Predictive Biomarkers and Personalized Medicine

Genetic Polymorphisms Associated with a Prolonged Progression-Free Survival in Patients with Metastatic Renal Cell Cancer Treated with Sunitinib

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Abstract

Purpose: The objective of this study was to identify genetic polymorphisms related to the pharmacokinetics and pharmacodynamics of sunitinib that are associated with a prolonged progression-free survival (PFS) and/or overall survival (OS) in patients with clear-cell metastatic renal cell cancer (mRCC) treated with sunitinib.

Experimental design: A retrospective multicenter pharmacogenetic association study was performed in 136 clear-cell mRCC patients treated with sunitinib. A total of 30 polymorphisms in 11 candidate genes, together with clinical characteristics were tested univariately for association with PFS as primary and OS as secondary outcome. Candidate variables with P < 0.1 were analyzed in a multivariate Cox regression model.

Results: Multivariate analysis showed that PFS was significantly improved when an A-allele was present in CYP3A5 6986A/G [hazard ratio (HR), 0.27; P = 0.032], a CAT copy was absent in the NR1I3 haplotype (5719C/T, 7738A/C, 7837T/G; HR, 1.76; P = 0.017) and a TCG copy was present in the ABCB1 haplotype (3435C/T, 1236C/T, 2677G/T; HR, 0.52; P = 0.033). Carriers with a favorable genetic profile (n = 95) had an improved PFS and OS as compared with noncarriers (median PFS and OS: 13.1 versus 7.5 months and 19.9 versus 12.3 months). Next to the genetic variants, the Memorial Sloan-Kettering Cancer Center prognostic criteria were associated with PFS and OS (HR, 1.99 and 2.27; P < 0.001).

Conclusions: This exploratory study shows that genetic polymorphisms in three genes involved in sunitinib pharmacokinetics are associated with PFS in mRCC patients treated with this drug. These findings advocate prospective validation and further elucidation of these genetic determinants in relation to sunitinib exposure and efficacy. Clin Cancer Res; 17(3); 620–9. ©2010 AACR.

Introduction

For decades, the treatment options of metastatic renal cell cancer (mRCC) have been limited and systemic treatment primarily consisted of immunotherapy with cytokines. Increasing knowledge of the underlying biology of renal cell cancer (RCC), in particular the clear-cell subtype, has expanded the treatment options for patients with mRCC (1). RCC is characterized by an inactivated von Hippel–Lindau (VHL) tumor suppressor gene. Inactivated VHL leads to elevated protein levels of hypoxia-induced factor-α which upregulates VEGF and platelet-derived growth factor (PDGF) genes and proteins. The development of targeted therapy against signaling of these proteins has significantly improved the perspectives of patients with mRCC.

Currently, sunitinib is the most widely prescribed drug for the treatment of mRCC and has been registered as first-line and second-line therapy. Sunitinib is an oral tyrosine kinase inhibitor (TKI) which targets several receptors including VEGF receptors-1,-2,-3, PDGF receptors (PDGFR)-α and –β, c-KIT, and FLT-3. In a randomized controlled trial, sunitinib significantly prolonged the progression-free survival (PFS) and overall survival (OS) as compared with interferon-α (2, 3). Although sunitinib can achieve partial response rates of up to 40% (3–5), approximately 35% of mRCC patients do not benefit from sunitinib treatment (4, 5). Because sunitinib treatment may also result in...
Translational Relevance

Currently, sunitinib is the most widely prescribed drug for the treatment of metastatic renal cell cancer. Unfortunately, only a part of treated patients will benefit from sunitinib therapy, despite the implementation of clinical prognostic criteria in the choice of therapy. As multiple systemic treatment modalities arise, a further refinement is needed to identify renal cell cancer patients who predispose to benefit from sunitinib treatment and patients who do not. One of the possible options to study the differential response to sunitinib treatment is to identify genetic polymorphisms related to the pharmacokinetics and pharmacodynamics of this drug. In the future, genetic variants may be added to the current prognostic criteria, enabling physicians to predict benefit from sunitinib in individual patients.

unnecessary toxicities (6, 7), pretreatment markers to identify mRCC patients with a favorable outcome to sunitinib treatment are warranted.

Sunitinib efficacy may be dependent on its exposure which is regulated by efflux pumps and metabolizing enzymes. After oral administration, the systemic exposure of sunitinib is initially determined by its absorption in the gastrointestinal tract (Fig. 1). This process may be regulated by active drug transport over the intestinal wall, as sunitinib may be a substrate for polyspecific efflux transporters, expressed on enterocytes (8, 9). The efflux transporters ABCB1 (ATP binding cassette member B1, formerly known as P-glycoprotein or MDR1) and ABCG2 (ATP binding cassette member G2, formerly known as breast cancer-resistance protein (BCRP) or mitoxantrone resistant protein (MXR)) are expressed in the intestine and liver, and are involved in the oral absorption and biliary secretion of several anticancer drugs (10).

Therefore, expression levels and functionality of these drug transporters may have important consequences for the efficacy of sunitinib.

The cytochrome P450 (CYP) 3A (CYP3A) family is the predominant drug metabolizing enzyme and CYP3A4 is thought to be the key enzyme for the biotransformation of sunitinib (11). CYP3A4 is predominantly found in the liver and its expression is regulated by the ligand-activated nuclear receptors NR1I2 [pregnane X receptor (PXR)] and NR1I3 [constitutive androstane receptor (CAR)] (12). In addition, other enzymes of the cytochrome P450 family (CYP3A5, CYP1A1, and CYP1A2) may metabolize sunitinib, as these enzymes are known to be involved in the metabolism of other TKIs (13).

Besides pharmacokinetic factors, pharmacodynamic factors may determine the efficacy of sunitinib. In RCC, sunitinib is thought to exert its major therapeutic effect by inhibition of the VEGFR on tumor-associated endothelium, leading to reduced tumor angiogenesis (14). In addition, inhibition of the PDGFR might increase the antiangiogenic effects of sunitinib by targeting pericytes, which are able to protect endothelial cells from apoptosis (15). As the main targets for sunitinib are thought to be located in the microenvironment of tumor cells, the efficacy of sunitinib treatment may be related to the genetics of the surrounding microenvironment (16). Particularly, genetic variation in VEGFR-2 may affect sunitinib activity, because VEGFR-2 is expressed in the normal endothelium (17) and the tumor vasculature may develop from preexisting vessels of the host (18).

Single nucleotide polymorphisms (SNP) in genes encoding for efflux transporters, metabolizing enzymes, and drug targets may affect the efficacy of sunitinib in mRCC, as SNPs in specific genes have previously been associated with sunitinib-induced toxicities in patients with RCC and gastrointestinal stromal tumors (19, 20). Therefore, SNPs may be useful markers for personalized treatment planning and may be candidate markers for selecting mRCC patients for sunitinib treatment. The objective of the current study was to identify SNPs involved in the pharmacokinetics and pharmacodynamics of sunitinib that are associated with a prolonged PFS and/or OS in mRCC patients.

Patients and Methods

Study population

In our previous study, 219 sunitinib-treated patients with various malignancies were included to investigate the association between SNPs and sunitinib-induced toxicities (19). In the present study, a subset of patients with histologically proven clear-cell RCC was selected for the analyses. A total of 136 consecutive mRCC patients who initiated sunitinib treatment between December 2005 and May 2008 were included. Sunitinib was administered orally at a dose of 50 mg daily, consisting of 4 weeks of treatment followed by a 2-week rest-period in cycles of 6 weeks. Dose reductions of sunitinib were allowed.
depending on the type and severity of adverse events according to the current guidelines (21). The study was approved by the medical ethics review board.

**Study design**

Demographic and clinical data of patients were reported on case record forms designed for data collection for this study. Patient characteristics considered relevant for PFS and OS analysis were age, gender, Eastern Cooperative Oncology Group (ECOG) performance status, prior systemic therapy, prior radiotherapy, the number of metastatic sites, and the risk factors according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria, which is based on 5 risk factors including low Karnofsky performance status (< 80%), high lactate dehydrogenase (LDH; >1.5 times the upper limit of normal), low hemoglobin level, high corrected serum calcium (> 10 mg/dl), and time from initial diagnosis to treatment < 1 year (22). Residual blood or serum samples taken for routine patient care were stored at −20°C at the local hospital laboratory. Of each patient one whole blood or serum sample was collected from the participating hospitals. 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).

**Genetic polymorphisms**

Nineteen polymorphisms in 7 genes involved in the pharmacokinetics and 11 polymorphisms in 4 genes involved in the pharmacodynamics of sunitinib were selected (Supplementary Table S1). Selection criteria for the polymorphisms were a minor allelic frequency > 0.2 in Caucasians and an assumed clinical relevance based on previously reported associations or the assumption that nonsynonymous amino acid change leads to changed protein functionality.

The 11 candidate genes were selected on their potential relation with the pharmacokinetics and pharmacodynamics of sunitinib (Fig. 1). First, the candidate genotypes were selected by literature review. If there was no available data in the literature review, we referred to the SNPs from the dbSNP database (http://www.ncbi.nlm.nih.gov/sites/entrez). ABCB1, ABCG2, NR1I2, NR1I3, CYP3A5, CYP1A1, and CYP1A2 were selected for the pharmacokinetic pathways, whereas VEGFR-2, VEGFR-3, PDGFR-α, and FLT-3 were selected for the pharmacodynamic pathways. The most common functional SNPs in human ABCB1 are the synonymous 3435C > T and 1236C > T changes and the nonsynonymous 2677G > T change. As functional studies have shown that the haplotype of these three SNPs is a silent mutation and alters the function of the efflux transporter including its substrate specificity (23), the haplotype of ABCB1, instead of the three individual SNPs, was included in the analysis. Although VEGFR-1 is a target of sunitinib, and CYP3A4 is an important enzyme for metabolism of sunitinib (11), no polymorphisms of VEGFR-1 and CYP3A4 were analyzed, as no functional polymorphisms met the criteria for SNP selection.

Methods for genotyping assay validation and haplotype estimation have been described previously (19). Briefly, germline DNA was isolated from 1 ml. of serum or EDTA-blood with the Magnapure LC (Roche Diagnostics). Polyorphic sites in genomic DNA were analyzed with TaqMan assays (Applied Biosystems).

**Statistical design and data analysis**

For PFS and OS, data collection was closed on August 31, 2009. The primary outcome measures of this study, PFS, was defined as the time between the first day of sunitinib and the date of progressive disease (PD) according to Response Evaluation Criteria in Solid Tumors (24), clear clinical evidence of PD or death due to PD within 12 weeks after the last response evaluation. If a patient had not progressed, PFS was censored at the time of the last follow-up. If the PD date was unknown or a patient died due to PD later than 12 weeks after the last response evaluation, PFS was censored at the last adequate tumor assessment. OS was the secondary outcome and was defined as the time between the first day of sunitinib treatment and the date of death or the date at which patients were last known to be alive.

All patient characteristics were tested univariately against the primary outcome using Kaplan–Meier and Cox-regression analysis, depending on the tested variables. The polymorphisms and haplotypes were tested univariately against PFS and OS using the Kaplan–Meier method. For this initial analysis, the general model was used. Given the explorative nature of this study, variables with a P < 0.1 were selected as candidate variables for multivariate Cox-regression analyses. Data were fitted to the most appropriate model (multiplicative, dominant or recessive) and tested in the multivariate Cox regression survival analysis with PFS and OS as depending variables. Additional patient characteristics were introduced in the multivariate analyses based on univariately tested results if P < 0.1. Hazard ratios (HR) were generated considering patients with the most common clinical factor or genotype as the reference group. Missing data were kept as missing except for factors in the MSKCC score and the ECOG performance status. Patients with missing performance status (n = 2), LDH (n = 2), hemoglobin (n = 1), and baseline calcium values (n = 2) were assumed to be part of the worse prognosis scores. Accordingly, MSKCC scores were increased with one risk factor in 5 patients and with two risk factors in one patient. As a result 3 patients were categorized into the intermediate risk group, whereas 3 other patients were categorized into the poor risk group. Patients with missing ECOG performance statuses (n = 2) were scored as ECOG = 1. To test these assumptions, the multivariate analyses were performed with and without the replacement of the patients with missing factors in the MSKCC score. Similar results were generated, indicating that the replacement was legitimate. All statistical analyses were performed using SPSS 16.0 software. A 20% improvement (HR, 0 = 0.44) in PFS.
at ~1 year in patients with sunitinib was judged to be clinically meaningful by the investigators designing the study. Forty-six events with disease progression were estimated to be needed to detect such an improvement using a two-sided, unstratified log-rank test with an overall significance level of 0.05 and power of 0.80. All results from the multivariate analyses with \( P < 0.05 \) were considered significant. Because this is an explorative study, no correction for multiple testing was made.

**Results**

**Study population**

The main patient characteristics are presented in Table 1. Thirty-one (22.8%) patients had one metastatic site, 47 (34.6%) patients had two metastatic sites, and 58 (42.6%) patients had at least 3 metastatic sites. According to the MSKCC prognostic criteria most patients (59.6%) were categorized into the intermediate risk group, whereas 24.3% and 16.2% of the patients were categorized into the favorable and poor risk group, respectively.

At the time of the analysis, 47 (34.6%) patients were alive and 92 (67.6%) patients had disease progression. Overall, the median PFS time was 10.0 months (range, 7.6–12.4 months) and the median OS time was 16.3 months (range, 13.5–19.2 months). Of the clinical characteristics, the MSKCC risk factors had the largest contribution to PFS and OS (\( P = 0.001 \) and \( P < 0.001 \), respectively; Tables 2 and 3). In addition, the number of metastatic sites, age, and the ECOG performance status were prognostic for PFS (\( P = 0.019, 0.047, \) and \( 0.049 \), respectively), whereas only the ECOG performance status and the number of metastatic sites were also prognostic for OS (\( P = 0.004 \) and \( 0.058 \), respectively).

Baseline characteristics entered into the multivariate Cox models included the MSKCC risk factors, the number of metastatic sites, and age for PFS analyses, and the MSKCC risk factors and the number of metastatic sites for OS analyses. The ECOG performance status was excluded from the multivariate analyses due to colinearity with the MSKCC prognostic criteria (22).

**Pharmacogenetic factors for sunitinib and progression-free survival**

Among the 30 studied polymorphisms, only polymorphisms related to the pharmacokinetics of sunitinib were predictive of PFS (Table 2). A prolonged PFS was found in the univariate analysis of patients with presence of the A-allele in \( \text{CYP3A5} \) 6986A/G (\( P = 0.017 \)), absence of a CAT copy in \( \text{NR1I3} \) haplotype (\( P = 0.021 \)), presence of the C-allele in \( \text{NR1I2} \) 8055C/T (\( P = 0.025 \)), presence of the C-allele in \( \text{NR1I2} \) -25385C/T (\( P = 0.032 \)), presence of a TCG copy in the \( \text{ABCB1} \) haplotype (\( P = 0.072 \)) and presence of the A-allele in \( \text{ABCG2} \) 34G/A (\( P = 0.077 \)). Together with the MSKCC risk factors, the numbers of metastatic sites and age, these polymorphisms were entered into the multivariate Cox model.
Multivariate analysis confirmed the following factors as significant (<0.05) predictors of improved PFS: the MSKCC risk factors (HR: 1.988; 95% CI, 1.394–2.837), the number of metastatic sites (HR: 1.400; 95% CI, 1.042–1.880), age (HR: 1.031 per year increase; 95% CI, 1.003–1.060), presence of the A-allele in CYP3A5 6986A/G (HR: 0.266; 95% CI, 0.079–0.892), absence of a CAT copy in the NR1I3 haplotype (HR: 1.758; 95% CI, 1.108–2.790), and the presence of a TCG copy in the ABBC1 haplotype (HR: 0.522; 95% CI, 0.287–0.950).

### Pharmacogenetic factors for sunitinib and overall survival

In univariate analysis, polymorphisms related to the pharmacokinetics and pharmacodynamics of sunitinib were predictive of OS (Table 3). Of the pharmacokinetic...
polymorphisms, presence of the C-allele in NR1I2-25385C/T ($P = 0.017$), presence of a TCG copy in the ABCB1 haplotype ($P = 0.097$) and presence of the A-allele in ABCG2 34G/A ($P = 0.072$) were associated with a prolonged OS. In addition, univariate analyses identified two pharmacodynamic polymorphisms including two GCGT copies in the PDGFR-$\alpha$ haplotype and presence of the A-allele in VEGFR-2 1718T/A as factors for a prolonged OS ($P = 0.002$ and 0.016, respectively).

Multivariate analysis confirmed the MSKCC risk factors (HR: 2.273; 95% CI, 1.595–3.238) and the presence of the A-allele in VEGFR-2 1718T/A (HR: 2.907; 95% CI, 1.224–6.903) as significant ($< 0.05$) predictors of a prolonged OS.

In multivariate analysis, there was a trend toward an improved OS for patients with a TCG copy in the ABCB1 haplotype (HR: 0.593; 95% CI, 0.332–1.061; $P = 0.078$) or presence of the A-allele in ABCG2 34G/A (HR: 0.416; 95% CI, 0.162–1.070; $P = 0.069$).

### Table 3. Univariate and multivariate analyses of overall survival in mRCC patients treated with sunitinib

<table>
<thead>
<tr>
<th>Factors</th>
<th>No.</th>
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<th>95% CI</th>
<th>$P$</th>
<th>HR $^b$</th>
<th>95% CI</th>
<th>$P$</th>
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<td>0</td>
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<td>&lt;0.001</td>
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<td>1–2</td>
<td>81</td>
<td>14.8</td>
<td>11.8–17.7</td>
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<td>2.273</td>
<td>1.595–3.238</td>
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<td>$\geq$3</td>
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<td>10.9</td>
<td>7.2–14.7</td>
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<td>No. of metastatic sites</td>
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<td>1</td>
<td>31</td>
<td>28.8</td>
<td>15.3–42.2</td>
<td>0.058</td>
<td>1.273</td>
<td>0.957–1.693</td>
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<td>2</td>
<td>47</td>
<td>15.6</td>
<td>13.5–17.7</td>
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<td>$\geq$3</td>
<td>58</td>
<td>13.2</td>
<td>9.5–16.9</td>
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<td>CC + CT versus TT</td>
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<td>17.1</td>
<td>12.9–21.2</td>
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<td>24.2</td>
<td>17.2–31.2</td>
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<td>0.002</td>
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<td>0.108</td>
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<tr>
<td>AA + AT versus TT</td>
<td>125</td>
<td>16.3</td>
<td>12.4–20.2</td>
<td>0.022</td>
<td>1.458</td>
<td>0.920–2.310</td>
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<td>AA + AT versus TT</td>
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<td>2.907</td>
<td>1.224–6.903</td>
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$^a$Only factors with $P < 0.1$ level are presented; factors with $P < 0.1$ in the univariate analyses were selected for multivariate analyses.
$^b$HR $< 1.0$ indicates that the factor associates with improved OS, HR $> 1.0$ associates with worse OS.
$^c$Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria [based on the 5 risk factors: low Karnofsky performance status (<80%), high LDH (>1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/dL), and time from initial diagnosis to treatment <1 year] (22).
$^d$Multinomial model, HR per increase in MSKCC class or number of disease sites class.

Description haplotypes: $^e$ = ABCB1 3435C/T, 1236C/T and 2677G/T; $^f$ = PDGFR-$\alpha$ 1580T/C -1171C/G -735G/A -573G/T.

Favorable genetic profiles and outcome
Polymorphisms that were associated with an improved PFS were combined in a predictive model. Patients were categorized as carriers of the favorable genetic profiles when they had at least an A-allele in CYP3A5, a TCG copy in the ABCB1 haplotype or a missing CAT copy in the NR1I3 haplotype. Carriers with a favorable genetic profile...
(n = 95) had an improved PFS and OS as compared with noncarriers (median PFS: 13.1 versus 7.5 months, \( P = 0.001 \) and median OS: 19.9 versus 12.3 months, \( P = 0.009 \)). Multivariate analysis including the clinical factors showed consistent predictive value of the model for PFS and showed a trend for OS (HR: 0.541; 95% CI, 0.340–0.860, \( P = 0.009 \) and HR: 0.667; 95% CI, 0.420–1.058, \( P = 0.085 \), respectively; Fig. 2).

Discussion

In mRCC patients treated with sunitinib, the MSKCC risk groups and the number of metastatic sites are clinical factors that are usually associated with PFS and OS (2,3,7). However, these clinical factors are prognostic criteria that are associated with the extent of the disease and do not necessarily predict antitumor efficacy of a specific drug. As an increasing number of drugs is currently available for the treatment of mRCC (25), tools are needed to identify patients who predispose to benefit from sunitinib treatment and to select individual mRCC patients for treatment with this drug. The efficacy of sunitinib may be influenced by multiple genes encoding for enzymes, efflux transporters and targets related to the pharmacokinetics and pharmacodynamics of sunitinib. Therefore, we analyzed whether SNPs in the pharmacokinetic and pharmacodynamic pathways of sunitinib were predictive of PFS and OS in patients with clear-cell mRCC. Our study showed that next to 3 clinical characteristics (MSKCC prognostic criteria, number of metastatic sites, and age), 3 genetic variants in the CYP3A5, NR1I3, and ABCB1 genes were predictive factors for PFS. In addition, a role of an A-allele in VEGFR-2 1718T/A for a prolonged OS as a secondary outcome was found.

Clinical benefit from sunitinib treatment may depend on systemic exposure to sunitinib. Sunitinib is metabolized primarily to the active N-de-ethylated metabolite SU12662, which reaches similar plasma concentrations and has equipotent biochemical activity as the parent compound (26). Thereafter, SU12662 undergoes a second N-de-ethylation step, which occurs at a slower rate, to the inactive metabolite SU14335. Recently, a meta-analysis of pharmacokinetic data in 443 patients treated with sunitinib, showed that higher plasma levels of sunitinib and its active metabolite SU12662 were associated with a prolonged time–to–tumor progression and OS (27). Currently, it is not clear which underlying factors account for the observed interindividual differences in plasma levels of sunitinib and its active metabolite SU12662. Interindividual differences in sunitinib exposure may be the result of variations in sunitinib absorption, metabolism, distribution, and excretion through metabolizing enzymes and transporter proteins. Concerning the pharmacokinetics of sunitinib, the present study identified variants in three genes (CYP3A5, NR1I3, and ABCB1) as predictive factors for PFS in mRCC patients treated with sunitinib. Although these polymorphisms were not predictive of OS, there was a trend toward a prolonged OS for patients with a TCG copy in the ABCB1 haplotype. Additional treatment after discontinuation of sunitinib treatment may explain the discrepancy between the results of the PFS and OS analyses, as 26% of patients were subsequently treated with at least one other agent, including sorafenib (22%), temsirolimus (2%), and everolimus (4%).

Figure 2. Favorable (—) and nonfavorable genetic profile (…) in mRCC patients treated with sunitinib for progression-free (A) and overall survival (B) using multivariate Cox regression analysis. Patients were categorized as carriers of the favorable genetic profiles when they had at least an A-allele in CYP3A5, a TCG copy in the ABCB1 haplotype or a missing CAT copy in the NR1I3 haplotype.
CYP3A4 is the major key enzyme for the biotransformation of sunitinib (11). However, no polymorphisms of CYP3A4 were analyzed in the present study, as there are no functional polymorphisms in CYP3A4 that meet our described criteria for SNP selection yet. The CYP3A5 enzyme is another important enzyme for the metabolism of several TKIs including erlotinib, gefitinib, and imatinib (13). Similarly, the CYP3A5 enzyme may metabolize sunitinib and was therefore included in the analysis, though the sunitinib-metabolizing capacity of CYP3A5 has to be confirmed. In the current study, presence of the A-allele in CYP3A5, an SNP which leads to the CYP3A5 expressor phenotype (28, 29), was a predictive factor for a prolonged PFS. As the CYP3A5 expressor phenotype may lead to increased metabolism of sunitinib, these findings suggest that the prolonged PFS in patients with presence of the A-allele in CYP3A5 may be caused by increased levels of the active metabolite SU12662, which has a longer half-life than the parent compound (80–100 hours versus 40–60 hours; ref. 26). Furthermore, polymorphisms in other genes (NR1I2 and NR1I3) that regulate the expression of CYP3A4 (12) were identified as predictive factors for outcome in sunitinib-treated mRCC patients.

The efflux transporters ABCB1 and ABCG2 play an important role in drug absorption, excretion, cellular accumulation, and resistance (10). Consequently, polymorphisms in ABCB1 and ABCG2 may affect drug absorption and excretion of sunitinib. In the present study, the found associations between polymorphisms in the ABCB1 haplotype (a TCG copy) and the ABCG2 gene (presence of the A-allele), and improved outcome suggest that these polymorphisms may lead to reduced efflux transport of sunitinib into the gastrointestinal lumen and bile, resulting in increased systemic exposure of sunitinib. In vitro studies have reported conflicting data on the affinity of sunitinib for ABCB1 and ABCG2 (8, 9). Hu and colleagues (8) found a moderate affinity of sunitinib for ABCB1 and a negligible transport of sunitinib in cells overexpressing ABCG2, whereas another study reported higher sunitinib affinity for ABCG2 compared with ABCB1 (9). Furthermore, sunitinib reversed ABCG2-mediated multidrug resistance by inhibiting the drug efflux function of ABCG2 (30). This inhibitory capacity of sunitinib on ABCG2 appeared to be sensitive for the ABCG2 1291T > C genotype (31). In addition, another SNP in ABCG2 (421C > A) was associated with increased sunitinib exposure (32).

The ABC transporters may contribute to multidrug resistance in tumors by actively extruding drugs from cancer cells. In RCC, an increase in ABCB1 expression (33, 34) and activity (35) has been reported, suggesting a contribution of ABCB1 to the resistance of RCC to some anticancer drugs. Although polymorphisms in ABCB1 and ABCG2 may be associated with the development of RCC (36, 37), it is currently not known whether polymorphisms in ABCB1 and ABCG2 are associated with the expression and function of these transporters at the somatic level in renal cancer cells (38). Nevertheless, the role of efflux transporters in tumor cells may be limited for acquired resistance to sunitinib, which may develop after an initial response to sunitinib, as acquired resistance to sunitinib may be more related to physiological changes in the microenvironment of tumors, allowing reestablishment of angiogenesis during sunitinib treatment (16).

Clinical efficacy of treatment with TKIs may also be related to specific mutations in drug targets, as was shown for imatinib and gefitinib (39–40). Currently, it is not known which targets in RCC predict response to sunitinib or whether the somatic polymorphisms of targets in RCC correlate with genetic polymorphisms obtained from germline cells. Of the studied pharmacodynamic polymorphisms of sunitinib, only a polymorphism of VEGFR-2 1718T/A was associated with a decreased OS in multivariate analysis, whereas the presence of two GCGT copies in the PDGFR-α haplotype was associated with a prolonged OS in univariate analysis. However, no significant association between these polymorphisms and PFS was found. These findings may suggest that polymorphisms in VEGFR-2 and PDGFR-α may be associated with the nature of the disease and may therefore be prognostic instead of predictive. However, prospective validation in an independent mRCC cohort that is not treated with sunitinib is necessary to determine whether the associated polymorphisms of the present study are predictive markers of sunitinib activity or prognostic markers of mRCC disease.

In our previous study, several polymorphisms in genes involved in the pharmacokinetic and pharmacodynamic pathways of sunitinib were associated with sunitinib-induced toxicity (19). Polymorphisms of NR1I3 (absence of a CAG copy in the haplotype), ABCB1 (presence of a TTT copy), and VEGFR-2 (T allele in 1191 C/T) were significantly related with an increased risk for leukocytopenia, hand-foot syndrome, and any toxicity > grade 2, respectively (19). In the present study however, other genotypes or haplotypes in NR1I3, ABCB1, and VEGFR-2 were associated with clinical outcome. It is currently not clear how the different genotypes and haplotypes between these studies are related, but it is conceivable that severity and prevalence of some sunitinib-induced toxicities may basically depend on inhibition of a specific molecular pathway rather than variation in exposure, whereas other sunitinib-induced toxicities may mainly depend on exposure to the drug. In addition, the discrepancy between these two studies may be the result of different study populations, as patients with different malignancies were included in our previous study. Recently, Houk and colleagues (27) have shown a relationship between sunitinib exposure and the probability of grade ≥ 1 fatigue, the absolute neutrophil count, and the changes in diastolic blood pressure. However, these sunitinib-induced toxicities cannot be extrapolated to our previous toxicity study (19), as different toxicities and grades of toxicity were analyzed in both studies.

A potential limitation of the study is the retrospective design. As a result, pharmacokinetic data were not available to correlate polymorphisms of CYP3A5, NR1I3, and ABCB1
with plasma levels of sunitinib and its active metabolite SU12662. If future studies reveal a relation between sunitinib exposure and presence of an A-allele in CYPIA1, 6986A/G, absence of a CAT copy in the ABCB1 haplotype, or presence of a TCG copy in the ABCB1 haplotype, the sunitinib starting dose may be adjusted to dose-escalation of sunitinib > 50 mg/daily for patients without these genotypes and haplotypes. The nonbeneficial genetic profile may be used to select patients who may be eligible for alternative dosing schedules with intensive monitoring of plasma levels of sunitinib and its active metabolite SU12662. Before this genetic profile can be implemented, prospective validation in an independent patient population is necessary.

In conclusion, pharmacokinetic but not pharmacodynamic polymorphisms were independent predictive factors for PFS in patients with clear-cell mRCC who were treated with sunitinib. Patients with an A-allele in CYPIA1, 6986A/G, absence of a CAT copy in the NR1I3 haplotype, or presence of a TCG copy in the ABCB1 haplotype had a prolonged PFS. These polymorphisms may be valuable factors to identify patients with reduced exposure to sunitinib in order to improve treatment strategies in these patients. The findings of this study advocate more pharmacokinetic studies in patients treated with sunitinib to further elucidate the role of these genetic determinants in sunitinib exposure and efficacy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Pharmacogenetics in Sunitinib-Treated mRCC Patients


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