Published in final edited form as:

Urol Clin North Am. 2016 February ; 43(1): 95–104. doi:10.1016/j.ucl.2015.08.009.

Prognostic Biomarkers for Response to VEGF Targeted Therapy for Renal Cell Carcinoma

Andrew G. Winer¹, Robert J. Motzer², and A. Ari Hakimi¹

¹Urology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

²Genitourinary Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract

Our improved understanding of renal carcinoma disease biology over the past three decades has led to the discovery and FDA-approval of five novel therapies targeting specific molecules in the VEGF biochemical pathway. In order to properly select patients who will receive maximal therapeutic benefit from such targeted agents, biomarker studies attempting to predict response to VEGF targeted therapies have largely focused on circulating proteins, tissue based molecules and germline polymorphisms. Thus far, such studies have yielded conflicting results that require prospective validation; therefore no definitive biomarker has yet been integrated into the clinician's armamentarium. However, early analyses featuring genomic biomarkers have generated promising findings. This review will provide an overview of available biomarkers that have been evaluated with respect to VEGF-targeted therapies in patients with advanced renal cell carcinoma.

Keywords

biomarker; renal cell carcinoma; tyrosine kinase inhibitor; VEGF; targeted therapy

Introduction

Renal cell carcinoma (RCC) has an annual estimated incidence of 64,000 cases in the United States, of which the clear cell (ccRCC) histology represents the most common and aggressive subtype¹. The incidence of this disease appears to be rising, which is largely thought to be a result of improved quality and more frequent use of cross-sectional imaging leading to a stage migration towards smaller, lower stage tumors². However, despite the observed stage migration over the past two decades, this trend has not translated into

Corresponding Author: A. Ari Hakimi, MD, 353 E 68th St, Department of Surgery, Urology Service, Memorial Sloan Kettering Cancer Center, New York, NY 10065, Tel: 212-639-7900, Fax: 646-888-3266, hakimia@mskcc.org.
Contact email addresses for contributing authors: Andrew G. Winer- winera@mskcc.org, Robert J. Motzer- motzerr@mskcc.org

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest: none

population-level improvements in survival in those diagnosed with RCC^{2,3}. In fact, roughly 30% of patients with RCC present with advanced disease, which remains largely incurable⁴.

Our enhanced understanding of RCC disease biology in the past several decades has led to the discovery of novel therapies targeted at specific biochemical pathways involved in renal tumorigenesis. Specifically, the identification of molecular disturbances in the von Hippel-Lindau (*VHL*) gene, which, when altered, causes aberrant stabilization of the hypoxia-inducible factor (HIF) alpha subunit with consequent upregulation of pro-angiogenic downstream molecules, such as vascular endothelial growth factor (VEGF). From this framework spawned the development and FDA approval of four inhibitor molecules directed at various targets in the tyrosine kinase signaling pathway involved in VEGF modulation, termed tyrosine kinase inhibitors (TKIs) and one monoclonal antibody directed specifically at the VEGF receptor (Table 1).

While several clinical trials with such targeted agents have demonstrated improved outcomes in patients with metastatic RCC, a clear understanding of which patients will respond remains uncertain. To address this challenge, several biomarkers have been identified to aid in both patient selection for particular therapies as well as prediction for therapeutic response. In this review, we will provide an overview of available biomarkers that have been tested and used with respect to VEGF-targeted therapies in patients with metastatic RCC.

Circulating/Blood-based biomarkers

VEGF and VEGF related proteins

Under both normal as well as disease conditions, VEGF is a critical regulator of angiogenesis and lymphangiogenesis⁵. On a cellular level, VEGF is persistently upregulated in ccRCC as a direct result of *VHL* gene inactivation. Given the relationship between VEGF and RCC tumorigenesis, the prognostic value of circulating VEGF levels and response to VEGF targeted therapy has been extensively evaluated with conflicting results (Table 2). Biomarker analysis from two sunitinib trials demonstrated that low baseline levels of soluble VEGFR-3 and VEGF-C were found to be favorably correlated with longer progression free survival (PFS)^{6,7}. Conversely, in the AVOREN Phase III trial comparing IFN- α alone vs IFN- α plus bevacizumab, the PFS benefit observed in the bevacizumab arm was not significantly different between patients with baseline VEGF levels above or below the median⁸. In a separate phase III randomized controlled trial, higher pretreatment levels of VEGF were associated with a trend toward improved PFS in sorafenib treated patients compared to placebo ($p=0.096$)^{9,10}. The same study investigated the predictive significance of changes in VEGF and soluble VEGFR-2 after 3 or 12 weeks of treatment with sorafenib, however there was no association identified. This lack of association was similarly found between decreases in circulating VEGF-2 and response in patients treated with sunitinib¹¹. On the contrary, biomarker analysis from a phase II sunitinib study revealed that patients with objective tumor responses experienced substantially larger changes in VEGF, soluble VEGFR-2 and soluble VEGFR-3 levels compared to those patients exhibiting tumor progression ($p<0.05$)¹². The conflicting nature of the results to date may be a direct consequence of inconsistent biomarker detection methods in addition to the effect that therapy may have on our ability to accurately measure the concentrations of drug targets.

Cytokine and Angiogenic Factors (CAFs)

Numerous cytokines and other proteins involved in the angiogenic cascade have also been evaluated as biomarkers for response to VEGF targeted therapies (Table 2). Using data from phase II and phase III pazopanib trials, the authors identified interleukin 6 (IL-6), interleukin 8 (IL-8), VEGF, osteopontin (OPN), E-selectin, and hepatocyte growth factor (HGF) to be associated with tumor shrinkage and PFS. In the validation set of samples from the phase III trial, patients in the pazopanib arm with elevated concentration of interleukin 8 (IL-8) ($p=0.006$), osteopontin (OPN) ($p=0.0004$), hepatocyte growth factor (HGF) ($p=0.010$), and TIMP-1 ($p=0.006$) had shorter PFS than did those with low concentrations. They also found that elevated levels of IL-6 were correlated with improved PFS in the pazopanib treated arm as compared with placebo arm ($p=0.009$)¹³. Additional analysis from this study generated a seven factor angiogenic signature (IL6, IL8, HGF, OPN, TIMP1, VEGF, and E-Selectin) and patients were categorized into high and low groups, which were then correlated with PFS. They found that patients with higher signature scores were associated with significantly shorter PFS in both the placebo ($p=.001$) and pazopanib ($p=.001$) arms¹⁴. In a separate investigation of CAFs as biomarkers predicting therapeutic response to sorafenib, two distinct clusters of patients emerged that were characterized either by elevated levels of proangiogenic or proinflammatory factors. A panel of 6 baseline mediators (OPN, VEGF, carbonic anhydrase IX (CAIX), collagen IV, VEGFR-2, and TNF-related -apoptosis-inducing ligand (TRAIL)) was then correlated with PFS after sorafenib. Patients negative for the 6-marker signature benefitted from an improved PFS (HR 0.20 vs 2.25 in the signature negative versus positive, respectively; $P=0.0002$)¹⁵.

Inflammatory markers

Several circulating markers of inflammation have been investigated as prognostic markers for advanced RCC treated with targeted agents (Table 2). In a small series of 41 metastatic RCC (mRCC) patients treated with sunitinib, c-reactive protein (CRP) was considered as a possible biomarker for therapeutic response¹⁶. They found that patients with normal CRP levels had a significantly higher partial response plus stable disease rate (84.6% vs 35.7%, $p=0.002$) and significantly longer PFS (median 19.0 vs 6.0 months, $p=0.036$) than patients with an elevated level of C-reactive protein. CRP was identified as an independent predictor of objective response ($p=0.016$) on multivariate analysis. Non-elevated levels of CRP ($<8\text{mg/l}$) was also found to be an independent predictor of improved overall survival (OS) ($p=0.003$) in another small study ($n=52$) that included treatment with both sunitinib and sorafenib¹⁷. A larger retrospective report evaluating 200 patients who received sunitinib as first-line therapy substantiated the previous findings in smaller subsets of treated patients. They found that elevated baseline CRP levels conferred a clear disadvantage with respect PFS (8 months vs. 25 months, HR 2.48) and OS (12 months vs 50 months, HR 3.17). Increasing baseline CRP levels was independently associated with inferior PFS in a multivariate model accounting for the variables included in the International Metastatic RCC Database Consortium (IMDC) model (HR 1.14 for each doubling in CRP)¹⁸.

Neutrophil to lymphocyte ratio (NLR) is an inflammatory response marker that has demonstrated prognostic value in several cancer types including RCC. This ratio has been recently been examined as a tool for predicting response to VEGF targeted agents in mRCC

patients. The first study analyzed 109 sunitinib treated patients and compared pretreatment NLR to post-treatment outcomes¹⁹. They found NLR <3 to be associated with improved PFS (HR=0.285, $p<0.001$) and OS (HR = 0.38, $p = 0.043$). Similarly, another analysis correlated pretreatment NLR with PFS and OS in 100 patients²⁰. They demonstrated an improved PFS ($p=0.009$) and OS (0.004) after VEGF directed therapy in patients with a lower baseline NLR (< 3.04). The median OS was 16 months versus 29 months, in patients with NLR > 3.04 versus < 3.04 , respectively ($P = .004$). This association was further corroborated in a recent analysis that demonstrated high baseline NLR to be correlated with lower response rates to TKIs²¹.

Additional circulating proteins have been studied as potential biomarkers although in a limited capacity. In a small prospective predictive marker trial of 13 mRCC patients on TKI therapy, the authors found that the plasma granulocyte macrophage colony-stimulating factor (GM-CSF) concentrations were significantly higher in the therapy responsive group when compared to the patients who either progressed or remained stable post-treatment ($p=0.012$)²². In a biomarker sub-analysis from a phase II randomized controlled trial comparing sunitinib dose schedules, the authors correlated drug efficacy with selected serum markers. Of the 45 proteins evaluated, only two of the proteins showed statistically significant correlations with tumor response (both complete and partial response vs. stable and progressive disease): lower angiopoietin-2 (Ang-2) concentrations ($p = 0.0215$) and higher matrix metalloproteinase-2 (MMP-2) concentrations ($p = 0.0180$)²³. Lastly, in a phase III sunitinib trial, elevated lactate dehydrogenase (LDH) was found to be independently associated with shorter PFS (HR 1.575) and OS (HR 2.01), however the fact that LDH is a robust prognostic marker in advanced RCC independent of treatment call these findings into question²⁴.

Tissue-based biomarkers

VEGF and VEGF related proteins

Circulating levels of proteins often vary at any given time making them difficult to accurately evaluate. Another approach to assess the relationship between VEGF related protein levels and treatment response has been through tumor tissue profiling. Several studies have investigated the expression levels of VEGF and VEGF related proteins in RCC tissue in order to identify factors predicting susceptibility to VEGF directed agents (Table 3). One such study performed immunohistochemical (IHC) staining for 10 molecular markers on 40 patients undergoing cytoreductive nephrectomy for mRCC and compared expression levels to response to sunitinib. Only strong expression of VEGFR-2 was found to be correlated with improved PFS on multivariate analysis ($p=0.039$)²⁵. Another study evaluated tissue biomarkers using reverse transcription polymerase chain reaction (RT-PCR) on RNA extracted from 23 primary tumors in patients with mRCC treated with sunitinib to predict response. Of the 16 biomarkers analyzed, increased expression levels of soluble isoforms of VEGF (VEGF(121) and VEGF(165)) were correlated with therapeutic responses to sunitinib ($P = 0.04$ for both)²⁶.

The *VHL* downstream effector proteins in the HIF family have been demonstrated to be independent poor prognostic markers in RCC²⁷. However, less is known about the predictive

ability of these proteins to be used as a marker of response to VEGF targeted therapy. One analysis in which IHC of 8 key hypoxia related proteins was performed in 67 primary ccRCC samples from patients with advanced RCC who received first-line sunitinib. They demonstrated that elevated expression of HIF-2 α ($p = 0.024$) and platelet derived growth receptor beta (PDGFRB) ($p = 0.026$) were correlated with higher rates of objective response according to RECIST criteria and increased VEGFR3 was associated with improved PFS ($P = 0.012$), while elevated VEGFA was associated with shorter ($P = 0.009$) and HIF-2 α with longer ($P = 0.048$) OS²⁸. Further studies are needed to validate the findings of this one investigation.

CA-IX

Carbonic anhydrase IX (CAIX) is a transmembrane protein that is used a cellular marker for hypoxia and is frequently overexpressed in *VHL*-mutated tumors. Similar to HIF family proteins, it has been demonstrated to be independent poor prognostic marker in RCC but its role as a predictor of response has been less well established²⁷ (Table 3). One study evaluated tumor CAIX expression in 94 mRCC patients treated with VEGF directed therapy to predict response. They found that patients with high versus low tumor CAIX expression experienced similar tumor shrinkage rates ($p=0.38$). Additionally, CAIX expression levels were not associated with response rate ($p=1.0$), treatment duration ($p=0.23$) and OS ($p=0.43$)²⁹. Another study by the same authors evaluated the predictive value of CAIX in predicting response to sorafenib versus placebo in the pivotal TARGET trial. CAIX expression was evaluated in paraffin embedded tumor samples for 133 patients. They found that the degree of CAIX expression was not correlated with PFS (5.5 months vs 5.4 months, high CAIX vs. low CAIX respectively, $p = 0.97$) or median tumor shrinkage (-14.9% vs. -12.6% , high CAIX vs. low CAIX respectively, $p = 0.63$) in the sorafenib treatment arm³⁰. Conflicting data from another study found that among several protein biomarkers that were associated with improved outcomes on univariate analysis, only positive immunostaining of CAIX was an independent predictor of prolonged OS in patients receiving sunitinib on multivariate analysis³¹.

VHL gene functional status

Given its central role in the development of ccRCC, the functional status of the *VHL* gene has been investigated as a possible predictive biomarker for TKI therapy response (Table 3). One such study compared *VHL* activation status via PCR with clinical response in 43 patients receiving IFN- α plus bevacizumab. They found that patients harboring *VHL* loss via methylation or a mutation predicted had a longer median time to progression (TTP) of 13.3 months compared to 7.4 months in patients with *VHL* wild-type ($P = 0.06$)³². In a similar study in 123 mRCC patients receiving VEGF targeted therapies, the patients with loss of function *VHL* mutations had higher response rates (52% vs 31%, $p = 0.04$), which was also significant in a multivariate analysis as an independent predictor of improved response to therapy. PFS and OS were not significantly different in the *VHL* mutated vs wild-type cohorts³³. Tissue from a phase II pazopanib trial of 78 patients was used to analyze biomarkers for response in the *VHL/HIF* pathway. Aberrations in the *VHL* gene were identified in 70 (90%) patients via PCR, status of HIF-1 α and HIF-2 α were assessed via IHC, and isolated RNA was used to evaluate gene expression in a HIF-1 α transcriptional

signature. They found that *VHL* gene status was not associated with objective response or PFS. Likewise, expression levels of HIF-1 α , HIF-2 α and the HIF-1 α transcriptional signature did not correlate with therapeutic response or PFS³⁴. Given the virtually ubiquitous presence of *VHL* inactivation within ccRCC, it is unclear if the observed relationships between mutation and outcomes can reliably be used as a clinically relevant tool to predict response in this setting.

SNPs as biomarkers for response

Multiple studies have been performed in order to identify genomic polymorphisms that are associated with patient response to VEGF targeted therapy (Table 4). Germline alterations in key genes encoding proteins related to the pharmacokinetics (*CYP3A5*, *NR1/3* and *ABCB1*) and pharmacodynamics (*VEGFR1*, *VEGFR3*) of sunitinib as well as pro-angiogenic pathways (*FGFR2*) were associated with improved outcomes after sunitinib treatment^{35,36}. In patients with mRCC receiving pazopanib, three polymorphisms in *IL8* and *HIF-1 α* and five polymorphisms in *HIF-1 α* , *NR1/2* and *VEGFA* showed a marginally significant association with PFS and clinical response rate³⁷. Another study rigorously tested 27 SNPs in 13 genes from pazopanib or sunitinib treated patients and found that specific polymorphisms in *IL8* are associated with poorer OS compared to those with the reference allele³⁸. In the phase III AXIS trial comparing axitinib versus sorafenib in the 2nd line setting, germline SNPs were evaluated as predictors of response to either therapy. On univariate analysis, polymorphisms in *VEGF-A* in the axitinib treated cohort and *VEGFR2* in the sorafenib treated group were associated with prolonged OS (HR 0.39 and 0.41, respectively). However, on multivariate analysis, no single SNP predicted axitinib outcomes whereas polymorphisms in *VEGFR2* predicted both improved PFS (p=0.005) and OS (p=0.003) for sorafenib treated patients³⁹. In a validation cohort of 333 sunitinib-treated patients, 2 of the previously reported SNP associations maintained significance; namely polymorphisms in *CYP3A5* which was associated with drug dose reductions (p=0.039) and polymorphisms in the *ABCB1* haplotype which was correlated with increased PFS (p<0.001)⁴⁰. Another SNP biomarker effort attempted to validate findings from earlier reports by retrospectively analyzing 16 SNPs in 10 genes in 88 mRCC patients treated with sunitinib⁴¹. The authors confirmed significant associations between sunitinib response and SNPs in *ABCB1*, *NR1/2*, *NR1/3* and *VEGFR3*, however prospective studies are required to corroborate these findings.

Future directions

Genomic profiling has become increasingly precise and broadly applicable with the advent of next-generation sequencing. Although the current literature is relatively scarce, there have been a few recent analyses that have attempted to employ such technology in order to identify a molecular biomarker of response and resistance to VEGF targeted therapy in mRCC patients beyond the *VHL* gene alone. One such study utilized whole exome sequencing data on 28 tumor samples from two phenotypes of response to therapy; extreme responders versus refractory patients. Specific mutations or copy number alterations were then compared with response to therapy. In this small pilot study, the authors identified that mutations in *PBRM1* were associated with extreme response to VEGF therapy on univariate

analysis ($p=0.03$)⁴². In a larger cohort of 260 patients that received sunitinib in the RECORD-3 trial⁴³, associations between somatic mutations and treatment efficacy were investigated⁴⁴. The authors found that mutations in the *KDM5C* gene were associated with longer PFS within the sunitinib arm (median PFS 20.6 months in mutated vs 8.4 months in wild-type group; $p=0.05$). If such findings can be reproduced in additional studies, this may suggest that epigenetic modifications within the tumor affect therapeutic response to VEGF targeted therapy. Finally, a unique study using global transcriptomic data from patients treated with sunitinib in the first-line setting identified 4 ccRCC molecular subtypes which were predictive for response to therapy and could potentially be used for proper selection of patients for TKI-directed treatments⁴⁵. This area of investigation, while promising, requires further studies to identify and validate predictive genomic biomarkers.

Summary

The ability to accurately predict response to VEGF targeted therapy in patients with mRCC is of critical import. Although an extensive amount of work has been performed on this topic, to date no definitive biomarker has been identified that would guide the proper patient selection to receive TKI therapy that would allow for maximal therapeutic efficacy. A major pitfall of the available literature lies in the heterogeneous methodology of these studies. Such variability, both in study design, laboratory assay and data analysis often precludes the ability to reproduce and validate results. Thus, the first step in overcoming this challenge may lie in the standardization of our approach to biomarker evaluation. Given recent advances in genomic sequencing, development of a comprehensive molecular level assay is warranted.

Acknowledgments

Funding: Supported by the Sidney Kimmel Center for Prostate and Urologic Cancers.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin.* 2014; 64:9–29. [PubMed: 24399786]
2. Kane CJ, Mallin K, Ritchey J, Cooperberg MR, Carroll PR. Renal cell cancer stage migration: analysis of the National Cancer Data Base. *Cancer.* 2008; 113:78–83. [PubMed: 18491376]
3. Russo P, et al. Survival rates after resection for localized kidney cancer: 1989 to 2004. *Cancer.* 2008; 113:84–96. [PubMed: 18470927]
4. Motzer RJ, et al. Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. *J Clin Oncol.* 1999; 17:2530–2540. [PubMed: 10561319]
5. Tugues S, Koch S, Gualandi L, Li X, Claesson-Welsh L. Vascular endothelial growth factors and receptors: anti-angiogenic therapy in the treatment of cancer. *Mol Aspects Med.* 2011; 32:88–111. [PubMed: 21565214]
6. Harmon CS, et al. Circulating proteins as potential biomarkers of sunitinib and interferon-alpha efficacy in treatment-naïve patients with metastatic renal cell carcinoma. *Cancer Chemother Pharmacol.* 2014; 73:151–161. [PubMed: 24220935]
7. Rini BI, et al. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. *J Clin Oncol.* 2008; 26:3743–3748. [PubMed: 18669461]

8. Escudier B, et al. Update on AVOREN trial in metastatic renal cell carcinoma (mRCC): efficacy and safety in subgroups of patients (pts) and pharmacokinetic (PK) analysis. ASCO Annual Meeting Proceedings. 2008; 26:5025.
9. Pena C, Lathia C, Shan M, Escudier B, Bukowski RM. Biomarkers predicting outcome in patients with advanced renal cell carcinoma: Results from sorafenib phase III Treatment Approaches in Renal Cancer Global Evaluation Trial. Clin Cancer Res. 2010; 16:4853–4863. [PubMed: 20651059]
10. Escudier B, et al. Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J Clin Oncol. 2009; 27:3312–3318. [PubMed: 19451442]
11. Gruenewald V, et al. Circulating endothelial cells are an early predictor in renal cell carcinoma for tumor response to sunitinib. BMC Cancer. 2010; 10:695. [PubMed: 21194438]
12. Deprimio SE, et al. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. J Transl Med. 2007; 5:32. [PubMed: 17605814]
13. Tran HT, et al. Prognostic or predictive plasma cytokines and angiogenic factors for patients treated with pazopanib for metastatic renal-cell cancer: a retrospective analysis of phase 2 and phase 3 trials. Lancet Oncol. 2012; 13:827–837. [PubMed: 22759480]
14. Liu Y, et al. Circulating baseline plasma cytokines and angiogenic factors (CAF) as markers of tumor burden and therapeutic response in a phase III study of pazopanib for metastatic renal cell carcinoma (mRCC). JOURNAL OF CLINICAL ONCOLOGY. 29 (AMER SOC CLINICAL ONCOLOGY 2318 MILL ROAD, STE 800, ALEXANDRIA, VA 22314 USA, 2011).
15. Zurita AJ, et al. A cytokine and angiogenic factor (CAF) analysis in plasma for selection of sorafenib therapy in patients with metastatic renal cell carcinoma. Ann Oncol. 2012; 23:46–52. [PubMed: 21464158]
16. Fujita T, et al. C-reactive protein as a prognostic marker for advanced renal cell carcinoma treated with sunitinib. Int J Urol. 2012; 19:908–913. [PubMed: 22671963]
17. Yasuda Y, et al. Prognostic impact of pretreatment C-reactive protein for patients with metastatic renal cell carcinoma treated with tyrosine kinase inhibitors. Int J Clin Oncol. 2013; 18:884–889. [PubMed: 22886358]
18. Beuselinck B, et al. Prognostic impact of baseline serum C-reactive protein in patients with metastatic renal cell carcinoma (RCC) treated with sunitinib. BJU Int. 2014; 114:81–89. [PubMed: 24215209]
19. Keizman D, et al. The association of pre-treatment neutrophil to lymphocyte ratio with response rate, progression free survival and overall survival of patients treated with sunitinib for metastatic renal cell carcinoma. Eur J Cancer. 2012; 48:202–208. [PubMed: 22018713]
20. Cetin B, et al. Is the pretreatment neutrophil to lymphocyte ratio an important prognostic parameter in patients with metastatic renal cell carcinoma? Clin Genitourin Cancer. 2013; 11:141–148. [PubMed: 23083797]
21. Templeton AJ, et al. Neutrophil to lymphocyte ratio (NLR) and its effect on the prognostic value of the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) model for patients treated with targeted therapy (TT). JOURNAL OF CLINICAL ONCOLOGY. 32 (AMER SOC CLINICAL ONCOLOGY 2318 MILL ROAD, STE 800, ALEXANDRIA, VA 22314 USA, 2014).
22. Yamada D, et al. Granulocyte macrophage colony-stimulating factor as a predictor of the response of metastatic renal cell carcinoma to tyrosine kinase inhibitor therapy. Mol Clin Oncol. 2014; 2:1023–1027. [PubMed: 25279192]
23. Motzer RJ, et al. Investigation of novel circulating proteins, germ line single-nucleotide polymorphisms, and molecular tumor markers as potential efficacy biomarkers of first-line sunitinib therapy for advanced renal cell carcinoma. Cancer Chemother Pharmacol. 2014; 74:739–750. [PubMed: 25100134]
24. Patil S, et al. Prognostic factors for progression-free and overall survival with sunitinib targeted therapy and with cytokine as first-line therapy in patients with metastatic renal cell carcinoma. Ann Oncol. 2011; 22:295–300. [PubMed: 20657034]

25. Terakawa T, Miyake H, Kusuda Y, Fujisawa M. Expression level of vascular endothelial growth factor receptor-2 in radical nephrectomy specimens as a prognostic predictor in patients with metastatic renal cell carcinoma treated with sunitinib. *Urol Oncol*. 2013; 31:493–498. [PubMed: 21478036]
26. Paule B, et al. Soluble isoforms of vascular endothelial growth factor are predictors of response to sunitinib in metastatic renal cell carcinomas. *PLoS One*. 2010; 5:e10715. [PubMed: 20502715]
27. Klatte T, et al. Hypoxia-inducible factor 1 alpha in clear cell renal cell carcinoma. *Clin Cancer Res*. 2007; 13:7388–7393. [PubMed: 18094421]
28. Garcia-Donas J, et al. Prospective study assessing hypoxia-related proteins as markers for the outcome of treatment with sunitinib in advanced clear-cell renal cell carcinoma. *Ann Oncol*. 2013; 24:2409–2414. [PubMed: 23788753]
29. Choueiri TK, et al. Carbonic anhydrase IX and pathological features as predictors of outcome in patients with metastatic clear-cell renal cell carcinoma receiving vascular endothelial growth factor-targeted therapy. *BJU Int*. 2010; 106:772–778. [PubMed: 20230385]
30. Choueiri TK, et al. Carbonic anhydrase IX as a potential biomarker of efficacy in metastatic clear-cell renal cell carcinoma patients receiving sorafenib or placebo: analysis from the treatment approaches in renal cancer global evaluation trial (TARGET). *Urol Oncol*. 2013; 31:1788–1793. [PubMed: 23141780]
31. Dornbusch J, et al. Analyses of potential predictive markers and survival data for a response to sunitinib in patients with metastatic renal cell carcinoma. *PLoS One*. 2013; 8:e76386. [PubMed: 24086736]
32. Rini BI, et al. Clinical response to therapy targeted at vascular endothelial growth factor in metastatic renal cell carcinoma: impact of patient characteristics and Von Hippel-Lindau gene status. *BJU Int*. 2006; 98:756–762. [PubMed: 16827904]
33. Choueiri TK, et al. von Hippel-Lindau gene status and response to vascular endothelial growth factor targeted therapy for metastatic clear cell renal cell carcinoma. *J Urol*. 2008; 180:860–895. discussion 865-6. [PubMed: 18635227]
34. Choueiri TK, et al. The role of aberrant VHL/HIF pathway elements in predicting clinical outcome to pazopanib therapy in patients with metastatic clear-cell renal cell carcinoma. *Clin Cancer Res*. 2013; 19:5218–5226. [PubMed: 23881929]
35. van der Veldt AA, et al. Genetic polymorphisms associated with a prolonged progression-free survival in patients with metastatic renal cell cancer treated with sunitinib. *Clin Cancer Res*. 2011; 17:620–629. [PubMed: 21097692]
36. Garcia-Donas J, et al. Single nucleotide polymorphism associations with response and toxic effects in patients with advanced renal-cell carcinoma treated with first-line sunitinib: a multicentre, observational, prospective study. *Lancet Oncol*. 2011; 12:1143–1150. [PubMed: 22015057]
37. Xu CF, et al. Pazopanib efficacy in renal cell carcinoma: evidence for predictive genetic markers in angiogenesis-related and exposure-related genes. *J Clin Oncol*. 2011; 29:2557–2564. [PubMed: 21576632]
38. Xu CF, et al. IL8 polymorphisms and overall survival in pazopanib- or sunitinib-treated patients with renal cell carcinoma. *Br J Cancer*. 2015; 112(Suppl):1190–1198. [PubMed: 25695485]
39. Escudier B, et al. Genotype Correlations With Blood Pressure and Efficacy From a Randomized Phase III Trial of Second-Line Axitinib Versus Sorafenib in Metastatic Renal Cell Carcinoma. *Clin Genitourin Cancer*. 2015
40. Diekstra MH, et al. CYP3A5 and ABCB1 Polymorphisms as Predictors for Sunitinib Outcome in Metastatic Renal Cell Carcinoma. *Eur Urol*. 2015
41. Beuselinck B, et al. Single-nucleotide polymorphisms associated with outcome in metastatic renal cell carcinoma treated with sunitinib. *Br J Cancer*. 2013; 108:887–900. [PubMed: 23462807]
42. Fay AP, et al. Whole-exome sequencing (WES) predicting two extreme phenotypes of response to VEGF-targeted therapies (VEGF-TT) in patients with metastatic clear cell renal cell carcinoma (mRCC). *ASCO Meeting Abstracts*. 33:422.
43. Motzer RJ, et al. Phase II Randomized Trial Comparing Sequential First-Line Everolimus and Second-Line Sunitinib Versus First-Line Sunitinib and Second-Line Everolimus in Patients With Metastatic Renal Cell Carcinoma. *Journal of Clinical Oncology*. 2014

44. Hsieh JJ, C D, Wang P, Chen YB, Redzematovic A, Marker M, Patel P, Chevinsky M, Bhanot U, Pinciroli P, Bouvier N, Huberman KH, Berger MF, Socci ND, Cheng E, Lee W, Knox JJ, Voss MH, Voi M, Motzer RJ. Identification of efficacy biomarkers in a large metastatic renal cell carcinoma (mRCC) cohort through next generation sequencing (NGS): Results from RECORD-3. *Journal of Clinical Oncology*, 2015 ASCO Annual Meeting. 2015; 33(15_suppl):4509. (May 20 Supplement).
45. Beuselinck B, et al. Molecular Subtypes of Clear Cell Renal Cell Carcinoma Are Associated with Sunitinib Response in the Metastatic Setting. *Clin Cancer Res*. 2015

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Key Points

- Biomarker studies in advanced renal cell carcinoma attempting to predict response to VEGF targeted therapies have largely focused on circulating proteins, tissue based molecules and germline polymorphisms
- To date, such devoted studies have yielded conflicting results therefore no definitive biomarker has emerged
- The heterogeneity in findings may relate to inter-study inconsistencies in design, laboratory assay and data analysis
- Information from high-throughput molecular analyses has improved our understanding of renal cell carcinoma and is presently being used to create prediction models for response to VEGF targeted therapies.

Table 1

Summary of FDA-approved VEGF directed therapies for renal cancer.

Drug	Brand name	Company	Target	Year of FDA approval
Sorafenib	Nexavar	Bayer	Inhibits VEGFR-2, VEGFR-3, PDGFR- β , Flt-3, RAF-1 and c-KIT	2005
Sunitinib	Sutent	Pfizer	Inhibits VEGFR-2, Flt-3, c-KIT and PDGFR- β	2006
Pazopanib	Votrient	GSK	Inhibits VEGFR-1, VEGFR-2, VEGFR-3, PDGFR- α , PDGFR- β and c-KIT	2009
Axitinib	Inlyta	Pfizer	Inhibits VEGFR-1, VEGFR-2, and VEGFR-3	2012
Bevacizumab	Avastin	Roche	Recombinant monoclonal antibody to VEGF-A	2009

Table 2

Summary of circulating/blood-based biomarkers for response to VEGF targeted therapy.

Circulating Biomarker	No. of Patients	Treatment	Description of biomarker levels	Outcome	Ref.
<i>VEGF and VEGF related proteins</i>					
VEGF	363	Sorafenib	High	Trend toward prolonged PFS (HR 0.64; p=0.096)	10
	63	Sunitinib	Change after treatment	Larger change in patients with PR compared to those with SD or PD (HR NR; p<0.0001)	12
VEGFR-2	63	Sunitinib	Change after treatment	Larger change in patients with PR compared to those with SD or PD (HR NR; p<0.0001)	12
VEGFR-3	33	Sunitinib	Low	Increased PFS (HR 2.40; p=0.01) and OS (HR 1.68; p=0.07)	6
	59	Sunitinib	Low	Increased PFS (HR 0.45; p=0.006)	7
	63	Sunitinib	Change after treatment	Larger change in patients with PR compared to those with SD or PD (HR NR; p<0.0001)	12
VEGF-C	59	Sunitinib	Low	Increased PFS (HR 0.37; p=0.0006)	7
<i>CAFs</i>					
IL-6	344	Pazopanib	High	Increased PFS (HR 0.55; p=0.009)	13
<i>Inflammatory markers</i>					
CRP	41	Sunitinib	Low	Increased PFS (HR NR; p=0.036)	16
	52	Sunitinib, Sorafenib	Low	Improved OS (HR 1.79; p=0.01)	17
	200	Sunitinib	High	Shorter PFS (HR 1.14; p= 0.01) and OS (HR 1.29; p< 0.001)	18
NLR	109	Sunitinib	NLR<3	Improved PFS (HR 0.285, p<0.001) and OS (HR 0.3; p = 0.043)	19
	100	Sunitinib, Sorafenib, Pazopanib	NLR<3	Improved PFS (HR NR, p=0.009) and OS (HR NR; 0.004)	20
LDH	375	Sunitinib	High	Shorter PFS (HR 1.6; p=0.003) and OS (HR 2.0; p<0.001)	24

Table 3

Summary of tissue-based biomarkers for response to VEGF targeted therapy.

Tissue Biomarker	No. of Patients	Treatment	Tissue analysis technique	Description of biomarker	Outcome	Ref.
VEGF	23	Sunitinib	PCR	Increased expression	Correlated with therapeutic responses to sunitinib (HR NR; p = 0.015)	26
VEGFR-2	40	Sunitinib	IHC	Strong staining	Improved PFS on multivariate analysis (HR NR; p=0.039)	25
VEGFR-3	67	Sunitinib	IHC	Strong staining	Improved PFS (HR 0.4; P = 0.012)	28
HIF-2 α	67	Sunitinib	IHC	Strong staining	Higher ORR (HR 0.11; p=0.024) and longer OS (HR 0.39; P = 0.048)	28
PDGF	67	Sunitinib	IHC	Strong staining	Higher rates of ORR (HR 0.04; p = 0.026)	28
CA-IX	94	Sunitinib, Sorafenib, Valatenib, Bevacizumab	IHC	Strong staining	Not associated with ORR (HR NR; p=1.0) or OS (HR NR; p=0.43)	29
	133	Sorafenib	IHC	Strong staining	Not correlated with PFS (HR NR; p=0.97)	30
	42	Sunitinib	IHC	Strong staining	Independent prognostic marker for OS (HR 0.174; p = 0.011)	31
VHL gene	43	Bevacizumab	PCR	Mutated or methylated gene	Longer TTP compared patients with wild-type VHL (HR NR; P = 0.06)	32
	123	Sunitinib, Sorafenib, Axitinib, Bevacizumab	PCR	Mutated gene	Higher ORR (HR NR; p = 0.04)	33
	78	Pazopanib	PCR	Mutated or methylated gene	Not associated with ORR (HR NR; p=0.17)	34

Table 4

Summary of germline biomarkers for response to VEGF targeted therapy compared to wild type.

Biomarker Gene	SNP ID	No. of Patients	Treatment	Gene analysis technique	Outcome	Ref.
<i>VEGFR2</i>	rs2071559	146	Sorafenib	TaqMan assay	Predicted PFS (HR 2.22; p=0.0053) and OS (HR 2.58; p=0.0027)	39
<i>VEGFR3</i>	rs307826 rs307821	89	Sunitinib	KASPar SNP genotyping system	Reduced PFS (HR 3.57; p=0.0079) Reduced PFS (HR 3.31; p=0.014)	36
	rs307826 rs307821	88	Sunitinib	Sequenom MassArray platform	Reduced OS (HR NR; p=0.013) Reduced PFS (HR NR; p=0.032) and OS (HR NR; p=0.011)	41
<i>FGFR2</i>	rs2981582	88	Sunitinib	Sequenom MassArray platform	Reduced PFS (HR NR; p=0.031)	41
<i>IL8</i>	rs1126647 rs4073	397	Pazopanib	TaqMan assay	Reduced PFS (HR 1.8; p=0.009) Reduced PFS (HR 1.7; p=0.01)	37
	rs1126647	1,059	Pazopanib, Sunitinib	Multiple platforms	Reduced OS (HR 1.32; p=8.8 × 10 ⁻⁵)	38
<i>HIF-1α</i>	rs11549467	397	Pazopanib	TaqMan assay	Reduced PFS (HR 1.8; p=0.03)	37
<i>NRI/2</i>	rs2276707	88	Sunitinib	Sequenom MassArray platform	Reduced PFS (HR NR; P=0.047)	41
<i>NRI/3</i>	rs2307424, rs2307418, rs4073054	136	Sunitinib	TaqMan assay	Improved PFS (HR 1.76; P = 0.017)	35
	rs4073054 rs2307424	88	Sunitinib	Sequenom MassArray platform	Reduced PFS (HR NR; P=0.025) and OS (HR NR; P=0.035) Reduced OS (HR NR; P=0.048)	41
<i>CYP3A5</i>	rs776746	136	Sunitinib	TaqMan assay	Improved PFS (HR 0.27; P = 0.032)	35
<i>ABCB1</i>	rs1045642, rs1128503, rs2032582	136	Sunitinib	TaqMan assay	Improved PFS (HR 0.52; P = 0.033)	35
	rs1128503	88	Sunitinib	Sequenom MassArray platform	Reduced PFS (HR NR; P=0.027) and OS (HR NR; P=0.025)	41
	rs1128503, rs2032582, rs1045642	333	Sunitinib	Multiple platforms	Increased PFS (HR 1.9; p< 0.001)	40

PFS- progression free survival; **OS**- overall survival; **TTP**- time to progression; **SD**- stable disease; **PD**- progressive disease; **HR NR**- hazard ratio not reported; **ORR**- objective response rate; **IHC**- immunohistochemistry; **PCR**- polymerase chain reaction

Author Manuscript Author Manuscript Author Manuscript Author Manuscript