitinib [7–10] or pazopanib [11,12].

The main objective of the present study was to analyze the impact of SNPs in selected genes potentially linked to sunitinib pharmacokinetics (*ABCB1, NR1/2, NR1/3* and *CYP3A5*) and the occurrence of dose reductions during treatment. Additionally, we analyzed the impact of SNPs in two genes encoding sunitinib targets (*VEGFR1* and *VEGFR3*), linked to sunitinib efficacy, and the occurrence of dose reductions.

Materials and methods

For the purpose of this retrospective study, germline DNA samples were collected from the "CIT-rein" kidney tumor bank and from patients treated at the University Hospitals Leuven. The French-Belgian multicentric "CIT-rein" kidney tumor bank contains more than 250 frozen pathologically confirmed RCC tumor samples collected at 20 academic hospitals. In the "CIT-rein" kidney tumor bank, we selected the samples of patients treated in first-line with sunitinib at a starting dose of 50 mg/day four weeks on, two weeks off and of whom frozen normal kidney tissue as well as data on dose reductions were available. In order to extend the series, we sampled peripheral blood in all the RCC patients treated at the University Hospitals Leuven from July 2011 to December 2012 applying the inclusion criteria. Eligible patients could have received cytokines as systemic treatment for kidney tumors, but they could not have received any other TKI or mammalian target of rapamycin (mTOR) inhibitor before starting sunitinib.

Dose reduction policy and timing of clinical radiological assessments were left to the discretion of the attending doctors in accordance with current local practice guidelines. Usually, whenever necessary for tolerance issues, in a first step, the dose is reduced to 37.5 mg/day and, if necessary, in a second step to 25 mg/day. In some patients, sunitinib is definitively stopped for tolerance issues.

The endpoint of this study was time-to-dose-reduction (TTDR), calculated as the time between the start of sunitinib and the occurrence of a dose reduction to 37.5 mg/day or of definitive stop of sunitinib for tolerance issues. Therefore, in this study, the SNPs were primarily evaluated as toxicity-related markers, although it was also foreseen to check our previous findings on associations between these SNPs and outcome in this patient series in order to show the inverse correlation between TTDR and outcome. If a patient's regimen was switched from 50 mg/day to 37.5 mg/day continuously because of flare-up during the two weeks off sunitinib, this was

not considered as a dose reduction for adverse event and the censing was closed at the moment of dose adaptation.

For the statistical analysis, the genotypes were combined as much as possible, as it was done in the original publications. Details and exceptions to this rule are documented in the legend of Table IV.

For those SNPs that were significantly associated with TTDR, we also analyzed their association with PFS and OS. For this efficacy analysis, only patients with clear cell RCC were considered as previous publications on associations between polymorphisms and efficacy were only reported in clear cell RCCs. Moreover, for the efficacy analysis, all the patients had to complete at least one cycle of sunitinib and had to reach at least the first evaluation by computed tomography (CT) scan. Response evaluation was done by RECIST in most of the cases.

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 utilization of remaining tissue was foreseen by the institutional board.

SNPs with potential relevance for sunitinib dose reductions were selected from the literature (Table IA and B). In particular, we included SNPs in genes linked to sunitinib or pazopanib pharmacokinetics or pharmacodynamics associated with efficacy and/or dose reductions in previous publications with sunitinib [7–10] or pazopanib [11,12].

DNA was isolated 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 with the Qiagen DNA kit (Qiagen) and the final DNA concentration was quantified with Nanodrop (Nanodrop, Wilmington, DE, USA). High-throughput SNP genotyping was performed using the Sequenom MassArray platform [13]. Investigators blinded for the clinical data performed the genotyping analysis.

Table IA. Analyzed SNPs linked to sunitinib pharmacokinetics.

Polymorphism	Number of patients therapy	Reasons for selection of SNPs for this project
ABCB1		
rs1128503 1236C>T	88 pts Sunitinib	Better PFS (19 vs. 8 months; p = 0.027) and OS (34 vs. 21 months; p = 0.025) in the CC/CT-genotype compared to the TT-genotype in rs1128503 [9].
rs1045642 3435C>T	89 pts Sunitinib	Trend to better PFS (HR 1.42; p = 0.089) and better OS (HR 1.75; p = 0.055) in favor of the CC- and CT-genotype in rs1128503 [8].
rs2032582 2677G>T or G>A	129 pts Sunitinib	Better PFS (15.2 vs. 8.4 months; p = 0.033) and a tendency for prolonged OS (23.9 vs. 15.4 months; p = 0.078) in presence of a TCG haplotype (rs1045642 – rs1128503 – rs2032582) in ABCB1 (thus a CC-genotype in rs112503) [7].
	241 pts Pazopanib	Better OS (28 vs. 20 months, p = 0.009) in the CC-genotype compared to the TT-genotype in rs1128503 [12].
CYP3A5		
rs776746 6986G>A	128 pts Sunitinib	Better PFS (not reached vs. 9.3 months) for the AA- and AG-genotypes compared to the GG-genotypes (p = 0.032) [7].
	84 pts Sunitinib	More dose-reductions (HR 3.75; p = 0.022) in the AG-genotype compared to the GG-genotype [8].
NR1/2		
rs3814055 25385C>T	136 pts Sunitinib	Better PFS (10.8 vs. 6.7 months; p = 0.025) and better OS (17.1 vs. 10.2 months; p = 0.017) for the CT- and CC-genotypes compared to the TT-genotype [7].
	241 pts Pazopanib	Better OS for the CC-genotype: 29 vs. 22 vs. 23 months for the CC-, CT- and TT-variants, respectively (p = 0.03) [11].
NR1/2		
rs2276707 8055C>T	136 pts Sunitinib	Better PFS (10.8 vs. 6.7 months) in the CC- and CT-genotypes compared to the TT-genotype (p = 0.025) [7].
	83 pts Sunitinib	Better PFS (18 vs. 7 months; p = 0.047) and trend for better OS (31 vs. 12 months; p = 0.08) in the GG- and GT-genotype compared to the TT-genotype [9].
NR1/3		
rs4073054 7837T>G	135 pts Sunitinib	Better PFS (13.3 vs. 8.0 months) if a CAT-copy was absent in the NR1/3 haplotype composed of rs2307424, rs2307418 and rs4073054 (thus no TT-genotype in rs4073054) (p = 0.017) [7].
	87 pts Sunitinib	Better PFS (21 vs. 12 months; p = 0.025) and OS (35 vs. 22 months; p = 0.035) in the GG- and GT-genotype compared to the TT-genotype [9].

OS, overall survival; PFS, progression-free survival; Pts, patients; SNP, single nucleotide polymorphism.

Table IB. Analyzed SNPs linked to sunitinib pharmacodynamics.

Polymorphism	Number of patients therapy	Reasons for selection of SNPs for this project
VEGFR1		
rs9582036 319A>C	91 pts Sunitinib	Better PFS (18 vs. 10 months; p = 0.06) and better OS (31 vs. 14 months; p = 0.008) in the AA- and AC-genotypes compared to the CC-genotype [10].
VEGFR3		
rs307826 1480A>G	89 pts Sunitinib	Better PFS (13.7 vs. 3.6 months; p = 0.0079) in the AA-genotype compared to the AG-genotype [8].
	241 pts Pazopanib	OS of 26, 23 and 3.2 months for the AA-, AG- and GG-genotypes, respectively (p = 0.04) [12].
	88 pts Sunitinib	Better PFS (19 vs. 10 months; p = 0.051) and OS (31 vs. 22 months; p = 0.013) in the AA-genotype compared to the AG- and GG-genotype [9].

OS, overall survival; PFS, progression-free survival; Pts, patients; SNP, single nucleotide polymorphism.

Overall, the 11 selected SNPs were successfully genotyped with success rates ≥ 92% for each SNP and an overall average success rate of 98%. For most of the SNPs, genotypes were analyzed in the same way as they were described in the original reports (i.e. according to dominant, recessive or co-dominant genetic models or in the context of a specific haplotype). Details are given in Table IV.

Clinical data were collected at 12 different sites in France (11) and Belgium (1). TTDR, PFS and OS were calculated by Cox regression. Based on the data of Houk et al. [4], we considered that gender, age and general shape of the patient as reflected by his IMDC prognostic score could influence tolerance and as a consequence TTDR. These factors were tested in univariate analysis, except patient weight which was not available. Any parameter related to TTDR in univariate analysis by Kaplan-Meier with a p-value < 0.2 was included in the multivariate model (Cox regression). Without correction for multiple testing, results with a p-value of < 0.05 were considered as significant. However, correction for multiple testing by Bonferroni, taking into account the fact that the correlation with 11 SNPs was analyzed, indicated a p-value of < 0.005 as the threshold for significance. Statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) and XLSTAT software (Addinsoft, Paris, France).

Results

We used tissue and clinical information from 96 patients who started sunitinib between November 2005 and November 2012 and closed the follow-up database in June 2013. For 72 patients, frozen normal kidney samples from the "CIT-rein" kidney tumor bank were used and for 24 additional patients treated in Leuven, peripheral blood was used. The data of 81 of these patients were used in a previous publication on the impact of *ABCB1* polymorphisms on outcome by our own group [8]. Table II shows the clinical characteristics of these patients. Mean age at diagnosis was 59 years (range 25–84). The majority of patients (> 95%) were of Caucasian origin. According to the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) prognostic criteria [14], 14% of patients were categorized into

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

At initial diagnosis	Total
Male	72% (69/96)
Mean age	59 years
Ethnic origin	
Caucasian	95% (91/96)
Unknown	5% (5/96)
Synchronous metastasis	51% (47/93)
Fuhrman grade	
1–3	46% (43/94)
4	54% (43/94)
Clear cell histology	92% (88/96)
At the start of sunitinib	
ECOG PS > 0	42% (40/96)
Neutrophils > 4.500/mm³	43% (40/94)
Platelets > 400.000/mm³	13% (12/96)
Hemoglobin low [< 11.5 g/dl (women) or < 13 g/dl (men)]	43% (41/96)
LDH > 1.5 ULN	9% (8/94)
Corrected Calcium > 10 mg/dl	8% (7/93)
Time from nephrectomy to systemic treatment < 12 months	63% (90/96)
Immunotherapy before sunitinib	25% (24/96)
Site of metastasis	
Lung	79% (76/96)
Liver	20% (19/96)
Bone	39% (37/96)
Brain	7% (7/96)
IMDC prognosis	
Favorable	14% (13/96)
Intermediate	59% (57/96)
Poor	27% (26/96)

ECOG PS, Eastern Cooperative Oncology Group Performance Status; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; LDH, lactate deshydrogenase; ULN, upper limit of normal.

the favorable risk group, 59% had intermediate and 27% poor risk.

Forty-nine of 96 patients (51%) required dose reductions or definitive stop of sunitinib after a median TTDR of five cycles (46 dose reduction and 3 treatment withdrawal). Median TTDR in all patients, i.e. in those undergoing dose reduction and those not undergoing dose reduction, was nine cycles. The most frequent reason for dose reduction were hand foot skin reactions (17 patients), followed by diarrhea (14 patients), fatigue (9 patients), arterial hypertension (5 patients) and thrombocytopenia (5 patients). Less common reasons for dose reductions were anorexia, cardiotoxicity, mucositis, nausea and neutropenia. At the time of analysis, 71 (74%) patients had progressed and 59 (61%) had died. The median follow-up was 59 months (range 2–89 months) after the start of sunitinib. The median PFS of the whole study population was 15 months and the median OS 29 months. Best response could be evaluated in 91 patients. Seven of 91 (7.7%) patients had a complete response (CR), 33/91 (36.3%) patients a partial response (PR), 37/91 (40.7%) stable disease (SD) and 14/91 (15.4%) PD as best response.

For each of the 11 genotyped polymorphisms the respective genotypes, allele frequencies and changes at the amino acid level are described in Table III. The observed allele frequencies for each polymorphism were similar as previously reported in the dbSNP database (dbSNP build 136) or 1000 Genomes Project, except for SNPs rs2276707.

The association between these polymorphisms and TTDR, as assessed by univariate analysis, are reported in Table IV and displayed in Figures 1–3. We observed increased TTDR in patients carrying the rs1125803 TT-genotype compared to patients carrying the CC- or CT-genotypes in *ABCB1* (19 vs. 7 cycles; $p = 0.031$). Likewise, we observed increased TTDR in patients carrying the TT/TA-variant in *ABCB1* rs2032582 (19 vs. 7 cycles; $p = 0.046$), but rs1128503 and rs2032582 were in high linkage disequilibrium ($r^2 = 0.984$) with each other. We also observed increased TTDR in patients with the TT-genotype in NR1/2 rs2776707 compared to patients with CC- and CT-genotypes (41.5 vs. 7 cycles; $p = 0.027$). We could not observe any association between SNPs in *NR1/3, CYP3A5, VEGFR1* and *VEGFR3* and TTDR.

In view of an adjusted p-value and of the multivariate analysis, we checked TTDR in female and male (10 vs. 9 months; $p = 0.13$) and in IMDC good and intermediate versus poor risk patients (11 vs. 7 months; $p = 0.054$). Age at start of sunitinib (under or above the median age of 61 years) had no influence on TTDR [HR 0.89 (95% CI 0.49–1.60); $p = 0.69$]. Unfortunately, patient weight at start of sunitinib therapy was not available in a considerable part of the patients. Taking in to account gender and IMDC, the adjusted p-value is 0.014 for rs1128503 in *ABCB1*, 0.025 for rs2032582 in *ABCB1* and 0.063 for rs2776707 in *NR1*/2.

In a next step, we introduced the other polymorphism in the multivariate analysis. Including gender,

Table III. Genotype and allele distribution of selected SNPs.

RS ID	Polymorphism	Location or functional consequence	n	Wildtype/ Wildtype n (%)	Wildtype/ Variant n (%)	Variant/ Variant n (%)	Observed minor allele frequency (%)	Minor allele frequency in dbSNP (%)
ABCB1								
rs1045642	3435C>T	I1154I	96	27 (28)	49 (51)	20 (21)	46.3	53.4
rs1128503	1236C>T	G412G	95	36 (39)	43 (45)	16 (17)	39.5	45.1
rs2032582	2677G>T or G>A	A893S	89	32 (36)	42 (47)	15 (17)	40.5	41.7
CYP3A5								
rs776746	6986G>A	Affecting splicing	88	78 (89)	11 (8)	0 (0)	6.3	3.6
NR1/2								
rs3814055	25385C>T	UTR-5	93	36 (39)	39 (42)	18 (19)	40.3	33.6
rs2276707	8055C>T	Intron	91	60 (66)	25 (27)	6 (7)	20.3	9.3
NR1/3								
rs2307424	5719C>T	P151P	96	49 (51)	37 (39)	10 (10)	29.6	33.6
rs2307418	7738A>C	Intron	96	71 (74)	25 (26)	1 (1)	14.1	15.9
rs4073054	7837T>G	Intron	96	38 (40)	43 (45)	15 (16)	39.1	40.7
VEGFR1								
rs9582036	319A>C	Intron	96	46 (48)	41 (43)	9 (9)	30.7	31.3
VEGFR3								
rs307826	1480A>G	T494A	96	72 (75)	22 (23)	2 (2)	13.5	10.2

n, number of patients with successful determination of polymorphisms. Note that rs2307424 and rs4073054 in NR1/3 were analyzed because of their involvement in the CAT-haplotype [6]. rs2032582 and rs1045642 in ABCB1 were analyzed because of their involvement in the TCG-haplotype [6].

Table IV. Univariate analysis: association between SNPs and time of dose reduction.

Gene (a) SNP ID	Polymorphism	No. of patients	Median TTDR (cycles)	p (UV)	HR	95% CI of HR
ABCB1						
rs1128503	CT + CC	73	7	0.031	2.278	1.077–4.820
1236C > T	TT	15	19			
ABCB1						
rs1045642	CC	27	12	0.26	NA	NA
3435C > T	CT	49	7			
	TT	20	19			
	CC + CT	76	9	0.47	NA	NA
	TT	20	19			
ABCB1						
rs2032582	GG	32	11	0.048	NA	NA
2677G > T or G > A	GT/GA	42	5			
	TT/TA	15	19			
	GG + GT/GA	74	7	0.046	2.106	1.015–4.371
	TT/TA	15	19			
CYP3A5						
rs776746	GG	78	9	0.23	NA	NA
6986G > A	AG	10	NR			
NR1/2						
rs3814055	CC + CT	75	10	0.35	NA	NA
25385C > T	TT	18	5			
NR1/2						
rs2276707	CC + CT	85	7	0.027	2.954	1.132–7.707
8055C > T	TT	6	41.5			
NR1/3						
rs2307424	CC	49	11	0.90	NA	NA
5719C > T	CT + TT	47	7			
NR1/3						
rs2307418	AA	71	10	0.92	NA	NA
7738A > C	AC + CC	25	7			
NR1/3						
rs4073054	TT	38	7	0.92	NA	NA
7837T > G	TG + GG	58	9			
VEGFR1						
rs9582036	AA + AC	87	9	0.46	NA	NA
319A > C	CC	9	5			
VEGFR3						
rs307826	AA	72	9	0.85	NA	NA
1480A > G	AG + GG	24	9			

p-values were calculated by a log-rank test. HR, hazard ratio; NA, not applicable; 95% CI, 95% confidence interval; TTDR, time-to-dose-reduction; UV, univariate analysis.

For ABCB1 rs1128503, we analyzed genotype TT against and the combination of genotype CC and CT, because four previous publications clearly associated the TT-variant with poor outcome. For ABCB1 rs1045642 and rs2032582, we analyzed the three genotypes separately and then combined the genotypes in function of the obtained graphs, isolating the groups that were associated to the longest TTDR. In case of CYP3A5, there were no AA-variants in our series. For NR1/2, the variants were combined as it was done in the original publications isolating the patients with TT-genotype, associated with poorer outcome. For NR1/3 rs2307424, the analysis of the three genotypes separately did not result in a significant difference in TTDR. We report the combination of the genotypes as it was done in previous publications. For NR1/3 rs2307418, there was only one patient with the CC-genotype. For NR1/3 rs4073054, we compared the TT-genotype to the TG- and GG-genotype because the TT-genotype was associated to poorer survival. For VEGFR1 rs9582036, the genotypes were pooled as it was done in the original publication. For VEGFR3 rs307826, there were only two patients with the GG-genotype: they were pooled with the GA-genotype patients.

IMDC (good and intermediate vs. poor), rs1128503 in *ABCB1* and rs2776707 in NR1/2, the p-value for the association between these SNPs and TTDR were 0.012 and 0.058, respectively. With rs2032582 (in *ABCB1* instead of rs1128503) and rs2776707, the p-value for the association between these SNPs and TTDR were 0.024 and 0.060, respectively. Note that rs1128503 and rs2032582 were not included in the same multivariate analysis, because of their high linkage disequilibrium ($r^2 = 0.984$).

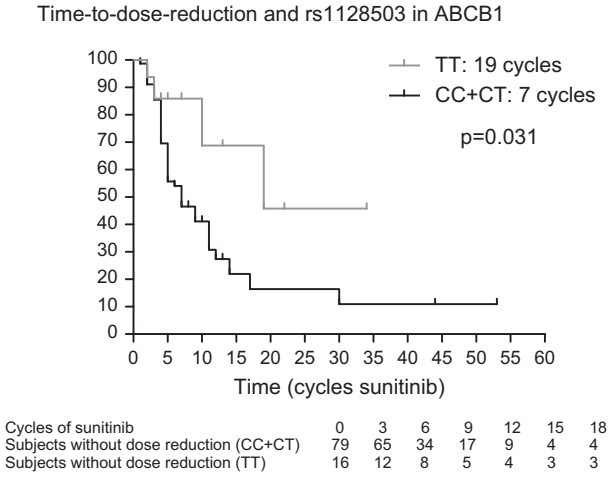

Figure 1. Impact of ABCB1 rs1128503 variants on timing of dose reductions.

In a previous publication, we showed the impact of SNP rs1128503 in *ABCB1* on outcome in mRCC treated with sunitinib. In order to show the inverse correlation between TTDR and outcome, we checked the impact of the SNPs associated with dose reductions on outcome. As there is no complete overlap with the previously published series, we report the outcome data of the present patient series. In patients with clear cell histology, we have found a trend to a shorter PFS (11.5 vs. 16 months, $p = 0.078$) and a shorter OS (24 vs. 34 months, $p = 0.016$) in patients with the TT-genotype compared to patients with the CC- and CT-genotypes in rs1125803 in *ABCB1*, a trend to a shorter PFS (15 vs. 18 months, $p = 0.094$) and a shorter OS (26 vs. 41 months, $p = 0.012$) in patients with the TT/TA-genotype compared to patients with the GG- and GA/GT-genotype in rs2032582 in *ABCB1* and a shorter PFS (7 vs. 18 months; $p = 0.011$) and a trend to a shorter OS (12 vs. 31 months; $p = 0.14$)

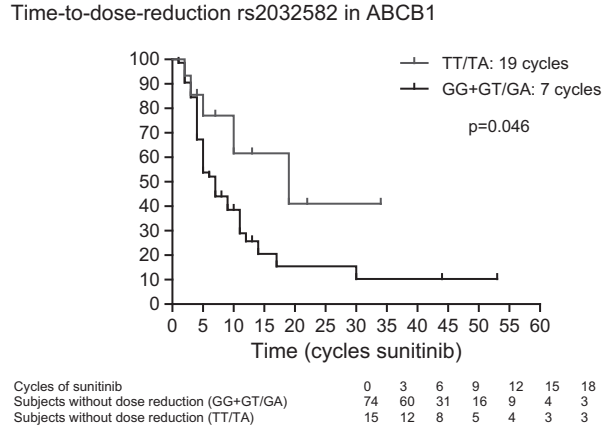

Figure 2. Impact of ABCB1 rs2032582 variants on dose reductions.

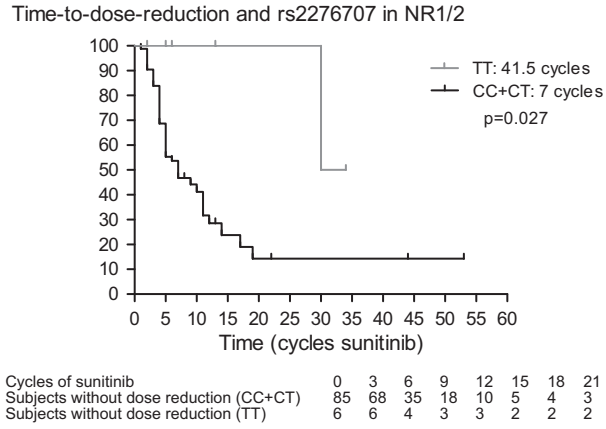

Figure 3. Impact of NR1/2 rs2776707 variants on dose reductions.

in patients with the TT-genotype compared to patients with the CC- and CT-genotypes in rs2776707 in *NR1*/2 (Figures 4 and 5).

Discussion

The main objective of the present study was to analyze the impact of SNPs in selected genes potentially linked to sunitinib pharmacokinetics (*ABCB1, NR1/2, NR1/3* and *CYP3A5*) and the occurrence of dose reductions during treatment. We hypothesized that patients carrying genotypes that reduce absorption of sunitinib or increase metabolism of sunitinib – through lower sunitinib plasma levels and less frequent adverse events – less frequently require dose reductions.

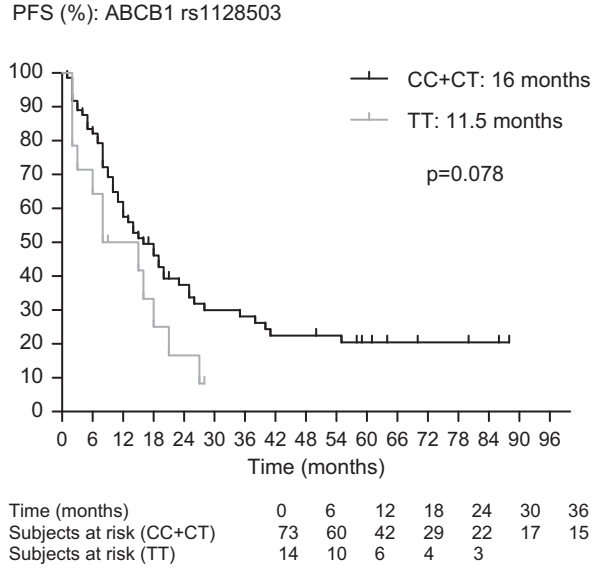

Figure 4. Impact of ABCB1 rs1128503 variants on progression-free survival.

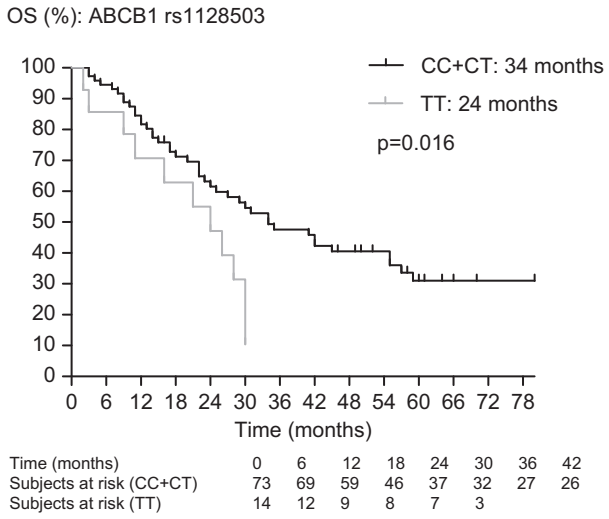

Figure 5. Impact of ABCB1 rs1128503 variants on overall survival.

In a series of 96 mRCC patients treated with sunitinib as first-line targeted therapy, we observed an association between SNP rs1128503 in *ABCB1*, rs2032582 in *ABCB1* as well as SNP rs2776707 in *NR1*/2 and the time point of dose reductions during sunitinib treatment, although the latter was not confirmed on multivariate analysis. Our time-to-event approach enabled us to avoid lead-time bias, which could easily have occurred if we would have merely compared the incidence of dose reductions in subgroups with significantly different treatment durations. The impact of these SNPs on PFS and OS was also analyzed on the patients' series, showing an inverse correlation between efficacy and dose reductions. Note that in a previous publication, we had already reported on the association between these SNPs and outcome on a patient series including 81 patients of the present study. With the lack of a placebo-treated control group, we cannot define if these SNPs have a prognostic or a predictive value for outcome, although the fact that these genes are involved in sunitinib pharmacokinetics points toward a predictive value.

At the start of therapy, anti-VEGFR-TKIs are generally administered at a fixed dose irrespective of the age, gender, weight or length of the patient. In the case of sunitinib, the starting dose is 50 mg/day for four weeks, followed by two weeks off-treatment. Many patients require dose modifications, i.e. dose reductions to 37.5 mg/day or even 25 mg/day due to tolerance issues. In the pivotal sunitinib trial, 38% of patients had dose interruptions and 32% had dose adaptations due to toxicity [1]. Remarkably, Houk et al. observed that when doses are lowered to 37.5 mg/day or subsequently even to 25 mg/day due to tolerance issues, or even when the dose of sunitinib is increased to 62.5 mg/day of sunitinib in patients with good tolerance but in need for an increased antitumor activity, the plasma levels of sunitinib are remarkably similar in all patients irrespective of dose adaptation [5]. These data suggest that individual patient characteristics that influence TKI absorption, excretion and metabolism may indeed influence TKI plasma levels and as a consequence determine the time and frequency of a dose reduction.

The efflux transporter *ABCB1* (ATP binding cassette member B1, formerly known as P-glycoprotein or MDR1) is expressed in the intestine and liver and involved in the oral absorption and biliary secretion of several anticancer drugs [15]. This transporter may contribute to multidrug resistance in tumors by actively extruding drugs from cancer cells, particularly in RCC [16,17]. As a consequence, expression levels and functionality of these drug transporters, i.e. due to polymorphisms, may have important consequences for the efficacy of sunitinib. The most common functional SNPs in *ABCB1* are the synonymous 3435C>T (rs1045642) and 1236C>T (rs1128503) changes and the non-synonymous 2677G>T change (missense A893S/T rs2032582). Functional studies have shown that the haplotype of these three SNPs (rs1046542 – rs1128503 – rs2032582) alters the function of the efflux transporter including its substrate specificity. There are four publications showing an association between rs1128503 in *ABCB1* and treatment outcome on anti-VEGFR-TKIs in mRCC (Table I) favoring patients with CT- and CC-variants [7–9,12]. As a consequence, the TT-genotype could lead to a more active efflux pump or more affinity of the pump for sunitinib, leading to lower sunitinib plasma levels. Our data suggesting an association between the TT-genotype and a delay in dose reductions supports this hypothesis.

Although Garcia-Donas, on a series of 89 mRCC patients treated with sunitinib, did not observe a higher risk for dose reductions in patients with the *ABCB1* rs1128503 TT-variant or the rs2032582 TT/TA-variant, he observed less hypertension in patients with these variants: HR for the development hypertension was 0.41 (95% CI 0.20–0.81; $p = 0.011$) for the rs1128503 TT-variant and 0.42 (95% CI 0.21–0.84; $p = 0.014$) for the rs2032582 TT/TA-variant [8]. Moreover, on a series of 115 mRCC patients treated with sunitinib, the TT-variant in rs2032582 in ABCB1 and the TT-variant of rs1128503 in ABCB1 were associated with a higher plasmatic sunitinib clearance ($p = 0.02$ and 0.05, respectively) [18].

After absorption, sunitinib is converted to an equipotent metabolite, SU12662 [19]. Both sunitinib and SU12662 are metabolized predominately

by cytochrome (CYP) 3A4, and elimination is primarily via the feces. The expression of cytochrome 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) [20,21]. There is evidence that polymorphisms in *NR1*/2 and *NR1*/3 might be associated with outcome in mRCC treated with anti-VEGFR-TKIs (Table I). Patients with the TT-genotype in rs2276707 in *NR1/2,* leading to a higher expression of *CYP3A4*, seem to have a shorter PFS and OS. Our findings of an association between the TT-genotype and a decreased delay in dose reductions support this hypothesis.

We could not find any association between the TT-variant in rs4073054 in NR1/3 and TTDR despite the fact that patients with this variant tend to have a worse outcome. Neither could we find any association between SNP rs776746 in *CYP3A5* and TTDR, although it was shown that the AA- and AG-genotypes were link to improved treatment outcome [7] and to increased need for dose reductions [8].

These findings, when validated, could have interesting clinical applications. In fact, a patient whose disease is primarily or secondarily resistant to sunitinib 50 mg/day, who has few side effects and who has the *ABCB1* rs1128503 TT-variant, the rs2032582 TT-variant or the *NR1*/2 rs2776707 TT-variant, could be a good candidate for a trial with sunitinib dose escalation to 62.5 mg/day or even 75 mg/day. There is evidence for the positive impact of dose escalation of some anti-VEGFR-TKIs on treatment outcome in mRCC. In a randomized phase II trial with sorafenib, dose escalation of sorafenib from 2×400 mg/d to 2×600 mg/d was foreseen. Forty-three (66%) of 65 patients who progressed on sorafenib 2×400 mg/d had their dose escalated and 42% of these patients achieved a reduction in tumor size and disease stabilization. The median PFS was 3.6 months for patients escalated to sorafenib 2×600 mg/d. The PFS of escalation of sorafenib was more effective than placebo in this setting [22].

Our study has several potential limitations: 1) it was a retrospective analysis of patients treated in several centers without a central protocol dictating schedule and dose modifications or timing and method of radiological assessments; 2) the clinical sites did not report precise data on different side effects with National Cancer Institute Common Toxicity Criteria scoring, only the date of dose reduction and the reason for it were reported. Nevertheless, we assume that in most cases, the dose was reduced for grade 3 toxicity; 3) sunitinib plasma level were not available; 4) correction for multiple testing by Bonferroni, taking into account that the correlation with 11 SNPs was analyzed, indicated a p-value of < 0.005 as the threshold for significance. Our results did not reach this level of significance, probably due to the small number of patients in our series; 5) finally, there was better treatment outcome in our series (PFS 15.0 and OS 29.0 months) compared to the outcome on sunitinib in the pivotal trial (PFS 11.0 and OS 26.0 months [1]). This difference is likely due to patient selection: all the patients had to complete at least one cycle of sunitinib and had to reach at least the first evaluation by CT scan.

Conclusion

Polymorphisms in the *ABCB1* efflux pump are associated with the incidence of dose reductions in mRCC patients treated with sunitinib. Prospective validation of these findings including the association with sunitinib plasma levels is warranted and ongoing (EudraCT: 2011-006085-40/MetaSun).

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