Title:

Single Nucleotide Polymorphisms associated with response and toxicity in advanced renal carcinoma patients treated with first line sunitinib: an observational, prospective, multicenter study

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Brief title: SNPs associated with sunitinib outcome in renal cancer patients

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ABSTRACT

Background: Sunitinib is a tyrosine kinase inhibitor with proven efficacy in renal cell carcinoma (RCC). However, properly validated molecular predictors of response and toxicity are still lacking. This study aims to identify genetic markers of outcome focussing on the pharmacokinetic and pharmacodynamic pathways of the drug.

Methods: 101 patients with RCC were recruited into this observational, prospective study conducted in 15 institutions of the Spanish Oncology Genitourinary Group. Overall response rate, progression free survival (PFS), overall survival and the toxicity profile of clear cell RCC patients treated with first line sunitinib were investigated for association with 16 key polymorphisms in nine genes: *VEGFR2* (rs2305948,rs1870377), *VEGFR3* (rs307826, rs448012, rs307821), *PDGFR-α* (rs35597368), *VEGF-A* (rs2010963, rs699947, rs1570360), *IL8* (rs1126647), *CYP3A4* (rs2740574), *CYP3A5* (rs776746), *ABCB1* (rs1045642, rs1128503, rs2032582), and *ABCB2* (rs2231142).

Findings: In total 95 patients were included for toxicity and 89 for efficacy analyses. Multivariable analysis showed that two *VEGFR3* missense polymorphisms, rs307826 and rs307821, were associated with worse sunitinib response, hazard ratios (HRs) (*per allele*) of 8·8 (95%CI=1·9-40·3, P=0·005) and 7·1 (95%CI=1·5-35·0, P=0·015), respectively, and shorter PFS, HRs (*per allele)* of 3·6 (95%CI=1·7-7·3, P=0·0005) and 3·3 (95%CI=1·6-6·7, P=0·0008), respectively. The associations with PFS remained significant after correcting for the number of polymorphisms tested (Bonferroni P cut-point of 0·003). The *CYP3A5*1* (rs776746) high metabolizing allele was associated in a multivariable analysis with an increased risk of dose reductions due to toxicity, HR (*per allele*) of 3·7 (95%CI=1·7-8·4, P=0·001). The results

remained significant after Bonferroni correction for multiple testing. None of the other SNPs tested had statistically significant adjusted P values.

Interpretation: Our results suggest that two *VEGFR3* polymorphisms define a subset of RCC patients with a decreased sunitinib efficacy. In addition, patients carrying *CYP3A5*1* allele exhibited a higher risk of sunitinib dose reductions due to toxicity. If confirmed, these results should promote interventional studies testing alternative therapeutic approaches for these two specific populations.

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INTRODUCTION

Sunitinib malate is an orally administered tyrosine kinase receptor inhibitor that targets VEGF receptors, PDGFR, KIT, FLT-3, CSF-1R, and RET. A randomized, phase III pivotal study in advanced clear cell renal cell carcinoma (ccRCC) comparing first-line interferon-α and sunitinib showed a superior progression free survival (PFS) for sunitinib (5 *versus* 11 months; *P* <0·001), and nowadays sunitinib is a standard treatment.[1] These findings were further supported by data demonstrating an impact on overall survival (OS) (21·9 months with interferon-α *versus* 26·4 months with sunitinib),[2] and an expanded access trial with more than 4500 patients worldwide confirmed similar outcomes.[3] However, some important caveats still remain, namely, lack of efficacy where 20% of patients develop early progression of the disease, and adverse events that, though manageable in most cases, can lead to dose suspensions, reductions and, delays (8, 32, and 38%, respectively).[1] Since drug exposure has been correlated with efficacy, toxicity may not only impact patients' quality of life but also lead to dose modifications that could jeopardize treatment outcomes.[4] Biomarkers that can identify patients more likely to be resistant to sunitinib or at higher risk of toxicity could lead to new therapeutic approaches and improved results. Though different mechanisms of resistance have been proposed,[5] the genetic background of the patient could play an important role, particularly for drugs like sunitinib, that interact with the microenvironment of the tumor and non-malignant endothelial cells instead of having a direct cytotoxic activity. Recent description of hypertension as a reliable marker of sunitinib outcome supports the hypothesis that sunitinib sensitivity and resistance could be, at least in part, a host-dependent condition.[6] Against this background, we designed a multicentric, prospective study aimed at identifying single nucleotide polymorphisms (SNPs) in the

pharmacokinetic and pharmacodynamic pathways of the drug that were associated with response and toxicity in ccRCC patients treated with first line sunitinib.

PATIENTS AND METHODS

Study population

Eligible patients were 18 years old or above, with a pathologically confirmed diagnosis of renal cell carcinoma with a component of clear cell histology, having local or distant advanced disease, who had not received any systemic treatment for kidney tumor, including cytokines, and who were scheduled for sunitinib in a daily clinical practice setting (Table 1). The protocol study was approved by the medical ethics review board of all participating institutions, and written, signed consent was obtained in all cases.

Study design

This was an observational, prospective, multicentric study performed in 15 hospitals, all members of the Spanish Oncology GenitoUrinary Group (SOGUG). Drug schedule, policy for dose reductions and timing for radiological assessments were decided by the attending physicians in accordance with current, local practice guidelines. Demographic and clinical data were recorded on specific case record forms and periodically reviewed by an external monitor. Samples were anonymized, and molecular analysis was performed blinded to clinical data. Study recruitment started the 10th October 2007 and finished the 13th December 2010, and the database was closed for follow-up in May 2011. The authors designed the study, analyzed and held the data, wrote the manuscript, made the decision to submit the manuscript for publication, and vouch for the accuracy and completeness of the data and analyses.

SNP selection and genotyping

Sixteen SNPs were selected in nine genes potentially relevant for sunitinib action, metabolism and transport (Table 2). The polymorphisms were selected based on functionality evidence from previously reported associations, those leading to amino acid changes and with reported minor allele frequencies greater than 5%. The selected polymorphisms potentially influencing sunitinib pharmacodynamics were in genes encoding sunitinib targets and ligand *VEGFR2, VEGFR3, PDGFR-*α and *VEGF-A,*[7] and in *IL8* gene.[8] From these SNPs, six were putatively functional missense polymorphisms in the receptors, the *VEGF-A* 5'UTR and promoter variants corresponded to SNPs previously associated with bevacizumab response and tumor prognosis,[9,10] and the *IL8* 3'UTR variant corresponded to a SNP previously associated with PFS in renal cancer patients treated with pazopanib.[8] The polymorphisms potentially affecting sunitinib pharmacokinetics were in genes relevant for its metabolism, *CYP3A4* and *CYP3A5,*[11] and transport, *ABCB1* and *ABCG2*.[12] *CYP3A5*1* is a high-activity allele,[13] while the SNP corresponding to *CYP3A4*, the major sunitinib metabolizing enzyme, is a promoter variant with contradictory published results concerning its activity.[14] Concerning the transporters, the *ABCG2* variant has been reported to increase sunitinib exposure[15] and the three *ABCB1* SNPs have been repeatedly associated with an altered P-glycoprotein activity.[16]

DNA was isolated either from peripheral blood or saliva collected from the patients. For peripheral blood, DNA was isolated using the FlexiGene DNA Kit (Qiagen, Valencia, USA) according to the manufacturer's recommended protocols. For DNA extractions from saliva, Oragene DNA Self-Collection Kits (DNA Genotek Ottawa, Canada) were used. The final DNA concentration was quantified by PicoGreen (Invitrogen, Carlsbad, USA). SNPs were genotyped using the KASPar

SNP Genotyping System (Kbiosciences, Herts, UK). The sequence Detection System 7900HT (Applied Biosystems, Foster City, USA) was used for fluorescence detection and allele assignment.

Statistical analyses

PFS was defined as the time between the first day of treatment with sunitinib and the date of radiological progression of the disease (PD), clear clinical evidence of PD or death. Patients who had not progressed at database close were censored at final follow-up. If the date of PD was unknown, PFS was censored at the last tumor assessment. OS was defined as the time between the first day of sunitinib treatment and the date of death or last date of follow-up. Objective response was assessed by treating physicians, according to RECIST criteria: complete response (CR), partial response (PR), stabilization of the disease (SD) and PD. Since this study was performed in a daily clinical practice setting, timing for assessments was dictated by individual institution policy. All adverse effects were graded by the attending physicians according to CTCAE v3·0 (Supplementary Table 1). Mucositis, hand-foot syndrome (HFS), hypertension, anemia and thrombocytopenia were selected for analysis, based on clinical relevance and grading objectiveness, together with grade 3/4 adverse events. Additionally, toxicity events leading to dose reductions and the date on which they occurred were also recorded.

The SNP genotypes were tested against PFS and OS, using Cox-regression, and against PD as best objective response, using a logistic regression. A multivariable analysis was performed by including as covariates clinical factors associated with $P<0{\cdot}1$ to PFS, OS or response (Table 3). According to the MSKCC prognostic classification (see Table 1), patients were divided into favourable,

intermediate and poor prognosis groups, and this variable was used for the multivariable analysis. Center was not included as a covariate in the statistical analysis because the number of patients recruited in each of the 15 institutions was small (≤17). SNPs associated to PFS and OS in the multivariable analysis were further analysed by the Kaplan-Meier method.

Genotypes associated with an increased risk of sunitinib dose reduction due to toxicity were tested using the Cox-regression method modelling the number of days of sunitinib treatment until the reduction of dose due to toxicity. Patients with no dose reductions were censored at final follow-up (Table 4). For multivariable analysis, we used as covariates clinical factors associated with dose reductions with P<0·1. SNPs associated to sunitinib dose reduction in the multivariable analysis were further analysed by the Kaplan-Meier method. The analysis was performed using the subset of patients that initiated treatment with 50 mg sunitinib (n=84) and all patients (n=95). Associations between specific sunitinib toxicities and genotypes were studied using logistic regression analysis, with toxicity development as a dichotomous end point. Multivariable logistic regression analyses included as covariates clinical factors associated with the corresponding outcome with P<0·1 (Table 4). All genotypes were tested using an additive genetic model.

Missing data were kept as missing except for the MSKCC prognostic factors. Variables needed to assign the MSKCC score were missing for 18 patients (basal Ca^{2+} corrected value in 11 cases, performance status in six cases, and LDH value in one case). The most likely value, based on the data from the rest of the serie, was assigned to these cases (2·5% (2-79) of the patients had an elevated Ca^{2+} corrected value, 9·9% (8-81) had high performance status and 6·9% (6-86) had elevated LDH). Note that some patients could be correctly assigned to a MSKCC group even with

some data missing. As a result 15 patients were assigned to the good prognosis group and three patients to the intermediate group. Multivariable analyses were performed with and without the replacement of the missing factors. Similar results were found, indicating that the replacement did not skew the results. The level of statistical significance was set at $P<0{\cdot}05$, and the adjustment for multiple testing was performed using Bonferroni's method. Taking into account that 16 polymorphisms were selected for the analysis, only P values $< 0{\cdot}0031$ were statistically significant after Bonferroni correction at the 95% confidence level. All statistical analyses were carried out using RStudio version 0·93.

RESULTS

Study population

From the initial 101 patients, six were excluded from the study (three because no blood or saliva could be collected, two were confirmed as non-clear cell kidney cancers and one did not receive sunitinib). In six other cases malignant kidney tumors were confirmed but subtype could not be determined. These latter cases were excluded from the sunitinib efficacy analysis but were included for the toxicity analysis. Thus, 89 and 95 patients were used for the efficacy and toxicity analyses, respectively. The main characteristics of the patients are presented in Table 1. Most patients, 66 (70%) of 95, had multiple metastases with the lung as the site of most frequent localisation. Regarding the MSKCC prognostic factors, most patients were classified in the intermediate and favourable groups, 53% (50-95) and 44% (42-95), respectively. All patients were Spanish Caucasians, except for one African and one Asian.

With a median follow-up of 21·2 months (95% CI=13·6-28·9), the median PFS of the 89 evaluable patients was 12·3 months (95% CI=9·1-15·4) and 62 (70%) were alive by the time of the analysis. Objective response was assessed in 78 patients with measurable disease: 1 had CR (1%), 36 PR (46%), 26 SD (33%) and 15 PD (19%). Concerning overall toxicities, the most common were asthenia, mucositis, diarrhea, neutropenia, and HFS (Supplementary Table 1). From the 95 patients 41 (43%) developed grade 3 adverse events and 47 (49%) required dose reductions due to toxicity (Supplementary Table 1). No grade 4 toxicity was reported.

MSKCC risk factors were significantly associated with worse OS and overall response rate and showed a trend with PFS. In addition, women had worse

outcomes (Table 3). Regarding sunitinib toxicities, women had a significantly higher risk of needing sunitinib dose reductions due to adverse events than men (Table 4).

Polymorphisms associated with sunitinib efficacy

The minor allele frequencies of the 16 polymorphisms genotyped (Table 2) were similar to those previously described for Caucasians (dbSNP database) and all SNPs were in Hardy-Weinberg equilibrium (P>0·05). The results of the multivariable Cox regression analysis for the 16 polymorphisms genotyped are summarized in Table 3. As shown, two polymorphisms in *VEGFR3,* rs307826 and rs307821, were significantly associated with an increased risk of having PD as best sunitinib response, with estimated HRs (*per allele*) of 8·8 (95%CI=1·9-40, P=0·005) and 7·1 (95%CI=1·5-35·0, P=0·015), respectively. These polymorphisms were also associated with a worse PFS with estimated HRs (*per allele*) of 3·6 (95%CI=1·7-7·3, P=0·0005) and 3·3 (95%CI=1·6-6·7, P=0·0008), and remained significant after Bonferroni correction for multiple testing (Table 3 shows uncorrected P values, adjusted P values are 0·0078 and 0·014 for rs307826 and rs307821, respectively). These *VEGFR3* polymorphisms are missense variants in relatively high linkage disequilibrium (r^2=0.55) and few patients carried these variants independently. Thus, we constructed a model including both polymorphisms that shows the PFS of the four patients carrying only one of the variants (Supplementary Figure 1). Three additional polymorphisms in *ABCB1, ABCG2* and *VEGFR2*, (rs1128503, rs2231142 and rs1870377) showed trends towards worse sunitinib PFS and/or OS (Table 3).

Polymorphisms associated with sunitinib dose reductions and toxicities

Most dose reductions (94%; 47 of 50) were caused by adverse events (Supplementary Table 2 shows toxicities associated with dose reductions). To be able to detect factors relevant for dose reductions, we performed a Kaplan-Meier analysis modelling the time of sunitinib treatment up to the reduction of dose due to toxicity. We found that women required dose reductions earlier than men (P=0·005), and multivariable analysis identified *CYP3A5* rs776746 as an important risk factor (Figure 2), with a HR of 3·7 (95%CI=1·7-8·4, P=0·001) (Table 4). This association remained significant after correction for multiple testing. The analysis was performed considering only patients with a 50 mg sunitinib starting dose (n=84) and also considering all patients (n=95), with similar results (data not shown). Concerning specific toxicities, *VEGFR2* rs1870377 and *VEGF-A* rs699947 and rs1570360 variant alleles were significantly associated with an increased risk of hypertension (P=0·0058, P=0·0074, and P=0·035, respectively, Table 4), while *ABCB1* rs1128503 and rs2032582 variant alleles were associated with hypertension protection (P=0·0073, and 0·014, respectively). *VEGFR2* rs2305948 variant allele was associated with an increased risk of developing HFS (P=0·014) and *VEGF-A* rs2010963 variant was associated with mucositis protection (P=0·034).

DISCUSSION

This is, to our knowledge, the first prospective evaluation of SNPs as predictors of efficacy and toxicity of sunitinib in first line, naïve, ccRC patients. Two *VEGFR3* missense polymorphisms were strongly associated with shorter PFS and one functional polymorphism in *CYP3A5* was associated with an increased risk of sunitinib dose reductions due to toxicity, These associations, although not validated in an independent series, remained significant after correction for multiple testing. This data suggests that alternative therapeutic approaches for these populations should be promoted.

The increasing therapeutic options for kidney cancer with four approved antiangiogenic drugs (sorafenib, sunitinib, bevacizumab and pazopanib), and at least three others in advanced stage of development (axitinib, tivozanib and dovitinib, and not taking into account mTOR inhibitors), has made the identification of biological markers of response and toxicity for these drugs a key step for moving forward and improving patient care. Therefore, we initiated this multicenter study aimed at the identification of polymorphisms as potential markers of outcome, focussing on genetic variants potentially altering the pharmacokinetics and pharmacodynamics of sunitinib (Table 2). Our data suggest that variants in *VEGFR3* and *CYP3A5* could account for part of the lack of response and low tolerability found in some patients.

VEGFR3 is a transmembrane tyrosine kinase receptor of VEGF that has been primarily associated with lymphangiogenesis.[17] Though initially its expression was considered to be restricted to lymphatic vessels in adulthood, growing evidence has not only confirmed the expression of VEGFR3 in tumor blood vessels but also pointed to VEGFR3 as being a key mediator of other proangiogenic factors.[18,19] Preclinical models have even suggested that VEGFR3 could be more relevant than

VEGFR2 for the development of lymphatic and distant metastases.[20] Additionally, sunitinib administration has been shown to alter plasma-soluble VEGFR3 levels and this alteration has been associated with sunitinib efficacy in patients with kidney carcinoma.[21,22] This study shows an association between two *VEGFR3* missense SNPs, rs307826 (T494A), and rs307821 (R1324L) with worse overall response and PFS (Table 3), with this latter association remaining statistically significant after multiple testing correction. Van der Veldt *et al.* did not find statistically significant results for rs307826 and sunitinib PFS.[23] However, this discrepancy could be due to differences in the patients studied, in particular the use of previous medical treatments, not allowed in this study but frequent (41% of the patients) in the van der Veldt study.[23] Since rs307826, and rs307821 are in moderate linkage disequilibrium (Supplementary Figure 1 shows in more detail the association of these SNPs with PFS), it is difficult to determine which is the causal SNP, or if both could have an effect on sunitinib response. rs307826 and rs307821 affect moderately and weakly conserved nucleotides, respectively, with T494A located in the immunoglobulin homology domain 5 (D5) of VEGFR3, while R1324L is in the C-terminal region of the protein. Bioinformatic tools (SIFT and Align GVGD) in both cases predicted amino acid changes that would affect protein function. However, since D5-D5 interactions have been suggested to contribute to dimer stabilization and activation of VEGFR3[24] and this study shows a stronger association for this SNP with sunitinib response and PFS, it suggests that this could be the causal variant. It cannot be conclusively determined if the identified *VEGFR3* variation could also be influencing prognosis, however its association with sunitinib overall response suggests a major effect in drug efficacy.

The risk of dose reductions due to toxicity was significantly associated, even after correction for multiple testing, with *CYP3A5*1* (rs776746), which determines CYP3A5 enzyme expression[13] (Figure 2 and Table 4). CYP3A5 shares substrate specificity with CYP3A4[25], the major enzyme catalysing sunitinib metabolism.[11] Thus, *CYP3A5*1* might metabolise sunitinib and result in an increased production of the active and longer-acting metabolite SU12662 leading to toxicity. If confirmed, the large ethnic differences in *CYP3A5*1* allele frequency, more common in Africans and Asians than in Caucasians,[13] might be an underlying cause of the higher sunitinib toxicity observed in Asians.[26, 27] Interestingly, van der Velt *et al.* found an association between *CYP3A5*1* and a better PFS.[23] The sunitinib pharmacokinetic profile of *CYP3A5*1* carriers should be investigated in future studies to clarify this point. Concerning specific toxicities (Table 4), the most relevant results corresponded to hypertension, with three SNPs in *VEGFR2, VEGF-A,* and *ABCB1* showing P values <0·0075. A recently published study by Rini et al. has established hypertension as a sunitinib predictor of response,[6] thus, these SNPs could also be surrogate markers of sunitinib efficacy. In agreement with this, the *ABCB1* rs1128503 variant T-allele, protective for hypertension, showed a trend for worse OS and PFS (Table 3). However, methodological differences in blood pressure assessment preclude a direct comparison between both studies.

Up to date only one serie has been published assessing sunitinib pharmacogenetic for toxicity[29] and efficacy.[23] Though authors reported challenging results, both studies were considered exploratory and no correction for multiple testing was performed. Other relevant differences with this study were allowance of patients with prior treatments, retrospective collection of data and inclusion of tumors other than renal cancer in toxicity analysis. Despite those differences, some of the

associations have shown similar trends in our serie (*ABCB1* rs1128503 and worse PFS and OS and *VEGFR2* rs1870377 and worse OS). Regarding our study some limitations must be highlighted. Schedule and dose modifications were not dictated by central protocol, and timing for radiological assessments was performed according to each institution's policy. Thus, courses of treatment were not standardized for the study and outcomes were evaluated as it is actually used in practice. Heng et al. established hemoglobin, corrected calcium, performance status, time from diagnosis, neutrophils and platelets, as clinical predictors of outcome for mRCC treated with antiangiogenic agents.[28] However, our study started before this work was published and basal neutrophils were not recorded precluding the application of this model to our population. The SNPs associated to sunitinib outcome, although with significant adjusted P values, have a relatively low allele frequency (9, 8 and 6% for rs307821 and rs307826 and rs776746, respectively) decreasing the power of the study. Finally, this study lacks a prospective, external validation. Since the patients included in this study were mainly Caucasians, the relevance of these polymorphisms should also be determined in other ethnic groups. However, including only patients with clear cell cancer component tumors, exclusion of any prior treatment, even cytokines, and the fact that clinical data were externally reviewed by an independent monitor, have contributed to the quality of data and homogeneity of the population. These are probably the major contributors for the robustness of our results, with three statistically significant associations after correction for multiple testing.

To summarize, this study provides strong evidence supporting *VEGFR3* rs307826 and rs307821 variants as predictive factors of sunitinib PFS, and that *CYP3A5* rs776746 is associated with dose reductions due to toxicity. These results warrant pharmacokinetic studies to better understand the molecular mechanisms

leading to dose reductions and further validation in independent series. If confirmed, these genetic variants could provide the basis for an individualized renal cancer treatment.

RESEARCH IN CONTEXT

Systematic review

At the time of protocol development we searched PubMed and American Society of Clinical Oncology databases using key words related to renal carcinoma, sunitinib and polymorphisms and found no published studies on SNPs as markers of sunitinib outcome. PubMed was searched to identify genes relevant for sunitinib pharmacokinetic and pharmacodynamic pathways. Variants in those genes were selected after revising for each gene the polymorphisms listed in dbSNP database. During recruitment one retrospective study focused on the determination of polymorphism associated to sunitinib-induced toxicity was published.[29] This serie was also used to identify polymorphisms associated to sunitinib PFS and OS.[23]

Interpretation

This first prospective evaluation of SNPs as predictors of efficacy and toxicity of sunitinib, in first line, naïve, ccRC patients, finds a strong association between two missense *VEGFR3* polymorphisms and sunitinib PFS. It also shows that *CYP3A5*1* allele is associated with an increased risk of sunitinib dose reductions due to toxicity. If our findings are confirmed, these polymorphisms could be used to identify subsets of patients that could benefit form alternative therapeutic options.

CONTRIBUTORS

JG-D and CR-A designed the study; JG-D, LJL-G, CR-A participated in the collection and assembly of data; JG-D, LJL-G, MR and CR-A participated in data analysis, interpretation and manuscript writing; JG-D, EE, DEC, AGA, MAC, JAA, EG, JP, JB, BM, EM, FM and AF participated in the provision of study material or patients. All authors critically reviewed the manuscript and approved the final version.

CONFLICTS OF INTEREST

JG-D and JB have consultant and advisory board fees from Pfizer. The other authors declare that they have no conflicts of interest.

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This study has been supported by an unrestricted educational grant from Pfizer that played no role on study design, collection, analysis or interpretation of data, writing the report or the decision to submit the paper for publication.

JG-D, LJL-G and CR-A had access to raw data.

JG-D, LJL-G, DC and CR-A had final responsibility to submit for publication.

ETHICS COMMITTEE APPROVAL

The protocol study was approved by the medical ethics review board of all participating institutions, and written, signed consent was obtained in all cases.

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TABLES

Table 1. Patient and clinical characteristics.

Characteristic	Nr.	%
Age at sunitinib (y)	65	
Range	56-73	
Sex		
Male	65	68
Female	30	32
ECOG		
0	25	26
1	56	59
2	8	8
3	0	0
Missing	6	6
Previous nephrectomy		
Yes	76	80
No	19	20
Nr. of metastatic sites		
0	2	2
1	27	28
2	44	46
3	16	17
4	5	5
6	1	1
Common metastasis sites		
Lung	66	69
Lymph nodes	43	45
Bone	24	25
Kidney	17	18
Liver	13	14
MSKCC risk factors[a]		
0 (favorable)	42	44
1-2 (intermediate)	50	53
≥3 (poor)	3	3
Initial sunitinib dose		
50 mg	84	88
37.5 mg	9	9
25 mg	2	2

[a] Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic factors: ECOG performance status >1, high LDH levels (> 1·5 times upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/ dL) and no nephrectomy.[30]

Table 2. Polymorphisms genotyped and allele frequency found in the patients.

Gene	SNP	Variation	Number patients[a]	Homozygous wild type	Heterozygous	Homozygous variant	MAF[b]
VEGFR2	rs2305948 C>T	V297I	95	78	15	2	0·100
VEGFR2	rs1870377 T>A	Q472H	95	53	34	8	0·263
VEGFR3	rs307826 A>G	T494A	95	80	15	0	0·079
VEGFR3	rs448012 C>G	H890Q	94	32	48	14	0·404
VEGFR3	rs307821 G>T	R1324L	95	78	16	1	0·095
PDGFR-α	rs35597368 T>C	S478P	95	77	16	2	0·105
VEGF-A	rs2010963 G>C	5'UTR	95	47	37	11	0·311
VEGF-A	rs699947 A>C	Promoter	95	27	47	21	0·468
VEGF-A	rs1570360 G>A	Promoter	95	45	41	9	0·311
IL8	rs1126647 A>T	3' UTR	94	35	48	11	0·372
CYP3A4	rs2740574 A>G	Promoter	94	89	5	0	0·027
CYP3A5	rs776746 G>A	Splicing	94	82	12	0	0·064
ABCB1	rs1045642 C>T	I1145I	94	27	51	16	0·441
ABCB1	rs1128503 C>T	G412G	95	36	45	14	0·384
ABCB1	rs2032582 G>T	A893S	92	38	39	15	0·375
ABCG2	rs2231142 C>A	Q141K	95	85	10	0	0·053

[a] Number of patients with a successful genotype.

[b] MAF (Minor Allele Frequency)..

Table 3. Multivariable analyses of response, PFS and OS in renal cancer patients treated with sunitinib.

Factors[a]	PD as best response			PFS			OS		
	HR	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Clinical factors									
MSKCC risk factors[b]	4·14	1·30-13·2	**0·016**	1·64	0·96-2·81	0·072	2·21	1·00-4·41	**0·025**
Sex	3·66	1·37-11·7	**0·030**	1·70	0·91-3·16	0·094	2·80	1·30-6·03	**0·009**
Polymorphisms[c]									
VEGFR3 rs307826	8·79	1·92-40·3	**0·0051**	3·57	1·74-7·29	**0·00049** [d]	1·77	0·65-4·84	0·26
VEGFR3 rs307821	7·14	1·46-35	**0·015**	3·31	1·64-6·68	**0·00085** [d]	1·24	0·41-3·75	0·71
ABCB1 rs1128503	1·25	0·46-3·38	0·67	1·42	0·95-2·12	0·089	1·75	0·99-3·12	0·055
ABCG2 rs2231142	2·96	0·96-9·15	0·99	3·00	0·85-10·5	0·087	1·45	0·42-4·98	0·56
VEGFR2 rs1870377	1·32	0·47-3·70	0·59	1·09	0·68-1·74	0·71	1·74	0·91-3·32	0·092

[a] Only factors with P values < 0·1 are shown.

[b] According to the number of MSKCC prognostic factors patients were classified into favourable, intermediate and poor prognosis groups.

[c] Multivariable analyses for response, PFS and OS include as covariates MSKCC risk groups and sex.

[d] P values that remain statistically significant after Bonferroni correction. Adjusted P values are 0.0078 and 0.014 for rs307826 and rs307821, respectively.

Table 4. Genetic factors associated with sunitinib dose reductions and toxicities.

Genetic factors [a]	HR	95% CI	*P* value
Dose reduction due to toxicity [b]			
CYP3A5 rs776746	3·75	1·67-8·41	**0·0014** [c]
Hypertension			
VEGFR2 rs1870377	2·62	1·32-5·20	**0·0058**
ABCB1 rs1128503	0·41	0·20-0·81	**0·0073**
VEGF-A rs699947	2·43	1·27-4·66	**0·0074**
ABCB1 rs2032582	0·42	0·21-0·84	**0·014**
VEGF-A rs1570360	2·04	1·05-3·96	**0·035**
Hand-foot syndrome			
VEGFR2 rs2305948 [d]	3·94	1·33-11·7	**0·014**
Mucositis			
VEGF-A rs2010963	0·50	0·27-0·95	**0·034**

[a] Only toxicities influenced by a polymorphism with a P value <0·05 are shown.

[b] Cox regression analysis for time of sunitinib treatment until dose reduction, in patients with initial doses of 50 mg sunitinib (n=84). The multivariable analysis includes as covariate sex (this was the only clinical factor with P value <0·1. The results for sex in univariable analysis were HR=2·6, 95%CI=1·3-5·4, P=0·005).

[c] P value that remains statistically significant after Bonferroni correction. Adjusted P value = 0·022.

[d] Multivariable analysis that includes as covariate MSKCC risk groups (this was the only clinical factor with P value <0·1. The results for MSKCC risk groups in univariable analysis were: HR=0·48, 95%CI=0·2-1·0, P=0·063).

FIGURE LEGENDS

Figure 1. Kaplan-Meier comparison for PFS of renal cancer patients treated with sunitinib by rs307826 and rs307821 genotypes in *VEGFR3*. A) Patients grouped according to rs307826. Median PFS for the wild type (AA) and heterozygous (AG) group were 13·7 and 3·6 months, respectively. B) Patients grouped according to rs307821. Median PFS for the wild type (GG) and heterozygous (GT) group were 13·7 and 6·7 months, respectively. The P values shown correspond to the multivariable analysis, unadjusted P values from the univariable log-rank test were 0·00070 and 0·0047 for rs307826 and rs307821, respectively.

Figure 2. Kaplan-Meier comparison for time of sunitinib treatment up to dose reduction due to toxicity, by rs776746 genotype in *CYP3A5*. Patients included in the analysis had a sunitinib initial dose of 50 mg on a 4 weeks on, 2 weeks off schedule (n=84). Groups rs776746 GG and rs776746 AG include 72 and 11 patients, respectively, 1 patient did not have genotype data for this SNP. Unadjusted P value from the log-rank test was 0·0012.

Figure 1

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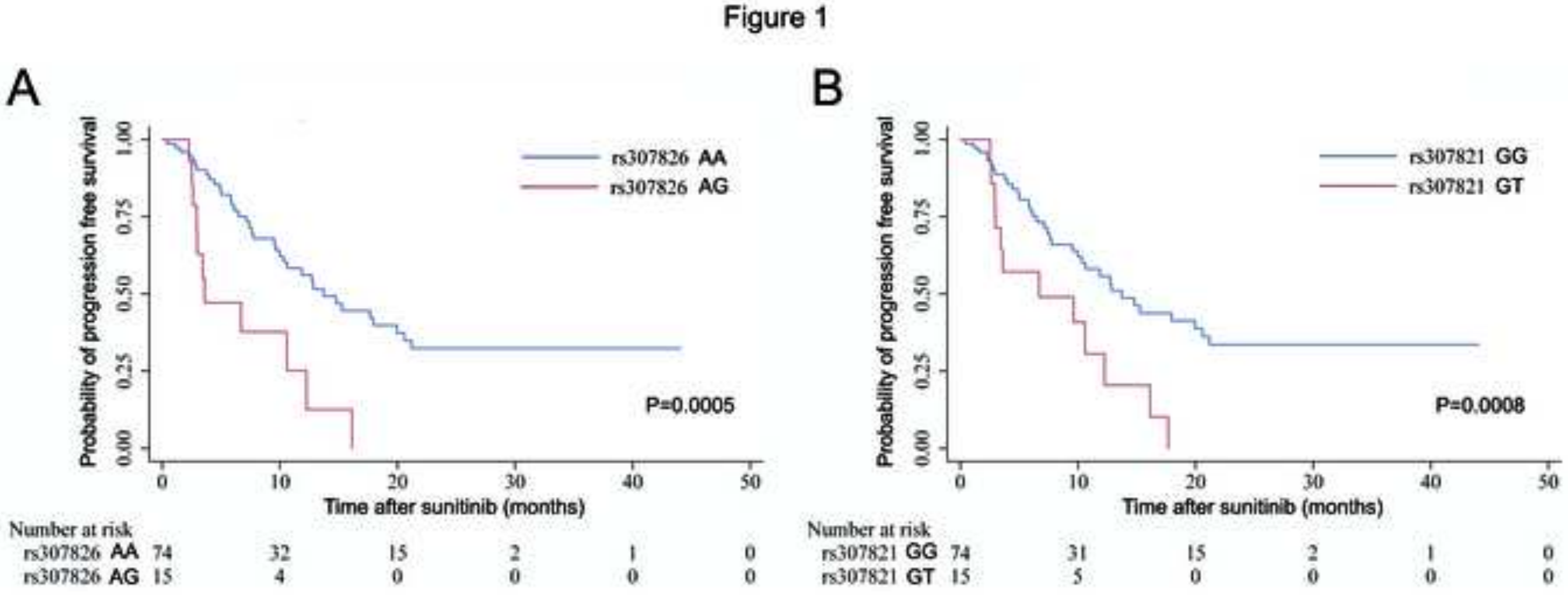

Figure 2

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