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Review

Clinical Cancer Research

Molecular Biomarkers in Advanced Renal Cell Carcinoma

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Abstract

The availability of agents directly targeting tumorigenic and angiogenic pathways has significantly improved the outcomes of patients with advanced renal cell carcinoma (RCC) in recent years. However, all patients eventually become resistant and a substantial percentage experience immediate disease progression with first-line targeted therapy. In addition, patients have variable clinical benefit and/or tolerance to different agents, including drugs within the same class. Thus, the choice of therapy for an individual patient remains empiric at present. Upon this landscape, several molecular biomarkers have been investigated with the purpose of guiding therapy. This review discusses prognostic biomarkers correlating with the outcome of patients independent of therapy, and predictive biomarkers of treatment response, including circulating biomarkers (such as VEGF and VEGF-related proteins, cytokine and angiogenic factors, and lactate dehydrogenase), and tissue-based biomarkers (such as single-nucleotide polymorphisms). Many potential prognostic and predictive molecular biomarkers have now been identified in RCC, although none has yet entered into clinical practice, and all require prospective validation in appropriately designed randomized studies. In the near future, however, validated biomarkers may become integral to management strategies in RCC, enabling tailored treatment for individual patients to improve clinical outcomes. *Clin Cancer Res; 20(8); 2060–71. ©2014 AACR.*

Introduction

Therapy for advanced renal cell carcinoma (RCC) has been dramatically changed by agents directly targeting tumorigenic and angiogenic pathways. First-line treatments, including sunitinib, pazopanib, temsirolimus, and bevacizumab plus IFN-α, and second-line options such as axitinib, sorafenib, and everolimus, are associated with substantial improvements in median progression-free survival (PFS; refs. 1–6, 7). Several biomarkers have been identified or are under investigation to better select patients for specific treatments (8). Prognostic biomarkers predict clinical outcomes independent of therapy and predictive biomarkers can be used to optimize treatment selection (9), either from baseline (static markers)—in terms of the likelihood of response or toxicity—or during therapy, as an ongoing marker of treatment response (dynamic markers). Predictive biomarkers of response (or toxicity) are markers that are associated with clinical benefit (or toxicity), and may be followed during treatment.

This review discusses prognostic and predictive biomarkers of response and toxicity under investigation in patients with advanced RCC, and their potential implications for guiding therapy.

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Prognostic and/or Predictive Biomarkers

Molecular biomarkers can be grouped according to their physiologic location; circulating biomarkers include VEGF and VEGF-related proteins, cytokine and angiogenic factors (CAF), circulating endothelial cells (CEC), and lactate dehydrogenase (LDH). Tissue-based biomarkers include single-nucleotide polymorphisms (SNP) and biomarkers related to the von Hippel–Lindau (VHL) and mammalian target of rapamycin (mTOR) pathways. Tables 1 and 2 summarize circulating and tissue-based predictive biomarkers and observations with respect to clinical outcome in patients with advanced RCC (4, 10–28).

Circulating biomarkers

VEGF and VEGF-related proteins. VEGF proteins regulate vascular and lymphatic function (29). There are five mammalian VEGF ligands and three primary VEGF receptor tyrosine kinases (VEGR-1, -2, and –3; ref. 29). Ligand binding to specific VEGF receptors (VEGFR) results in receptor dimerization and signal transduction. The most studied ligand, VEGF-A (hereafter referred to as VEGF), encodes an endothelial mitogen that has numerous roles, including inducing angiogenesis, vasculogenesis, and endothelial cell growth; increasing vascular permeability; promoting cell migration; and inhibiting apoptosis (29). VEGF is persistently upregulated in clear-cell RCC due to inherent *VHL* tumor suppressor gene inactivation and drives tumor angiogenesis, facilitating tumor growth and metastasis (30).

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Table 1. Summary of molecular biomarker status for predicting response in RCC (4, 10–21)

Biomarker	Associated outcomes	Comments
Circulating biomarkers		
VEGF and VEGF-related proteins		
Elevated baseline VEGF	Elevated pretreatment VEGF (>median) associated with trend to prolonged PFS with sorafenib compared with placebo (5.5 vs. 2.7 mo with placebo; HR, 0.48, 95% CI, 0.38–0.62) than low pretreatment VEGF (≤median; 5.5 vs. 3.3 mo with placebo; HR, 0.64; 95% CI, 0.49–0.83; *P* for interaction between VEGF and treatment arm = 0.096; *n* = 712; ref. 10)	VEGF levels correlate inversely with PFS and OS in RCC but higher baseline VEGF levels may be associated with better clinical outcome with sorafenib therapy
Low baseline VEGFR-3	Increased PFS (21.7 vs. 10.9 mo; HR, 2.40; *P* = 0.01) and OS (NR vs. 23.3 mo; HR, 1.68; *P* = 0.07) in sunitinib recipients with low baseline sVEGFR-3 (*n* = 33; ref. 11)	Low baseline sVEGFR-3 and VEGF-C levels may predict improved outcome following sunitinib treatment
Low baseline VEGFR-3 and VEGF-C	Longer PFS in patients with sunitinib in bevacizumab-refractory mRCC with VEGFR-3 <median versus >median (36.7 vs. 19.4 wks; HR, 0.4457; *P* = 0.0060) and VEGF-C <median versus >median (46.1 vs. 21.9 wks; HR, 0.3662; *P* = 0.0006), and significantly lower baseline VEGFR-3 and VEGF-C in patients with PR compared with those with SD or PD (*n* = 59; ref. 4)	
VEGF, sVEGFR-2, and sVEGFR-3	Larger changes over first 28 days of treatment in VEGF (*P* = 0.0001), sVEGFR-2 (*P* = 0.0003), and sVEGFR-3 (*P* = 0.042) levels in sunitinib recipients with objective response versus those with SD or PD (*n* = 63; ref. 12) On-treatment reduction in sVEGFR-2 levels did not correlate with PFS in sunitinib-treated patients (*n* = 26; ref. 13) On-treatment increase in VEGF was greater in patients with PD than in those with clinical benefit during sunitinib therapy (*n* = 39; ref. 14)	Sunitinib inhibition of VEGF signaling via receptor blockade results in modulation of plasma levels of circulating VEGF proteins. The association of degree of modulation with clinical outcome is unclear
CAFs		
Elevated baseline IL-6	Increased PFS with pazopanib versus placebo (HR, 0.32 in the high IL-6 group and 0.57 in the low IL-6 group; *P* value for interaction = 0.009; *n* = 344; ref. 15)	Only IL-6 was predictive of PFS benefit of the CAFs evaluated; IL-6 was both a prognostic marker and a predictive marker for pazopanib therapy
LDH		
Elevated serum LDH	Increased OS in temsirolimus versus IFN-α recipients in patients with an elevated baseline LDH (>ULN; 6.9 vs. 4.2 mo; *P* < 0.002; *n* = 404; ref. 16)	LDH is a known prognostic marker in RCC. Baseline serum LDH is a potential predictive biomarker for OS in patients with poor-risk RCC treated with temsirolimus
Tissue-based biomarkers		
VHL pathway		
VHL mutation	No association with clinical outcome to VEGF-targeting agents (*n* = 123; ref. 17)	
VHL and *c-myc* combination	Elevated c-myc activity and enhanced proliferation found (*in vivo*) in pVHL-deficient tumors expressing HIF-2α (*n* = 160; ref. 18)	pVHL status, HIF-α, and c-myc expression may have value as predictive biomarkers of response to targeted therapy in RCC

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Table 1. Summary of molecular biomarker status for predicting response in RCC (4, 10–21) (Cont'd)

Biomarker	Associated outcomes	Comments
	Increased PFS in sunitinib recipients with c-myc negative versus c-myc–positive primary tumors (median PFS, 11.4 vs. 5.4 mo; $P = 0.0062$; $n = 58$; ref. 19)	
mTOR pathway		
Elevated phospho-S6 expression	Increased ORR ($P = 0.02$) in patients treated with temsirolimus. No patient ($n = 20$) without high expression of phospho-S6 experienced an ORR (20)	This was a very small study that has not been replicated
Elevated pAKT expression	With every percentage increase in pAKT, decreases in PFS (HR, 1.04; $P = 0.0411$) and OS (HR, 1.15; $P = 0.0173$) were observed in sorafenib (±IFN-α) recipients ($n = 40$; ref. 21)	pAKT expression is a potential prognostic factor which may affect survival through angiogenic pathways

Abbreviations: NR, not reached; PD, progressive disease; PR, partial response; pVHL, von Hippel Lindau protein; s, soluble; SD, stable disease.

VEGF as a prognostic factor. Data from a randomized, placebo-controlled phase III study of sorafenib in previously treated patients with metastatic RCC (mRCC) suggested that baseline VEGF is an independent prognostic factor for PFS and overall survival (OS) in placebo-treated patients (10). This prognostic value was preserved in multivariate analyses. In addition, a small subset analysis of circulating protein biomarkers from the phase III sunitinib versus IFN-α study suggested that high baseline VEGF correlated with poor PFS and OS in both treatment arms (11). Hypoxia-inducible factor (HIF)-1α and -2α regulate VEGF expression, and HIF-1α expression has been shown in one series to be an independent prognostic factor for OS in a subset of patients with mRCC, although further investigation is required (31).

VEGF and related proteins as predictive biomarkers. High pretreatment VEGF was associated with a trend toward longer PFS in patients treated with sorafenib compared with placebo (5.5 vs. 2.7 months) than in patients with low pretreatment VEGF (5.5 vs. 3.3 months; *P* for interaction between VEGF and treatment arm = 0.096; refs. 10, 32). However, changes in VEGF or in soluble VEGFR-2 concentrations from baseline to week 3 or 12 of sorafenib did not predict PFS, somewhat conflicting with the notion that the effect of sorafenib on VEGF is the main mediator of this observation. With regard to the predictive value of on-treatment changes in VEGF, a separate study (12) found that patients with an objective response to sunitinib had significantly larger fluctuations in VEGF ($P = 0.0005$), soluble VEGFR-2 ($P = 0.0003$), and soluble VEGFR-3 ($P = 0.010$) than those without a response. However, smaller studies of sunitinib failed to demonstrate a correlation between reduction in soluble VEGFR-2 concentration on treatment and PFS (13). In addition, low baseline soluble VEGFR-3 and VEGF-C levels were significantly associated with longer PFS following sunitinib in the phase III sunitinib versus IFN-α study (11) and in patients achieving an objective response in a phase II study of sunitinib in patients with bevacizumab-refractory mRCC (4).

Of note, sunitinib-induced dose-dependent and reversible increases in circulating plasma VEGF have been observed in nontumor-bearing mice (33) and in healthy humans (34); such nontumor-induced increases in VEGF (and potentially VEGF-related proteins) may mask differences attributable to tumor-induced protein changes in responding versus nonresponding patients. Larger studies are required and the influence of previous treatments and of different VEGF detection methods needs to be carefully considered when assessing VEGF/VEGFR biomarker studies (33, 35, 36). Importantly, some angiogenesis inhibitors may interfere with VEGF detection; for example, bevacizumab, a monoclonal antibody that binds human VEGF, can limit the ability of ELISA-based methods to detect VEGF (35, 36).

CAFs. As tumor angiogenesis is regulated by an array of pro- and antiangiogenic factors, blockade of angiogenesis with agents that inhibit the VEGF pathway can also affect their balance. As such, baseline levels of CAFs have been studied. Of note, however, a recent study examined current multiplex assays for cytokine detection, and found that these assays vary in their ability to measure serum and/or plasma concentrations of cytokines, and that reproducibility over an extended time frame or among multiple laboratories may be limited (37). This again highlights the need for standardized methodology and may explain some of the inconsistencies in current data on CAFs.

In a retrospective analysis of a small, randomized, phase II study comparing first-line sorafenib with sorafenib plus IFN-α in advanced RCC (38), evaluation of multiple CAFs identified two distinct patient groups: one with elevated proangiogenic and hypoxia-regulated factors and the other group with elevated levels of interleukins and proinflammatory mediators. PFS benefit with sorafenib correlated

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Table 2. Summary of SNPs associated with response and toxicity in RCC (22–28)

Biomarker	Associated outcomes	Data corrected for multiple testing?	Comments
SNPs associated with response			
CYP3A5 6986 AG or AA versus GG	Increased PFS in sunitinib recipients (HR, 0.27; $P = 0.032$; $n = 136$; ref. 22)	No	Polymorphisms that influence sunitinib drug levels could affect clinical outcome
COMT V158M Met/Val versus Met/Met and Val/Val	PFS and OS differ depending on COMT V158M polymorphism in sunitinib recipients: Met/Val: PFS = 15 mo, OS = 17.2 mo; Val/Val: PFS = 3.3 mo, OS = 4.4 mo; Met/Met: PFS and OS, NR; $P = 0.005$ (PFS) and $P = 0.003$ (OS; $n = 30$; ref. 23)	Not stated	Specific COMT V158M polymorphisms seem to be genetic markers of efficacy in sunitinib recipients with mRCC
VEGFA 634GG versus CC or CG; *VEGFA* 1498 CC versus CT versus TT; *VEGFA* 2578 AA versus AC versus CC	Decreased ORR in pazopanib recipients ($P = 0.03$ for *VEGFA* 634GG vs. CC or CG; $P = 0.02$ for *VEGFA* 1498 CC vs. CT vs. TT; $P = 0.02$ for *VEGFA* 2578 AA vs. AC vs. CC; $n = 397$; ref. 24)	No	Predictive for RR but not associated with PFS. The −2578 and −1498 alleles are associated with increased expression and therefore reduced response to pazopanib
VEGFR2 1718 T versus A	Increased OS in sunitinib recipients with an A allele (16.3 vs. 9.4 mo; HR, 2.9; $P = 0.016$; $n = 136$; ref. 22)	No	No effect on PFS; therefore, may be a prognostic rather than predictive factor. Prospective validation in patients not treated with sunitinib is required
VEGFR3 1323 GG versus AT	Decreased PFS in sunitinib recipients with either of two missense polymorphisms (rs307826: HR, 3.57; $P = 0.00049$; and rs307821: HR, 3.31; $P = 0.014$; $n = 89$; ref. 25)	Yes	VEGFR-3 pathway alterations may play a role in sunitinib efficacy
IL-8 2767 TT versus AA; *IL-8* 251 AA versus TT	Decreased PFS in patients treated with pazopanib but not placebo ($P = 0.009$ for *IL-8* 2767 TT vs. AA; $P = 0.01$ for *IL-8* 251 AA vs. TT; $n = 397$; ref. 24)	No	*IL-8* may drive an alternative proangiogenesis pathway in the presence of VEGF blockade promoting resistance
HIF1A 1790 AG versus GG	Decreased ORR in patients treated with pazopanib ($P = 0.02$); decreased PFS in patients treated with pazopanib but not placebo ($P = 0.03$; $n = 397$; ref. 24)	No	*HIF1A* is a high-activity variant allele possibly associated with increased angiogenesis capability and therefore reduced response to pazopanib
NR1I2 CC versus CT or TT	Increased ORR in patients treated with pazopanib ($P = 0.03$; $n = 397$; ref. 24)	No	The *NR1I2T* allele may increase pazopanib clearance, reducing systematic exposure via CYP3A4
Absence of *NR1I3* CAT haplotype; presence of *ABCB1* TCG haplotype	Increased PFS in sunitinib recipients: absence of *NR1I3* CAT haplotype: 13.3 vs. 8.0 mo; HR, 1.8; $P = 0.017$; presence of *ABCB1* TCG haplotype: 15.2 vs. 8.4 mo; HR, 0.5; $P = 0.033$; $n = 136$; ref. 22)	No	*NR1I3* CAT predictive for outcome of sunitinib therapy via regulation of CYP3A4 *ABCB1* TCG haplotype predictive for improved PFS via reduced sunitinib efflux
SNPs associated with toxicity			
CYP1A1 2455 G versus A	Increased leucopenia (6.2-fold higher risk; $P = 0.029$) and mucosal inflammation (4.0-fold higher risk; $P = 0.021$) in sunitinib recipients when the G allele was present in *CYP1A1* 2455A/G ($n = 219$; ref. 26)	No	A relationship between the development of sunitinib toxicity and polymorphisms in specific genes encoding for metabolizing enzymes, efflux transporters, and drug targets is suggested in this exploratory study

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Table 2. Summary of SNPs associated with response and toxicity in RCC (22–28) (Cont'd)

Biomarker	Associated outcomes	Data corrected for multiple testing?	Comments
CYP1A2 and CYP2C19	Increased risk of dose reductions due to toxicity in sunitinib recipients: with CYP1A2, median time to dose reduction: 2.33 versus NR; $P < 0.006$; with CYP2C19, median time to dose reduction: 2.8 vs. 9.73 mo; $P < 0.021$; $n = 30$; ref. 23	Not stated	*CYP1A2* and *CYP2C19* SNPs may be associated with toxicity in patients with RCC treated with sunitinib (preliminary analysis)
FLT3 738 CT or CC versus TT	Decreased leucopenia (2.8-fold reduction in risk; $P = 0.008$) in sunitinib recipients ($n = 219$; ref. 26); less reduction in thrombocyte counts in sunitinib recipients (TT vs. CT/CC): mean thrombocyte count ratios after 4 weeks of sunitinib: 0.54 versus 0.65; $P = 0.024$ ($n = 193$; ref. 27)	No (26) Not stated (27)	The FLT-3 738C allele may have a protective effect against sunitinib-induced thrombocytopenia. Together with the findings relating to leucopenia, the FLT-3 738C4T polymorphism seems to have a role in sunitinib-induced bone marrow toxicities
VEGFR2 1191CT or TT versus CC	Increased toxicity (grade >2; 2.4-fold higher risk; $P = 0.046$) in sunitinib recipients ($n = 219$; ref. 26)	No	
Absence of NR1I3 CAG haplotype	Increased leucopenia (1.7-fold higher risk; $P = 0.041$) in sunitinib recipients ($n = 219$; ref. 26)	No	
Absence of ABCB1 TTT haplotype	Decreased hand–foot syndrome (2.6-fold lower risk; $P = 0.035$) in sunitinib recipients ($n = 219$; ref. 26)	No	
Presence of 1 or 2 copies of ABCG2 TT haplotype	Increased toxicity (grade >2; 2.6-fold higher risk; $P = 0.016$) in sunitinib recipients ($n = 219$; ref. 26)	No	
VEGR 634GG versus CC or CG	Greater likelihood of hypertension in sunitinib recipients (OR, 13.62; $n = 63$; ref. 28)	No	Of a panel of VEGF and VEGFR-2 SNPs, VEGF 634 was associated with the development of hypertension in patients with mRCC receiving sunitinib
CYP3A5 6986A GG versus AG	Increased length of time on sunitinib before requiring dose adjustment due to toxicity (HR, 3.75; $P = 0.022$; $n = 95$; ref. 25)	Yes	One functional polymorphism in *CYP3A5* may result in increased production of the active metabolite, SU12662, which has a longer half-life than sunitinib and could lead to toxic effects

Abbreviation: NR, not reached.

with a candidate baseline 6-marker CAF proangiogenic signature [osteopontin (OPN), VEGF, carbonic anhydrase IX (CAIX), collagen IV, VEGFR-2, and TRAIL; HR, 2.25]; patients negative for the signature had a 5-fold reduction in PFS benefit (HR, 0.20; $P = 0.0002$).

In patients treated with pazopanib in a phase II study ($n = 215$), those with higher levels of E-selectin and lower levels of interleukin (IL)-6 and hepatocyte growth factor (HGF) seemed to have a longer median PFS (83.9 weeks) than the overall study population (39.3 weeks; $P = 0.0016$; ref. 39). A 7-factor signature (IL-6, IL-8, HGF, OPN, TIMP-1, VEGF, and E-selectin) correlated with outcome in a similar analysis in pazopanib-treated patients in the phase III trial (vs. placebo). However, as a high versus low expression of the signature was associated with differing outcomes in both the placebo (median PFS: 11 vs. 24 weeks, respectively; $P = 0.001$) and pazopanib arms (25 vs. 48 weeks, respectively; $P = 0.001$), prognostic versus predictive CAFs could not be clearly separated (40). In a further analysis from this trial, after adjusting for two clinical variables predictive of short PFS [hemoglobin <lower limit of normal and neutrophils >upper limit of normal (ULN)], only OPN and IL-6 in the placebo arm, and OPN alone in the pazopanib arm, were prognostic (41).

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In a separate analysis from the phase III pazopanib trial, high IL-6 levels were predictive of improved PFS with pazopanib compared with placebo ($P = 0.009$; ref. 39). In addition, high concentrations of IL-8, OPN, HGF, and TIMP-1 were significantly associated with shorter PFS on pazopanib. Strong prognostic markers of shorter PFS in placebo recipients were high levels of IL-6 ($P < 0.0001$), IL-8 ($P = 0.002$), and OPN ($P < 0.0001$; ref. 39). These analyses of the phase III pazopanib trial are overlapping, but they point to IL-6 as potentially both adversely prognostic in placebo-treated patients and predictive of pazopanib benefit in RCC.

CECs and progenitors. Increased levels of CECs and circulating endothelial progenitors (CEP) are normally associated with vascular injury, repair, and neovascularization. Several studies have also demonstrated their contribution to tumor vascularization. Given these findings, their utility as prognostic biomarkers is being examined (42). In one study, CEPs were elevated in RCC, but not in patients with VHL syndrome without RCC (43). The role of CECs and CEPs in prognosis or as markers of treatment efficacy in RCC remains to be established.

LDH. Currently, LDH is more widely recognized as a prognostic biomarker, being one of the five specific risk factors by which prognosis is evaluated within the Memorial Sloan-Kettering Cancer Center (MSKCC) classification system (44). LDH is regulated by the phosphoinositide 3-kinase (PI3K)/AKT pathway and tumor hypoxia/necrosis (45). In the phase III trial of temsirolimus versus IFN-α, the group of patients with high LDH at baseline (>1 × ULN) had an HR for OS of 0.56 ($P = 0.002$), with a median survival time of 6.9 months for patients treated with temsirolimus versus 4.2 months for patients treated with IFN-α. A beneficial effect of temsirolimus on OS versus IFN-α was not observed in patients with LDH ≤1 × ULN (HR, 0.90; $P = 0.51$; OS 11.7 months for temsirolimus compared with 10.4 months for IFN; ref. 16). These data support a prognostic and potentially predictive role of baseline LDH for mTOR inhibitors in RCC. The biologic assumption is that LDH elevation connotes a greater activation of the mTOR pathway in these tumors and thus enhanced clinical effect with mTOR inhibition. However, a decline in LDH on therapy was only associated with improved outcome in the IFN arm, not in the temsirolimus arm, refuting the hypothesis that temsirolimus-induced reductions in baseline high LDH/mTOR-driven tumors are responsible for the observed improved OS. In the phase III trial of sunitinib versus IFN-α, elevated serum LDH was an independent predictor of poor PFS [HR, 1.575; 95% confidence interval (CI), 1.166–2.129; $P = 0.003$] and OS (HR, 2.009; 95% CI, 1.540–2.621; $P < 0.001$) in the sunitinib group (46). An independent association between LDH and clinical outcome was also seen in the IFN-α group, suggesting that this observation was related to the prognostic role of LDH in RCC.

Tumor Tissue Biomarkers

Predictive factors. *VEGF and VEGF-related proteins.* Pretreatment tumor expression of VEGFR-2 (either moderate or strong immunohistochemical staining intensity in >10% of tumor cells) has been independently associated with increased PFS in 40 patients with advanced RCC treated with sunitinib (HR, 2.91; 95% CI, 1.15–7.41; $P = 0.0025$; ref. 47). A small exploratory study of primary tumors from 23 patients with mRCC also found that pretreatment gene expression of *VEGF* isoforms $VEGF_{121}$ and $VEGF_{165}$ was associated with response to sunitinib at 3 months ($P = 0.04$ for both; ref. 48). These data from small, retrospective series are not definitive in providing insight into the role of tissue VEGF expression and clinical outcome in RCC, and further study is required.

VHL gene status. Data on somatic *VHL* mutation events and their impact on prognosis has been variable, due in part to small sample-size studies, variations in factors such as treatment regimen and stage of tumors analyzed, and a lack of understanding of how *VHL* mutations may impact other pathways, for example HIF regulation (49). The first study to demonstrate an association of *VHL* changes with a prognostic factor linked *VHL* alteration (mutation or hypermethylation) to a poor risk factor (pT3 tumor stage; $P = 0.009$; ref. 50). However, many other studies have not found correlations between *VHL* mutational status and common clinical prognostic factors (51–53). In a study of 123 patients with mRCC treated with VEGFR-targeting agents (49% of whom had *VHL* mutations), there was no significant association between *VHL* inactivation and either objective response rate (ORR) or PFS. The authors hypothesized which specific *VHL* mutations would be "loss of function" mutations and, while patients with these mutations had a greater ORR, these data can be considered hypothesis generating only, as there was no functional testing of *VHL* mutations (17). Furthermore, no association was found between *VHL* mutation status and clinical outcomes in patients treated with pazopanib (54). Given the nearly universal presence of *VHL* mutations in clear-cell RCC, it may be that VHL status is not sufficiently differentiating to be associated with clinical outcome. Several mutations present in RCC have recently been described including *polybromo-1 (PBRM1)* gene in 40% of RCC cases, *BAP-1* in 15%, and SETD2 in 10% of cases (55–58). These genes are also located on chromosome 3p and initial studies largely in localized disease have identified prognostic relevance. *BAP-1* and *PBRM1* mutations seem to be mutually exclusive, with *BAP-1* mutation conferring a worse prognosis than *PBRM1* mutation (59). A retrospective analysis of 145 patients with primary clear-cell RCC showed a median OS of 4.6 years versus 10.6 years for patients with *BAP-1* and *PBRM1* mutations, respectively (HR, 2.7; 95% CI, 0.99–7.6; $P = 0.044$); a similar risk ratio was shown using data from a second independent cohort ($n = 327$) from The Cancer Genome Atlas (58, 59). No predictive data about these mutations are currently available. Clearly, however, genes located on the short arm of chromosome 3 are integral to the biology of RCC.

Carbonic anhydrase IX. Carbonic anhydrase IX (CAIX) is implicated in regulating cell proliferation in response to hypoxia and is upregulated in approximately 70% of renal

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tumors (60). The predictive value of CAIX was assessed in treatment-refractory patients receiving sorafenib or placebo in the phase III TARGET trial (61). Despite suggestive retrospective evidence (60), data from the TARGET study did not find CAIX expression to be of predictive or prognostic value in patients with mRCC treated with sorafenib. Similarly, there was no association between CAIX expression and clinical outcomes in patients treated with temsirolimus (20).

Marker combinations: VHL and c-myc. An *in vivo* analysis of *VHL* genotype and HIF-α expression in primary clear-cell RCC tumors defined three subgroups of patients with differential HIF-1α and HIF-2α expression: those who express both HIF-1α and HIF-2α, those who do not express either, and a third group of patients expressing only HIF-2α. p*VHL*-deficient clear-cell RCC tumors expressing only HIF-2α showed elevated c-myc activity, resulting in enhanced proliferation. Patients whose RCC tumors express HIF-2α alone may, therefore, be uniquely resistant to current targeted therapies, although this hypothesis has not been tested prospectively (18). Consistent with the *in vivo* analysis described above, expression of c-myc correlated with outcome in patients with mRCC ($n = 80$) treated with sunitinib in a recent observational prospective study. Median PFS was 5.4 months versus 11.4 months in patients with c-myc–positive versus c-myc–negative primary tumors, respectively (HR, 2.54; $P = 0.0062$; ref. 19).

Predictive factors of response to mTOR inhibitors

Temsirolimus and everolimus are derivatives of rapamycin and are primarily allosteric inhibitors of mTORC1 function. In a small, retrospective analysis of a phase II trial of patients with advanced RCC treated with temsirolimus, objective response to therapy was associated with elevated phospho-S6 expression ($P = 0.02$) and possibly pAKT expression ($P = 0.07$; ref. 20). However, because of the small sample size, these correlations should be regarded as hypothesis generating.

One small study suggested that *KRAS* mutations have been associated with lack of response to everolimus. In a mutational analysis of patients with cancer who had received everolimus in phase I and II studies, the presence of *KRAS* mutations was associated with a significant reduction in clinical benefit from everolimus; of 12 patients with *KRAS* mutant tumors, 11 (92%) experienced disease progression as their best response. In comparison, only 16 of 31 (52%) patients with wild-type *KRAS* tumors experienced disease progression as their best response ($P = 0.0171$; ref. 62).

SNPs

SNPs are the most common type of genetic variation and occur throughout an individual's DNA. Germline SNPs occur in a proportion of the population, although single nucleotide variation can also be acquired during tumorigenesis. Genome-wide association studies can be used to identify germline polymorphisms that are associated with clinical outcome.

SNPs as predictors of efficacy. Several studies have investigated SNPs in specific genes involved in sunitinib pharmacokinetics and pharmacodynamics. A retrospective study in 136 patients with advanced clear-cell RCC treated with sunitinib evaluated the association between genetic polymorphisms and clinical response (22). Patients ($n = 95$) with polymorphisms in all of three specific genes relating to the pharmacokinetics of sunitinib (encoding, respectively, CYP3A5, NR1/3, and ABCB1) had significantly improved median PFS (13.1 vs. 7.5 months; $P = 0.001$) and median OS (19.9 vs. 12.3 months; $P = 0.009$) compared with those who did not. Pharmacokinetic but not pharmacodynamic polymorphisms were found to be independent predictive factors for PFS. As clinical benefit from sunitinib may depend on systemic exposure to the drug, with higher plasma levels associated with prolonged PFS (63), these findings suggest that polymorphisms of CYP3A5, NR1/3, and ABCB1 may increase drug exposure by reducing metabolism and excretion (22). Pharmacokinetic data were not available in this retrospective analysis to specifically correlate SNPs with plasma levels of sunitinib and its active metabolite, and additional studies are needed.

In a prospective exploratory study that analyzed 92 SNPs in 34 genes involved in drug pharmacokinetic and pharmacodynamic pathways ($n = 25$), significantly greater PFS and OS were found in patients with a *catechol-O-methyl transferase* (*COMT*) G472A SNP, resulting in a Met/Val polymorphism (PFS, 15 months; OS, 17.2 months) than in those with a Val/Val polymorphism [PFS, 3.3 months; OS, 4.4 months; $P = 0.005$ (PFS) and $P = 0.003$ (OS; ref. 23)]. COMT is involved in metabolism of catecholamines and other substances but its role in the pharmacokinetics of RCC therapies is unknown.

A prospective observational study evaluated the impact of 16 SNPs from nine genes on outcome in 89 evaluable patients who received first-line sunitinib for advanced clear-cell RCC. Two *VEGFR-3* missense polymorphisms (A1559G and G4050T) were significantly associated with reduced PFS on multivariate analysis (A1559G: HR, 3.57; $P = 0.0079$; A1559G: HR, 3.31; $P = 0.014$; Fig. 1; ref. 25). The authors speculate that those patients with unfavorable polymorphisms have less VEGFR-3 dependence and are thus less susceptible to the VEGFR-3–inhibiting effects of sunitinib. This hypothesis, however, is not supported by observations from clinical trials that patients with low sVEGFR-3 have a more favorable outcome to sunitinib therapy (albeit after prior treatment with bevacizumab). In addition, polymorphisms in *VEGFR3* and *CYP3A5*1* were associated with a trend to reduced PFS and OS in response to sunitinib (25).

Another recent retrospective study in 63 patients with mRCC found an association between poor OS and the presence of the combination of a *VEGF* SNP C936T, located in the 3′ untranslated region (UTR), and *VEGFR2* SNP G889A, located in exon 7, after adjustment for prognostic risk group ($P = 0.03$), although no single SNP tested (six VEGF and two VEGFR-2 SNPs) correlated with clinical outcome (28).

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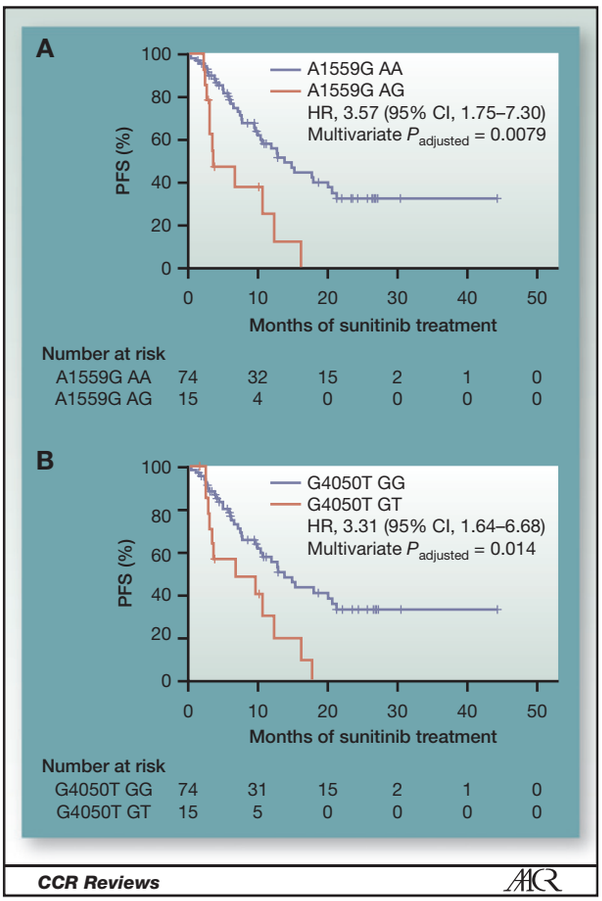

Figure 1. Kaplan–Meier analysis of PFS in first-line sunitinib recipients with advanced RCC according to *VEGFR3* A1559G AA versus AG polymorphism (A) and *VEGFR3* G4050T GG versus GT polymorphism (B). Clinical factors associated with PFS or OS and MSKCC prognostic classification were included as covariates in the multivariate analysis. *P* values were adjusted for multiplicity using the Bonferroni method. Reprinted from Garcia-Donas et al. (25). © 2011, with permission from Elsevier.

The predictive value of selected SNPs in patients with RCC treated with pazopanib has also been explored (24). Twenty-seven functional polymorphisms in 13 genes were prospectively correlated with PFS and ORR in 397 patients treated with pazopanib and 96 patients treated with placebo across three clinical trials. Patients with genotypes associated with increased angiogenesis capability and/or increased pazopanib clearance, including polymorphisms in the *IL-8* (2767TT) and *HIH1A* (1790AG) genes, had inferior PFS. Importantly, there was no association between the *IL-8* and *HIF1A* variants and PFS in placebo-treated recipients, suggesting that these markers are predictive of pazopanib efficacy and are not simply prognostic. Genetic analysis of the COMPARZ phase III trial comparing pazopanib with sunitinib attempted to validate this association of *IL-8* polymorphisms and survival. Significant associations were found between *IL-8* polymorphisms (rs1126647 and rs4073) and both PFS and OS in sunitinib- but not pazopanib-treated patients; however, the association with OS was also significant for the combined treatment population, and HRs for genetic effects were not significantly different between sunitinib- and pazopanib-treated patients (64).

SNPs as predictors of toxicity. A multicenter pharmacogenetic association study was performed in 219 patients (including 159 mRCC patients) treated with sunitinib. Investigation of the association between 31 SNPs in 12 genes and toxicity found that genetic polymorphisms in specific genes encoding metabolizing enzymes (CYP1A1 and NR1/3), efflux transporters (ABCG2 and ABCB1), and drug targets (FLT3 and VEGFR-2) of sunitinib were significantly associated with increased risk of adverse events, including leucopenia, thrombocytopenia, mucosal inflammation, hand–foot syndrome, and any toxicity grade >2 (26, 27). In addition, the *VEGFR* G634T (a 5′-UTR SNP) genotype was found to be independently predictive for the prevalence and duration of hypertension by multivariate analysis ($P \leq 0.05$) in patients with mRCC treated with sunitinib (28), a finding also noted in patients with metastatic breast cancer treated with bevacizumab (65). A prospective observational study in sunitinib-treated patients with advanced clear-cell RCC found that a specific SNP (G6986A) in the *CYP3A5* gene was associated with significantly increased time on sunitinib before requiring a dose reduction because of toxicity (25).

In addition, an exploratory analysis of data from two clinical studies of pazopanib in patients with RCC ($n = 115$) identified two markers in the hemochromatosis (*HFE*) gene that may be associated with reversible ALT elevation in pazopanib-treated patients (66). An analysis of patients with RCC who had received pazopanib in phase II ($n = 116$) or phase III ($n = 130$) clinical studies found that the Gilbert's uridine-diphosphoglucuronate glucuronosyltransferase IAI (UGT IAI) polymorphism was frequently associated with pazopanib-induced hyperbilirubinemia (67).

In summary, several groups have explored candidate SNPs and association with efficacy and toxicity to various VEGF-targeted therapies. Unfortunately, the specific SNPs examined have been largely nonoverlapping and across different agents, and to date, the results have not consistently identified specific SNPs associated with clinical outcome or toxicity in large populations. Results of individual studies that test multiple hypotheses should also be considered in the light of whether corrections for multiple testing were performed in an effort to reduce the false positive rate (Table 2).

Discussion and Implications for Therapy

Despite many exploratory studies, which have identified potential prognostic and predictive biomarkers in RCC, there are currently several obstacles to their clinical use. Potential biomarkers require prospective validation in randomized and adequately powered studies (68). However, few (if any) of the existing studies on tumor biomarkers fully conform to the reporting recommendations for tumor marker prognostic studies guidelines (REMARK; ref. 69), which state that univariate and multivariate analyses should

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be conducted, and there remain several challenges for the conduct of future studies. Currently, most studies have been performed in sunitinib- and pazopanib-treated patients; more research on biomarkers with other targeted therapies is needed. Furthermore, studies to date have enrolled predominantly Caucasian patients, which may impact the generalizability of their conclusions given pharmacogenomic ethnic differences (22, 25, 70). Methodologic factors, such as adequate and appropriate controls and optimum sample collection, storage, and processing (71, 72), may impact biomarker stability and study results. The standardization of techniques will be essential for further validation studies. In addition, there are further challenges specific to mRCC, such as difficulty in routinely obtaining biopsies for biomarker analysis, and tumor heterogeneity (73). Robust and specific assays for clinically useful biomarkers with multiparametric, placebo-controlled validation are the goal of continuing research (71). Furthermore, due to the relatively high prevalence of clear-cell RCC, clinical trials of targeted agents have typically excluded those with nonclear cell histology. Addressing biomarkers of response in nonclear cell RCC will be an important additional area for future study.

To date, the strongest biomarker evidence in mRCC is from independent prognostic markers or independent predictors of response in phase III studies, or pooled data from multiple studies. Baseline VEGF has been identified as an independent prognostic marker in two randomized phase III studies (10, 11). Moreover, a three-step approach for screening, confirmation, and validation of prospective CAF biomarkers with data from a phase II and a phase III trial of pazopanib treatment identified an association between VEGF and PFS in patients treated with pazopanib (39). Baseline LDH was an independent predictor of response to temsirolimus (16) in phase III studies, and a prognostic factor in the MSKCC classification system (44). There is a large body of evidence investigating SNPs as potential biomarkers, although the research is still in its infancy and requires further investigation. In addition, data are emerging to suggest that specific CAFs or multi-CAF signatures may have predictive value as biomarkers of response to VEGF inhibitors, although large prospective studies are required to validate these preliminary findings. New biomarkers are also emerging. It has been shown that programmed death-1 (PD-1) receptor and programmed death ligand-1 (PD-L1)–positive renal cancers are associated with poorer prognoses than those that are PD-1/PD-L1 negative (74, 75), and agents inhibiting various elements on the PD-1/PD-L1 pathway are currently in clinical development. Whether tumor expression of PD-L1 is predictive of response to these agents in RCC is currently unknown but will be critical to explore as clinical development proceeds. MicroRNA and circulating tumor cells (CTC) as biomarkers are other expanding fields that have not yet been well explored with advanced RCC treatments; preliminary studies indicate that circulating miR-1233 may be a potential biomarker for patients with RCC (76). Moreover, detection of cytokeratin 8/18-expressing CTCs in peripheral blood correlated with poor OS in a study of 154 patients with RCC ($P = 0.048$; ref. 77). Further studies are required to fully understand the potential of these markers as predictive and prognostic biomarkers in RCC. Progress in validating individual biomarker candidates is likely to be linked to the development of future immunotherapeutics or molecularly targeted agents.

The combination of molecular or genetic biomarkers into a signature may also be valuable for differentiating patient groups in terms of response or potential for toxic effects. Several ongoing studies are analyzing high-throughput genomics to allow the identification of genes functionally required for axitinib (PREDICT-A; NCT01693822), everolimus (PREDICT-E), and sunitinib (PREDICT-S) response and biomarkers of therapeutic outcome (78). An assay (PREDICT-TOR) is also in development to measure panels of biomarkers to predict patient response to drugs that target, or are influenced by, the PI3K/AKT/mTOR signal transduction pathway.

Targeted agents have significantly improved outcomes for patients with RCC, to the extent that advanced RCC may be considered a chronic treatable condition in some cases (79); however, there is a clear need to further incorporate molecular factors in clinical decisions. Although specific treatment guidelines will be required, the development of validated clinical and molecular biomarkers should facilitate patient management and further improve clinical outcomes by allowing more specific tailoring of treatment to the individual patient.

Disclosure of Potential Conflicts of Interest

P. Maroto reports receiving speakers bureau honoraria from Novartis and Pfizer and is a consultant/advisory board member for Pfizer and GlaxoSmithKline.

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