Clinical pharmacology of the tyrosine kinase inhibitors imatinib and sunitinib

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Pharmacogenetic Pathway Analysis for determination of Sunitinib-induced Toxicity

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Abstract

Purpose: To identify genetic markers in the pharmacokinetic and pharmacodynamic pathways of sunitinib that predispose for development of toxicities; thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome and any toxicity according to National Cancer Institute Common Toxicity Criteria higher than grade 2.

Patients and Methods: A multicenter pharmacogenetic association study was performed in 219 patients treated with single-agent sunitinib. A total of 31 single nucleotide polymorphisms in 12 candidate genes, together with several nongenetic variants, were analyzed for a possible association with toxicity. In addition, genetic haplotypes were developed and related to toxicity.

Results: The risk for leukopenia was increased when the G-allele in *CYP1A1* 2455A/G (odds ratio [OR], 6.24; *P* = .029) or the T-allele in *FLT3* 738T/C (OR, 2.8; *P* = .008) were present or CAG in the *NR1I3* (5719C/T, 7738A/C, 7837T/G) haplotype (OR, 1.74; *P* = .041) was absent. Any toxicity higher than grade 2 prevalence was increased when the T-allele of *VEGFR-2* 1191C/T (OR, 2.39; *P* = .046) or a copy of TT in the *ABCG2* (-15622C/T, 1143C/T) haplotype (OR, 2.63; *P* = .016) were present. The risk for mucosal inflammation was increased in the presence of the G-allele in *CYP1A1* 2455A/G (OR, 4.03; *P* = .021) and the prevalence of hand-foot syndrome was increased when a copy of TTT in the *ABCB1* (3435C/T, 1236C/T, 2677G/T) haplotype (OR, 2.56; *P* = .035) was present.

Conclusion: This exploratory study suggests that polymorphisms in specific genes encoding for metabolizing enzymes, efflux transporters, and drug targets are associated with sunitinib-related toxicities. A better understanding of genetic and nongenetic determinants of sunitinib toxicity should help to optimize drug treatment in individual patients.

Introduction

The oral, multitargeted tyrosine kinase inhibitor sunitinib (sunitinib malate; Sutent; Pfizer Pharmaceuticals Group, New York, NY) is known to inhibit vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3, platelet-derived growth factor receptor (PDGFR) α and β, KIT, Fms-like tyrosine kinase 3 receptor (FLT3), and the receptor encoded by the ret proto-oncogene (RET).[1-4] Sunitinib is approved for first-line treatment of metastatic renal cell carcinoma (mRCC) and imatinib-resistant metastatic gastrointestinal stromal tumors (GIST).[4-6] Targeted cancer therapies are generally considered to be less toxic than conventional chemotherapy since they specifically inhibit tyrosine kinase receptors that are frequently overexpressed or mutated in various types of tumor cells.[7] Tyrosine kinases, however, are also present in normal tissues and toxic effects are therefore difficult to eliminate. The 4 weeks on 2 weeks off dosing schedule of sunitinib was selected for the first phase I study on request of the health authorities to allow patients to recover from potential bone marrow and adrenal toxicity observed in animal models, indicating that toxicity was regarded as a serious problem.[3, 8] Although the proportion of patients with grade 3 to 4 adverse events was relatively low in the recent phase III studies, a dose interruption appeared to be necessary in 38% of patients with mRCC and in 28% of patients with GIST whereas a dose reduction was required in 32% and 11%, respectively. Similar percentages were reported in other studies.[2, 4, 9] Disease- and sunitinib-related toxicities can be distinguished based on results of a phase III trial in which the toxicity profile of sunitinib-treated patients has been compared with events in the placebo-treated patients.[2] Adverse events that preferentially occurred in the group treated with sunitinib were diarrhea, hand-foot syndrome, mucositis, vomiting, hypertension, leukopenia, neutropenia, and thrombocytopenia.[2-4, 9-13] Less common, but specific toxicities related to sunitinib were cardiotoxicity and hypothyroidism.[5, 14, 15]

Sunitinib is used as palliative therapy with no standard therapeutic options available after failure of the therapy. It is therefore relevant for patients to adhere to sunitinib therapy while their quality of life is not unnecessarily reduced by drug toxicity. To date, it is not completely clear which patient characteristics render an individual patient at risk for sunitinib-induced toxicity. The aim of the present study is to identify genetic markers in sunitinib disposition, metabolism, and mechanism of action that predispose for development of common sunitinib related toxicities: thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome and any higher than grade 2 National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) toxicity.

Patients and methods

A total of 219 patients from five Dutch medical centers were analyzed in this study. The study was approved by the medical ethics review board. Patients were treated at the Erasmus University Medical Center (n=74), the Netherlands Cancer Institute (n=51), Leiden University Medical Center (n=37), VU University Medical Center (n=36), and the University Medical Center Groningen (n=21). The collection of DNA and patient data was performed between June 2004 and May 2008. A total number of 159 mRCC, 50 GIST, and 10 patients with other tumors were included in this study. Of them, 77 patients with mRCC and 26 patients with GIST were treated according to an expanded access programme of sunitinib. Eligible patients were those treated with single agent sunitinib for at least one treatment cycle (4 consecutive weeks of 50 mg per day followed by a two-week period of rest).

Study design

Sunitinib toxicity was evaluated during the first treatment cycle by CTCAE version 3.0.[16] Toxicity scores were assessed by analysis of adverse events, physical examination and laboratory assessments carried out at baseline (before starting sunitinib), after 4 weeks of sunitinib therapy, and after 6 weeks (just before starting the second cycle). Demographic and clinical data of patients were reported on case record forms designed for data collection in this study. Patient characteristics considered relevant for experiencing toxicity were: age, gender, ethnicity, body-surface area (BSA), Eastern Cooperative Oncology Group (ECOG) performance status, tumor type, renal, liver and bone marrow function (serum creatinine, total bilirubin, albumin, ALT, AST, hemoglobin, leukocytes and thrombocytes). Residual blood or serum samples taken for routine patient care were stored at -20ºC at the local hospital laboratory. Of each patient one whole blood or serum sample was collected from the participating centers. All samples were anonymized 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).

Definition of toxicity

All adverse events were graded by independent physicians of the participating medical centers. Four- and 6-week reported toxicities were compared to baseline conditions. The primary outcome measures of this study were thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome and any toxicity higher than grade 2. Toxicities were selected based on objectivity, clinical relevance and manageability of the symptoms. Thrombocytopenia and leukopenia were scored from blood cell counts and are thus objective endpoints. In case of any toxicity higher than grade 2, a dose interruption and, depending on the kind of toxicity, a resumed treatment with 25% dose reduction is advised in the drug label of sunitinib. Moreover, mucosal inflammation and hand-foot syndrome are frequently reported and poorly manageable and therefore dose reduction is relatively soon considered. In addition, dose reduction of at least 25% according to the drug label (data complete for 187 patients) which is applied because of safety or tolerability issues, after cycle 1 to 3 was related to the toxicity outcomes.

Genetic Polymorphisms

Nineteen polymorphisms in seven genes involved in the pharmacokinetics and 12 polymorphisms in five genes involved in the pharmacodynamics of sunitinib were selected. Selection criteria for the polymorphisms were an allelic frequency higher than 0.2 in whites and an assumed clinical relevance based on previously reported associations or the assumption that nonsynonymous amino acid change leads to changed protein functionality. The selected polymorphisms are listed in Table 1.

Genotyping of selected polymorphisms

Germline DNA was isolated from 1 ml of serum or EDTA-blood with the Magnapure LC (Roche Diagnostics, Almere, The Netherlands). DNA concentrations were quantified on the nanodrop (Isogen, IJsselstein, The Netherlands). Taqman assays were obtained from Applied Biosystems (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). All Single Nucleotide Polymorphisms (SNPs) were initially determined on the Biomark 48.48 Dynamic Array (Fluidigm, San Fransisco, CA, USA) according to the manufacturer's protocol. Failed samples were repeated on the TaqMan 7500 (Applied Biosystems), according to standard procedures. For serum samples, a pre-amplification step was necessary. Briefly, a dilution of all TaqMan assays in a total volume of 1.25 μL and 2.5 μL of pre-amplification mastermix (Applied Biosystems) was added to 1.25 μL of serum-DNA, and subsequently amplified by polymerase chain reaction. This mixture was 20 times diluted and 2.5 μL was used in the Biomark array according to the protocol.

Genotyping assay validity

The overall average success rate of the assays and the individual samples was 98%. The lowest success rate in our study was 93.5%. As a quality control, all DNA samples were genotyped in duplicate for 12 of 31 SNPs, and three DNA samples were genotyped in duplicate for all 31 SNPs. No inconsistencies were observed. In addition negative controls (water) were used. The allelic frequencies of the 31 single nucleotide polymorphisms were tested for Hardy-Weinberg equilibrium (HWE). Six genotype assay results did not meet HWE. However, of four of these, frequencies were compared with allelic frequencies as reported on the National Center for Biotechnology Information website (NCBI) for white population and found similar to the reported frequencies. Of the two remaining SNPs no frequencies were available on the

Table 1 Polymorphisms genotyped in the pharmacokinetic and pharmacodynamic pathway of sunitinib

	Gene	Polymorphism	rs-number
Pharmacokinetic pathway	NR1I2	-25385C/T	rs3814055
		-24113G/A	rs2276706
		7635A/G	rs6785049
		8055C/T	rs2276707
		10620C/T	rs1054190
		10799G/A	rs1054191
	NR1I3	5719C/T	rs2307424
		7738A/C	rs2307418
		7837T/G	rs4073054
	CYP3A5	6986A/G	rs776746
	CYP1A1	2455A/G	rs1048943
	CYP1A2	-163A/C	rs762551
	ABCG2	421C/A	rs2231142
		34G/A	rs2231137
		-15622C/T	*
		1143C/T	rs2622604
	ABCB1	3435C/T	rs1045642
		1236C/T	rs1128503
		2677G/T	rs2032582
Pharmacodynamic pathway	PDGFRα	1580T/C	rs35597368
		-1171C/G	rs1800810
		-735G/A	rs1800813
		-573G/T	rs1800812
	VEGFR2 (=KDR)	-604T/C	rs2071559
		-92G/A	rs1531289
		54T/C	rs7692791
		1191C/T	rs2305948
		1718T/A	rs1870377
	VEGFR3 (=FLT4)	1501A/G	rs307826
	RET	2251G/A	rs1799939
	FLT3	738T/C	rs1933437

* No rs-number assigned yet

NCBI website (www.ncbi.nlm.nih.gov). The homozygotic wildtype frequencies of both SNPs exceed the HWE and were therefore allowed for the analysis.

Haplotype estimation

Polymorphisms within a gene were tested to detect linkage disequilibrium (LD). If LD between SNPs was present, haploblocks (with several haplotypes) were determined. The uncertainty measure R_h^2 was calculated. R_h^2 gives us information on the uncertainty in the prediction of common haplotypes from unphased SNP genotypes [17]. A haplotype was considered to be present if the haplotype uncertainty measure R_h^2 was greater than 0.98 as tested with the software program CHAPLIN [18]. Haplotypes with an uncertainty measure $R_h^2 \leq 0.7$ in CHAPLIN were not considered for further analysis since the data provided no information on haplotypes in our population. All haplotypes with uncertainty ($0.7 < R_h^2 \leq 0.98$) and without uncertainty ($R_h^2 > 0.98$) were computed and assigned per individual using gPLINK [19]. Rare haplotypes (< 2%) were combined into one group of other haplotypes in the association analysis. The haplotypes used in this study had no phase uncertainty ($R_h^2 > 0.98$). The *VEGFR-2* gene had a large phase uncertainty ($R_h^2 \leq 0.7$) indicating that in our population *VEGFR-2* polymorphisms could not be defined as a haplotype. The following SNPs were combined for further analysis: *ABCG2*; 1143C/T and -15622C/T; *PDGFR*α; -573G/T, -1171C/G, -735G/A, 1580T/C; *NR1I3*; 5719C/T, 7738A/C, 7837T/G; *NR1I2 ;* 10620C/T, 10799G/A and *ABCB1*; 3435C/T, 1236C/T, 2677G/T.

Statistical design and data analysis

For the analysis of toxicity, we used dichotomous end points expressed as increased toxicity (yes or no) or any toxicity higher than grade 2 (yes or no). All demographic and clinical variables were tested univariately against the selected primary outcomes using *t* test, the Mann-Whitney *U* test or the χ^2 test, depending on the tested variables. A χ^2 test was also used to detect linkage disequilibrium (LD). The polymorphisms were initially tested with 2 *df*. If the initial 2 *df* tests resulted in $P \leq .1$, the polymorphisms were fitted and the most appropriate model (multiplicative, dominant, or recessive) was selected. The number of copies of each haplotype was used as parameter in the analysis. The polymorphisms and haplotypes were tested univariately against the selected primary outcomes using a χ^2 test. Candidate variables with $P \leq .1$ were selected for the multiple logistic regression analysis with toxicity as depending variable. All multivariate logistic regression analyses were corrected for age, gender and ECOG performance status. Additional patient characteristics were introduced in the multivariate analyses based on univariate tested results if $P \leq .1$. Missing data were kept as missing data except for BSA and ECOG performance status. Missing BSA values (n=15) were replaced for the median BSA ($1.93m^2$) and missing ECOG performance status (n=7) were replaced for the median ECOG performance status (1). To test this action, the multivariate analyses were performed with and without the replacement of the patients with missing BSA and ECOG performance status. Similar results were generated, indicating that the replacement was legitimate. All statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL, USA). With the sample size of our study, an increase in toxicity of 17% could be measured between two groups with a power of 80% and a confidence interval of 99%. All results from the multivariate analyses with P less than .05 were considered significant. Since this was an exploratory study, no correction for multiple testing was done.

Results

Patients

Nineteen out of 219 patients had to be excluded from analysis for several reasons including progressive disease (PD) during the first treatment cycle resulting in early death (n=4), discontinuation of sunitinib in the first treatment cycle due to adverse events (hypertension grade 3, headache grade 3 and rash grade 3, respectively; n=3) and no acceptable genotyping success rate due to poor DNA quality (n=12). For toxicity analyses, a total of 200 patients were evaluable (Table 2). For the endpoint any toxicity higher than grade 2, the three patients who stopped therapy due to adverse events were included (n=203).

Table 2 Patient characteristics (N=203)

Characteristic		Value
Age (years)		
Median (range)		60 (20-84)
Sex		
Male		129 (63.5%)
Female		74 (36.5%)
Body Surface Area (square meters)		
Median (range)		1.93 (1.47-2.51)
ECOG performance status		
0		81 (39.9%)
1		90 (44.3%)
2		17 (8.4%)
3		8 (3.9%)
Missing		7 (3.4%)
Ethnicity		
Caucasian		190 (93.6%)
Blacks		6 (3.0%)
Asian		2 (1.0%)
Latin-American		2 (1.0%)
Middle East		3 (1.5%)
Tumor types		
Renal cell carcinoma		152 (74.9%)
Gastrointestinal stromal tumor		46 (22.7%)
Other		5 (2.5%)
Previous medical treatments		
Yes*		116 (57.1%)
No		87 (42.9%)
First treatment regimen (N=116)*		
Interferon-alpha (INF-α)		46 (39.7%)
Imatinib		46 (39.7%)
Sorafenib		5 (4.3%)
Others		19 (16.4%)
Dose reduction after sunitinib cycle 1 – 3		
Yes	Renal cell carcinoma	58 (28.6%)
	GIST	14 (6.9%)
	Other tumor	1 (0.5%)
No	Renal cell carcinoma	94 (46.3%)
	GIST	32 (15.8%)
	Other tumor	4 (2.0%)
Baseline chemistry and hematology		
Creatinine (μM)		
Median (range)		96.0 (40-176)
Total bilirubin (μM)		
Median (range)		7 (3-32)
Albumine (gram/L)		
Median (range)		40 (23-52)
ALT (units/L)		
Median (range)		18 (3-210)
AST (units/L)		
Median (range)		24 (9-190)
Hemoglobin (mM)		
Median (range)		7.6 (5.2-10.4)
Leukocytes (*10^9/L)		
Median (range)		7.5 (3.6-56.5)
Thrombocytes (*10^9/L)		
Median (range)		284.0 (92-864)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ECOG, Eastern Cooperative Oncology Group. CR, complete response ; PR, partial response; SD stable disease ; PD, progressive disease.

Toxicities

The hematological toxicities scored in this analysis were thrombocytopenia (40% any grade), leukopenia (59%, any grade). Non-hematological toxicities were primarily any toxicity higher than 2 (22%), mucosal inflammation (44%) and hand-foot syndrome (19%; Table 3). Dose reduction after cycle 1 to 3 was related to mucosal inflammation ($P = .002$) and any toxicity higher than grade 2 ($P < .001$)

Table 3 Number (No) of patients (%) according to the distribution of increased toxicity grades

Toxicity	No/Yes	Grade	No (%)
Thrombocytopenia (n=198)	No		118 (59.0)
	Yes	1	58 (29.0)
		2	14 (7.0)
		3	7 (3.5)
		4	1 (0.5)
Leukopenia (n=198)	No		81 (40.5)
	Yes	1	91 (45.5)
		2	22 (11.0)
		3	4 (2.0)
Any toxicity > 2 (n=203)		0, 1, 2	158 (77.8)
		3, 4	45 (22.2)
Mucosal inflammation (n=199)	No		112 (56.0)
	Yes	1	57 (28.5)
		2	25 (12.5)
		3	5 (2.5)
Hand-foot syndrome (n=199)	No		162 (81.0)
	Yes	1	27 (13.5)
		2	8 (4.0)
		3	2 (1.0)

Pharmacogenetic risk factors for sunitinib-induced toxicity

The results of the multivariate logistic regression analysis for the selected endpoints thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome and any toxicity higher than grade 2 are summarized in Table 4. For thrombocytopenia, an increase in age (P = .030) and ECOG performance status (P = .050) were independently significant in the multivariate logistic model. The factors associated with development of leukopenia were: *CYP1A1* 2455A/G; the presence of the G allele in an additive model was related to a 6.2-fold increase in the risk for leukopenia during the first treatment cycle (P = .029); the presence of the *FLT3* 738C allele (dominant model) was related to a 2.8-fold reduction in the risk for leukopenia (P = .008); the absence of the *NR1I3* CAG haplotype was related to a 1.7-fold increased risk for leukopenia (P = .041) and 4); one grade increase in ECOG performance status, implicating a worse clinical condition, was related to a 1.8-fold reduction in the risk of leukopenia (P = .016). The presence of the *VEGFR-2* 1191T-allele (additive model) was related to an increased risk of 2.4-fold for the development of any toxicity higher than grade 2 (P = .046), while the risk for this toxicity was 2.6-fold higher when 1 or 2 copies of TT in the *ABCG2* haplotype were present (P = .016). For mucosal inflammation only *CYP1A1* 2455A/G was independently related; the G-allele (additive model) resulted in a 4.0-fold higher risk for mucosal inflammation (P = .021). The occurrence of hand-foot syndrome was related to the *ABCB1* haplotype; the absence of copies of the TTT haplotype was protective and was related to a 2.6-fold lower risk to experience hand-foot syndrome as compared to patients with copies of the TTT haplotype (P=.035). The explained variance (R^2) of the patient characteristics, without taking the polymorphisms into account, in the multivariate analyses was between the 2 to 10% of the total variance. After adding the selected polymorphisms the explained variance increased to 10 to 23% of the total variance.

Discussion

To the best of our knowledge, this is the first study exploring the relationship between drug-induced toxicity and genetic polymorphisms in genes encoding for enzymes, efflux transporters and targets involved in the pharmacokinetics and pharmacodynamics of sunitinib.

Sunitinib is metabolized by cytochrome P450 3A4 (CYP3A4) and CYP3A5. In addition, affinity of sunitinib for the ATP-binding cassette transporters ABCG2 and ABCB1 has also recently been reported.[20] The transcription of CYP3A4 is regulated by members of the NR1I nuclear receptor subfamily.[21] Metabolism through CYP1A1 and CYP1A2 is hypothesized since these enzymes appear to be involved in the metabolism of multiple tyrosine kinase inhibitors (eg, imatinib, erlotinib).[22, 23] Both genes encoding the sunitinib targets, as well as genes encoding the enzymes (except for CYP3A4, in which no functional polymorphisms have been identified) and efflux transporters involved in sunitinib's disposition and metabolism are highly polymorphic and may be related to the differential toxicity response in patients treated with sunitinib.

Although the nature and incidence of adverse events related to sunitinib are currently well recognized and described, data regarding determinants of toxicity are still scarce.[2, 4, 5, 14, 24, 25] So far, only one study has described factors (low BSA, high age, female gender) that are associated with the development of severe toxicities, defined as dose reduction or permanent discontinuation of sunitinib therapy.[9] That study, however, was limited to patient characteristics and no genetic determinants were investigated. In our study, these patient characteristics, and another (performance status), were included as covariates in the data analysis. We should emphasize, however, that the definition of the endpoint severe toxicity is different in both studies as well as the observed study period (whole sunitinib treatment period *v* first treatment cycle in our study).

Table 4 Factors relevant for sunitinib-induced toxicity, defined as thrombocytopenia, leukopenia, any toxicity > grade 2, mucosal inflammation or hand-foot syndrome

	Data corrected for patient characteristics[1]				Uncorrected data[1]	
	Factor	**Genotype**	**OR (95% CI)**	***P-value***	**OR (95% CI)***	***P-value***
Thrombocytopenia (n=177)	Age		1.04 (1.00–1.07)	**.030**		
	Gender		1.93 (0.97–3.85)	.063		
	ECOG		0.60 (0.37–1.00)	**.050**		
	NR1I2 -24113 G/A	GG *vs*	1		1	
		AG + AA	0.60 (0.28–1.28)	.187	0.51 (0.25–1.05)	0.067
	CYP1A2 -163 A/C	AA *vs*	1		1	
		AC + CC	0.67 (0.34–1.32)	.251	0.65 (0.34–1.23)	0.186
	ABCG2 421 C/A	CC *vs*	1		1	
		CA + AA	2.09 (0.88–4.96)	.096	1.93 (0.85–4.42)	0.118
	ABCG2 haplotype ξ	TT-TT + TT-other *vs*	1		1	
		Other-other	1.93 (0.96–3.86)	.065	1.77 (0.91–3.46)	0.093
	PDGFRα haplotype ψ	TGAT-TGAT + TGAT-other	1		1	
		vs Other-other	0.44 (0.17–1.18)	.103	0.36 (0.14–0.92)	**0.033**
Leukopenia (n=188)	Age		1.01 (0.98–1.04)	.423		
	Gender		0.81 (0.41–1.60)	.536		
	ECOG		0.57 (0.36–0.90)	**.016**		
	VEGFR-2 -92G/A	GG → GA → AA	0.74 (0.44–1.23)	.241	0.74 (0.45–1.22)	.235
	VEGFR-2 1718 T/A	TT → AT → AA	1.49 (0.84–2.66)	.172	1.47 (0.83–2.60)	.188
	CYP1A1 2455A/G	AA → AG → GG	6.24 (1.20–32.42)	**.029**	4.87 (1.06–22.29)	**.042**
	FLT 3 738T/C	TT *vs*	1		1	
		CT + CC	0.36 (0.17–0.77)	**.008**	0.41 (0.20–0.85)	**.016**
	NR1I3 haplotype Ω	CAG-CAG → CAG-other → other-other	1.74 (1.02–2.96)	**.041**	1.81 (1.07–3.04)	**.026**
	NR1I3 haplotype Ω	TCT-TCT → TCT-other → Other-other	0.54 (0.27–1.06)	.074	0.55 (0.28–1.06)	.075
Any toxicity > grade 2 (n=183)	Age		1.03 (0.99–1.07)	.140		
	Gender		1.71 (0.69–4.26)	.248		
	ECOG		1.31 (0.79–2.19)	.299		
	BSA		0.22 (0.02–2.49)	.220		
	VEGFR-2 1191 C/T	CC → TC → TT	2.39 (1.02–5.60)	**.046**	2.31 (1.07–4.99)	**.033**
	ABCG2 haplotype ξ	TT-TT + TT-other *vs*	1		1	
		Other-other	0.38 (0.17–0.83)	**.016**	0.40 (0.19–0.84)	**.016**
Mucosal Inflammation (n=193)	Age		1.00 (0.97–1.03)	.956		
	Gender		1.54 (0.82–2.88)	.177		
	ECOG		1.31 (0.86–1.99)	.212		
	NR1I2 -24113G/A	GG *vs*	1		1	
		AG + AA	0.58 (0.30–1.13)	.110	0.55 (0.29–1.04)	.064
	CYP1A1 2455A/G	AA → AG → GG	4.03 (1.24–13.09)	**.021**	4.15 (1.29–13.36)	**.017**
	ABCG2 34G/A	GG → AG	2.45 (0.74–8.17)	.144	2.41 (0.75–7.76)	.140
	NR1I3 haplotype Ω	TCT-TCT → TCT-other → Other-other	0.78 (0.42–1.44)	.420	0.77 (0.42–1.42)	.404
Hand-Foot Syndrome (n=182)	Age		0.99 (0.96–1.02)	.563		
	Gender		1.22 (0.56–2.68)	.612		
	ECOG		0.76 (0.43–1.33)	.336		
	ABCB1 haplotype ∞	TTT-TTT + TTT-other	1		1	
		vs Other-other	0.39 (0.16–0.94)	**.035**	0.39 (0.16–0.92)	**.032**
	ABCB1 haplotype ∞	CTT-CTT → CTT-other → Other-other	0.38 (0.11–1.32)	.126	0.36 (0.10–1.27)	.114

Abbreviations: OR, odds ratio; CI, confidential interval; ECOG, Eastern Cooperative Oncology Group. Multiplicative model is indicated with (→) between the genotypes. Dominant and recessive models are indicated with (vs) between the two groups of genotypes.

1: All toxicity outcomes in the corrected analysis are corrected for; age, gender and ECOG performance status. Additional correction with body surface area (BSA) was done for Any toxicity > grade 2. Under the uncorrected data only the genotypes are included in the multivariate analysis. Description haplotypes: ξ = ABCG2 -15622C/T and 1143C/T; ψ = PDGFRα -573G/T, -1171C/G, -735G/A and 1580T/C; Ω = NR1I3 5719C/T, 7738A/C and 7837T/G; ∞ = ABCB1 3435C/T, 1236C/T and 2677G/T.

P-value < .05 is regarded as significant and printed bold.

Description of polymorphisms and rs-numbers: See table

To our knowledge, we report for the first time herein that the *ABCB1* TTT haplotype was related to hand-foot syndrome. The TTT haplotype as well as the T genotype in 3435C/T and the T polymorphism in 1236C/T separately have been associated with higher exposures to drugs transported by ABCB1 due to a decreased expression of the ABCB1 transporter.[26-31] Also, for the other ABC-transporter investigated, ABCG2, the TT haplotype was related to the development of increased toxicity (eg, any toxicity > grade 2). This haplotype has been associated with increased erlotinib exposure, a tyrosine kinase inhibitor that uses metabolic and predisposition pathways similar to those of sunitinib.[32] Thus, our results concerning *ABCB1* and *ABCG2* are in line with previously reported functional consequences of the studied genetic variants and might lead to an increased systemic exposure to sunitinib resulting in dose-limiting toxicities. Certainly, to confirm our findings, further studies that relate pharmacogenetics to pharmacokinetics and pharmacodynamics are required.

Thus far, the extrahepatic CYP1A1 enzyme has not been described as being involved in the metabolism of sunitinib. For other receptor tyrosine kinase inhibitors, such as erlotinib, imatinib and gefitinib affinity for CYP1A1 has been demonstrated in in vitro studies.[22, 23] Therefore, we also included genetic variants of *CYP1A1* in the present study. The polymorphism studied in *CYP1A1* resulting in an amino acid change of isoleucine 462 Valine was found to be related to the occurrence of mucosal inflammation and leukopenia. This suggests that CYP1A1 may also play a role in the metabolism of sunitinib *in vivo*.

In addition, we investigated genetic polymorphisms in the *NR1I3* gene, encoding the constitutive androstane receptor. This nuclear receptor plays an important role in the regulation of multiple drug detoxification genes, such as *CYP3A4*. The functionality of polymorphisms in *NR1I3* is not yet fully elucidated, however we found a relationship between the absence of the CAG haplotype in this gene and an increased risk for leukopenia[33]. Obviously, it would be interesting to relate this polymorphism with sunitinib exposure levels in future studies.

The *VEGFR-2* 1191CT and TT genotypes were found to be predictive for the development of coronary heart disease due to a lower binding efficiency of VEGF to the polymorphic VEGFR-2.[34] In our study, these genotypes were related to the development of any toxicity higher than 2, which predominantly included fatigue, thrombocytopenia, and hypertension. The polymorphic receptor might therefore be involved in sunitinib-induced cardiac toxicity and the development of hypertension.

The importance of the FLT3 receptor has been described in relation to the development of several subtypes of leukemia such as acute myeloid leukemia, acute lymphocytic leukemia, and chronic myeloid leukemia, in which FLT3 is frequently overexpressed and/or mutated.[35, 36] However, the association between *FLT3* 738T/C polymorphism and a reduction in the risk of leukopenia has not previously been described. Since sunitinib-induced leukopenia could be regulated strongly by this polymorphic receptor the clinical relevance should be further investigated.

In our study, a large number of candidate polymorphic loci were evaluated and multiple analyses of each genetic polymorphism were performed. This introduces the potential problem of multiple testing which increases the risk to find false-positive relations. However, our study was designed to explore associations that should be confirmed in an independent group of patients. The presented odds ratios and CIs facilitate comparisons of replicate studies with our data.

The ECOG performance status was not consistently related to the occurrence of toxicities in our study. The quantified performance status is multifactorial and is dependent on subjective interpretation of the physician. Moreover, in our study patients with poor performance status had relatively high baseline thrombocyte and leukocyte counts resulting in a small number of reported leukopenia and thrombocytopenia in this group in the first treatment cycle.

Toxicities in the first treatment cycle of sunitinib were used as outcome measure. The rationale was that signs of clinical deterioration from disease progression in later cycles could be misinterpreted and would interfere with the drug-induced toxicity outcome. We hypothesized that patients that suffer from relatively mild (grade 1 or 2) toxicities in the first treatment cycle were at risk for developing more severe toxicity during further treatment cycles because the two weeks of rest would not be sufficient for patients to recover to baseline conditions. This cumulative effect is underscored by measured blood cell counts and the observed dose reduction after cycle 1 to 3. Indeed, we found for leukocyte count and to a lesser extent also for thrombocyte count, that 91% and 73%, respectively, of the patients had not returned to baseline values (defined as > 90% of baseline counts) at cycle 2 day 1 (data not shown). In addition, we found that mucosal inflammation and any toxicity higher than grade 2 were strongly related to a dose reduction after cycle 1 to 3, indicating that these toxicities are regarded as clinically relevant to the treating physicians.

Together, the genetic, clinical and demographic determinants in this exploratory study explain between 10 and 23% of the total variance in toxicity response. Although it indicates that the major part of the variability is left unexplained, it also shows that pharmacogenetics may make a greater contribution to explaining variability in sunitinib toxicity as compared to the nongenetic determinants in our study. From this study we cannot conclude whether the genetic variants are prognostic or predictive markers, due to the absence of a placebo-treated control group of patients. However in the future, pharmacogenetics may help to select patients which need a priori dose reduction to prevent toxicities.

In conclusion, this study suggests a relationship between polymorphisms in the genes *CYP1A1*, *ABCB1*, *ABCG2*, *NR1I3*, *VEGFR-2* and *FLT3* and the development of sunitinib toxicity. The next step will be to validate our data with the aim to better understand the determinants of sunitinib toxicity.

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