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Association of VEGF and VEGFR2 Single Nucleotide Polymorphisms with Hypertension and Clinical Outcome in Metastatic Clear Cell Renal Cell Carcinoma Patients Treated With Sunitinib

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Abstract

PURPOSE—Biomarkers that predict response or toxicity to antiangiogenic therapy are sought to favorably inform the risk/benefit ratio. This study evaluated the association of vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR2) genetic polymorphisms with the development of hypertension (HTN) and clinical outcome in meta-static clear cell renal cell carcinoma (MCCRCC) patients treated with sunitinib.

PATIENT AND METHODS—Sixty-three MCCRCC patients receiving sunitinib (50 mg 4/2) with available blood pressure (BP) data and germline DNA were retrospectively identified. A panel of candidate VEGF and VEGFR2 single nucleotide polymorphisms (SNPs) were evaluated for associations with the development of hypertension and clinical outcome.

RESULTS—VEGF SNP –634 genotype was associated with the prevalence and duration of sunitinib-induced hypertension (as defined by systolic pressure ≥150 mmHg and/or diastolic pressure ≥90 mmHg) in both univariable analysis ($P = .03$ and $.01$, respectively) and multivariable analysis, which adjusted for baseline BP and use of antihypertension medication ($P = .05$ and $.02$, respectively). Patients with the GG genotype were estimated to have a greater likelihood of being hypertensive during treatment compared with patients with the CC genotype (odds ratio of 13.62, 95% confidence interval [CI] 3.71–50.04). No single VEGF or VEGFR SNPs were found to correlate with clinical outcome. However, the combination of VEGF SNP 936 and VEGFR2 SNP 889 were associated with overall survival after adjustment for prognostic risk group ($P = .03$).

CONCLUSIONS—In MCCRCC patients treated with sunitinib, VEGF SNP –634 is associated with hypertension and a combination of VEGF SNP 936 and VEGFR2 SNP 889 genotypes is associated with overall survival.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

Keywords

VEGF; VEGFR2; sunitinib; renal carcinoma; SNPs

INTRODUCTION

Because angiogenesis is central to tumor growth, strategies for inhibiting this pathway have been a focus for drug development.¹ Early success to potentially blunt vascular endothelial growth factor (VEGF) signaling was realized with bevacizumab currently approved for use in the treatment of breast, colorectal, lung, brain, and kidney cancer.¹ Complexity of the VEGF pathway has led to the development of VEGF receptor (VEGFR) targeted tyrosine kinase inhibitors (TKIs) that have rapidly entered the clinical arena because of their efficacy in treating cancers, particularly renal cancer.² The efficacy of these TKIs in inhibiting several receptor tyrosine kinases (RTKs) in the angiogenesis signaling pathway may be responsible for both their efficacy and associated toxicity.³⁻⁵

In the clinical setting there is a wide heterogeneity in therapeutic efficacy and degree of toxicity experienced by patients treated with the TKIs, and thus far, predictors of clinical response and/or toxicity that would aid in maximizing therapeutic index remain largely unknown. In exploring individual susceptibility, genetic variations, specifically single nucleotide polymorphisms (SNPs) that reside within both coding and noncoding regions within VEGF and VEGFR2, have shown promising potential as biomarkers for clinical response or toxicity with VEGF pathway targeted therapy.⁶⁻⁹

Hypertension (HTN) caused by the TKIs has been shown to be dependent on the potency of these inhibitors against VEGFR2,³ and data suggest that host susceptibility, such as preexisting HTN, can also affect the induction of HTN during treatment with these agents. Correlation between HTN and inhibitory effects on VEGFR2 is strengthened by the recent finding that treatment with a specific VEGFR2 antibody in vivo leads to a sustained and rapid increase in blood pressure (BP).¹⁰ Based on this observation it was inferred that VEGF, acting through VEGFR2, can play a critical role in BP control by promoting nitric oxide synthase expression and nitric oxide activity.¹⁰

Thus, given the central role of VEGF and VEGFR2 in angiogenesis as a mechanism for development of HTN, it was hypothesized that SNPs within VEGF and VEGFR2 may provide the genetic basis for the variability in response and HTN seen in patients treated with VEGF pathway inhibitor therapy. This study involved analysis of a panel of 6 VEGF and 2 VEGFR2 SNPs in germline DNA in mRCC patients during sunitinib therapy and their correlation to HTN as well as clinical outcome, in particular, progression free survival (PFS) and overall survival.

MATERIALS AND METHODS

Patients

Patients with metastatic clear cell carcinoma treated with sunitinib (50 mg daily on a 4 weeks on/2 weeks off schedule) from 8/2004 to 9/2008 with available BP data obtained in

clinic were retrospectively identified. Computed tomography (CT) scans were obtained at baseline and on average after every 2 cycles (12 weeks) of therapy according to treating physician discretion. Dose modifications for toxicity were according to treating physician decision. Objective response was recorded per investigator assessment according to the RECIST criteria, version 1.0.¹¹ Patients signed a written, institutional review board-approved informed consent for peripheral blood collection at a single time point during their treatment period.

DNA Extraction from Blood and Tumor Tissues

Genomic DNA from peripheral blood or frozen tissue was isolated using the Puregene Blood DNA Isolation kit (Qiagen, Valencia, Calif) according to the manufacturer's protocol. For DNA extraction from paraffin embedded tissue 5- μ M-thick sections from microdissected tumor was prepared as previously described.¹²

SNP Analysis

A panel of candidate VEGF and VEGFR2 SNPs that has demonstrated an association between VEGF/VEGFR genotype and HTN or overall survival among breast cancer patients treated with the anti-VEGF agent, bevacizumab, has been reported by Schneider et al.⁹ Based on the clinical activity of sunitinib in the management of metastatic clear cell renal cell carcinoma (MCCRCC), and the documented effects of sunitinib on the VEGF/ VEGFR2 signaling pathway, we sought to investigate a possible association between an expanded panel of VEGF/VEGFR SNPs and toxicity and outcome with sunitinib treatment. An overview of the panel of VEGF and VEGFR2 SNPs analyzed in this study with a description of location, reference sequence (rs)-numbers and forward and reverse primers used for polymerase chain reaction (PCR) amplification is outlined in Table 1. PCR-based amplification of the sequences flanking each SNP was performed on samples of genomic DNA derived from peripheral blood samples and available tumor samples. Sequencing results were confirmed by reamplification and resequencing of DNA in the reverse direction. The PCR mix contained, 100 ng genomic DNA, 1.5 mM MgCl₂, 0.625 mM dNTPs, and 1 $\times$ PCR buffer (20 mM TrisHCl, pH 8.4, 50 mM KCl), 0.75 U Taq polymerase, and 0.5 pmol of the forward and reverse primers in a total of 15 μ L. All PCR reactions, before submission to the core sequencing facility, were run on a 6% nondenaturing polyacrylamide gel to check for amplification of a single product at the appropriate size with the aid of a marker ladder (New England Biolabs, Ipswich, Mass). Negative no DNA template controls did not amplify. The amplicons were sequenced using an ABI377 automated sequencer (Applied Biosystems, Foster City, Calif) at the Cleveland Clinic Foundation's Genomic Core at the Lerner Research Institute using either the forward or reverse PCR primer. There were several types of positive controls. These included simply repeating the PCR and sequencing and obtaining the same genotype, or using identical samples that had been previously successfully amplified for other SNPs not reported in this study, or in the case of SNP 889 VEGFR, using different primer sets to amplify the same 889 SNP and getting the same genotype. Sequences derived from the amplified samples were compared with the respective wild-type VEGF or VEGFR2 using LaserGene software (DNASTar, Perkin-Elmer, Foster City, Calif) to identify and characterize the polymorphisms.

Definition of HTN

HTN was defined before data review as systolic blood pressure (SBP) ≥ 150 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg. These cutoff values were chosen based on the following criteria: 1) NCI CTCAE version 3.0 designation of SBP > 150 mmHg as grade I HTN, and 2) improved outcome in metastatic renal cell cancer (RCC) patients with axitinib-related DBP ≥ 90 mm Hg as demonstrated in a prior analysis.¹³ Blood pressure (BP) measurements used were obtained upon patient arrival to an outpatient clinic at baseline and generally on day 28 of each sunitinib cycle. Home BP assessments were not used in the present data analysis.

For this study, 2 HTN variables were analyzed. First, prevalence of HTN on sunitinib therapy was evaluated. Prevalence was defined as a binary indicator of whether or not patients in each VEGF SNP genotype cohort exhibited SBP ≥ 150 and/or DBP ≥ 90 at anytime during the course of therapy. Second, duration of HTN on sunitinib therapy was also evaluated and was defined as the cumulative proportion of the total treatment period that patients in each VEGF SNP genotype cohort exhibited SBP ≥ 150 mm Hg throughout and/or DBP ≥ 90 .

Analysis of VEGF and VEGFR-2 Haplotype

Unphased SNP genotypes were assembled into phased haplotypes and SNPs missing directly observed genotypes were assigned “most likely” genotypes using Phase v2.1.1.^{14,15} Haploview v4.1 software was used to calculate linkage disequilibrium (LD) relationships between the SNPs and to analyze haplotype blocks.¹⁶

Statistical Analysis

The primary endpoints of this investigation were 2 measures of HTN (prevalence and duration, as described above), PFS, and overall survival. PFS was measured from the date sunitinib therapy started to the date of documented progression or death, whichever came first. Overall survival was measured from the start of treatment to death or last follow-up. The panel of SNPs was summarized by the frequency of each genotype and all SNPs were considered in all analyses. However, as the VEGF SNPs -2578 and -2459 were found to be in complete LD ($r^2 = 1$ with the $-2578A$ allele in *cis* with the -2459 18-nt insertion allele), VEGF -2459 was not analyzed independently. Directly observed genotypes obtained from the SNP analysis described above were used for data analysis for most patients. However, in 37% of patients, genotypes could not be determined for 1 or more SNPs (primarily VEGF SNP -1154). In those cases best predicted genotypes derived from the PHASE program were used in the analysis.

The relationship between individual SNPs and the prevalence of HTN was analyzed in univariable analysis using the Cochran-Armitage trend test and Fisher exact test. Linear regression models and proportional hazards models were used to assess their association with the duration of HTN and PFS and overall survival, respectively. The joint effect of multiple SNPs was assessed using logistic (HTN prevalence), linear (HTN duration), and proportional hazards (PFS and overall survival) models. In all cases the models addressing HTN included terms for baseline SBP and DBP and the use of antihypertensive medications;

and the models addressing clinical outcome included a term for risk group status based on the Cleveland Clinic TKI classification system.¹⁷ A backward selection algorithm that initially included all SNPs and used $P = .10$ as the criteria for retention in the model was used to identify which, if any, SNPs or SNP combinations were independent prognostic factors for the outcome being analyzed. No adjustments were made for P values for multiple comparisons, and all data analyses were conducted using SAS version 8 (SAS Inc., Cary, NC).

RESULTS

Patient Characteristics

A total of 63 patients were identified. Patient characteristics are listed in Table 2. This was a typical metastatic renal cell carcinoma (RCC) population of predominantly male patients with a median age of 60 years. All patients had clear cell histology RCC, with 89% of patients having undergone prior nephrectomy and 63% of patients having had prior therapy with either cytokines and/or VEGFR TKIs. 86% of patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Sixty-two percent of patients progressed at a median of 20.6 months (95% confidence interval [CI] 16.3–33.9) and 38% (24/63) have died. Median overall survival has not been reached; however, the estimated 1- and 2-year survival rates were $84\% \pm 5\%$ and $70\% \pm 6\%$, respectively.

Baseline BP characteristics of 63 patients in the study are summarized in Table 2. Median SBPs and DBPs at baseline were 139 mm (range, 93–190 mm) and 80 mm (range, 47–103 mm), respectively; 38% of patients had SBP >150 mm and/or DBP >90 mm. A total of 57% of patients (36 of 63) were being treated with median of 1 (range, 0–4) antihypertensive medications at baseline.

VEGF SNPs and HTN

Table 3 summarizes the frequency and duration of HTN during treatment for each of the analyzed SNPs. Consistent with the results of ECOG study E2100,⁹ there was a significant association between the prevalence of HTN and both VEGF SNPs –634 and –1498 in univariable analysis; with patients having the more favorable genotypes (–634 CC and –1498 TT) having less HTN, $P = .03$ in both cases. In addition, no association was seen between the prevalence of HTN and VEGF SNPs –1154 or 936. In contrast to E2100, however, SNP –2578 was also seen to be significantly associated with HTN; all 11 patients with an AA genotype having HTN compared with 89% (34 of 63) of those with an AC genotype, and 71% (10 of 14) of patients with a CC genotype, $P = .03$. Neither of the VEGFR2 SNPs analyzed was associated with the prevalence of HTN.

In addition to being associated with the prevalence of HTN, VEGF SNP –634, was also associated with HTN duration in univariable analysis. Patients with the CC genotype were hypertensive for a median of 8.9% of the treatment period compared with 10.2% for patients with a CG genotype, and 27.2% for those with a GG genotype, $P = .01$ (Fig. 1). None of the other SNPs analyzed were associated with HTN duration.

To assess the joint impact of the SNP panel on HTN, and to take into account the fact that 38% of patients were considered hypertensive pretreatment and 57% were on antihypertensive medications, multivariable logistic (prevalence), and linear regression (duration) models were assessed. As described in the methods, all 7 SNPs summarized in Table 3 were initially included in the models, as were terms for pretreatment SBP and DBP and concurrent antihypertensive therapy. For the prevalence of HTN after dropping SNPs unrelated to this outcome, VEGF –634 was the only SNP found to have independent prognostic value ($P = .05$). Adjusting for pretreatment SBP and DBP, and antihypertensive use, patients with the less favorable GG genotype were estimated to have an approximately 13- to 14-fold greater likelihood of being hypertensive during treatment compared with patients with the more favorable CC genotype (odds ratio of 13.62; 95% CI, 3.71–50.04), whereas patients with a CG genotype were estimated to have a 3–4-fold greater likelihood (odds ratio, 3.69; 95% CI, 1.01–13.51). VEGF –634 remained significantly associated with the prevalence of HTN after restriction to the subset of patients who were neither hypertensive at baseline nor on antihypertensive medications pre-treatment ($n = 24$, $P = .05$). Small subsets precluded analysis by use or type of antihypertensive medication initiated during sunitinib therapy.

In a similarly conducted analysis VEGF SNP –634 was also the only SNP found to be an independent predictor of the duration of HTN ($P = .02$). Adjusting for the effects of antihypertensive therapy and pretreatment SBP and DBP patients with the GG genotype were estimated to be hypertensive 21% longer on average than patients with the CC genotype (95% CI, 12%–29%). A similar trend was seen in the subset of patients who at pretreatment were normotensive and not taking antihypertensive medications, although the effect was not statistically significant.

Adjusting for the effects of prognostic risk group,¹⁷ none of the SNPs examined either alone or in combination was associated with PFS. However, there was some suggestion that VEGF SNP 936 ($P = .16$) and/or VEGFR SNP 889 ($P = .08$) may be associated with overall survival; patients with 936 CC and 889 GG genotypes having a poorer prognosis. In multivariable analysis, which was conducted in a manner similar to the analyses of HTN, the joint effect of these 2 SNPs was seen to be an independent predictor of overall survival $P = .03$, even after adjusting for the impact of CCF-TKI risk group ($P = .001$). That is, patients who were CC for 936 and GG for 889 were estimated to have an approximately 3-fold greater risk of death than patients with other genotypes (hazard ratio of 3.18; 95% CI, 1.12–8.99) (Fig. 2). This is in contrast to E2100, which found an association between overall survival and VEGF SNPs –2578 and –1154.

VEGF Haplotype Analysis

Linkage disequilibrium plots from analysis of VEGF SNPs in genomic DNA from peripheral blood lymphocytes, paired tumor from patients with lymphocyte DNA and RCC cell lines (13 with clear cell and 1 with papillary histologies) are given in Figure 3. Strong evidence of LD is apparent in lymphocytic DNA, tumor DNA and renal carcinoma cell lines. In addition, independent analysis of the various VEGF SNPs in DNA from lymphocytes, tumor, or cell lines revealed an identical predominant haplotype with

comparable frequency. It is also important to note that SNPs –2578, –2549, –1498, and –634, 3 of which were the SNPs most strongly associated with HTN in univariable analysis, demonstrated strong evidence for linkage disequilibrium; and is the likely reason –2578 and –1498 were not found to be independent prognostic factors in the multivariable analysis.

DISCUSSION

This is the first report identifying VEGF and VEGFR2 SNPs associated with HTN or outcome in MCCRCC patients treated with sunitinib. A key finding is the role of VEGF SNP –634 with treatment-induced HTN as observed here with sunitinib therapy and by Schneider et al⁹ in breast cancer patients treated with bevacizumab. The present analysis suggests VEGF SNP –634 that is potentially involved in translational efficiency of VEGF¹⁸ is an independent predictor of the prevalence and duration of HTN during sunitinib therapy for RCC patients. Further, analysis of –634 (and other SNPs) from tumor-derived DNA showed near 100% concordance with germline DNA genotypes, suggesting that the VEGF genotypes from tumor would correlate similarly to both occurrence and duration of HTN during sunitinib therapy in this patient cohort. VEGF SNP –1498, which was significantly associated with HTN in E2100,⁹ was also associated with HTN in the present study, but only in univariable analysis. This lack of independent prognostic value may be due to several reasons including the strong linkage disequilibrium with –634 seen with genomic DNA analyzed from lymphocytes, renal tumor, and RCC lines, inherent difference in the downstream effect of VEGF and VEGFR inhibition in targeting angiogenesis in different tumor types, effects of small sample size and the retrospective nature of this study with variable timing of BP attainment and management of sunitinib-induced HTN. VEGF SNP –2578, which was not associated with HTN in E2100, was similarly prognostic in the present study in univariable but not multivariable analysis, possibly for the same reasons as described above.

Interestingly, for VEGF SNP –634, a higher proportion of patients (57.1%) with RCC in the current study were G/G compared with 42.4% in patients with nonsmall-cell lung cancer¹⁹ and 44.6% in a control population.¹⁹ The etiology for this discrepancy is unclear. However, 1 hypothesis that may be derived from previous studies is the possible correlation between VEGF polymorphism and the development and extent of RCC. Kawai et al,²⁰ for example, reported in a patient population of Japanese origin with RCC that, although the –634 polymorphism was not found to have a clinical effect, VEGF SNPs –2578 and –1154 were associated with metastases, pathologic tumor stage, and survival. More recently Bruyere et al²¹ reported that –460 VEGF polymorphism was a risk factor for renal cancer in Caucasian subjects. It is difficult to reconcile these results with the present study, however, because their focus was on risk/prognosis for renal cancer and not treatment-related toxicity or outcome. Further, the small sample size of the present cohort makes the estimation of genotype frequency imprecise.

Analysis of VEGF SNP genotypes obtained from available tumor tissue (30 patients with paired lymphocyte DNA) highly correlated (>98%) with those derived from the peripheral blood. This is in agreement with prior studies demonstrating that variability seen in common polymorphic sites is usually inherited,⁹ suggesting that DNA from either peripheral blood or

tumor is useful for analysis of VEGF SNPs associated with response or toxicity to sunitinib therapy in mRCC. The present study did not measure circulating VEGF levels, but the -634 polymorphism has been reported to affect VEGF production¹⁸ and carriers of the -2578C, 460T, and 405 C alleles were found to have significantly higher serum VEGF levels in patients with ovarian cancer.²²

Although individually no single VEGF or VEGFR2 SNPs was associated with overall survival, it was of interest that VEGF 936 and VEGFR2 889 combined did have an impact. Because VEGFR2 SNP 889 is located in the VEGF binding domain, the polymorphism may have a differential effect on VEGF ligand binding and its downstream signaling through VEGFR2. The lack of association with PFS implies that either this genotype is prognostic in RCC and independent of treatment effect, or that the cohort size lacked adequate power to detect a treatment effect. As with several of the hypotheses generated from these data, further prospective testing in a larger cohort, preferably with a control group, either untreated or treated with alternative agents, is required. Although this study did not find any association of VEGFR2 SNP 889 with HTN, van Erp et al²³ reported that the T allele was associated with increased risk for development of any toxicity greater than grade 2. Interestingly, the unfavorable VEGF 936 CC genotype and VEGFR2 889 GG genotype was found in >65% and 78%, respectively, of the RCC cell lines analyzed in this study. Because the effect of sunitinib in inducing HTN is possibly related to inhibition of VEGFR2, and antibodies to VEGFR2 can induce HTN,¹⁰ inhibitory effects of sunitinib on VEGFR2 and circulating VEGF levels affected by VEGF SNP -634 and 936^{18,24} may be important determinants of treatment-induced HTN and/or outcome. This hypothesis requires prospective testing in a larger cohort of patients.

Although results from this study provide evidence on VEGF and VEGFR2 SNPs as potential biomarkers of toxicity and outcome in MCCRCC patients treated with sunitinib, limitations include the retrospective nature of the present study, limited number of patients, and variability in timing of BP as well as tumor measurements between patients. Ideally, it would have been advantageous to have information on continuous monitoring of BP or at least daily monitoring during therapy. Such frequent assessment, however, was not part of the prescribed monitoring schedule for these patients, and is a shortcoming of the study. In addition, because not all patients with MCCRCC treated with sunitinib during this time had blood collected for genotyping (eg, due to disease-related anemia), it is possible that some bias may have been introduced, for example, by implicitly excluding patients who were not genotyped, but who developed severe HTN while on sunitinib. Furthermore, management of HTN was physician-dependent as was sunitinib dose adjustments, both of which will need to be controlled in a uniform manner for prospective validation.

The impact of the VEGF and VEGFR2 SNPs identified in this study to be indicators of toxicity and outcome coupled with pharmacokinetic and pharmacodynamic studies that indicate impact of increased exposure to sunitinib on outcome and adverse events²⁵ or biomarker response to sunitinib²⁶ could provide a rational basis for improving clinical benefit of treatment in MCCRCC patients. The overlap in VEGF SNPs correlated with HTN after bevacizumab or sunitinib treatment suggest that effects on VEGF homeostasis and pharmacogenomics of VEGF/VEGFR2 are possibly involved in adverse toxicity observed

with the combination of bevacizumab plus sunitinib in patients with RCC.^{27–29} In summary, VEGF SNP –634 correlates with HTN and genotypes of VEGF SNP 936, and VEGFR2 889 correlate with overall survival during sunitinib therapy in this cohort of RCC patients. Prospective study in a larger cohort of patients is warranted to validate these findings as well as determine if the VEGF SNPs and VEGFR2 SNPs identified to impact HTN in patients treated with sunitinib are also relevant to treatment with other TKIs used in the clinical management of RCC.

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Figure 1. VEGF SNP –634 G/C (rs2010963) is associated with the duration of hypertension during treatment with sunitinib in patients with MCCRCC; $P = .01$.

Figure 2.

This figure shows overall survival and correlation with VEGF 936 C/T combined with VEGFR2 889 G/A SNPs in MCCRCC patients treated with sunitinib. — All other genotypes (n = 24); ---- VEGF 936 C/C and VEGFR2 G/G (n = 39), $P = .03$.

Table 1

Primers for VEGF and VEGFR2 SNPs

SNP ^a	Location	rs Number	Forward Primer	Reverse Primer
VEGF				
–2578 C/A ^c	Promoter	699947	5'-CTG CAT TCC CAT TCT CAG TC-3'	5'-TGC CCC AGG GAA CAA AGT TG-3'
–2459ins/del18 ^b	Promoter	35569394	5'-CTG CAT TCC CAT TCT CAG TC-3'	5'-TGC CCC AGG GAA CAA AGT TG-3'
–1498 C/T ^c	Promoter	833061	5'-TCT TCG AGA GTG AGG ACG TG-3'	5'-GGG ACA CAC AGA TCT ATT GG-3'
–1154 G/A ^c	Promoter	1570360	5'-CGG GCC AGG CTT CAC TGA GC-3'	5'-CTC CCC GCT ACC AGC CGA CT-3'
–634 G/C ^c	5'UTR	2010963	5'-GCT CCA GAG AGA AGT CGA GG-3'	5'-GGT GTC TGT CTG TCT GTC CG-3'
936 C/T ^c	3'UTR	3025039	5'-ACT CTG CGC AGA GCA CTT TG-3'	5'-TTC CGG GCT CGG TGA TTT AG-3'
VEGFR				
889 G/A ^c	Exon 7	2305948	5'-TCT TGG TCA TCA GCC CAC TG-3'	5'-AAA CCC AGT CTG GGA GTG AG-3'
1416 A/T ^c	Exon 11	1870377	5'-CTG GAA GTC CTC CAC ACT TC-3'	5'-TAC CAT GGT AGG CTG CGT TG-3'

Abbreviation: SNP, single nucleotide polymorphism.

^a Nomenclature as reported in Schneider et al.⁹^b Identical primers were used for PCR and sequence analysis for SNP-2459 and –2578. These SNPs are 119 bp apart. The size of the PCR product that harbors these 2 SNPs is 143 bp.^c The Hardy Weinberg P values for VEGF SNPs rs699947, rs833061, rs1570360, rs2010963, and rs3025039 were 0.06, 0.61, 0.10, 0.20, and 0.76, respectively; Hardy Weinberg P values for VEGFR2 SNPs rs2305948, and rs1870377 were 0.34 and 0.68, respectively.

Table 2

Patient Characteristics (N = 63)

Characteristic	N (%)
Gender	
Male	49 (78)
Female	14 (22)
Age (y)	
Median	60
Range	35–80
Histology	
Clear cell	63 (100)
Prior nephrectomy	
Yes	56 (89)
No. of systemic therapies	
0	23 (37)
1	24 (38)
2	16 (25)
Prior therapy	
Cytokines	30 (48)
TKIs	15 (24)
ECOG PS^a	
0	42 (67)
1	12 (19)
2	1 (2)
CCF TKI risk group	
Favorable	23 (37)
Intermediate	18 (29)
Unfavorable	13 (21)
Unknown	9 (14)
Tumor reduction (%)	
Median	30.0
Range	–35–100
PFS (mo)	
Median	20.4
Baseline SBP (mmHg)	
Median	139
Range	93–190
Baseline SBP >150 mmHg	18 (29)
Baseline DBP (mmHg)	
Median	80
Range	47–103
Baseline DBP >90 mmHg	13 (21)

Characteristic	N (%)
Baseline SBP >150 and/or DBP >90 mmHg	24 (38)
On Anti-HTN med(s) at baseline	
Yes	36 (57)

Abbreviations: CCF TKI, Cleveland Clinic Foundation tyrosine kinase inhibitor; DBP, diastolic blood pressure; ECOG PS, Eastern Cooperative Oncology Group performance status; HTN, hypertension; Med(s), medications; PFS, progression free survival; SBP, systolic blood pressure.

Table 3
VEGF and VEGFR2 SNPs and the Frequency and Duration of Hypertension During Treatment

SNP/Genotype ^a	N	N (%)	<i>p</i> ^b	Frequency of HTN During Treatment	Median	Range	<i>p</i> ^c
-2578							
AA	11	11 (100%)			33.8%	2.6%–100%	
CA	38	34 (89%)			15.3%	0%–74.8%	
CC	14	10 (71%)	.03		11.1%	0%–100%	.22
-1498							
CC	14	14 (100%)			19.8%	2.6%–100%	
TC	34	30 (88%)			17.0%	0%–74.8%	
TT	15	11 (73%)	.03		11.6%	0%–100%	.31
-1154							
AA	35	32 (91%)			20.4%	0%–100%	
AG/GA	26	22 (85%)			12.2%	0%–85.4%	
GG	2	1 (50%)	.14/.45^{d,e}		23.4%	0%–46.8%	.87/.88^e
-634							
GG	36	34 (94%)			27.2%	0%–100%	
CG/GC	21	17 (81%)			10.2%	0%–71.6%	
CC	6	4 (67%)	.03		8.9%	0%–28.1%	.01
936							
TT	1	1 (100%)			85.4%	—	
CT/TC	12	11 (92%)			21.3%	0%–71.3%	
CC	50	43 (86%)	.52/.10^{d,f}		14.0%	0%–100%	.27/.61^f
889							
GG	51	45 (88%)			14.3%	0–100%	
GA	12	10 (83%)	.14^d		21.3%	0–100%	.44
1416							
TT	3	3 (100%)			2.7%	0–42.4%	
AT	18	15 (83%)			12.5%	0–100%	
AA	42	37 (88%)	.98/.29^{d,g}		21.4%	0–100%	.30/.34^g

Abbreviations: HTN, hypertension; SNP, single nucleotide polymorphism.

^aPredicted genotype from Haploview used if missing: -2578, n = 3; -1498, n = 0; -1154, n = 17; -634, n = 0; -936, n = 7; 889, n = 7; 1416, n = 8.

^bCochran-Armitage trend test unless otherwise noted.

^cFrom linear regression model with the specified SNP as the only independent variable unless otherwise noted.

^dFisher's exact test.

^eSecond P value compares AA to other genotypes.

^fSecond P value compares CC to other genotypes.

^gSecond P value compares AA to other genotypes.