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Pharmacogenetics of sunitinib in metastatic renal cell carcinoma

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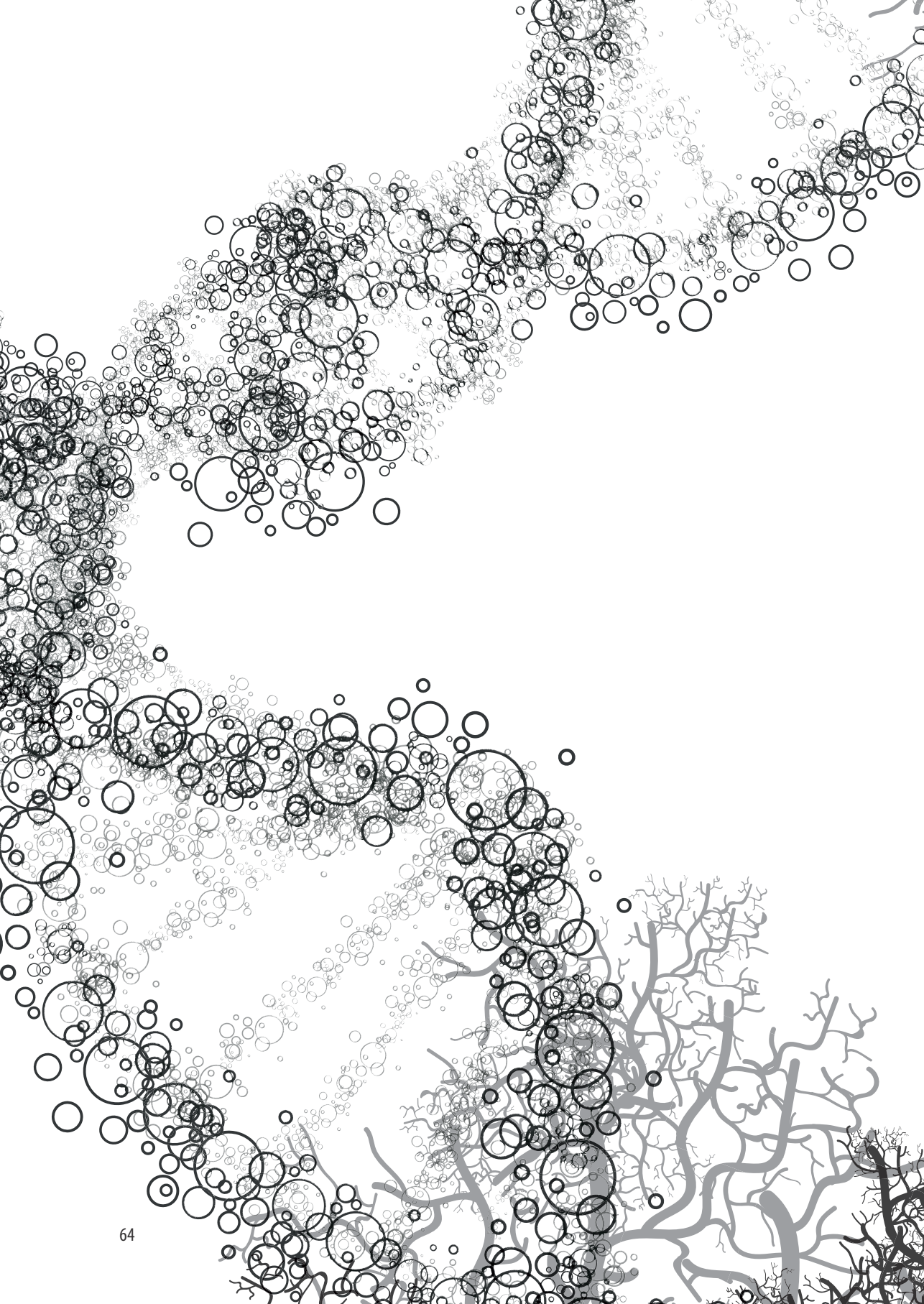


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CHAPTER 3

Association analysis of genetic polymorphisms in genes related to sunitinib pharmacokinetics, specifically clearance of sunitinib and SU12662

Diekstra MH, Klümpen HJ, Lolkema MPJK, Yu H, Kloth JSL, Gelderblom H, van Schaik RHN, Gurney H, Swen JJ, Huitema ADR, Steeghs N, Mathijssen RHJ.

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ABSTRACT

Interpatient variability in the pharmacokinetics (PK) of sunitinib is high. Single nucleotide polymorphisms (SNPs) in PK candidate genes have been associated with the efficacy and toxicity of sunitinib, but whether these SNPs truly affect the PK of sunitinib remains to be elucidated. This multicenter study involving 114 patients investigated whether these SNPs and haplotypes in genes encoding metabolizing enzymes or efflux transporters are associated with the clearance of sunitinib and its active metabolite SU12662. SNPs were tested as covariates in a population PK model. From univariate analysis, we found that the SNPs in *CYP3A4*, *CYP3A5*, and *ABCB1* were associated with the clearance of both sunitinib and SU12662. In multivariate analysis, *CYP3A4**22 was found to be eliminated last with an effect size of –22.5% on clearance. Observed effect sizes are below the interindividual variability in clearance and are therefore too limited to directly guide individual dosing of sunitinib.

INTRODUCTION

Sunitinib is an orally administered tyrosine kinase inhibitor that is approved as a systemic therapy for metastatic renal cell carcinoma, gastrointestinal stromal tumors after failure on treatment with imatinib, and neuroendocrine tumors of the pancreas.¹⁻⁶ Sunitinib is a multitargeted tyrosine kinase inhibitor that inhibits vascular endothelial growth factor receptors type 1, 2, and 3; platelet-derived growth factor receptor- α and - β ; Fms-related tyrosine kinase 3; colony stimulating factor-1 receptor; the cytokine receptor Kit; and the proto-oncogene tyrosine-protein kinase receptor Ret.¹⁻⁶

Cytochrome P450 (CYP)3A4 metabolizes sunitinib to its active *N*-desethyl metabolite, SU12662, and subsequently into SU14335 and other inactive metabolites (**Figure 1**).^{1,4,5,7-}

⁹ The involvement of CYP3A5, CYP1A1, and/or CYP1A2 in the metabolism of sunitinib has also been suggested, but no studies have yet confirmed this.¹ CYP3A4 expression is regulated by the pregnane X receptor and the constitutive androstane receptor, which are encoded by the *NR1I2* and *NR1I3* genes, respectively.^{7,9} Sunitinib is a substrate of the efflux transporters ATP-binding cassette transporter P-glycoprotein and the breast cancer resistance protein encoded by the *ABCB1* and *ABCG2* genes, respectively.⁸ No genetic polymorphisms affecting the distribution and excretion of sunitinib and SU12662 have been reported. Both sunitinib and SU12662 contribute to the total amount of active drug in plasma. When administered at the approved doses, sunitinib and SU12662 exhibit linear pharmacokinetics (PK).^{4,10} Yet interpatient variability in the PK of sunitinib and SU12662 is high, and a large variability in efficacy and toxicity of sunitinib has been reported.^{2-4,10} As a result, 32% of patients with renal cell carcinoma who were treated with sunitinib required a dose reduction for adverse events, including hypertension, fatigue, and hand-foot syndrome.^{1,2,10,11}

Exploratory studies have found that single nucleotide polymorphisms (SNPs) in candidate genes in the PK pathway of sunitinib are associated with the efficacy and toxicity of sunitinib. Van Erp *et al.*¹ reported that the presence of a copy of the TT allele in the *ABCG2* haplotype (rs2231142 and rs55930652) was associated with occurrence of any toxicity higher than grade 2 and that the presence of a copy of the TTT alleles in the *ABCB1* haplotype (rs1128503, rs2032582, and rs1045642) was associated with an increased prevalence of hand-foot syndrome. The G allele in *CYP1A1* (rs1048943) showed an increased risk for both mucosal inflammation and leukopenia.¹ The absence of a CAG copy in the *NR1I3* haplotype (rs2307424, rs2307418, and rs4073054) was associated with an increased risk for leukopenia. In a subsequent analysis, van der Veldt *et al.*³ reported an association between *CYP3A5**1 (presence of an A allele on rs776746) and an increased progression-free survival (PFS). The *CYP3A5**1 allele results in expression of the CYP3A5 enzyme, which is hypothesized to convert sunitinib to the active metabolite SU12662.

SU12662 has a longer half-life than sunitinib (80-110 vs. 40-60 h). This increased conversion into SU12662 could explain the observed increased occurrence of toxicities for *CYP3A5*1* carriers.² To maximize the potential for identifying novel associations, these studies did not correct for multiple testing, increasing the risk for inflation of type 1 errors and necessitating replication studies.¹⁻³

Recently, several novel SNPs affecting CYP3A4 activity have been identified. The *CYP3A4*22* SNP results in decreased CYP3A4 activity and is associated with decreased levels of clearance (CL) for several drugs as well as increased levels of paclitaxel-induced neurotoxicity.¹²⁻¹⁴ During treatment with midazolam or tacrolimus, an increased CYP3A activity was observed for *POR*28* (T allele carriers for rs1057868C/T) in the gene encoding P450 oxidoreductase (*POR*).^{12,13,15-19}

The reported associations between SNPs in candidate genes in the PK pathway of sunitinib and sunitinib-related outcomes may present an interesting opportunity to guide individualization of sunitinib treatment. However, replication of exploratory findings in an independent cohort is necessary, and it remains to be elucidated if these SNPs truly affect the PK of sunitinib and SU12662. Therefore, the aim of this study was to investigate, using CL as the PK parameter of interest, whether polymorphisms in candidate genes involved in the metabolism and absorption of sunitinib result in altered PK of sunitinib and its active metabolite, SU12662.

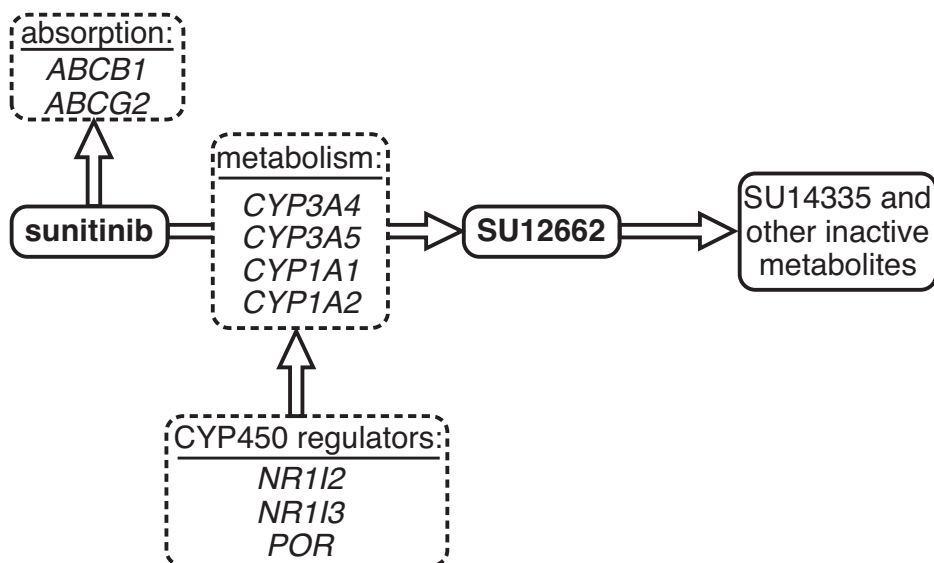


Figure 1 Pharmacokinetic pathway of sunitinib and its active metabolite, SU12662.

RESULTS

Population characteristics

Data from patients treated with monotherapy sunitinib were pooled from five prospective PK studies conducted at seven medical centers in the Netherlands and Australia (see **Supplementary Table S1** online).²⁰⁻²⁴ Both PK data and pharmacogenetics (PG) data were available for a total of 114 of the 122 patients. The majority (60.5%) of subjects were diagnosed with metastatic renal cell carcinoma (**Table 1**).

Table 1 Patient characteristics

Demographic	noted as <i>N</i> (%) or <i>N</i> (range)
Gender	
Male	75 (65.8%)
Female	39 (34.2%)
Age (years)	58.7 (range 27-81)
Ethnicity	
Caucasian	109 (95.6%)
Asian	3 (2.6%)
Middle-eastern	1 (0.9%)
Hispanic	1 (0.9%)
Tumor type	
(m)RCC	69 (60.5%)
(p)NET	14 (12.3%)
GIST	8 (7.0%)
other solid tumor type	23 (20.2%)
Sunitinib, daily dose (mg)	
25	5 (4.4%)
37.5	60 (52.6%)
50	48 (42.1%)
62.5	1 (0.9%)

ECOG, Eastern Cooperative Oncology Group; GIST, gastrointestinal stromal tumor; (m)RCC, metastatic or nonmetastatic renal cell carcinoma; (p)NET, neuroendocrine tumors of the pancreas or elsewhere.

Effect of SNPs on CL of sunitinib and SU12662

Parameter estimates of the base model are presented in **Table 2**. Association analysis was performed following a stepwise procedure that comprises genotype inclusion, univariate testing for potential association (forward inclusion), and, finally, elimination of the genotypes of least significance (backward elimination). The correlation results of SNPs and CL are listed in **Table 3**. The genotypes rs1048943 GG, rs35599367 TT, rs776746 AA, rs2231142 AA, and rs2307418 AA were excluded from the analysis due to a frequency <5% (**Table 3**).

The remaining 37 genotypes and 3 haplotypes were individually tested by inclusion in a base model (without any covariates). Genotypes CC and CT of SNP rs35599367 in *CYP3A4*, GG of SNP rs776746 in *CYP3A5*, TT of SNP rs2032582 in *ABCB1*, and CC of SNP rs1128503 in *ABCB1* showed a potential association ($P<0.05$) with CL of sunitinib and were included in the full model (**Table 3**). In the univariate analysis, a 29.0% increase in CL of sunitinib ($P=0.01$) was observed for the CC genotype of SNP rs35599367 (*CYP3A4*) as compared with the CT and TT genotypes. By contrast, genotype CT of SNP rs35599367 (*CYP3A4*) showed a 21.3% decrease in sunitinib CL ($P=0.02$) as compared with the CC and TT genotypes. *CYP3A5**3 (genotype GG of SNP rs776746) showed a decrease in sunitinib CL of 18.4% ($P=0.01$). The AG genotype of rs776746 in *CYP3A5* (*CYP3A5**1/*3) revealed potential association with CL of SU12662 with an effect size of 28.0% ($P=0.04$). None of the haplotypes showed an association with sunitinib or SU12662 CL (**Table 3**). **Figure 2a** shows an overview of the empirical Bayes estimates of CL for sunitinib and SU12662 plotted for the different tested genotypes in *CYP1A1*, *CYP3A4*, *CYP3A5*, *ABCB1*, *ABCG2*, *NR1I2*, *NR1I3*, and *POR*.

In the multivariate backward-elimination step, none of the tested SNPs reached the significance threshold of $P<0.0005$ (**Table 3**). SNP rs35599367 was the last SNP that was eliminated from the full model. In the presence of at least one T allele (*CYP3A4**22) of rs35599367, a decrease of 22.5% in CL of sunitinib was observed ($P<0.01$; **Table 3**). The interindividual variability in CL of sunitinib decreased with 1.3% (percentage of coefficient variation (%CV)) after inclusion of this genotype as a covariate in the model (**Table 2**).

Table 2 Parameter estimates of the base model for sunitinib and SU12662

Parameter	Estimate (RSE)	IIV _{%CV} (RSE)	IIV _{variance}
Sunitinib			
CL _p (l · h ⁻¹)	38.8 (3.4%)	32.2% (10.9%)	0.104
Ka _p , h ⁻¹	0.195	-	-
Vc _p , L	877 (16.9%)	64.7% (18.5%)	0.419
Q _p (l · h ⁻¹)	254 (12.5%)	-	-
V _{p,p} (l)	1670 (11%)	49.1% (12.8%)	0.241
Proportional error	0.0287 (20.7%)	-	-
Additive error (nmol/l)	0.50	-	-
SU12662			
CL _m (l · h ⁻¹)	79.6 (4.2%)	42.9% (6.5%)	0.184
V _{cm} (l)	309 (19.5%)	-	-
Proportional error	0.0332 (22%)	-	-
Additive error (nmol/l)	0.54	-	-

%CV, percentage of coefficient variation; CL_m, clearance of the active metabolite SU12662; CL_p, clearance of the parent drug sunitinib; IIV, interindividual variability; Ka_p, absorption rate constant; Q_p, intercompartment clearance; RSE, relative standard error; Vc_p, volume distribution of central compartment of the parent drug sunitinib; Vc_m, volume distribution of central compartment of the active metabolite SU12662; Vp_p, volume distribution of peripheral compartment of the parent drug sunitinib. From ref. 22.

Table 3 Results from association analyses of SNPs on sunitinib and SU12662 clearance

Gene(s)	rs Number	Genotype	N ^a	%	P value ^b (CL _p)	Effect size (%) ^c (CL _p)	P value ^d (CL _p)	P value ^b (CL _m)	Effect size (%) ^c (CL _m)	P value ^d (CL _m)
CYP1A1	rs1048943	AA (wt)	104	92.0	0.83	-2.5		0.34	-13.5	
		AG	8	7.1	0.86	-2.3		0.84	+3.0	
		GG	1	0.9	-	-		-	-	
CYP3A4	rs35599367	CC (wt)	99	88.4	0.01	+29.0	0.01	0.42	+11.0	
		CT	11	9.8	0.02	-21.3	0.84	0.58	-7.6	
		TT	2	1.8	-	-		-	-	
CYP3A5	rs776746	GG (wt)	92	82.9	0.01	-18.4	0.06	0.07	-17.7	
		AG	17	15.3	0.05	+19.0		0.04	+28.0	0.04
		AA	2	1.8	-	-		-	-	
ABCB1	rs1128503	CC (wt)	37	32.5	0.048	-4.8		0.45	-6.5	
		TC	52	45.6	0.35	-5.9		0.92	-0.8	
		TT	25	21.9	0.05	+17.0	0.66	0.33	+10.0	
ABCB1	rs2032582	GG (wt)	40	36.0	0.42	-5.3		0.46	-6.2	
		GT	41	36.9	0.23	-7.7		0.46	-6.3	
		TT	30	27.0	0.02	+18.0	0.02	0.20	+13.0	
ABCB1	rs1045642	TT (wt)	37	32.5	0.30	+7.0		0.94	+1.0	
		TC	51	44.7	0.69	+3.0		0.80	+2.0	
		CC	26	22.8	0.11	-11.5		0.71	-3.7	
ABCG2	rs2231142	CC (wt)	83	75.5	0.10	+13.0		0.86	+2.0	
		AC	24	21.8	0.14	-10.9		0.91	-1.2	
		AA	3	2.7	-	-		-	-	
ABCG2	rs55930652	CC (wt)	52	46.0	0.74	+2.0		0.58	-4.6	
		CT	52	46.0	0.28	-6.7		0.82	+2.0	
		TT	9	8.0	0.32	+13.0		0.32	+17.0	
ABCG2	rs2622604	CC (wt)	56	50.9	0.45	-4.8		0.17	-10.9	
		CT	47	42.7	0.84	-1.3		0.39	+8.0	
		TT	7	6.4	0.16	+21.0		0.08	+36.0	
NR1I2	rs3814055	CC (wt)	40	35.1	0.68	-2.7		0.77	+3.0	
		CT	56	49.1	0.19	+9.0		0.59	+5.0	
		TT	18	15.8	0.21	-10.5		0.26	-12.0	
NR1I3	rs2307424	CC (wt)	43	38.1	0.53	+4.0		0.57	-4.8	
		TC	55	48.7	0.30	-6.5		0.69	-3.3	
		TT	15	13.3	0.36	+9.0		0.07	+25.0	
NR1I3	rs2307418	AA (wt)	82	72.6	0.43	+6.0		0.56	-5.3	
		CA	29	25.7	0.25	-8.1		0.84	+2.0	
		CC	2	1.8	-	-		-	-	
NR1I3	rs4073054	TT (wt)	49	43.8	0.69	+3.0		0.14	+13.0	
		TG	47	42.0	0.50	-4.4		0.71	-3.1	
		GG	16	14.3	0.97	-0.2		0.40	-9.7	
POR*28	rs1057868	CC (wt)	53	46.9	0.87	-1.0		0.56	+5.0	
		CT	52	46.0	0.37	-5.6		1.00	-0.1	
		TT	8	7.1	0.10	+23.0		0.47	-11.0	
ABCB1	rs1128503	TTT - TTT	21	19.4	0.65	+3.0		0.90	+1.0	
	rs2032582	TTT - other	44	40.7						
	rs1045642	Other - other	43	39.8						

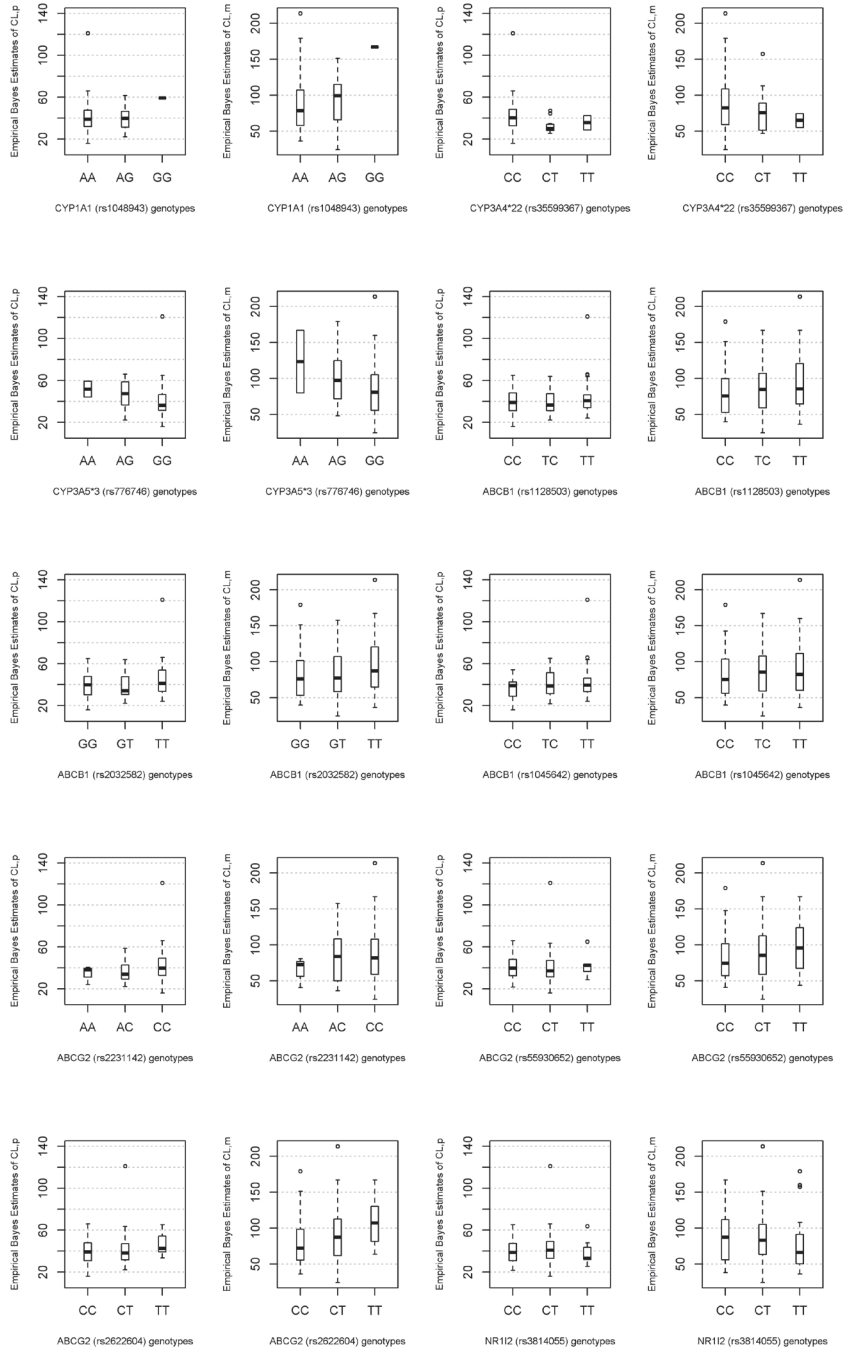
Table 3 Continued

Gene(s)	rs Number	Genotype	N ^a	%	P value ^b (CL _p)	Effect size (%) ^c (CL _p)	P value ^d (CL _p)	P value ^b (CL _m)	Effect size (%) ^c (CL _m)	P value ^d (CL _m)
ABCG2	rs55930652 rs2622604	TT - TT	7	6.4	0.71	+2.0		0.09	+15.0	
		TT - other	45	40.9						
		Other - other	58	52.7						
NR1I3	rs2307424 rs2307418	CAT - CAT	11	9.9	0.20	-7.9		0.34	-7.8	
		CAT - other	38	34.2						
		Other - other	62	55.9						
		CAG - CAG	16	14.4	0.49	-4.4		0.34	-7.7	
		CAG - other	47	42.3						
		Other - other	48	43.2						
CYP3A4	rs35599367	CT or TT vs. CC				-22.5	0.01		-9.9	
CYP3A5	rs776746	AG or AA vs. GG				+22.5	0.06		+21.5	
Genotypes	rs Number	CYP3A genotype- predicted phenotype	N ^a	%	P value ^b (CL _p)	effect size (%) ^c (CL _p)		P value ^b (CL _m)	Effect size (%) ^c (CL _m)	
CYP3A4 CT or TT and CYP3A5 GG	rs35599367 and rs776746	PM	12	11.0	0.01	-22.9		0.45	-9.7	
CYP3A4 CT or TT and CYP3A5 AG or AA	rs35599367 and rs776746	IM ₁	1	0.9	-	-		-	-	
CYP3A4 CC and CYP3A5 GG	rs35599367 and rs776746	IM ₂	78	71.6	1.00	0.0		0.31	-8.7	
CYP3A4 CC and CYP3A5 AG or AA	rs35599367 and rs776746	EM	18	16.5	0.02	+22.0		0.01	+35.0	

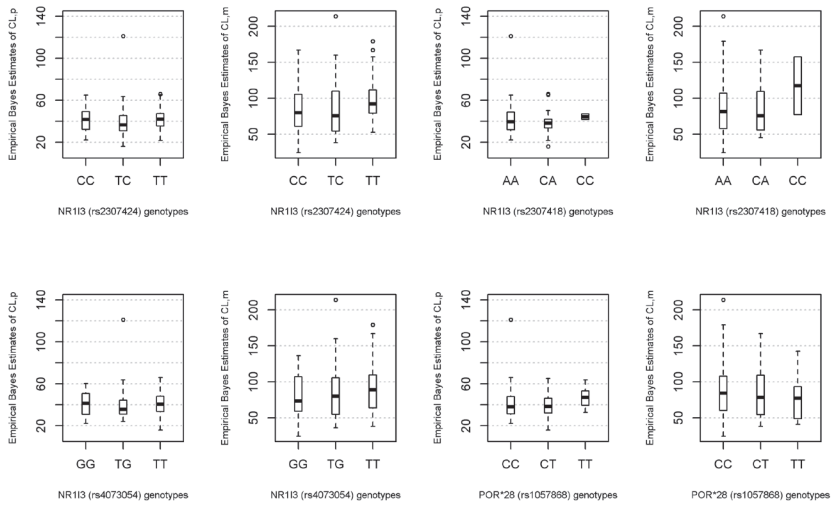
CL_m, clearance of SU12662; CL_p, clearance of sunitinib; EM, extensive metabolizer; IM1, intermediate metabolizer type 1; IM2, intermediate metabolizer type 2; PM, poor metabolizer; SNP, single nucleotide polymorphism; wt, wild type.

^aNumber of subjects. Numbers differ per SNP, haplotype, or phenotype because not all subjects were successfully genotyped for all SNPs. ^bP value for the influence of genotype or haplotype on clearance from the univariate analysis (forward inclusion). ^cEffect size of the genotype or haplotype on the clearance with presence of the genotype as compared with the clearance with absence of the genotype or haplotype. ^dP value for the influence of genotype or haplotype on clearance from the multivariate analysis (backward elimination). ^eEffect size of the phenotype on the clearance with presence of the phenotype as compared with the clearance with the presence of the IM1 + IM2 phenotypes.

A



A



B

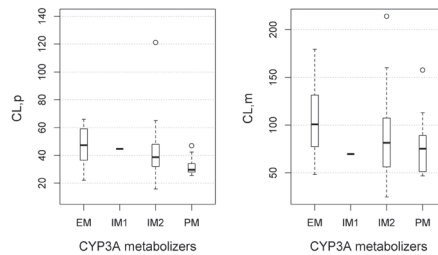


Figure 2 Clearance plotted against SNP genotypes. (a) Empirical Bayes estimates of clearance of sunitinib (CLp) and the active metabolite SU12662 (CLm) for base model stratified by different genotypes of single nucleotide polymorphisms or haplotypes in *CYP1A1*, *CYP3A4*, *CYP3A5*, *ABCB1*, *ABCG2*, *NR1H2*, *NR1H3*, and *POR*. The quartiles construct the upper and lower ranges of the boxes; the whiskers display the highest and lowest value within the 1.5 interquartile ranges; and the outliers are 1.5 interquartile ranges above the upper quartile or 1.5 interquartile ranges below the lower quartile. (b) Empirical Bayes estimates of clearance of sunitinib (CLp) and the active metabolite SU12662 (CLm) for the base model stratified by CYP3A phenotypes.

CYP3A genotype-predicted phenotype analysis

Because of a great similarity in CYP3A substrate specificity of CYP3A4 and CYP3A5, the CYP3A4 and CYP3A5 genotypes were combined as a CYP3A genotype-predicted phenotype (CYP3A phenotype).¹⁷ In a *post hoc* analysis, CYP3A genotype-predicted phenotypes (poor metabolizers (PMs), intermediate metabolizers (IMs), or extensive metabolizers (EMs)) were tested as covariates in our population PK model. The possible combinations, together with the frequencies of CYP3A4 and CYP3A5 genotypes, were linked to the corresponding metabolizer status (CYP3A phenotype) and are listed in **Table 3**. The aberrant CYP3A phenotypes (PMs and EMs) were compared with the most frequent CYP3A-predicted phenotype (IM type 1 with CT or TT genotype of CYP3A4 and AG or AA of CYP3A5 and IM type 2 with wild-type CC genotype of CYP3A4 and GG of CYP3A5). The observed effect sizes of these IMs were used as a reference point with respect to the observed effect sizes of PMs or EMs. For CYP3A PMs, a decrease of 22.9% in the CL of sunitinib was observed as compared with that of CYP3A IMs ($P=0.01$). For SU12662, a decrease in CL of 9.7% was observed for the group of CYP3A PMs as compared with CYP3A IMs ($P=0.45$), as shown in **Table 3**. For CYP3A EMs, a 22.0% increase in the CL of sunitinib was observed as compared with that of CYP3A IMs ($P=0.02$). For SU12662, a 35.0% increase in the CL of sunitinib was observed for the group of CYP3A PMs as compared with CYP3A IMs ($P=0.01$; **Table 3**). **Figure 2b** shows the empirical Bayes estimates of CL for sunitinib and SU12662 plotted for the different CYP3A phenotypes.

DISCUSSION

To our knowledge, this is the first PG study on the PK of sunitinib. Previous exploratory studies have directly related SNPs in candidate genes in the PK pathway of sunitinib to efficacy and toxicity, without involvement of plasma concentrations. Interestingly, none of the SNPs in candidate genes in the PK pathway of sunitinib that have been previously associated with outcomes of sunitinib treatment appear to be significantly associated with the CL of sunitinib and SU12662 in our population of 114 patients treated with sunitinib. Although not significant, CYP3A4*22 carriers showed a 22.5% decrease in the CL of sunitinib. By contrast, CYP3A5*1 carriers showed a 22.5% increase in the CL of sunitinib. When analyzed by CYP3A phenotype, PMs (defined as the concomitant presence of CYP3A5*3 GG and CYP3A4*22) showed a 22.9% decrease in the CL of sunitinib and a 9.7% decrease in the CL of SU12662 as compared with those of CYP3A IMs, and CYP3A EMs (defined as the concomitant presence of CYP3A5*3 AG or AA and CYP3A4 CC) showed a 22.0% increase in the CL of sunitinib and a 35% increase in the CL of SU12662 as compared with those of CYP3A IMs. Unfortunately, our data do not allow exact replication of previously reported

exploratory analyses because no toxicity or efficacy data were available for the current data set. In addition, SNPs related to the pharmacodynamic pathway were not included in this study.^{25,26} Furthermore, the small sample size of 114 patients limits the number of SNPs that could be investigated. Therefore, we selected only those SNPs and haplotypes that were significantly associated ($P < 0.05$) with toxicity, PFS, or overall survival as reported in published exploratory PG studies on sunitinib. The minor allele frequency for the tested SNPs was >0.1 , with the exception of the *CYP1A1* SNP rs1048943 (0.044), the *CYP3A4* SNP rs35599367 (0.067), and the *CYP3A5* SNP rs776746 (0.095).

The SNPs in *CYP3A4* (rs35599367) and *ABCB1* (rs2032582) deviated from Hardy-Weinberg equilibrium. However, the observed deviations were small, with $P = 0.02$ for *CYP3A4* (rs35599367) and $P = 0.01$ for *ABCB1* rs2032582. In addition, allele frequencies are highly similar to the frequencies reported in the HapMap-CEU The National Center for Biotechnology Information (NCBI) database. In this study, *ABCB1* rs2032582 showed an allele frequency of 45.5%, as compared with 46.9% reported in the NCBI database. For *CYP3A4**22, an allele frequency of 6.7% was found, as compared with 2.5% reported in the NCBI database.

The observed decrease of 22.5% in the CL of sunitinib resulting from *CYP3A4**22 has a similar order of magnitude as reported for the effect of *CYP3A4**22 and the *CYP3A* phenotype on CL of tacrolimus, midazolam, erythromycin, and erlotinib.^{12,13,27,28} The effect sizes of 22.5% (*CYP3A4**22) and 22.9% (*CYP3A* PMs) observed in this study are not large enough to guide individual sunitinib dosing in clinical practice because these effect sizes are smaller than the interindividual variabilities of 32.2 and 42.9% in CL of sunitinib and SU12662, respectively. Houk *et al.*⁴ reported an even larger interindividual variability of 37.9 and 52.2% in the CL of sunitinib and SU12662, respectively. In practice, the actual effect sizes of the studied SNPs on CL might be lower due to the effect on PK variability of environmental and other factors, such as smoking, alcohol use, body mass index, hormonal status, age, race, gender, and coadministration of other drugs.^{4,29,30}

The analysis of *CYP3A* genotype-predicted phenotypes in relation to the CL of sunitinib has not been performed previously. Earlier, it was observed that carriers of the *CYP3A5**1 allele have a prolonged PFS and increased toxicity.^{2,3} It was hypothesized that this might result from higher levels of SU12662, which has a longer half-life than sunitinib, resulting in increased exposure. Our data partly support this hypothesis by showing that carriers of a *CYP3A5**1 allele indeed have a 22.5% increased CL of sunitinib ($P = 0.05$) as compared with GG carriers, and thus increased formation of SU12662. However, the theory regarding increased exposure to SU12662 is refuted because our data indicate that carriers of a *CYP3A5**1 allele, in addition to having a higher CL of sunitinib, have a 21.5% ($P = 0.04$) higher CL of SU12662, resulting in a faster elimination and reduced exposure. Effect sizes of the PM *CYP3A* phenotype (22.9%) and *CYP3A4**22 (22.5%) on CL of sunitinib are comparable and

differ only by 0.4 percentage points. This can be explained by the low *CYP3A5*1* frequency in our study population, who are mainly Caucasians.

To date, studies looking for PG biomarkers for sunitinib-related outcomes have revealed a small set of potential markers. The available studies used the candidate-gene approach in retrospectively collected cohorts and thus are limited by our current knowledge of the PK and pharmacodynamics of sunitinib. Our results indicate that the currently available PG biomarkers cannot be used directly to guide individual dosing of sunitinib because they account for a limited part of the observed interindividual variability in CL of sunitinib and SU12662. Combinations of PG and environmental markers that predict the CL of this drug should be studied further. In addition, several other approaches to individualize sunitinib treatment remain to be explored. Therapeutic drug monitoring appears to be a promising method to guide sunitinib dosing that warrants further research.^{31,32} In addition, it would be quite interesting to explore other PG biomarkers by using an unbiased approach—for example, by conducting a genome-wide association study in a well-defined cohort of prospectively collected data.³³

In conclusion, of the 14 SNPs that are in the suggested PK pathway for sunitinib and that have been previously related to sunitinib outcome, none significantly influences the CL of sunitinib or SU12662 based on the *P* value <0.0005. The observed effect sizes are too small to directly guide individual dosing regimens of sunitinib.

METHODS

Study population

PK data were available from participating medical centers, including the Leiden University Medical Center, Academic Medical Center, University Medical Center Utrecht, Slotervaart Hospital, Erasmus MC Cancer Institute, Netherlands Cancer Institute-Antoni van Leeuwenhoek, Canberra Hospital, and the Westmead Hospital in Sydney.^{20–24} Patients were eligible if PK sampling of sunitinib was performed and blood for PG analysis was available. Patients were excluded because of missing dose levels (*N*=2), concomitant use of mitotane (a strong CYP3A4 inducer; *N*=2), and genotype call rates <80% (*N*=3). All patients received sunitinib orally, administered in doses ranging from 25.0 to 62.5 mg once daily in a continuous dosing regimen or an intermittent schedule (4 weeks on treatment followed by 2 weeks off treatment). Patients were diagnosed with metastatic or nonmetastatic renal cell carcinoma (*N*=69), neuroendocrine tumors of the pancreas (*N*=14), gastrointestinal stromal tumor (*N*=8), or another solid tumor type (*N*=23) (**Table 1**). The local medical ethical boards of the participating medical centers approved all studies, and informed consent was obtained from all patients.

PK data

Steady-state plasma concentrations of sunitinib and SU12662 were measured before start of sunitinib administration and at one or more (fixed or random) time points after dosing (see **Supplementary Table S1** online).²⁰⁻²⁴

SNP and haplotype selection

SNPs related to the sunitinib PK pathway were selected from seven published exploratory PG studies on sunitinib.^{1-3,12,15,18,19} SNPs and haplotypes significantly associated ($P < 0.05$) with toxicity response, PFS, or overall survival were included in the current study. Selected SNPs and haplotypes are listed in **Supplementary Table S2a,b** online.

Genotyping

Germ-line DNA was isolated from 400 µl whole blood, using the Maxwell 16 system (Promega, Leiden, The Netherlands), according to the manufacturer's protocol. Prior to analysis, DNA concentrations were measured using the Nanodrop ND-1000 spectrophotometer (Isogen Life Science, IJsselsteijn, The Netherlands). Genotyping was performed with TaqMan probes (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands) using the LightCycler480 (LC480) Real-Time PCR Instrument (Roche Applied Science, Almere, The Netherlands).

The mean genotype call rate was 98.4%, with a lowest observed call rate of 96% and a highest observed call rate of 100%. As a quality control, 5% of the samples were genotyped in duplicate for all assays, and no inconsistencies were observed. Patients with a genotype call rate of $< 80\%$ were excluded from analysis. Minor allele frequencies of all 14 SNPs were calculated and compared with reported minor allele frequencies for European populations (<http://www.ncbi.nlm.nih.gov/snp/>). All SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$), except for rs35599367 in *CYP3A4* ($P = 0.024$) and rs2032582 in *ABCB1* ($P = 0.007$) (see **Supplementary Table S3** online). The maximum likelihood estimates of haplotype probabilities were calculated using PLINK software, version 1.07.³⁴ Haplotype probabilities with a likelihood $\geq 95\%$ were included in statistical analysis. Haplotype likelihoods could be calculated for a mean percentage of patients of 97.3% (range: 96.3-98.2%).

Statistical analysis

Plasma concentration-time data and PG data were analyzed using the nonlinear mixed effect modeling software, NONMEM (version 7.1; ICON, Ellicott City, MD). The first-order conditional estimation method with interaction was used for the parameter estimation, and Piraña was used as the modeling environment. R (version 2.13.0; <http://cran.r-project.org>) was used for handling of data and results.

A two-compartment model for sunitinib and one-compartment model for SU12662 were structured as schematically shown in **Figure 3**.²² The base PK model was designated based on objective function value ($\Delta\text{OFV} > 3.84$; $P < 0.05$), successful minimization, successful covariance estimation, and goodness-of-fit plots. The goodness-of-fit plots are presented in the ClearSun study.²² The visual predictive checks are also described in the ClearSun study and shown in **Supplementary Figure S1** online.²² The bioavailability (F) was unknown and therefore the parameter estimates of the population PK model were noted as apparent terms proportional to F , except for the absorption rate constant of sunitinib ($K_{a,p}$). For CL, this means the parameter estimate is noted as CL/F . For SU12662, the PK parameters in the population PK model were estimated relative to F and f_m , which is the fraction of sunitinib metabolized to SU12662. The $K_{a,p}$ was set at 0.195/h based on the results of a population PK metaanalysis of sunitinib and SU12662.^{4,22} An exponential error term was used to define the interindividual variability of all PK parameters. This exponential error term was quantified as both %CV and variance (calculated as $(\%CV/100)$).² A combined error model with additive error terms fixed to the lower limit of quantification (**Table 2**) was used to model the residual error.^{4,22}

Once the base model was defined, genotypes and haplotypes were tested as covariates on both CL parameters in a stepwise manner. Genotype frequencies of SNPs or haplotypes that were smaller than 5% were considered as missing values and ignored in analysis. Other covariates, i.e. gender, age, and tumor type, were not included in the building of the model, because the main research question of the current study was to identify potential relationships between the genotypes of relevant drug-metabolizing enzymes and the CL of sunitinib and SU12662. Furthermore, considering the relatively small sample size and the large variability in the PK of sunitinib, inclusion of other covariates is unlikely to have substantial effects on the outcome. The different PK studies in which the included patients previously participated were regarded as “PK study covariates.” In the current analysis, these “PK study covariates” were considered during model building but were not included in the model. Instead, all goodness-of-fit diagnostics stratified on “PK study covariate” were evaluated in order to evaluate the influence of “PK study covariate” on PK parameters. No relevant differences between the different studies were identified.

First, a univariate forward-inclusion step was conducted. Covariates (genotypes or haplotypes) were screened by inclusion in a base model univariately using a power model, where $\text{CL}_{\text{typical value}} = \theta_1 \times (\theta_2)^{\text{pg}1}$, where pg is the genotype or haplotype of interest whereby the presence of the certain genotypes or haplotypes in the patients was scored 1 and the absence was scored 0. θ_1 is the population CL estimate and θ_2 is the covariate effect size estimate. The threshold of this step was set at $P < 0.05$ (likelihood ratio test, $\Delta\text{OFV} > 3.84$; degrees of freedom = 1). In the next step, all potentially related covariates were included in a full model.

Subsequently, in the backward-elimination step, covariates were removed one at a time from the full model, and significance was again assessed using the likelihood ratio test. The likelihood ratio test itself may have anticonservative properties, and to account for multiple testing, we applied a threshold P value of 0.0005 ($\Delta\text{OFV} > 12.12$; degrees of freedom = 1) in this step of the analysis. A separate analysis of combinations of the *CYP3A5**3 and *CYP3A4**22 genotypes (referred to as the CYP3A phenotype) was performed in order to evaluate correlations between each metabolizer group (PM, IM, or EM) and CLs. According to the literature, the presence of the T allele (CT or TT) in SNP *CYP3A**22 or the GG genotype in SNP *CYP3A5**3 results in decreased potency of metabolic enzymes.¹⁷ The subjects were grouped separately according to their genotypes, as presented in **Table 3**.

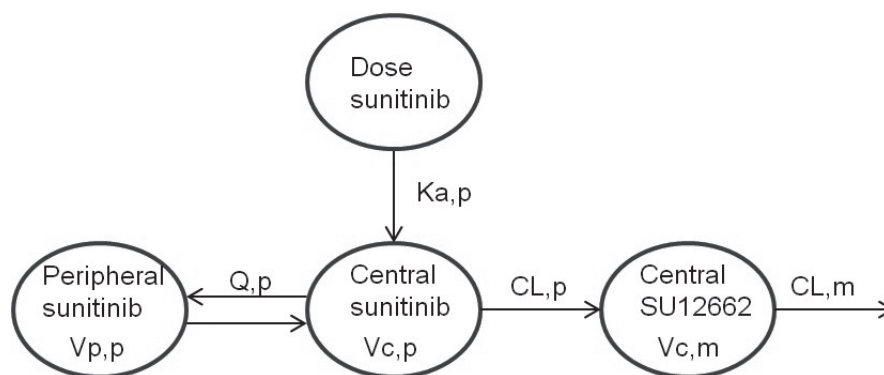


Figure 3 Pharmacokinetic model for sunitinib and SU12662. CL_m, apparent clearance of SU12662; CL_p, apparent clearance of sunitinib; K_{a,p}, absorption rate constant of sunitinib; Q_p, apparent intercompartmental flow of sunitinib; V_{c,p}, apparent central volume of distribution of sunitinib; V_{c,m}, apparent volume of distribution of SU12662; V_{p,p}, apparent peripheral volume of distribution of sunitinib.²²

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SUPPLEMENTARY MATERIAL

Supplementary table S1 Time points of blood sampling for included PK studies

Study acronym ^[REF]	Clinical trial registration number	Time points
NIB ²⁰	-	0 and one random sampling time point after start sunitinib
M10PKS ²¹	NCT01286896	0 and 24 hour(s)
Clearsun ²²	NCT01098903	0, 4, 8, and 24 hour(s)
Chrono ²³	NTR3526	0, 1, 2, 4, 6, 8, 12 and 24 hour(s)
Phenotyping study ²⁴	NCT01743300	0, 10, 30, 40 minutes and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 hour(s)

Supplementary Table S2a 14 selected SNPs related to sunitinib pharmacokinetics

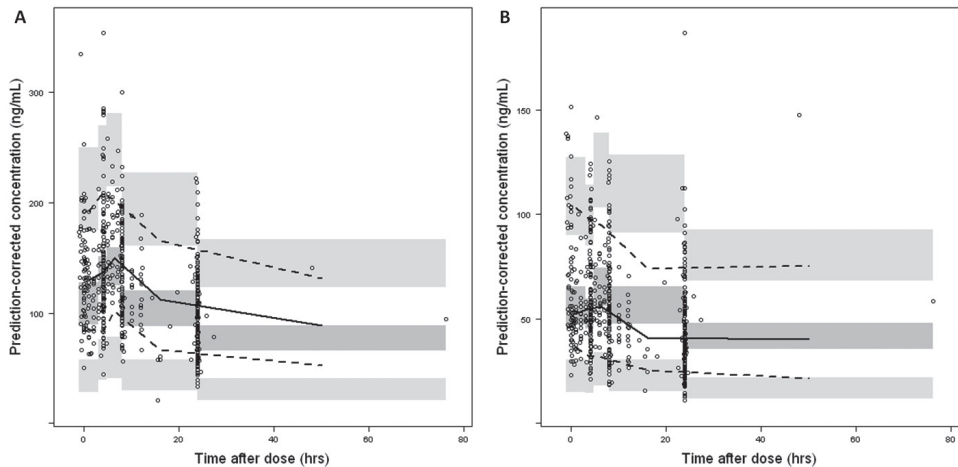
gene	rs number	Variant allele	P value	Associated effect	references
<i>CYP1A1</i>	rs1048943	G	0.029	increased risk for leukopenia and mucosal inflammation	van Erp NP <i>et al.</i> , J Clin Oncol, 2009 ¹
<i>CYP3A4*22</i>	rs35599367	T	0.018	decreased CYP3A4 activity; 33% lower dose requirements for carriers of the T allele than for CC patients. The calculated dose-adjusted C ₀ was lower for CC than for CT/TT patients.	Elens L <i>et al.</i> , Clin Chem, 2011 ¹²
<i>CYP3A5*3</i>	rs776746	A	0.022 0.032	dose reductions due to toxicity ¹ improved PFS ³	Garcia-Donas J <i>et al.</i> , Lancet Oncol, 2011 ²
<i>ABCB1</i>	rs1128503	T	0.09	less hypertension	van der Veldt AA <i>et al.</i> , Clin Cancer Res, 2011 ³
<i>ABCB1</i>	rs2032582	T	0.17 0.089 0.055	presence of T-allele: protective for hypertension worse PFS worse OS	van Erp NP <i>et al.</i> , J Clin Oncol, 2009 ¹ de Jonge H <i>et al.</i> , Pharmacogenomics, 2011 ¹⁵
<i>ABCB1</i>	rs1045642	C	0.22	less hypertension ($P=0.22$)	
<i>ABCG2</i>	rs2231142	A	0.088	PFS	Garcia-Donas J <i>et al.</i> , Lancet Oncol, 2011 ²
<i>ABCG2</i>	rs55930652	T	0.016	copy of TT: any toxicity higher than grade 2	van Erp NP <i>et al.</i> , J Clin Oncol, 2009 ¹
<i>ABCG2</i>	rs2622604	T			
<i>NR1I2</i>	rs3814055	T	0.03	response reduction	van Erp NP <i>et al.</i> , J Clin Oncol, 2009 ¹ Xu CF <i>et al.</i> , J Clin Oncol, 2011 ¹⁸
<i>NR1I3</i>	rs2307424	T	0.017	$P=0.017$ for haplblock; absence of CAT $P=0.041$ for haplblock; absence of CAG	van Erp NP <i>et al.</i> , J Clin Oncol, 2009 ¹
<i>NR1I3</i>	rs2307418	C	0.041		van der Veldt AA <i>et al.</i> , Clin Cancer Res, 2011 ³
<i>NR1I3</i>	rs4073054	G			
<i>POR*28</i>	rs1057868	T	0.04	increase in CYP3A activity	de Jonge H <i>et al.</i> , Pharmacogenomics, 2011 ¹⁵ Oneda B <i>et al.</i> , Pharmacogenet Genomics, 2009 ¹⁹

Supplementary Table S2b selected haplotypes related to sunitinib pharmacokinetics

gene	rs number	SNP	P value	Genotype and predicted functional change	references
ABCB1	1045642	C/T	0.035 (TTT)	presence of TTT : increased prevalence of hand-foot-syndrome TCG copy : improved PFS	van Erp NP <i>et al.</i> , J Clin Oncol, 2009 ¹ van der Veldt AA <i>et al.</i> , Clin Cancer Res, 2011 ³
	1128503	C/T	0.033 (TCG copy)		
	2032582	G/T			
ABCG2	rs55930652	C/T	0.016	copy of TT : any toxicity higher than grade 2	van Erp NP <i>et al.</i> , J Clin Oncol, 2009 ¹
	2622604	C/T			
NR1I3	2307424	C/T	0.017	Absence of CAG copy : leukopenia absence of CAT copy : improved PFS	van Erp NP <i>et al.</i> , J Clin Oncol, 2009 ¹ van der Veldt AA <i>et al.</i> , Clin Cancer Res, 2011 ³
	2307418	A/C	0.041		
	4073054	T/G			

SNPs rs1128503, rs2032582 and rs1045642 in the *ABCB1* gene and rs2231142 in *ABCG2* show an association of $P > 0.05$, but were integrated in haplotypes having an association outcome of $P < 0.05$ and therefore included in analysis.

PFS = progression free survival.



Supplementary Figure S1 Prediction-corrected visual predictive check of sunitinib (A) and SU12662 (B) concentration. Solid lines and dark grey areas represent the median observed values and predicted 90% confidence intervals (CIs). Dashed lines and light grey areas represent the 10% and 90% percentiles of the observed values and 90% CIs of the model predicted percentiles.²²

Supplementary Table S3 SNPs incorporated in haplotypes associated with functional changes

gene	rs number	genotype	N ¹	%	MAF (%)	HWE (P-value ¹)
<i>CYP1A1</i>	rs1048943	AA (wt)	104	92.0	G=4.4%	0.08
		AG	8	7.1		
		GG	1	0.9		
<i>CYP3A4</i>	rs35599367	CC (wt)	99	88.4	T=6.7%	0.02
		CT	11	9.8		
		TT	2	1.8		
<i>CYP3A5</i>	rs776746	GG (wt)	92	82.9	A=9.5%	0.27
		AG	17	15.3		
		AA	2	1.8		
<i>ABCB1</i>	rs1128503	CC (wt)	37	32.5	T=44.7%	0.41
		TC	52	45.6		
		TT	25	21.9		
<i>ABCB1</i>	rs2032582	GG (wt)	40	36.0	T=45.5%	0.01
		GT	41	36.9		
		TT	30	27.0		
<i>ABCB1</i>	rs1045642	TT (wt)	37	32.5	C=45.2%	0.30
		TC	51	44.7		
		CC	26	22.8		
<i>ABCG2</i>	rs2231142	CC (wt)	83	75.5	A=13.6%	0.44
		AC	24	21.8		
		AA	3	2.7		
<i>ABCG2</i>	rs55930652	CC (wt)	52	46.0	T=31.0%	0.42
		CT	52	46.0		
		TT	9	8.0		
<i>ABCG2</i>	rs2622604	CC (wt)	56	50.9	T=27.7%	0.49
		CT	47	42.7		
		TT	7	6.4		
<i>NR1I2</i>	rs3814055	CC (wt)	40	35.1	T=40.4%	0.82
		CT	56	49.1		
		TT	18	15.8		
<i>NR1I3</i>	rs2307424	CC (wt)	43	38.1	T=37.6%	0.69
		TC	55	48.7		
		TT	15	13.3		
<i>NR1I3</i>	rs2307418	AA (wt)	82	72.6	C=14.6%	0.75
		CA	29	25.7		
		CC	2	1.8		
<i>NR1I3</i>	rs4073054	TT (wt)	49	43.8	G=35.3%	0.39
		TG	47	42.0		
		GG	16	14.3		
<i>POR*28</i>	rs1057868	CC (wt)	53	46.9	T=30.1%	0.32
		CT	52	46.0		
		TT	8	7.1		

¹P-value for the test for HWE. HWE: Hardy Weinberg Equilibrium.

