Pharmacogenetics and cost-effectiveness of systemic treatment in soft tissue sarcoma

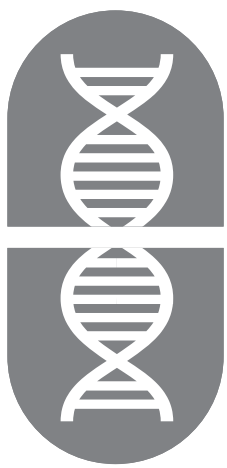
Verboom, M.C.

4

Genetic polymorphisms as predictive biomarker of survival in patients with gastro-intestinal stromal tumors treated with sunitinib

Michiel Verboom*, Jacqueline Kloth*, Jesse Swen, Tahar van der Straaten, Stefan Sleijfer, Anna Reyners, Neeltje Steeghs, Hans Gelderblom, Henk-Jan Guchelaar, Ron Mathijssen

* these authors contributed equally



The Pharmacogenomics Journal volume 18, pages 49-55 (2018)

Abstract

This study aimed to identify single-nucleotide polymorphisms (SNPs) that are associated with outcome to treatment with sunitinib in patients with advanced gastrointestinal stromal tumors (GIST). Forty-nine SNPS involved in the pharmacokinetic and pharmacodynamic pathway of sunitinib were associated with progression-free survival (PFS) and overall survival (OS) in 127 patients with advanced GIST who have been treated with sunitinib. PFS was significantly longer in carriers of the TT genotype in POR rs1056878 (hazards ratio (HR) 4.310, 95% confidence interval (CI):1.457-12.746, p= 0.008). The presence of the T-allele in SLCO1B3 rs4149117 (HR 2.024, 95% CI:1.013-4.044, p= 0.046), the CCC-CCC alleles in SLC22A5 haplotype (HR 2.603, 95% CI: 1.216-5.573, p= 0.014), and the GC-GC alleles in the IL4 R haplotype (HR 7.131, 95% CI:1.518-33.496, p= 0.013) were predictive for OS. This shows that polymorphisms in the pharmacokinetic and pharmacodynamic pathways of sunitinib are associated with survival in GIST. This may help to identify patients that benefit more from treatment with sunitinib.

Introduction

As the introduction of imatinib as first line treatment for advanced gastrointestinal stromal tumors (GIST), progression-free survival (PFS) and overall survival (OS) of patients with this malignancy has markedly improved. Unfortunately, eventually the vast majority of patients develop resistance to imatinib, mainly due to secondary mutations, while in others severe toxicity occurs, both resulting in the need to switch to second line treatment with sunitinib (Sutent; Pfizer Pharmaceuticals Group, New York, USA).[1] Sunitinib is a multi-targeted tyrosine kinase inhibitor.[2,3] Its clinical value in the treatment of patients with metastatic GIST has been shown in a randomized trial showing a median time to tumor progression of 27.3 weeks for patients treated with sunitinib, versus 6.4 weeks for patients treated with placebo.[1] However, there is a large interindividual difference in the efficacy of sunitinib in patients with GIST. This may in part be explained by the presence of specific mutations within the tumor but another factor that may contribute to the variability in efficacy may be germline genetic variation.[4] In patients treated with sunitinib for metastatic renal cell cancer, single-nucleotide polymorphisms (SNPs) in genes related to the pharmacokinetic and pharmacodynamic pathways of sunitinib have been associated with outcome in terms of PFS and OS.[5]

In patients with GIST, the role of germline genetic polymorphisms as biomarkers predicting outcome has never been investigated. To further personalize treatment in this group of patients, it is meaningful to get better insight into the factors predicting the efficacy of a drug before starting, especially when alternative treatment options exist such as in the case of advanced GIST. Therefore, a multicenter association analysis was performed to explore whether polymorphisms in candidate genes within the pharmacokinetic or pharmacodynamic pathway of sunitinib are associated with PFS and OS in patients with GIST.

Materials and methods

Study population and design

From a large multicenter Dutch cohort of 365 patients with GIST, those patients who have been treated with second line sunitinib were selected. Patients had started sunitinib treatment between March 2004 and June 2014 in the Erasmus MC Cancer Institute, Leiden University Medical Center, Netherlands Cancer Institute-Antoni van Leeuwenhoek, or University Medical Center Groningen. Sunitinib could be administered in a 4 weeks on/2 weeks off treatment scheme, or in a continuous dosing regimen (or both), with any dose of sunitinib. Patients who have had dose reductions or dose escalations were allowed to be included in this study.

Demographic data of patients was retrospectively collected in an electronic case record form, designed for this study. Collected patient characteristics were age, gender, self-declared ethnicity, Eastern Cooperative Oncology Group (ECOG) WHO performance score, weight, length, tumor characteristics (*i.e.* histology, mutation status, mitotic index (per 50 HPF), site of origin tumor, previous surgery), prior therapy and therapy after sunitinib, and survival estimates. For PFS and OS, data collection took place until August 2014.

From each patient one sample of whole blood, serum or tumor surrounding tissue containing germline DNA was collected for DNA isolation. Samples could be either residuals or prospectively obtained samples in a study approved by the local medical ethical board. Samples were stored at –20 °C or colder at the local hospital laboratory until further process. All samples were anonymized, according to the Codes for Proper use and Proper Conduct in the Self-Regulatory Codes of Conduct (www.federa.org).

Genetic polymorphisms and haplotype estimation

Forty-nine SNP in 23 genes involved in the pharmacokinetics and pharmacodynamics of sunitinib were selected for genotyping, based on literature (see Table 1). SNPs were selected from the genes *ABCB1, ABCC2, ABCG2, CYP1A1, CYP1A2, CYP3A4, NR1I2, NR1I3, POR* (Cytochrome P450 oxidoreductase), *SLCO1B3, SLC22A1, SLC22A4* and *SLC22A5* within the pharmacokinetic pathway and the genes *FLT1, FLT3, IL-4R, IL-8, KDR* (Kinase Insert Domain Receptor), *PDGFRA, RET* and *VEGFA* within the pharmacodynamic pathway.

Table 1: Selected polymorphisms within the pharmacodynamic and pharmacokinetic pathway of sunitinib

Gene	Protein	SNP	Allele change
Pharmacodynamic genes			
IL4	IL4	rs224350 (Chu *et al.*[9])	C/T
IL4R	IL4R	rs1801275 (Chu *et al.*[9]) rs1805010 (Chu *et al.*[9]) rs1805015 (Chu *et al.*[9])	A/G A/G T/C
IL8	IL8	rs4073 (Xu *et al.*[12]) rs1126647 (Xu *et al.*[12])	A/T A/T
IL13	IL13	rs1800925 (Chu *et al.*[9]) rs20541 (Chu *et al.*[9])	C/T G/A
FLT1	FLT1	rs7993418 (Beuselinck *et al.*[13])	A/G
FLT3	FLT3	rs1933437 (van Erp *et al.*[14])	T/C
FLT4	VEGFR3	rs6877011 (Scartozzi *et al.*[15])	C/G

KDR	VEGFR2	rs1870377 (Garcia-Donas *et al.*[16]) rs2071559 (van Erp *et al.*[14]) rs2305948 (Garcia-Donas *et al.*[16])	A/T C/T C/T
PDGFRA1	PDGFRA1	rs1800810 (van Erp *et al.*[14]) rs1800812 (van Erp *et al.*[14]) rs1800813 (van Erp *et al.*[14])	C/G G/T A/G
PDGFRA2	PDGFRA2	rs2228230 (Bruck *et al.*[17]) rs35597368 (Garcia-Donas *et al.*[16]; van Erp *et al.*[14])	C/T C/T
RET	RET	rs1799939 (van Erp *et al.*[14])	G/A
VEGFA	VEGFA	rs1570360 (Garcia-Donas *et al.*[16]) rs2010963 (Eechoute *et al.*[18]; Garcia-Donas *et al.*[16]) rs25648 (Scartozzi *et al.*[15]) rs3025039 (Kim *et al.*[19]) rs699947 (Eechoute *et al.*[18]; Garcia-Donas *et al.*[16]; Kim *et al.*[19]) rs833061 (Eechoute *et al.*[18]; Kim *et al.*[19])	G/A G/C C/T C/T A/C C/T
Pharmacokinetic genes			
ABCB1	ABCB1	rs1045642 (Maffioli *et al.*[20]; Takahashi *et al.*[21]) rs868755 (Angelini *et al.*[8]; Takahashi *et al.*[21]) rs28656907 (Loeuillet *et al.*[22])	C/T G/T C/T
ABCC2	ABCC2	rs717620 (Takahashi *et al.*[21])	C/T
ABCG2	ABCG2	rs2231137 (Angelini *et al.*[8]) rs2231142 (Angelini *et al.*[8]; Takahashi *et al.*[21])	G/A C/A
CYP1A1	CYP1A1	rs1048943 (van Erp *et al.*[14])	A/G
CYP1A2	CYP1A2	rs762551 (van Erp *et al.*[14])	A/C
CYP3A4	CYP3A4	rs2740574 (Angelini *et al.*[8])	A/G
NR1I2	NR1I2	rs3814055 (van Erp *et al.*[14]) rs1054191 (van Erp *et al.*[14])	C/T G/A
NR1I3	NR1I3	rs2307424 (van der Veldt *et al.*[5]; van Erp *et al.*[14]) rs2307418 (van der Veldt *et al.*[5]; van Erp *et al.*[14]) rs4073054 (van der Veldt *et al.*[5]; van Erp *et al.*[14])	C/T A/C G/T
POR	POR	rs1057868 (de Jonge *et al.*[23])	C/T
SLC1B3	OATP1B3	rs4149117 (Angelini *et al.*[8])	G/T
SLC22A1	hOCT1	rs628031 (Maffioli *et al.*[20];Takahashi *et al.*[21]) rs683369 (Angelini *et al.*[8]; Takahashi *et al.*[21]) rs6935207 (Maffioli *et al.*[20])	G/A C/G G/A
SLC22A4	OCTN1	rs1050152 (Angelini *et al.*[8])	C/T
SLC22A5	OCTN2	rs2631367 (Angelini *et al.*[8]) rs2631370 (Angelini *et al.*[8]) rs2631372 (Angelini *et al.*[8])	C/G T/C C/G

Selected polymorphisms within the pharmacodynamic and pharmacokinetic pathway of sunitinib

DNA isolation and genotyping were performed at the department of Clinical Pharmacy and Toxicology, Leiden University Medical Center. DNA was isolated from serum or whole blood using Magna Pure compact (Roche, Almere, The Netherlands), or from tumor surrounding tissue using Maxwell (Promega, Leiden, The Netherlands). DNA isolated from serum or tissue was pre-amplified as described before.[6]

SNPs were determined using the QuantStudio 12K Real-Time PCR System (Life Technologies, Bleiswijk, the Netherlands), with custom designed arrays. Custom designed pyrosequencing assays were used to enhance the call-rate above 90%. The mean genotype call-rate was 98.6% with a lowest call-rate of 93.2% and highest call rate of 100%. The allele frequencies of seven out of 49 SNPs were not in Hardy Weinberg equilibrium, but frequencies were comparable to the frequencies reported in the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov) and all SNPs were therefore kept within the analysis.

SNPs within a gene were tested for linkage disequilibrium (LD) using Haploview (Broad Institute). Haplotypes were estimated for polymorphisms with an LD of more than 95%. The maximum likelihood estimates of haplotype probabilities were calculated using PLINK software, version 1.7 (http://pngu.mgh.harvard.edu/purcell/plink/). Haplotype probabilities with a likelihood ≥ 95% were included in the statistical analysis. Haplotypes were formed from SNPs in *NR1I3* (rs2307418, rs2307424, rs4073054), *PDGFRA1* (rs1800810, rs1800812, rs1800813), *PDGFRA2* (rs2228230, rs35597368), IL8 (rs1126647, rs4073), *SLC22A5* (rs2631367, rs2631370, rs2631372), *VEGFA* (rs2010963, rs699947, rs833061), *IL4R* (rs1801275, rs1805015). Separate statistical analyses were performed for the SNPs and the haplotypes. In case a haplotype contained a certain SNP that was significant, the analysis of the SNP was dropped.

Statistics

PFS was defined as the time between the first day of sunitinib treatment, and the day of progressive disease (PD), or death due to PD, whatever came first. If PD had not occurred in a patient, or in those cases where a patient was lost to follow up, the patient was censored at the day of last follow up. OS was defined as the time between the first day of sunitinib treatment and the date of death. Patients who had not died or of whom that was unknown were censored at the last day of follow up.

All SNPs and haplotypes were univariately tested against PFS and OS using the Kaplan-Meier method with the log-rank test. Patient characteristics were also univariately tested against PFS and OS, using either the Kaplan-Meier method with the log-rank test, or Cox regression analysis, based on the type of data. Variables and SNPs or haplotypes with a P-value$\leqslant$0.10 in the univariate analysis were selected for inclusion

in a multivariate Cox regression analysis, using PFS and OS as dependent variables. For SNPs, the best fitted model (multiplicative, wild-type dominant or mutant dominant based on genotype distribution) was chosen to enter into the multivariate analysis, based on the univariate analyses. Missing data from baseline characteristics that were associated with PFS or OS in the univariate analysis, were randomly imputed before entering the variable in the multivariate regression model. Depending on the variable, 1-40% of data was imputed. Multivariate analysis were performed twice, with and without replacement of missing variables. If results were similar in size and direction of effect, replacement was considered legitimate.

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS, Chicago, IL, USA). Given the explorative nature of this study, all results from multivariate analysis with P-value $\leqslant 0.05$ were considered statistically significant and no correction for multiple testing was performed.

Results

Study population

The study population consisted of 127 patients with GIST treated with sunitinib, of whom 63% were men. The mean age at start of sunitinib was 61.2 ± 13.4 year. The stomach was the most frequent site of primary GIST location (38%). In 14 patients (11%) a *c-KIT* exon 9 mutation was found, and 58 patients (46%) had a tumor with an exon 11 mutation in *c-KIT* in the primary tumor. Other mutations were found in *c-KIT* exon 13 (*n*= 2), exon 14 (*n*= 1), exon 17 (*n*= 2) or in *PDGFR* exon 18 (*n*= 7). In 43 patients (33.8%) the mutation in the primary tumor was unknown. Most patients (76%) received sunitinib in an intermittent dosing scheme, starting sunitinib with 50 mg a day (*n*= 91, 72%) during the first 4 weeks, continued by 2 weeks off-dosing.

At the time of analysis, 110 patients had stopped sunitinib treatment. In 87 patients (85%), this was because of PD and in all other cases because of severe toxicity. In the entire population, the median PFS was 7.6 months (interquartile range 3.1-17.0 months) and the median OS was 18.3 months (interquartile range 9.7-29.3 months). The baseline characteristics of the study population are presented in Table 2.

Table 2: Baseline characteristics

Variable	N (%) or mean (sd)
Gender	
Male	80 (63)
Female	47 (37)
Age at start sunitinib (years)	61.2 (13.4)
Hospital	
LUMC	60 (47)
EMC	43 (34)
NKI	18 (14)
UMCG	6 (5)
Primary location tumor	
Stomach	48 (38)
Small bowel	36 (28)
Colon	7 (5)
Rectum	6 (5)
Unknown	30 (24)
Histology of primary tumor	
Spindle cell	70 (55)
Epitheloid	12 (9)
Mixed	21 (17)
Unknown	24 (19)
Mutation	
Exon 9	14 (11)
Exon 11	58 (46)
other mutation or wild type	32 (25)
Unknown	21 (16)
WHO PS at start sunitinib	
0-1	98 (77)
2-3	11 (9)
Unknown	18 (14)
Type of sunitinib treatment	
Intermittent	97 (76)
Continuous	28 (22)
Unknown	2 (2)
Dose of sunitinib at start treatment	
12.5 mg	1 (1)
25 mg	5 (4)
37.5 mg	28 (21)
50 mg	91 (72)
unknown	3 (2)
Reason to stop sunitinib	
Progressive disease	87 (69)
Toxicity	23 (18)
Continued treatment	17 (13)

Baseline characteristics. BSA: body surface area, LUMC: Leiden University Medical Center, EMC: Erasmus MC Cancer Institute, NKI: Netherlands Cancer Institute, UMCG: University Medical Center Groningen, WHO PS: World Health Organization Performance Score

Pharmacogenetic biomarkers for PFS

In the univariate analysis, PFS was longer for patients with the presence of the T-allele in *KDR* rs1870377 T/A (p= 0.033), the presence of the G-allele in *IL13* rs20451 G/A (p= 0.025), the presence of the C-allele in *VEGFA* rs25648 T/C (p= 0.014), and in the absence of two GCT copies in the *VEGFA* haplotype (p= 0.042) in the pharmacodynamic genes. With respect to the pharmacokinetic SNPs that were tested, the presence of the homozygous TT- allele in *POR* rs1057868 C/T (p= 0.008), and the absence of two CCC-copies in the *SLC22A5* haplotype (p= 0.007) were univariately associated with prolonged PFS. From the baseline characteristics length (per cm increase HR 1.028; 95% confidence interval (CI): 1.002-1.055, p= 0.032), mitotic index of the primary tumor (per unit increase HR 1.006, 95% CI: 1.000-1.012, p= 0.042), age at start of sunitinib (per year increase HR 0.986; 95% CI: 0.972-0.999, p= 0.037) and the reason to stop imatinib (PD 13.7 months, other than PD 29.9 months; p= 0.01) were included in the multivariate analysis.

Only the homozygous TT genotype in *POR* rs1057868 C/T (HR 0.232, 95% CI: 0.078-0.686, p= 0.008) was associated with PFS in the multivariate Cox regression analysis (Table 3). A trend toward shorter PFS was seen for the presence of 2 copies of the CCC *SLC22A5* haplotype, compared with 1 or 0 copies (HR 2.358, 95% CI: 0.978-5.684, p= 0.056).

Table 3: Univariate and multivariate analysis of progression free survival in patients with GIST treated with sunitinib (Continued on next page)

Factors		**Univariate analysis***			**Multivariate analysis****		
	No.	*Mean PFS (months)*	*95% CI*	*p value*	*HR*	*95% CI*	*p value*
Clinical factors							
Reason to stop imatinib				0.10			0.238
Progressive disease	102	13.7	11.3 - 16.1		1.565	0.744 - 3.929	
Other	23	29.9	14.9 - 45.0		1		
Length (HR 1.028)	96		1.002-1.055	0.032	1.008	0.994 - 1.007	0.582
Mitotic index (HR 1.006)	76		1.000-1.012	0.042	1.001	0.994 - 1.007	0.804
Age at start sunitinib (HR 0.986)	125		0.972-0.999	0.037	0.990	0.974 - 1.007	0.240
Genetic factors pharmacodynamic pathway							
KDR rs1870377				0.033			0.423
TT & TA	114	17.9	13.6 - 22.2		0.696	0.286 - 1.691	
vs AA	9	8.1	1.9 - 14.2		1		

Table 3: Continued

Factors		Univariate analysis*			Multivariate analysis**		
IL13 rs20541				0.025			0.756
GG & GA	113	18.0	13.7 - 22.3		0.870	0.362 - 2.090	
vs AA	11	8.0	4.8 - 11.3		1		
VEGFA rs25648				0.014			0.347
CC & CT	117	17.7	13.5 - 21.8		0.626	0.236 - 1.661	
vs TT	8	7.0	2.5 - 11.4		1		
VEGFA GCT-haplotype				0.042			0.081
GCT-GCT *vs*	1	3.0	3.0 - 3.0		6.488	0.793 - 53.06	
GCT-other & other-other	116	16.5	12.8 - 20.3		1		
Genetic factors pharmacokinetic pathway							
POR rs1057868				0.001			0.008
TT	9	46.5	17.6 - 75.4		0.232	0.087 - 0.686	
CC & CT	115	14.5	11.8 - 17.2		1		
SLC22A5 CCC-haplotype				0.007			0.056
CCC-CCC *vs*	15	7.7	4.3 - 11.1		2.358	0.987 - 5.684	
CCC-other or other-other	105	18.5	14.1 - 23.0		1		

Univariate and multivariate analysis of progression free survival in patients with GIST treated with sunitinib. 95% CI: 95% confidence interval

*Only factors with P-value < 0.10 level are presented; these were selected for multivariate analysis. PFS: progression free survival

**Hazard ratio. HR < 1 indicates that the factor is associated with improved PFS, HR > 1 indicated that the factor is associated with worse PFS.

Pharmacogenetic biomarkers for OS

In the univariate analysis two pharmacodynamic SNPs within *VEGFA* were predictive for longer OS (rs1570360 G/A, absence of the A allele; p= 0.005 and rs699947 C/A, presence of the C-allele; p= 0.036), as well as the presence of a CGG-copy in the *PDGFRA1* haplotype (p= 0.007) and the presence of the GC-other or other-other alleles in the *IL4R* haplotype (p= 0.008). Within the pharmacokinetic pathway, the presence of the C-allele in *ABCC2* rs717620 C/T (p= 0.006), as well as presence of the T-allele in *SLCO1B3* rs4149117 G/T (p= 0.054). Two haplotypes within the pharmacokinetic pathway were associated with longer OS: the absence of two CTT-copies in NR1I3 (Po0.0001) and the absence of two CCC-copies in *SLC22A5* (p= 0.001).

From the baseline characteristics that were univariately tested against OS, a better survival was seen in patients who stopped imatinib for another reason than PD (PD 25.8 months OS, other than PD 55.4 months OS, p= 0.001), the absence of liver metastasis at start of sunitinib (44.2 vs 27.4 months, p= 0.093), and the absence of metastases at the time of diagnosis (37.6 vs 25.8 months OS, p= 0.025). Multivariate Cox regression analysis

showed *SLCO1B3* rs4149117 G/T, the absence of a T-allele (HR 2.024, 95% CI: 1.013-4.044, p= 0.046), the presence of two copies of the CCC *SLC22A5* haplotype (HR 2.603, 95% CI: 1.216-5.573, p= 0.014), and the presence of two copies of the GC *IL4R* haplotype (HR 7.131, 95% CI: 1.518-33.496, p= 0.013) as predictors for OS, as well as PD as a reason to stop imatinib (HR 3.025, 95% CI: 1.358-6.742, p= 0.007) and the presence of metastases at the time of the primary diagnosis GIST (HR 1.773, 95% CI: 1.044-3.012, p= 0.034). Data are presented in Table 4.

Table 4: Univariate and multivariate analysis of overall survival in patients with GIST treated with sunitinib (continued on next page)

Factors		**Univariate analysis***			**Multivariate analysis****		
	No	Mean OS (months)	95% CI	p value	HR	95% CI	p value
Clinical factors							
Reason to stop imatinib				0.001			0.007
Progressive disease	102	25.8	21.8 - 29.8		3.025	1.358 - 6.742	
Other	24	55.4	37.5 - 73.3		1		
Metastasis at time of diagnosis				0.025			0.034
No	66	37.6	28.8 - 46.4		1		
Yes	59	25.8	19.5 - 32.2		1.773	1.044 - 3.012	
Liver metastasis at start sunitinib				0.093			0.127
No	37	44.2	28.1 - 30.3		1		
Yes	86	27.4	23.2 - 31.6		0.660	0.315 - 1.155	
Genetic factors pharmacodynamic pathway							
VEGFA rs1570360				0.005			0.128
GG *vs*	66	38.9	29.6 - 48.2		0.654	0.378 - 1.130	
GA & AA	58	22.0	18.1 - 25.9		1		
VEGFA rs699947				0.036			0.390
CC & CA	94	35.8	28.6 - 43.0		0.775	0.398 - 1.433	
vs AA	28	21.6	17.6 - 25.5		1		
PDGFRA CGG-haplotype				0.007			0.066
CGG-CGG & CGG-other	120	33.1	27.1 - 39.1		0.189	0.085 - 0.418	
vs other-other	6	13.7	6.6 - 20.7		1		
IL4R GC-haplotype				0.008			0.013
GC-GC *vs*	4	8.2	2.0 - 14.5		7.131	1.518 - 33.50	
GC-other & other-other	117	32.8	26.7 - 38.8		1		

Table 4: (continued)

Factors		Univariate analysis*			Multivariate analysis**		
Genetic factors pharmacokinetic pathway							
ABCC2 rs717620				0.006			0.168
CC & CT	121	32.7	26.8 - 38.6		0.248	0.090 - 0.682	
vs TT	5	10.2	8.5 - 11.8		1		
SLCO1B3 rs4149117				0.054			0.046
GG *vs*	97	28.1	23.3 - 32.9		2.024	1.013 - 4.044	
GT & TT	23	47.9	28.5 - 67.2		1		
NR1I3 CTT-haplotype				<0.001			0.062
CTT-CTT *vs*	4	9.1	3.1 - 15.0		4.599	0.927 - 22.81	
CTT-other & other-other	122	33.0	27.0 - 38.9		1		
SLC22A5 CCC-haplotype				0.001			0.014
CCC-CCC *vs*	14	15.6	10.5 - 20.8		2.603	1.216 - 5.573	
CCC-other & other-other	107	34.9	28.4 - 41.5		1		

Univariate and multivariate analysis of overall survival in patients with GIST treated with sunitinib. 95% CI: 95% confidence interval

*Only factors with P-value < 0.10 level are presented; these were selected for multivariate analysis. OS: overall survival

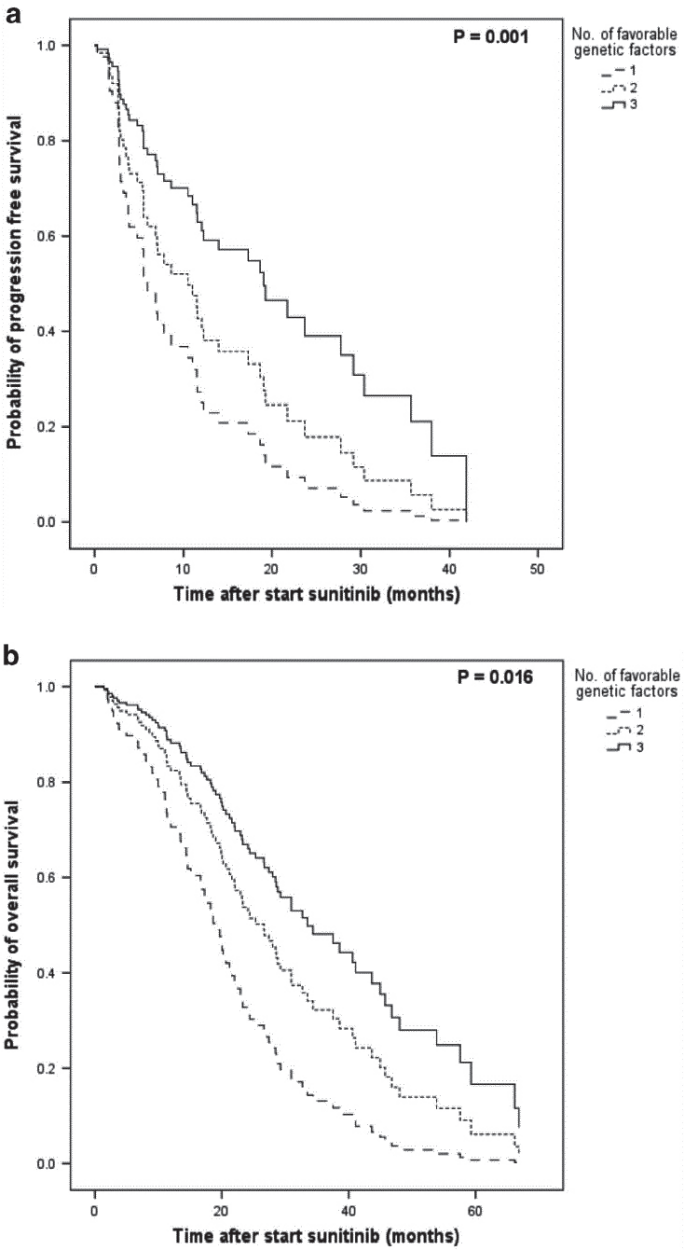
**Hazard ratio. HR < 1 indicates that the factor is associated with improved PFS, HR > 1 indicated that the factor is associated with worse PFS.

Favorable genetic profile

Polymorphisms and haplotypes that were significantly associated with OS (*SLCO1B3* rs4149117 G/T, the presence of the T-allele, the absence of a CCC-copy in the *SLC22A5* haplotype and the absence of a GC-copy in the *IL4R* haplotype) were combined in a favorable genetic profile for PFS and OS, using the number of favorable genetic factors. The number of favorable genetic factors was significantly associated with longer survival (PFS 9.2 vs 15.6 vs 28.4 months for respectively one, two or three favorable genetic factors, p= 0.005). There was only one patient with no favorable genetic factors in this population. In a multivariate regression model including the clinical factors (reason to stop imatinib, length and mitotic index of the primary tumor), this was confirmed (HR 0.654, 95% CI 0.512-0.836, p= 0.001, Figure 1a).

OS was significantly longer with an increasing number of positive predicting genetic factors (mean OS 16.0 vs 31.5 vs 49.5 months for respectively one, two or three positive predictive genetic factors, p= 0.001). This was confirmed in a multivariate regression analysis, including the amount of favorable genetic factors and the clinical factors reason to stop imatinib, metastasis at primary diagnosis and liver metastasis at the start of sunitinib (HR 0.359, 95% CI 0.156-0.826, p= 0.016, Figure 1b).

Figure 1: Progression free survival (a) and overall survival (b) in patients with GIST treated with sunitinib being carriers of one, two or three favorable genetic variations.



Discussion

Patients with GIST treated with sunitinib have a large inter-patient difference in PFS and OS. This may in part be explained by various tumor cell-related factors such as secondary mutations and by some clinical factors.[4] However, genetic polymorphisms within the pharmacokinetic and pharmacodynamic pathways may add to this, as the exposure to and the efficacy of the drug is affected, and thereby influence the outcome of treatment as well. In this explorative study in a population of 127 patients with GIST, it was shown that polymorphisms in both the pharmacokinetic (*SLCO1B3*, *SLC22A5* and *POR*) and the pharmacodynamic (*IL4R*) pathway of sunitinib are associated with PFS and OS in patients with advanced GIST treated with sunitinib.

These findings indirectly suggest that survival to sunitinib in patients with GIST is subjected to exposure to sunitinib and its active metabolite. Sunitinib is metabolized by CYP3A4 and CYP3A5 into its active metabolite SU12662. This is converted to several inactive compounds by the same enzymes. The activity of cytochrome P450-enzymes is regulated by P450 oxydoreductase (POR). In this study, rs1056878, otherwise known as *POR**28, was associated with prolonged PFS in sunitinib treated patients with GIST. Rs1056878 encodes for the amino acid variant A503V, and has been associated with lower activity of CYP1A2, CYP2D6, CYP3A5, but not of CYP3A4.[7] The finding that the polymorphic variant of rs1056878 is associated with better PFS suggests that carriers of this variant have a lower activity of metabolizing enzymes resulting in higher plasma concentrations.

Sunitinib is a substrate of the ATP-binding cassette ABCB1 and ABCG2 efflux transporters, playing a role in both uptake and efflux of sunitinib. However, none of the SNPs in these genes were associated with survival in this analysis. The precise role of members of the organic cation transporter novel (OCTN) family and the organic anion-transporting peptide (OATP) family in sunitinib absorption and elimination is unclear. However, SNPs in *SLC22A5*, which is the gene encoding for OCTN2, have been found to be associated with survival to imatinib in patients with GIST and CML.[8] Interestingly, the *SLC22A5* haplotype, consisting of rs2631367, rs2631370 and rs2631372, was found to be significantly associated with longer OS. Carriers of the two CCC-copies had significantly shorter OS than patients with other allelic combinations. This is consistent with the finding in imatinib treated patients with GIST.[8] Other members of the OCTN family that were tested in this study did not show a significant association with PFS or OS. In *SLCO1B3*, which encodes OATP1B3, rs4149117 was also associated with prolonged OS. Possibly, sunitinib is a substrate of these efflux transporters as well, but this needs to be elucidated.

The homozygous GC-copy in the *IL4R* haplotype consisting of rs1801275, rs1805015 (Ser478Pro and Gln551Arg) was significantly associated with longer OS. In a previous study, SNPs in *IL4R* have been associated with the development of renal cell carcinoma.[9] The finding that SNPs within *IL4R* are associated with OS in patients with GIST treated with sunitinib may be related to IL4R being involved in the tumor biology of GIST as well. A limitation of this study is that no pharmacokinetics of sunitinib as an intermediate endpoint were measured in this group of patients. Therefore, it can only be assumed that the effects of the SNPs on survival is caused by differences in pharmacokinetics. In a recent pharmacogenetic-pharmacokinetic study, *CYP3A4*22* was found to have an effect size of >20% on clearance.[10] However, this finding was not statistically significant.

Another limitation of this study is the sample size. Although this is the largest pharmacogenetic study in patients with GIST treated with sunitinib so far, the number of patients with specific genotypes is too small to draw conclusions from. Since this was an exploratory study, no formal correction for multiple testing was performed and results from the multivariate analyses with a p-value less than 0.05 were considered significant. Currently, the false discovery rate is frequently used to control for reporting false positives in exploratory studies. Therefore, false discovery rate values were calculated for each separate endpoint in a post hoc analysis. False discovery rate was below 10% for all SNPs with $P< 0.05$ indicating a low likelihood of false positive findings.

In this current study, SNPs that were found associated with prolonged PFS, were not associated with OS and *vice versa*. This is somewhat surprising, since PFS and OS can be expected to be related to each other. However, while PFS only includes the effects of sunitinib treatment, OS also embodies the effects of any subsequent lines of treatment. Patients in this study received sunitinib over a broad area of time. In the first years after the registration of sunitinib, no good third line of treatment was available, but patients were frequently offered other treatment in the context of clinical studies. Since recently, regorafenib has been approved for third line treatment of GIST after failure of imatinib and sunitinib.[11] This may have caused a bias in the OS in this analysis, as most patients did not receive this drug during earlier years. Still, it was shown in a large group of patients that genetic polymorphisms can serve as a biomarker for OS. In one of this previous studies; studying polymorphisms associated with survival in RCC, a favorable genetic profile was found, including mutations in *CYP3A5, NR1I3,* and *ABCB1*.[5] The only reason for the discrepancy with the current findings is the tumor type (GIST *versus* RCC). Progressive disease as the reason to stop imatinib treatment was univariately associated with both worsened PFS and worsened OS in this current study. In the multivariate analysis this was only confirmed for OS, but not for PFS. The existence of metastases at the time of the primary diagnosis was also associated with worse OS. Possibly, the tumor has a more aggressive behavior when metastasis are present at first diagnosis and when

the tumor has already progressed on imatinib, rather than the patient switched to sunitinib for other reasons, resulting in shorter OS.

Previously it has been described that primary mutations in *c-KIT* and *PDGFRA* may be predicting for the survival obtained by sunitinib in patients with GIST. This was not seen in this study. This may be explained by the fact that all patients were pre-treated with imatinib. It has been shown that during the treatment with imatinib, secondary mutations may arise, leading to imatinib-resistance.[4] Therefore, mutations that are found in the primary tumor may not be representative of the mutations within the tumor after treatment with imatinib. Moreover, not in all tumor samples mutations in *c-KIT* and *PDGFRA* were determined. A lack of correlation between *c-KIT* and *PDGFRA* in univariate analysis may be (partly) due to missing data.

Altogether it may be concluded that polymorphisms in genes encoding for proteins related to the pharmacokinetic and pharmacodynamic pathways of sunitinib may be associated with survival in patients with GIST treated with sunitinib. When validated in the future, this may be useful to predict which patient is going to respond to sunitinib therapy, and which patients may better respond to other treatment types.

Funding

Novartis provided an unrestricted grant which was used for mutation analysis, and the grant by Stichting Een Gift voor GIST was used for SNP genotyping.

Conflict of interest

J. Swen, H. Gelderblom and H.-J. Guchelaar have an unrestricted grant from Pfizer regarding pharmacogenetic research in patients treated with sunitinib.

Reference list

1. Demetri GD, van Oosterom AT, Garrett CR, et al: Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 368:1329-1338, 2006
2. Sunitinib prescribing information, [cited 21 Jul 2009], available at www.pfizer.com, 2009
3. Faivre S, Demetri G, Sargent W, et al: Molecular basis for sunitinib efficacy and future clinical development. Nat Rev Drug Discov 6:734-45, 2007
4. Heinrich MC, Maki RG, Corless CL, et al: Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. J. Clin. Oncol 26:5352-5359, 2008
5. Van der Veldt AA, Eechoute K, Gelderblom H, et al: Genetic polymorphisms associated with a prolonged progression-free survival in patients with metastatic renal cell cancer treated with sunitinib. Clin. Cancer Res 17:620-629, 2011
6. Baak-Pablo R, Dezentje V, Guchelaar HJ, et al: Genotyping of DNA samples isolated from formalin-fixed paraffin-embedded tissues using preamplification. J. Mol. Diagn 12:746-749, 2010
7. Elens L, Nieuweboer AJ, Clarke SJ, et al: Impact of POR*28 on the clinical pharmacokinetics of CYP3A phenotyping probes midazolam and erythromycin. Pharmacogenet Genomics 23:148-55, 2013
8. Angelini S, Pantaleo MA, Ravegnini G, et al: Polymorphisms in OCTN1 and OCTN2 transporters genes are associated with prolonged time to progression in unresectable gastrointestinal stromal tumours treated with imatinib therapy. Pharmacol. Res 68:1-6, 2013
9. Chu H, Wang M, Yan F, et al: Polymorphisms in the IL-13 and IL-4R genes are associated with the development of renal cell carcinoma. Ann Oncol 23:2114-21, 2012
10. Diekstra MH, Klumpen HJ, Lolkema MP, et al: Association analysis of genetic polymorphisms in genes related to sunitinib pharmacokinetics, specifically clearance of sunitinib and SU12662. Clin Pharmacol Ther 96:81-9, 2014
11. Demetri GD, Reichardt P, Kang YK, et al: Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 381:295-302, 2013
12. Xu CF, Bing NX, Ball HA, et al: Pazopanib efficacy in renal cell carcinoma: evidence for predictive genetic markers in angiogenesis-related and exposure-related genes. J Clin Oncol 29:2557-64, 2011
13. Beuselinck B, Karadimou A, Lambrechts D, et al: VEGFR1 single nucleotide polymorphisms associated with outcome in patients with metastatic renal cell carcinoma treated with sunitinib - a multicentric retrospective analysis. Acta Oncol 53:103-12, 2014
14. Van Erp NP, Eechoute K, van der Veldt AA, et al: Pharmacogenetic pathway analysis for determination of sunitinib-induced toxicity. J. Clin. Oncol 27:4406-4412, 2009

15. Scartozzi M, Bianconi M, Faloppi L, et al: VEGF and VEGFR polymorphisms affect clinical outcome in advanced renal cell carcinoma patients receiving first-line sunitinib. Br. J. Cancer 108:1126-1132, 2013

16. Garcia-Donas J, Esteban E, Leandro-Garcia LJ, et al: Single nucleotide polymorphism associations with response and toxic effects in patients with advanced renal-cell carcinoma treated with first-line sunitinib: a multicentre, observational, prospective study. Lancet Oncol 12:1143-50, 2011

17. Bruck P, Wassmann B, Lopez ER, et al: Development of hygromas or severe edema during treatment with the tyrosine kinase inhibitor STI571 is not associated with platelet-derived growth factor receptor (PDGFR) gene polymorphisms. Leuk Res 28:1153-7, 2004

18. Eechoute K, van der Veldt AA, Oosting S, et al: Polymorphisms in endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) predict sunitinib-induced hypertension. Clin Pharmacol Ther 92:503-10, 2012

19. Kim JJ, Vaziri SA, Rini BI, et al: Association of VEGF and VEGFR2 single nucleotide polymorphisms with hypertension and clinical outcome in metastatic clear cell renal cell carcinoma patients treated with sunitinib. Cancer 118:1946-54, 2012

20. Maffioli M, Camos M, Gaya A, et al: Correlation between genetic polymorphisms of the hOCT1 and MDR1 genes and the response to imatinib in patients newly diagnosed with chronic-phase chronic myeloid leukemia. Leuk. Res 35:1014-1019, 2011

21. Takahashi N, Miura M, Scott SA, et al: Influence of CYP3A5 and drug transporter polymorphisms on imatinib trough concentration and clinical response among patients with chronic phase chronic myeloid leukemia. J. Hum. Genet 55:731-737, 2010

22. Loeuillet C, Weale M, Deutsch S, et al: Promoter polymorphisms and allelic imbalance in ABCB1 expression. Pharmacogenet Genomics 17:951-9, 2007

23. de Jonge H, Metalidis C, Naesens M, et al: The P450 oxidoreductase *28 SNP is associated with low initial tacrolimus exposure and increased dose requirements in CYP3A5-expressing renal recipients. Pharmacogenomics 12:1281-91, 2011