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Pharmacogenetics of sunitinib in metastatic renal cell carcinoma

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Citation
Diekstra, M. H. M. (2017, September 13). *Pharmacogenetics of sunitinib in metastatic renal cell carcinoma*. Retrieved from https://hdl.handle.net/1887/55944

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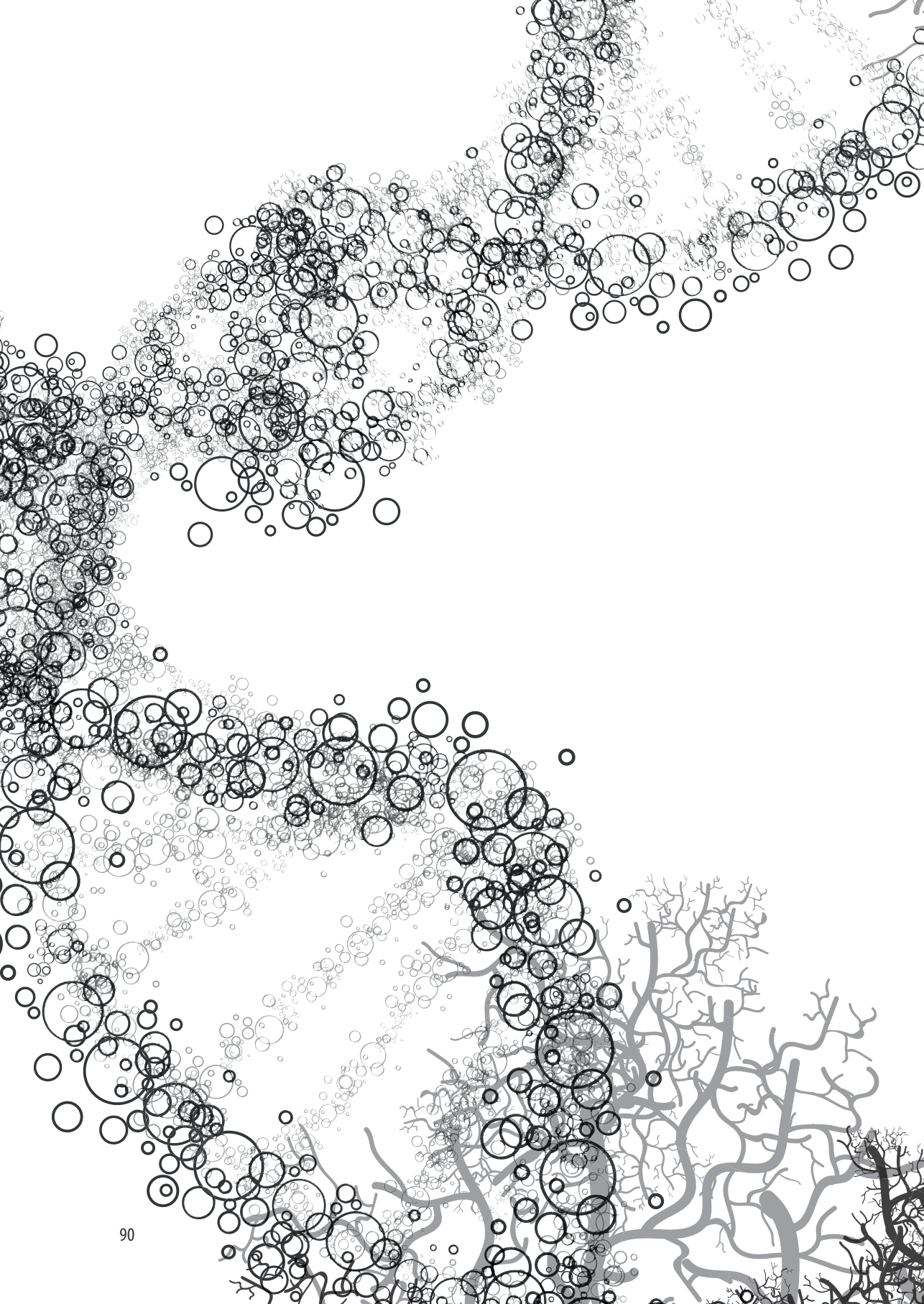
Author: Diekstra, Meta
Title: Pharmacogenetics of sunitinib in metastatic renal cell carcinoma
Date: 2017-09-13



CHAPTER 4

CYP3A5 and *ABCB1* polymorphisms as predictors for sunitinib outcome in metastatic renal cell carcinoma

Diekstra MH, Swen JJ, Boven E, Castellano D, Gelderblom H, Mathijssen RHJ, Rodríguez-Antona C, García-Donas J, Rini BI, Guchelaar H-J.

Eur Urol. 2015;68(4):621-629.

ABSTRACT

Background In our exploratory studies, we associated single nucleotide polymorphisms (SNPs) in candidate genes with the efficacy and toxicities of sunitinib in metastatic renal cell carcinoma (mRCC).

Objective To see whether previously reported associations of SNPs with sunitinib-induced toxicities and efficacy in mRCC can be confirmed in a large cohort of patients.

Design, setting, and participants The mRCC patients treated with sunitinib and a DNA sample available were pooled from three exploratory studies conducted in the United States, Spain, and the Netherlands. A total of 22 SNPs and 6 haplotypes in 10 candidate genes related to the pharmacokinetics and pharmacodynamics of sunitinib were selected for association testing.

Outcome measurements and statistical analysis SNPs and haplotypes were tested for associations with toxicity, dose reductions, progression-free survival (PFS), overall survival (OS), and best objective response.

Results and limitations A total of 333 patients were included. We confirmed 2 of the 22 previously reported SNP associations. The presence of *CYP3A5*1* was associated with dose reductions (odds ratio:2.0; 95% confidence interval [CI], 1.0-4.0, P=0.039). The presence of CGT in the *ABCB1* haplotype was associated with an increased PFS (hazard ratio: 1.9; 95% CI, 1.3-2.6; P<0.001) and remained significant after Bonferroni correction. These associations are consistent with prior observations.

Conclusions The confirmation of previously reported associations between polymorphisms in *CYP3A5* and *ABCB1* with sunitinib toxicity and efficacy, respectively, indicates that genotyping of these genetic variants will be useful for guiding sunitinib treatment. A prospective validation study is needed to confirm our findings on *ABCB1* and *CYP3A5* genetic polymorphisms.

Patient summary We confirmed that variants in genes involved in processing sunitinib through the body have an effect on sunitinib treatment outcome. These findings confirm the potential of testing for these genetic variants to improve individual patient care for patients with metastatic renal cell carcinoma treated with sunitinib.

INTRODUCTION

Sunitinib, a multitargeted tyrosine kinase inhibitor (TKI), is used as first- or second-line treatment of metastatic renal cell carcinoma (mRCC).[1-6] The drug is characterised by a wide interindividual variability in both pharmacokinetics and pharmacodynamics that are influenced by comedication, age, gender, and environmental factors. Increased sunitinib exposure results in an increase in time to tumour progression and overall survival (OS) accompanied by an increased risk of mild to moderate toxicities.[7] Dose reductions are needed in 32% of mRCC patients and may result in suboptimal therapeutic sunitinib levels.[8,9] Sunitinib-induced hypertension is associated with an improved clinical outcome.[10]

Exploratory analyses have reported statistically significant associations between single nucleotide polymorphisms (SNPs) in genes related to the pharmacokinetics and pharmacodynamics of sunitinib and the toxicity and efficacy of sunitinib in mRCC.[9,11-15] SNPs in *CYP1A1*, *ABCB1*, *ABCG2*, *VEGFR2* (synonym for *KDR*), *FLT3*, and *NR1I3* have been associated with an increased risk of leukopenia, mucosal inflammation, hand-foot syndrome (HFS), or the occurrence of any grade 3 or 4 toxicity.[9] The A allele in *CYP3A5* rs776746 (*CYP3A5*1*) has been associated with the need for more dose reductions of sunitinib due to toxicity.[12] Kim *et al.* reported that SNPs in *VEGF* and *VEGFR2* were associated with the prevalence and duration of sunitinib-induced hypertension.[14] Polymorphisms in *eNOS* (synonym for *NOS3*) and *VEGFA* were associated with grade 3 hypertension during the first cycle of sunitinib treatment.[15] For efficacy, SNPs in *VEGFR3* (synonym for *FLT4*) were associated with reduced progression-free survival (PFS)[12], and genetic polymorphisms in *NR1I3*, *ABCB1*, and *CYP3A5* were associated with an increased PFS.[13]

The SNP genotypes and haplotypes just listed are potential predictive biomarkers for sunitinib treatment outcome in mRCC patients and may enable individualised treatment. However, the exploratory studies applied fairly liberal statistical thresholds and had relatively small patient cohorts of 63-255 patients[9,12-15], increasing the risk for false-positive findings. Moreover, the selected SNP panels vary among studies. The objective of the present study is to see whether previously reported associations of SNPs with sunitinib-induced toxicities and efficacy in mRCC can be confirmed in a large cohort of patients.

METHODS

Study population

The mRCC patients treated with sunitinib were merged from three exploratory studies that were described in detail.[9,12-15] In brief, patients diagnosed with mRCC receiving 50.0 mg, 37.5 mg, or 25.0 mg sunitinib for at least 4 wk in a 4-wk-on/2-wk-off schedule or continuous dosing regimen were enrolled between 2004 and 2010 from the centres participating in the Dutch SUTOX consortium (five medical centres in the Netherlands), Spanish Oncology Genitourinary Group (SOGUG) medical centres (15 Spanish participating hospitals), and the Cleveland Clinic Foundation (CCF) Taussig Cancer Institute in the United States (**Figure 1**).[9,12-15] Patients with a non-clear cell histologic subtype of RCC were excluded. From each patient a blood sample had to be available. SUTOX samples were anonymised by a third party, according to the instructions stated in the Codes for Proper Use and Proper Conduct in the Self-Regulatory Codes of Conduct (www.federa.org). The medical ethical review boards of all participating centres approved the study.[9,12-15] Patients gave written informed consent.

Single nucleotide polymorphism selection and genotyping

Germline DNA was isolated from whole blood, serum, plasma, or peripheral blood mononuclear cell samples. Isolated DNA was genotyped for 22 SNPs and 6 haplotypes in genes *CYP1A1*, *CYP3A5*, *ABCB1*, *ABCG2*, *NR1I3*, *VEGFA*, *eNOS*, *VEGFR2*, *VEGFR3*, and *FLT3* (**Supplementary Table 1**).[9,12-15] Samples with a call rate <80% were excluded from the analysis. All SNPs achieved a SNP call rate >95%. Minor deviations from the Hardy-Weinberg equilibrium were observed for SNPs rs776746 in *CYP3A5* (P=0.01) and rs1045642 in *ABCB1* (P=0.041). A 98% concordance in SNP genotypes was observed between the Leiden University Medical Centre and the Spanish National Cancer Research Centre samples. A more detailed description of methods for SNP selection, genotyping, and quality control are described in **Supplement 1**.[9,12-17]

Study design

Toxicity and efficacy data for the present study were obtained by a retrospective review of the medical record of each patient and scored according to the protocol of the current study. Toxicity was assessed at baseline, week 4, and week 6 of each treatment cycle for the first four cycles. Toxicities were scored according to the National Cancer Institute-Common Terminology Criteria for Adverse Events v.3.0 or v.4.0. Toxicities of grade ≥3 were considered clinically relevant. Tested toxicities were thrombocytopenia, leukopenia, mucosal inflammation, HFS, hypertension, and any toxicity grade >2 in agreement with exploratory studies.[9,10,12,14,15] Data on dose reductions were also documented for the first four cycles of sunitinib treatment.

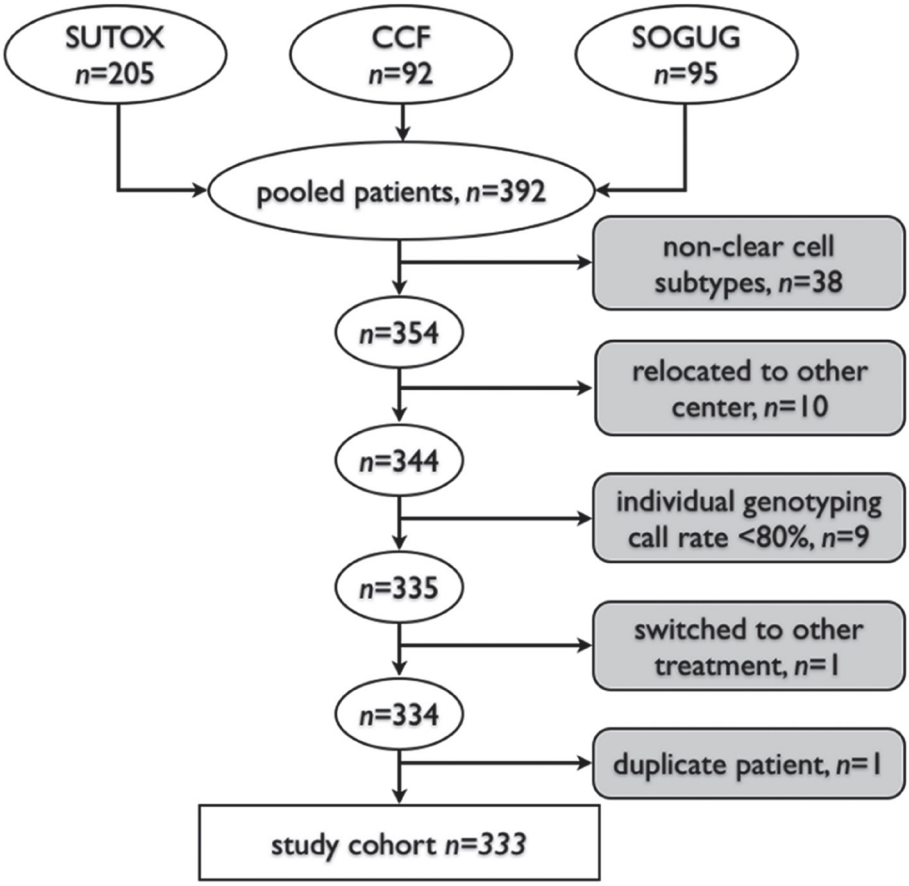


Figure 1 Patient flowchart on included patients. Fifty-nine patients had to be excluded from association analyses because of non-clear cell subtypes (N=38), relocation to another medical centre during follow-up (N=10), individual genotyping call rates <80% (N=9), double patient (N=1), or a change to another treatment than sunitinib directly after enrolment (N=1). A total of 333 sunitinib-treated metastatic renal cell carcinoma patients were available for analysis of toxicity and survival in the present study. CCF=Cleveland Clinic Foundation; SOGUG=Spanish Oncology Genitourinary Group; SUTOX=Dutch SUTOX consortium.

The primary outcome measure for efficacy analysis was PFS, defined as the time in months between the first day of sunitinib treatment and the date of progressive disease (PD) according to Response Evaluation Criteria in Solid Tumours (RECIST) v.1.0. If a patient had not progressed, PFS was censored at the time of the last follow-up. In addition, associations between SNPs and OS were tested. OS was defined as the time in months between the first day of sunitinib treatment and the date of death or the date at which patients were last known to be alive.

Statistical analysis

For toxicity analysis, baseline corrected toxicity scores were calculated by subtracting baseline values from the maximum recorded score in four cycles of treatment, and each toxicity end point was dichotomised as grade 0-2 versus grade 3-4. The end-point dose reduction was dichotomised as any dose reduction within cycle 1-4 or no dose reduction. Best response was dichotomised as PD versus other response (ie, stable disease, partial response, or complete response). SNPs and haplotypes, together with clinical covariates, were tested for associations with toxicities and dose reductions using an independent samples *t* test, chi-square test, or a Mann-Whitney *U* test depending on the type of data. Clinical variables and SNPs or haplotypes with $P \leq 0.1$ were included as covariates in the multivariate analysis. All SNPs and haplotypes were tested separately with adjustment for covariates using binary logistic regression analysis on the best fitting genetic model based on genotype distributions (additive, dominant, recessive, or general model). Association analysis on best response was performed using the covariates gender, Heng risk group, and study centre because these were $P \leq 0.1$ in the univariate analysis.

For survival analysis, SNPs and haplotypes were tested univariately using Kaplan-Meier survival analysis with the log-rank test. SNPs and haplotypes with $P \leq 0.1$ were included in the multivariate analysis. Dose reduction and prior treatment were tested for association with PFS and OS but not included in the multivariate analysis because of observed *P* values >0.1. Patients were assigned a prognostic risk group (favourable, intermediate, or poor) according to the Heng risk criteria.[18,19] SNPs and haplotypes together with Heng risk group and study centre were included as covariates in the Cox regression analysis. In addition, combined genotypes CC and GG in *VEGFA* rs3025039 and *VEGFR2* rs2305948 were tested for an association with OS.[14]

For multivariate analyses, associations with $P \leq 0.05$ were considered significant. Statistical analyses were performed using SPSS Statistics for Windows, v.20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Population characteristics

Data from 333 mRCC patients were available (**Figure 1**). **Table 1** presents the patient characteristics. Most of the patients had no toxicities at baseline (96-99% for the tested toxicities). Baseline grade 1-3 toxicities were observed for 0.3-3.9% of patients, and no baseline grade 4 toxicities were observed (**Table 2**). Dose reductions within cycles 1-4 of sunitinib were observed for 35% of the study population. For the 115 subjects who received a dose reduction, the reason for dose reduction was unknown for 54 subjects (47%) because of unavailable data. The remaining 61 patients (53%) received a dose reduction because of toxicity of grade 1 or 2 (N=18), grade 3 (N=39), or grade 4 (N=4). The distribution of response according to RECIST within cycles 1-4 is presented in **Table 1**. Overall, median follow-up times for PFS and OS analysis were 43 and 49 mo, respectively. Median PFS and OS of patients were 16 and 26 mo, respectively.

Table 1: Characteristics of metastatic clear cell renal cell carcinoma patients treated with sunitinib (N=333)

Characteristic	Value (quartiles: 25 and 75 percentiles)	%
Gender		
male	228	69
female	105	31
Age at start sunitinib (years)	61 (55, 69)	
BSA	1.97 (1.82, 2.13)	
Prior nephrectomy		
yes	281	84
no	48	14
unknown	4	1
WHO performance status		
0	146	44
1	156	47
2	23	7
3	1	0
unknown	7	2
Ethnicity		
Caucasian	321	96
Black	5	2
Asian	3	1
Latin-American	1	0
Arabian	3	1

Table 1: Continued

Characteristic	Value (quartiles: 25 and 75 percentiles)	%
Number of metastatic sites		
1	91	27
2	133	40
≥3	104	31
unknown	5	2
Metastatic sites		
Lung	228	70
Liver	62	19
Bone	95	29
Lymph nodes	152	46
Brain	19	6
kidney	77	23
Heng risk group*		
Good (0 risk factors)	67	20
Intermediate (1-2 risk factors)	176	53
Poor (3-6 risk factors)	90	27
Prior systemic antitumor treatment		
Yes	82	25
No	251	75
Duration of first prior treatment episode (months)**	6 (3, 11.5)	
Sunitinib, daily dose (mg) in first 4 cycles		
50 mg	315	95
37.5 mg	14	4
25 mg	4	1
Best response to sunitinib		
Progressive Disease	43	13
Stable Disease	129	39
Partial Response	130	39
Complete Response	11	3
unknown	20	6
Dose reduction after cycle 1, 2 or 3		
yes		
men	68	
women	47	
total	115	35
no		
men	153	
women	54	
total	207	62
unknown	11	3

Table 1: Continued

Characteristic	Value (quartiles: 25 and 75 percentiles)	%
Baseline chemistry and hematology		
SBP (mmHg)	138.5 (125.25, 150)	
DBP (mmHg)	80 (71, 88)	
LDH (units/L)	237 (170, 337)	
creatinine (µmol/L)	106.08 (88.4, 123.2)	
bilirubin (µmol/L)	6.84 (5.13, 9.00)	
albumin (g/L)	42 (38, 44.35)	
calcium (mmol/L)	2.38 (2.32, 2.48)	
AST (units/L)	20 (16, 27)	
ALT (units/L)	18 (13, 27)	
hemoglobin (mmol/L)	8 (7.3, 8.94)	
leukocytes ($x10^9$)	7.60 (6.46, 9.49)	
neutrophils ($x10^9$)	4.90 (3.83, 6.53)	
thrombocytes ($x10^9$)	264 (210, 353)	
MCV (fL)	88 (83, 91)	

ALT=alanine transaminase; AST=aspartate transaminase; BSA=body surface area; DBP=diastolic blood pressure; LDH=lactate dehydrogenase; MCV=mean corpuscular volume; SBP=systolic blood pressure; WHO=World Health Organisation. Values are presented as median unless otherwise indicated.

*Patients are grouped according to their Heng risk group based on the six Heng risk scores: deteriorated WHO performance status (≥2), low haemoglobin (lower than the lower limit of normal), high calcium (>2.5 mmol/l), time from initial diagnosis to treatment with sunitinib (<1 yr), neutrophil count (higher than the upper limit of normal), and thrombocytes (higher than the upper limit of normal).[18,19]

**Duration of first prior treatment episode refers to the number of months a patient has received any systemic anticancer treatment prior to start with sunitinib. In a case in which a patient had received more systemic antitumour treatments, only the duration of the first treatment was recorded.

Table 2: Distribution of delta toxicities within 4 cycles of sunitinib treatment

Type of toxicity	Toxicity grade	Number of patients (N)	Percentage of patients (%)
Thrombocytopenia	none	129	39
	grade 1	134	40
	grade 2	43	13
	grade 3	23	7
	grade 4	4	1
Leukopenia	none	169	51
	grade 1	95	28
	grade 2	60	18
	grade 3	9	3
Mucosal inflammation	none	144	43
	grade 1	107	32
	grade 2	67	20
	grade 3	15	5
Hand-foot-syndrome*	none	199	60
	grade 1	62	19
	grade 2	51	15
	grade 3	21	6
Any toxicity >grade 2	none	41	12
	grade 1	87	26
	grade 2	121	36
	grade 3	75	23
	grade 4	9	3
Hypertension grades	none	209	63
	grade 1	34	10
	grade 2	48	14
	grade 3	42	13

Baseline corrected toxicity scores were calculated by subtracting baseline values from the maximum recorded score in 4 cycles of treatment. In case the grade of toxicity was not recorded in the medical record of the patient, it was assumed that no toxicity had occurred (grade 0).
*For hand-foot syndrome, grade 3 is the highest possible grade according to the Common Terminology Criteria for Adverse Events v.4.0.

Single nucleotide polymorphism association results

All tested associations are presented in **Table 3** that include associations not previously reported. Two of the exploratory associations between individual SNPs and toxicities or efficacy were confirmed in the present study (**Table 4**). *CYP3A5*1* was associated with dose reductions (odds ratio [OR]:2.0; 95% confidence interval [CI], 1.0-4.0; *P*=0.039). PFS was improved in the case of the presence of CGT in the *ABCB1* haplotype (hazard ratio:1.9; 95% CI, 1.3-2.6; *P*<0.001), and the *P* value remained significant after Bonferroni correction (**Table 4**; **Figure 2**). Two of our current findings showed the same direction of effect and a similar effect size as observed in earlier exploratory studies but failed to meet the threshold for statistical significance in the present study. Presence of the G allele in *VEGFA* rs1570360 was associated with an increased risk for hypertension (OR:1.9; 95% CI, 0.8-4.5; *P*=0.17), and the T allele of *FLT3* rs1933437 was associated with an increased risk for leukopenia (OR:3.6; 95% CI, 0.8-15.6; *P*=0.088) (**Table 4**).

Table 3: Univariate and multivariate analyses of SNPs, haplotypes and other covariates on end-points of toxicity, dose reductions, response and survival during sunitinib treatment in mRCC.

Tested outcome	SNP or patient characteristic	Univariate analyses (*P*-value)	Multivariate analyses		
			P-value	OR/ HR	CI 95%
Thrombocytopenia	Age on start sunitinib	0.02	0.051	1.05	1.00-1.10
	Gender	0.05	0.6	1.28	0.48-3.38
	BSA	0.09	0.02	0.07	0.01-0.59
	SOGUG vs other centers		0.08	4.13	0.84-20.3
	CCF vs other centers		0.1	0.47	0.19-1.17
	rs1048943 in *CYP1A1*	0.07	0.1	3.04	0.72-12.7
	rs307826 in *VEGFR3*	0.07	0.2	1.76	0.69-4.44
Leukopenia	Age on start sunitinib	0.08	0.2	1.06	0.98-1.14
	BSA	0.04	>0.9	0.91	0.04-21.1
	rs2307418 in *NR1I3*	There are no patients with grade 3 or 4 leukopenia and the CA or CC genotype.			
	rs3025039 in *VEGFA*	0.07	**0.024**	5.42	1.25-23.5
	rs1933437 in *FLT3*	0.06	0.088	0.28	0.06-1.21
mucosal inflammation	Creatinine at baseline	0.03	0.3	1.01	0.99-1.03
	MCV at baseline	0.07	0.3	0.94	0.85-1.05
	SOGUG vs other centers		0.06	0.21	0.04-1.09
	CCF vs other centers	There are no patients from CCF having grade 3 or 4 mucosal inflammation.			
	rs1128503 in *ABCB1*	0.09	**0.028**	0.19	0.04-0.83
	rs2032582 in *ABCB1*	0.04	**0.048**	0.22	0.05-0.98
	rs2307418 in *NR1I3*	0.002	**0.013**	8.09	1.55-42.3
	rs1570360 in *VEGFA*	0.07	0.1	3.66	0.71-18.8

Table 3: Continued

Tested outcome	SNP or patient characteristic	Univariate analyses (*P*-value)	Multivariate analyses		
			P-value	OR/ HR	CI 95%
hand-foot syndrome	Age on start sunitinib	0.02	0.095	0.95	0.90-1.01
	Albumin at baseline	0.002	0.072	1.15	0.99-1.35
	Hb at baseline	0.09	0.5	1.20	0.72-2.02
	Leukocytes at baseline	0.014	0.2	0.83	0.64-1.08
	LDH at baseline	0.06	0.4	1.00	0.99-1.00
	rs2305948 in *VEGFR2*	0.03	**0.032**	2.84	1.09-7.38
	rs1933437 in *FLT3*	0.05	**0.037**	5.33	1.10-25.79
Tox > grade 2	Age on start sunitinib	0.03	0.077	1.03	1.00-1.07
	Gender	0.00	0.3	0.70	0.36-1.36
	Bilirubin at baseline	0.04	0.6	0.98	0.91-1.05
	Albumin at baseline	0.05	0.6	1.02	0.94-1.11
	Hb at baseline	0.00	0.015	0.65	0.46-0.92
	MCV at baseline	0.004	0.4	0.98	0.92-1.03
	rs1048943 in *CYP1A1*	0.06	**0.043**	3.65	1.04-12.8
	rs1045642 in *ABCB1*	0.04	0.4	1.43	0.65-3.15
	rs2307424 in *NR1I3*	0.02	**0.006**	0.46	0.27-0.80
	rs2010963 in *VEGFA*	0.08	0.3	1.28	0.81-2.04
	rs1570360 in *VEGFA*	0.05	0.093	0.65	0.39-1.08
	rs1933437 in *FLT3*	0.098	**0.037**	3.36	1.08-10.5
	haplotype *ABCB1* (rs1128503, rs2032582, rs1045642). Other-other vs Other-CGT + CGT-CGT	0.005	0.069	2.04	0.95-4.40
	haplotype *NR1I3* (rs2307424, rs2307418, rs4073054). Other-other (0) vs Other-CAT + CAT-CAT (1)	0.06	**0.045**	0.60	0.36-0.99
hypertension grades	BSA	0.011	0.1	3.92	0.71-21.6
	Albumin at baseline	0.001	0.001	1.30	1.12-1.51
	ALT at baseline	0.005	0.01	1.03	1.01-1.05
	Hb at baseline	0.08	0.2	0.77	0.50-1.18
	Leukocytes at baseline	0.04	0.4	0.95	0.84-1.08
	MCV at baseline	0.09	0.2	1.05	0.97-1.13
	SOGUG vs other centers		0.02	14.0	1.61-122.4
	rs776746 in *CYP3A5*	0.02	**0.009**	4.70	1.47-15.0
	rs2231142 in *ABCG2*	0.05	**0.040**	0.03	0.001-0.85
	rs55930652 in *ABCG2*	0.04	0.09	0.45	0.18-1.13
	rs2622604 in *ABCG2*	0.06	0.17	0.51	0.20-1.34
	rs1570360 in *VEGFA*	0.007	0.09	6.56	0.74-57.90
	rs3025039 in *VEGFA*	0.02	0.3	1.58	0.62-4.03
	haplotype *ABCG2* (rs55930652, rs2622604). Other-other vs TT-other+TT-TT	0.05	0.11	0.47	0.18-1.20

Table 3: Continued

Tested outcome	SNP or patient characteristic	Univariate analyses (*P*-value)	Multivariate analyses		
			P-value	OR/ HR	CI 95%
Dose reduction after cycle 1,2 or 3	Gender	0.006	0.13	1.59	0.88-2.87
	Hb at baseline	0.07	0.009	0.74	0.59-0.93
	SBP at baseline	0.095	0.13	0.99	0.97-1.00
	CYP3A5 rs776746	0.006	**0.039**	2.04	1.04-4.00
	ABCG2 rs2231142	0.07	**0.022**	0.36	0.15-0.86
	VEGFA rs699947	0.06	0.051	0.53	0.28-1.00
	VEGFA rs1570360	0.07	0.18	2.26	0.69-7.44
	NOS3 rs2070744	0.098	**0.014**	0.36	0.16-0.81
	haplotype *VEGFA* Other-other vs other-ACG + ACG-ACG	0.07	0.076	0.56	0.29-1.06
Response (PD vs other)	Gender	0.04	0.12	0.58	0.29-1.16
	Heng favorable risk (0)	0.00	0.004		
	Heng intermediate risk (1-2)		0.004	9.39	2.09-42.2
	Heng poor risk (3-6)		0.023	2.26	1.12-4.55
	CCF vs other centers		0.053	0.34	0.12-1.01
	rs55930652 in *ABCG2*	0.02	**0.045**	0.61	0.38-0.99
	rs2622604 in *ABCG2*	0.02	0.067	0.62	0.37-1.04
	rs833061 in *VEGFA*	0.087	0.086	0.44	0.17-1.12
	rs1933437 in *FLT3*	0.01	**0.008**	0.51	0.32-0.84
	haplotype *ABCB1 CGT* Other-other vs Other-CGT + CGT-CGT	0.077	**0.044**	2.83	1.03-7.79
	haplotype *ABCG2* Other-other > other-TT > TT-TT	0.03	0.069	0.62	0.37-1.04
	haplotype *NR1I3 CAT* Other-other(1) vs Other-CAT+ CAT-CAT (0)	0.01	**0.008**	3.79	1.41-10.2
PFS	Heng favorable risk (0)	0.00	<0.001		
	Heng intermediate risk (1-2)		<0.001	0.43	0.29-0.63
	Heng poor risk (3-6)		<0.001	0.53	0.40-0.72
	SOGUG vs other centers	0.04	0.3	0.84	0.62-1.14
	CCF vs other centers	0.002	0.016	1.52	1.08-2.13
	rs1128503 in *ABCB1*	0.02	**0.016**	1.42	1.07-1.90
	rs2032582 in *ABCB1*	0.05	**0.032**	1.36	1.03-1.81
	rs2622604 in *ABCG2*	0.04	0.082	1.26	0.97-1.65
	rs2070744 in *NOS3*	0.06	0.12	0.81	0.61-1.06
	rs1870377 in *VEGFR2*	0.04	**0.058**	0.59	0.35-1.02
	haplotype *ABCB1* (rs1128503, rs2032582, rs1045642). Other-other vs Other-CGT + CGT-CGT	0.002	**<0.001**	0.54	0.38-0.75
	haplotype *ABCG2* (rs55930652, rs2622604). Other-other vs Other-TT + TT-TT	0.042	0.074	1.28	0.98-1.67

Table 3: Continued

Tested outcome	SNP or patient characteristic	Univariate analyses (*P*-value)	Multivariate analyses		
			P-value	OR/ HR	CI 95%
OS	Heng favorable risk (0)	0.00	0.00		
	Heng intermediate risk (1-2)		0.00	0.32	0.21-0.49
	Heng poor risk (3-6)		<0.001	0.53	0.39-0.71
	SUTOX vs other centers	0.00	0.064	0.73	0.52-1.02
	CCF vs other centers	0.00	0.009	1.82	1.16-2.86
	rs1128503 in *ABCB1*	0.05	**0.021**	0.66	0.46-0.94
	rs2622604 in *ABCG2*	0.086	0.229	1.19	0.90-1.56
	rs1570360 in *VEGFA*	0.04	0.063	0.75	0.55-1.02
	rs1870377 in *VEGFR2*	0.089	**0.024**	0.50	0.28-0.92
	haplotype *ABCB1* (rs1128503, rs2032582, rs1045642). recessive: TTT-TTT vs Other-other + Other-TTT	0.06	**0.021**	0.63	0.43-0.93
	haplotype *ABCB1* (rs1128503, rs2032582, rs1045642). dominant: Other-other vs Other-CGT + CGT-CGT	0.05	**0.007**	0.63	0.45-0.88
	haplotype *ABCG2* (rs55930652, rs2622604). Other-other vs Other-TT + TT-TT	0.086	0.18	1.21	0.92-1.60

ALT=alanine transaminase; BSA=body surface area; CCF=Cleveland Clinic Foundation; CI=confidence interval; Hb=haemoglobin; HR=hazard ratio; LDH=lactate dehydrogenase; MCV=mean corpuscular volume; OR=odds ratio; OS=overall survival; PD=progressive disease; PFS=progression-free survival; SBP=systolic blood pressure; SNP=single nucleotide polymorphism; SOGUG=Spanish Oncology Genitourinary Group; SUTOX=Dutch SUTOX consortium. This table includes only SNPs, haplotypes, and other covariates with $P \leq 0.1$ in univariate analysis. Multivariate results with $P \leq 0.05$ are indicated in bold. Multivariate results are reported for a single base model including all patient characteristics as covariates without inclusion of SNPs. SNP results are presented for the singular SNP added to the base model of patient characteristics.

Table 4: **SNP association results from multivariate analysis.** This table presents an overview of single nucleotide polymorphisms (SNPs) and haplotypes in candidate genes related to the pharmacokinetics and pharmacodynamics of sunitinib tested for association with toxicity and efficacy of sunitinib in mRCC. Significant associated SNPs (P<0.05) from exploratory studies[9,12-15] are compared with the association results from the present study.

gene	rs number	Predicted functional change	Original association in exploratory studies				Results from present study		
			P-value	OR/HR	95% CI	ref	*P*-value	OR/HR	95% CI
CYP3A5	rs776746	A allele (*CYP3A5*1*) associated with dose reductions	0.0014	3.75	1.67-8.41	11	0.039	2.04	1.04-4.00
ABCB1	haplotype *ABCB1* (rs1128503, rs2032582, rs1045642)	CGT copy associated with increased PFS	0.033	1.92	1.05-3.45	12	<0.001	1.85	1.32-2.56
VEGFA	rs1570360	Increased risk for hypertension	0.035	2.04	1.05-3.96	11	0.17	1.86	0.76-4.52
FLT3	rs1933437	T allele associated with increased risk for leukopenia	0.008	2.78	1.30-5.88	9	0.088	3.57	0.83-15.6

CI=confidence interval; HR=hazard ratio; OR=odds ratio; OS=overall survival; PFS=progression-free survival. This table presents an overview of single nucleotide polymorphisms (SNPs) and haplotypes in candidate genes related to the pharmacokinetics and pharmacodynamics of sunitinib tested for association with toxicity and efficacy of sunitinib in metastatic renal cell carcinoma. Significantly associated SNPs (P<0.05) from exploratory studies[9,12-15] are compared with the association results from the present study.

DISCUSSION

Two of 22 previously reported pharmacogenetic biomarkers for efficacy or toxicity of sunitinib showed identical associations in a large cohort of 333 mRCC patients. *CYP3A5*1* is associated with dose reductions, and the presence of CGT in the *ABCB1* haplotype is associated with an improved PFS.[12,13] Compared with the exploratory studies, similar effect sizes were observed for the PFS increase in *ABCB1* CGT carriers, and a smaller effect size was observed for the chance of dose reductions in case of *CYP3A5*1* in the present study. Compared with the exploratory studies, smaller CIs were observed in the present study, and the association between *ABCB1* and improved PFS remains significant after

Bonferroni correction. These results indicate that genetic polymorphisms in the *CYP3A5* or the *ABCB1* gene play an important role in predicting the need for dose reductions and the length of PFS in individuals treated with sunitinib. Our observation that SNPs in *ABCB1* CGT are associated with sunitinib efficacy is supported by a recent study reporting that patients carrying the wild-type and heterozygote genotypes of rs2032582 and rs1128503 in *ABCB1* showed a decreased clearance of sunitinib and its active metabolite SU12662.[20] Another reinforcement of current findings in *ABCB1* was presented by Beuselinck *et al*., who reported an increased time-to-dose reduction for TT genotypes in rs2032582 and rs1128503.[21] For the same SNPs in *ABCB1*, Garcia-Donas *et al.* reported a protective effect for the T allele on hypertension.[12] These results could be explained by a dose-plasma concentration-effect relationship of sunitinib influenced by *ABCB1* genetic polymorphisms.[22] The studied variant genotypes in *ABCB1* result in an increased function of the ABCB1 efflux transporter leading to a decreased absorption or higher clearance of sunitinib and consequently a lower exposure to sunitinib, less hypertension, and a decrease in PFS in the absence of CGT or for TT variant homozygotes.[12,20,21] The *CYP3A5*1* allele results in CYP3A5 expression, and it was hypothesised by van der Veldt *et al.* that this may result in increased conversion from sunitinib to SU12662. This metabolite has a longer half-life than sunitinib resulting in increased exposure, and it could be responsible for any toxicity for which dose reduction is needed.[13,20] Our observation that *CYP3A5*1* is associated with more dose reductions agrees with this hypothesis and is supported by a recent study that reported a 22.5% increase in the clearance of sunitinib for *CYP3A5*1* carriers.[20] In contrast, Beuselinck *et al*. did not find any relation between *CYP3A5*1* and time-to-dose reduction.[21] This difference could be due to the time-to-event approach that is not the same end point as dose reductions or to the smaller number of subjects (96 patients) in their study.

The present study did not find any of the original exploratory association findings of SNPs in genes related to the pharmacodynamic pathway of sunitinib.[9,12-15] Scartozzi *et al*. reported significant associations of SNPs rs833061, rs699947, rs2010963, and rs6877011 in *VEGF* and *VEGFR-3* (*FLT4*) genes with PFS and OS on sunitinib.[23] Beuselinck *et al*. reported associations of the T allele in *VEGFR-3* rs307821 with a decrease in PFS and OS ($P<0.05$) compared with the GG genotype.[24] However, the studies of Scartozzi *et al*. and Beuselinck *et al*. only analysed 84 and 88 patients, respectively, which is rather limited for pharmacogenetic studies, and both studies emphasise the need for prospective validation of these findings in a larger patient cohort.[23,24] Moreover, the findings of Scartozzi *et al*. were based on genotyping results of tumour tissue instead of germline DNA, and expression of the *VEGF* gene is known to vary between tumour and normal tissue.[23] This could explain the lack of associations for SNPs in *VEGF* and *VEGFR3* in this large cohort of patients (N=333).

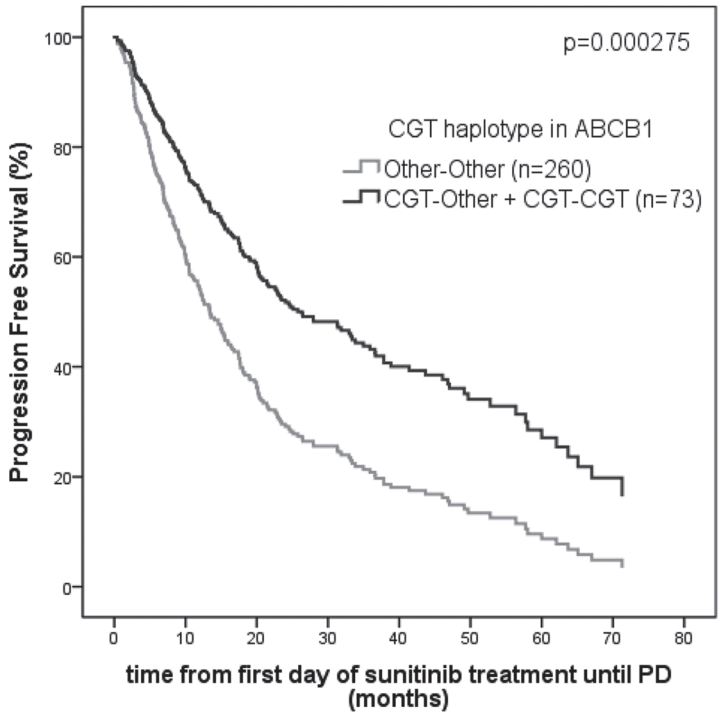


Figure 2 Multivariate Cox regression analysis for association of genetic polymorphisms in the *ABCB1* haplotype (rs1128503, rs2032582, and rs1045642) with progression-free survival in months, corrected for Heng risk group and study centre. PD=progressive disease.

Strengths of the current study are the large sample size for a pharmacogenomics study, the interrogation of significant SNPs from exploratory studies, and a technical validation on genotyping methods. In our analysis, toxicities were assessed during four cycles of sunitinib treatment, whereas most exploratory approaches only concerned one or two cycles.[9,13,15] The application of an extended time frame holds a small risk for bias of the number of sunitinib-induced toxicities by non-treatment-related effects such as withdrawal of patients due to early disease progression.

Survival analyses were adjusted for dose reductions by inclusion of a dichotomous covariate and regardless of starting dose. While this approach is valid, adjustment for the actual cumulative sunitinib dose would be preferable. Unfortunately, due to the retrospective character of the present study, available data were too sparse to calculate the cumulative dose accurately. It is conceivable that especially patients who are carriers

of a particular SNP genotype received dose reductions (eg, *CYP3A5*1*), thereby reducing sunitinib exposure. In this way, the potential effects of SNPs on survival outcomes could have been missed.

To date, most exploratory studies applied the candidate-gene approach limiting interrogated SNP panels to our current understanding of the biologic mechanisms of sunitinib. Consequently, relevant SNPs influencing sunitinib toxicity or efficacy may not yet have been identified. In contrast, a genomewide association study (GWAS) is an unrestricted hypothesis-free approach to find unbiased associations of SNPs with sunitinib treatment outcome.[25] Recently, a GWAS of sunitinib and pazopanib efficacy and toxicity in mRCC was reported. This study included 355 sunitinib-treated patients, and potential loci in *LOXL2*, *ENTPD4*, *IL2RA*, *LRRC2*, *ANAPC4*, and *SLC34A2* were identified.[26,27] However, no associations reached genomewide significance, and larger sample sizes are needed. A European collaborative project (EuroTARGET) was set up with the same objective and might provide additional information.[28]

CONCLUSIONS

Multiple targeted treatments are currently available for the treatment of mRCC in addition to sunitinib. Pharmacogenetic testing for *CYP3A5* and *ABCB1* may help make better informed decisions regarding TKI selection for individual patients and limit toxicity while increasing efficacy. A prospective validation study is needed to confirm our findings on *ABCB1* and *CYP3A5* genetic polymorphisms.

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SUPPLEMENTARY MATERIAL

Supplement 1 - Methods

SNP selection and genotyping

Twenty-two SNPs and six haplotypes in 10 genes (*CYP1A1*, *CYP3A5*, *ABCB1*, *ABCG2*, *NR1I3*, *VEGFA*, *eNOS*, *VEGFR2*, *VEGFR3* and *FLT3*) related to pharmacokinetics or pharmacodynamics of sunitinib were selected for this study because of reported associations ($P<0.05$) with toxicity, PFS or OS in previous exploratory studies (Supplementary Table 1).[9,12-15]

Genotyping

Germline DNA was isolated from whole blood, serum, plasma or peripheral blood mononuclear cell (PBMC) samples. SUTOX and CCF samples were genotyped at Leiden University Medical Center (LUMC) using Taqman probes (*Applied Biosystems, Nieuwerkerk aan den IJssel, the Netherlands)* on the LightCycler480 (LC480) Real-Time PCR Instrument (*Roche Applied Science, Almere, The Netherlands*). SOGUG samples were genotyped by the Spanish National Cancer Research Centre (CNIO) using a KASPar SNP genotyping system (Kbiosciences, Hoddesdon, UK) and the sequence Detection System 7900HT (Applied Biosystems, Foster City, CA, USA) for fluorescence detection and allele assignment. Haplotype analysis was performed using PLINK software, version 1.07. Haplotype maximum likelihood estimates ≥95% were included.[16]

Quality control

For CCF and LUMC samples, 5% of the samples were genotyped in duplicate and no inconsistencies were observed. At CNIO, two samples representing each the wild-type homozygote, heterozygote and variant homozygote groups were confirmed by sequencing obtaining concordant results. Samples with a call rate <80% were excluded from the analysis. All SNPs achieved a SNP call rate >95%. Minor deviations from the Hardy-Weinberg equilibrium (HWE) were observed for SNPs rs776746 in *CYP3A5* ($P=0.014$) and rs1045642 in *ABCB1* ($P=0.041$). These 2 SNPs were included in the analysis, since only minor deviations from HWE were observed and Minor Allele Frequencies (MAFs) were similar to those previously described (7.5% versus 3.6% for rs776746 and 51.2% versus 42.9% for rs1045642, for observed MAFs and NCBI Hap Map CEU MAFs respectively).[17]
To exclude inter assay variation between LUMC and CNIO, A selection of 132 anonymized SOGUG DNA samples were re-genotyped in Leiden. A 98% concordance in SNP genotypes was observed between the LUMC and CNIO samples.

Statistical analysis

SNPs and haplotypes, together with age, gender, Body Surface Area (BSA), WHO performance status, study centre (SUTOX, CCF or SOGUG) and baseline creatinine, total bilirubin, albumin, aspartate transaminase (AST), alanine transaminase (ALT), hemoglobin, leukocytes, thrombocytes, Mean Corpuscular Volume (MCV), calcium and lactate dehydrogenase (LDH) were tested for associations with toxicities and dose reductions using an independent samples t-test, χ^2 test or a Mann-Whitney *U* test depending on the type of data. Clinical variables and SNPs or haplotypes with $P \leq 0.1$ were included as covariates in multivariate analysis. For related variables (e.g. the transaminases AST and ALT) with $P \leq 0.1$, the most significant variable was retained as covariate in multivariate analysis.

Supplementary Table 1 – Overview of single nucleotide polymorphisms and haplotypes in candidate genes related to the pharmacokinetics and pharmacodynamics of sunitinib that were significantly associated ($P<0.05$) with toxicity and efficacy of sunitinib in metastatic renal cell carcinoma in exploratory studies.[9,12-15]

gene	rs number	*P*-value	OR/HR	95% CI	Previously found associations	Reference
CYP1A1	rs1048943	0.029	6.24	1.20-32.42	G allele associated with increased risk for **leukopenia**	9
		0.021	4.03	1.24-13.09	G allele associated with increased risk for **mucosal inflammation**	
CYP3A5	rs776746	0.032	0.27	0.08-0.89	A allele (*CYP3A5*1*) associated with an increased **PFS**	13
		0.0014	3.75	1.67-8.41	A allele (*CYP3A5*1*) associated with **dose reductions** due to toxicity	12
ABCB1	rs1128503	0.011	0.41	0.20-0.81	T allele is protective for **hypertension**	12
	rs2032582	0.014	0.42	0.21-0.84	T allele is protective for **hypertension**	
	haplotype *ABCB1* (rs1128503, rs2032582, rs1045642)	0.035	0.39	0.16-0.94	TTT copy associated with increased occurrence of **hand-foot syndrome**	9
		0.033	0.52	0.29-0.95	CGT copy associated with increased **PFS**	13
ABCG2	rs2231142	0.042	0.11	0.01-0.92	Protective for **hand-foot-syndrome**	12
	haplotype *ABCG2* (rs55930652, rs2622604)	0.016	0.38	0.17-0.83	TT copy associated with **any toxicity higher than grade 2**	9
NR1I3	haplotype *NR1I3* (rs2307424, rs2307418, rs4073054)	0.041	1.74	1.02-2.96	absence of CAG copy associated with an increased risk for **leukopenia**	9
		0.017	1.76	1.11-2.79	absence of CAT copy associated with an increased **PFS**	13

Supplementary Table 1 – Continued

gene	rs number	*P*-value	OR/HR	95% CI	Previously found associations	Reference
VEGFA	rs699947	0.0074	2.43	1.27-4.66	Increased risk for **hypertension**	12
	rs833061	0.03	N/A	N/A	Increased prevalence of **hypertension**	14
	rs2010963	0.03 and 0.01	13.62	3.71-50.04	Increased prevalence + duration of **hypertension**; GG genotype (rs2010963) associated with sunitinib-induced **hypertension** compared to CC	
	rs1570360	0.035	2.04	1.05-3.96	Increased risk for **hypertension**	12
	rs3025039	0.03	3.18	1.12-8.99	concomitant presence of CC in *VEGF* (rs3025039) and GG in *VEGFR2* (rs2305948) associated with decreased **OS (poor prognosis)**	14
	haplotype *VEGFA* (rs699947, rs833061, rs2010963)	0.031	0.59	0.34-1.03	ACG haplotype associated with the **incidence of grade 3 hypertension**	15
NOS3 (=eNOS)	rs2070744	0.045	2.62	1.08-6.35	C allele associated with increased risk for **grade 3 hypertension** during 1st cycle of sunitinib	15
KDR (=VEGFR2)	rs2305948	0.046	2.39	1.02-5.60	presence of the T allele associated with **any toxicity > grade 2**	9
		0.03	3.18	1.12-8.99	concomitant presence of CC in *VEGF* (rs3025039) and GG in *VEGFR2* (rs2305948) associated with decreased **OS**	14
	rs1870377	0.0058	2.62	1.32-5.20	Increased risk for **hypertension**	12
FLT-4 (=VEGFR3)	rs307826	0.00049	3.57	1.75-7.30	Associated with reduced **PFS**	12
	rs307821	0.00085	3.31	1.64-6.68	Associated with reduced **PFS**	12
FLT3	rs1933437	0.008	0.36	0.17-0.77	T allele was associated with an increased risk for **leukopenia**	9

Abbreviations: OR = Odds Ratio; HR = Hazard Ratio; CI = Confidence Interval, OS = Overall Survival, PFS = Progression Free Survival