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Molecular Marker For Predicting Treatment Response in Advanced Renal Cell Carcinoma: Does the Promise Fulfill Clinical Need?

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Abstract

Renal cell carcinoma (RCC) is largely diagnosed incidentally on imaging taken for unrelated reasons. The management of localized lesions is primarily extirpative with excellent results. Treatment of advanced RCC has evolved over recent years with the use of targeted therapies such as tyrosine kinase inhibitors, mammalian target of rapamycin inhibitors, and antibody-mediated therapies. The treatment response to these targeted therapies is highly variable, with no clear clinical method of identifying patients who will benefit from or not tolerate therapy. The field of molecular markers has evolved significantly in the last decade, with a multitude of markers identified that predict treatment response and drug toxicity. The following review critically evaluates those molecular markers that have been assessed for their utility in predicting treatment response in patients with advanced/metastatic renal cell carcinoma (mRCC). Identifying the ideal treatment for these patients will improve responses to therapy, minimize morbidity, and save significant healthcare dollars.

Keywords

Metastatic renal cell carcinoma; Tyrosine kinase inhibitors; Prognostic markers; Sunitinib; Sorafenib

Introduction

Renal cell carcinoma (RCC) is diagnosed largely via imaging technologies such as ultrasound (US) or computed tomography (CT). Over the last decade, the ubiquity of renal imaging for various indications has led to a significant increase in the detection of incidental, often small, renal masses. The classic triad of flank pain, gross hematuria, and an abdominal mass is uncommon and suggests advanced disease [1]. No effective screening tests

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Conflict of Interest

Dr. Michael Garcia-Roig, Dr. Nicolas Ortiz, and Dr. Vinata Lokeshwar declare that there are no conflicts of interest relevant to this article.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

have been devised for RCC, as its low incidence in the general population makes screening impractical.

mRCC is present at the time of diagnosis in approximately 30 % of patients. About 20–40 % of patients presenting with localized disease ultimately progress to metastasis. Advanced RCC, as defined by metastasis, carries a poor, 10-year overall survival (OS) of 5 % [2, 3]. Metastases are more common in larger and/or poorly differentiated tumors. Metastatic lesions in RCC are primarily identified by imaging, and a workup for such lesions is recommended for all renal masses, regardless of size, by performing an abdominopelvic CT scan and chest X-ray [4, 5]. Magnetic resonance imaging (MRI) may be used alternatively in the setting of contrast allergy or pregnancy, or to further characterize a tumor thrombus. Further workup is recommended for patients with suspicious pulmonary or bony lesions [4, 5].

In RCC, molecular markers have been described to help characterize tumor type and predict the likelihood of progression and metastasis [6]. These markers can help create more accurate tumor staging and prognostication [7]. Advances in more effective chemotherapy for advanced RCC make it more important to identify these patients early on in the disease process. For example, markers can be useful in stratifying patients into responders and non-responders for a variety of targeted treatments that are available for metastatic RCC. If non-responders are identified at the beginning or early stages of treatment, it would avoid any delay in administration of alternate treatment(s) and avoid unnecessary side effects due to a treatment that will not be effective.

A variety of targeted treatments directed towards molecular determinants of metastatic RCC, including tyrosine kinase inhibitors (TKIs) and vascular endothelial growth factor (VEGF), have become available for mRCC. The role of the Von Hippel Lindau (VHL) gene in the development of clear cell RCC (ccRCC) has been extensively described. Inactivation of this gene leads to overexpression of pro-angiogenic elements such as VEGF, which plays a critical role in RCC tumor development. TKIs targeting this pathway are the mainstay of treatment for advanced ccRCC, and are therefore the most widely studied. Four FDA-approved targeted drugs are currently available in the United States: Sunitinib, Sorafenib, Pazopanib, and Axitinib. Several targets of the angiogenic cascade have been evaluated as predictors of prognosis and response to TKIs; however, treatment response to targeted therapies is highly variable and predicting this response would help to guide treatment. Below, we review the current literature on tumor markers in advanced renal cell carcinoma, relative to predicting treatment response to the various options for targeted therapy.

Methods

Medline databases were searched with a combination of the following terms: renal cell carcinoma, metastasis, advanced, molecular markers, targeted therapy, systemic therapy, tyrosine kinase inhibitor (TKI), mammalian target of rapamycin (mTOR), vascular endothelial growth factor (VEGF), carbonic anhydrase IX (CA IX), Sunitinib, Sorafenib, Bevacizumab, VHL, circulating endothelial cells, serum amyloid alpha (SAA), neutrophil gelatinase-associated lipocalin (NGAL), erythrocyte sedimentation rate (ESR), metalloproteinase 9 (MMP-9) and tumor necrosis factor (TNF)-α, and lactate dehydrogenase (LDH). Articles were selected based on study size, uniqueness, and importance of contribution to the field.

Treatments for Advanced Renal Cell Carcinoma

Surgery

Surgical management of metastatic renal cell carcinoma is the primary treatment modality for localized RCC and also plays a significant role in mRCC for patients who are acceptable surgical candidates. Cytoreductive nephrectomy is the mainstay of surgical management for advanced RCC. Two randomized studies evaluated the benefits of cytoreductive surgery and interferon (IFN)-α2b. When compared to IFN-α2b treatment alone, a survival benefit of 6 months (13.6 vs. 7.8 months) was noted among patients undergoing both surgery and interferon treatment for mRCC [8]. It is not yet understood how incomplete tumor removal improves survival, although some hypothesize that the effect may be due to changes in cytokines or growth factors, thereby enhancing tumor immunity, or from the reduction in tumor bulk itself. Patients with a good performance status were found to benefit most from surgical intervention, as they were at the lowest risk for operative-related complications [9]. Minimally-invasive cytoreductive nephrectomy may be an option for appropriately selected patients [10]. To date, the role of cytoreductive nephrectomy in the setting of targeted therapy has not been investigated. However, several groups have described the use of neoadjuvant TKIs to shrink tumor size prior to partial nephrectomy in patients with multiple tumors or a solitary kidney [11, 12].

In the setting of solitary localized metastasis or solitary tumor recurrence, primary tumor excision with complete resection of the metastasis improves survival to 30–47 % at 5 years in appropriately selected patients [13]. One group identified risk factors for poor outcome in a series of 175 patients with solitary pulmonary metastasis. These factors included pleural infiltration, synchronous presence of primary RCC, metastasis > 3 cm, mediastinal and hilar node status, and completeness of metastasectomy [14]. Several other groups have identified similar predictors of outcome in the setting of metastasis [15, 16]. Moreover, a patient's disease-free interval plays a significant role in predicting post-metastasectomy survival [17]. No studies have reported on prospectively treated patients; however, this surgical extirpation of solitary RCC metastasis is currently offered to such patients who are surgical candidates.

Systemic Therapy

Patients with residual tumor after resection for mRCC are offered systemic therapy. Immunotherapy with IFN-α (or interleukin (IL)-2 was initially used for patients with advanced disease with significant overall response rates of 6.5–18.6 % at 1 year, either alone or in combination [18]. More recently, treatments targeting the VEGF pathway have become the mainstay of systemic therapy, due to improved response to therapy and reduced toxicity compared with immunotherapy. VEGF is the end product of the VHL gene deregulation pathway in ccRCC. VHL modulates the hypoxia-inducible factor (HIF) pathway, normally activated in the setting of hypoxia to promote oxygen delivery through angiogenesis and red blood cell production via VEGF and platelet-derived growth factor (PDGF) axes [19]. Both VEGF and PDGF promote endothelial cell proliferation, motility, and hence, neovascularization by binding to their respective cell surface receptors on endothelial cells and stimulating signaling cascades downstream. Several treatment options have emerged that inhibit VEGF production or signaling, and therefore, abrogate angiogenesis and tumor growth. Therapy targeting the VHL–VEGF pathway falls into three classes: mTOR inhibitors, TKIs, and monoclonal antibodies against VEGF. The class of mTOR inhibitors include Temsirolimus and Everolimus, the latter being an orally bioavailable form. The mTOR pathway plays a role in growth factor-related signaling cascades. mTOR was found to be directly involved in HIF-1α transcription and stability, in which blocking its production halts tumor growth [20].

TKIs have downstream targets in the VHL-growth factor axis, including VEGF and PDGF pathways. Four TKIs are currently available in the United States: Sunitinib, Sorafenib, Pazopanib, and Axitinib. mRCC. All of these TKIs have multiple targets, including VEGF receptor-1, 2 and 3, PDGF-receptor, c-KIT, and FLT-3 [21]. Their efficacy was confirmed in two trials by Motzer and colleagues that established improved outcomes. In a phase-II trial for patients with mRCC who had failed immunotherapy, Sunitinib provided progression-free survival for 8.5 months, with 35 % experiencing response [22]. In a follow-up, randomized, phase-III study, Sunitinib outperformed interferon with a progression-free survival benefit of 11 months vs. 5 months, and a response rate of 31 % vs 5 % based on the guidelines of Response Evaluation Criteria In Solid Tumors (RECIST) [23].

Bevacizumab is a humanized, anti-VEGF, monoclonal antibody that inhibits angiogenesis by blocking the signaling cascade produced by the aberrant VHL activation that is common to RCC [24]. This antibody has demonstrated efficacy in many tumor types because it directly targets the VEGF signaling pathway [25]. Based on several randomized trials aimed at determining efficacy, this agent is used as second-line therapy in combination with IFN for patients who have failed one of the first-line TKIs, as described above [9, 26–28].

Markers in Targeted Therapy

Clinical parameters have been the mainstay of management and prognosis upon disease diagnosis, which relies on imaging in the case of RCC. The recent rise in the incidental diagnosis of small renal masses is due to frequent abdominal imaging, and ultimately, the choice of surgical approach is based on imaging parameters. Upon tumor removal, follow-up imaging is recommended to rule out recurrent renal masses. RECIST is used to gauge the response to treatment in mRCC, among other advanced tumors, and relies upon imaging, among several other parameters, to track tumor size during treatment. These criteria have been used for three decades and are validated for evaluating tumor response to treatment [29].

Role of Markers in Targeted Therapy

Biomarkers are used in oncology as indicators of tumor status and may be involved in the entire management process for certain malignancies. A tumor marker may allow for efficient screening, leading to diagnosis, surveillance for recurrence after treatment, and/or prediction of response to treatment. Screening markers preferably should be noninvasive and easy to test. For RCC, a diagnostic tumor marker may distinguish between benign and malignant lesions. Such distinction and early detection of RCC is crucial since up to 1/3 of patients have metastatic disease at the time of diagnosis. However, the use of a marker for screening the general population for RCC is not feasible, since the incidence of RCC in the general population is only about 0.01 %. However, markers that detect mRCC early and predict treatment response can improve clinical outcome in terms of cancer-specific survival, while avoiding unnecessary side effects from treatments that are ineffective against a patient's disease. To date, most prognostic tumor markers that have been evaluated for RCC are tissue-based. Markers such as those in the hyaluronic acid family and certain chemokine and cheomkine receptors have shown efficacy in predicting the development of metastasis in the future, based on the their expression in primary tumors [6, 7]. Molecular markers predictive of treatment response can be used to monitor response during administration of therapy. Further, pretreatment molecular characterization can help design personalized treatment regimens based on a tumor's molecular profile. An ideal tumor marker should have high sensitivity and specificity, and it should be easy to perform.

Evaluating the efficacy of markers in advanced RCC is challenging because the disease has a heterogeneous response to the targeted therapies available. Evaluation of the cellular architecture of tumors by histology does not account for the molecular heterogeneity

inherent to advanced RCC. These molecular differences account for a heterogeneous tumor behavior and treatment response to targeted therapy. Several groups have confirmed that molecular markers may predict tumor behavior, in addition to its response to treatment. Predicting this response saves time associated with treatment failure. Moreover, targeting the most effective treatment has the potential to save significant healthcare dollars [30].

Tissue-based Markers

Immunohistochemistry is used to elucidate an unclear diagnosis or classify tumor subtype, among other uses. Certain immunohistochemical markers not only have diagnostic potential, but may also accurately predict metastasis and/or response to prognosis and treatment [6, 7]. These tissue-based markers may be used at the time of initial surgery or biopsy, however, they are limited beyond the time of diagnosis, as one cannot follow treatment response by repeatedly taking tissue samples from the tumor bed or yet-to-be-discovered sites of micrometastasis.

CA-9

Carbonic anhydrase 9 (CA9) is a common immunohistochemical marker in the diagnosis of clear cell RCC (ccRCC). It is a cell-surface enzyme that is overexpressed in upwards of 90 % of ccRCC cases, and its expression is regulated by HIF-1α and the inactivation of VHL [31–33]. Specifically, it contributes to maintaining a neutral intracellular pH and acidic extracellular space. CA9 expression can be detected directly in tissues or in tissue sampling through fine needle aspiration by immunohistochemistry or real-time PCR, and in the serum using a CA9 ELISA. Several groups have retrospectively correlated CA9 expression to prognosis. Bui and colleagues correlated CA9-stained tumor tissue with outcome and found that a low CA9 expression was associated with a poor prognosis in high-risk patients (T stage >2 and Fuhrman grade >1). Specifically, high-risk patients with <85 % CA9 expression had a shorter survival than those with >85 % expression [34]. Several groups have confirmed that low expression of CA9 on tumor specimens correlates with a lower disease-specific survival, and correlates with other prognostic molecular and clinical markers [31, 35, 36]. Moreover, in one study, VHL gene status was also found to correlate with CA9 levels, demonstrating that a complete absence of the VHL gene carries a worse prognosis than a VHL mutation when combined with low CA9 expression [31].

Genetic markers

Genetic factors may play an important role in regulating treatment tolerability and efficacy in mRCC. Specific genetic abnormalities may be associated with deficiencies along a pathway specifically targeted by a treatment. The knowledge of such genetic alternations (i.e., mutations, amplifications, deletions, chromosomal rearrangements) may be exploited to identify individuals predisposed to treatment failure or toxicity, and to subsequently select an ideal regimen prior to starting treatment. Since TKIs target specific pathways within tumor cells, genetic alterations may result in variable responses. A review of the literature identifies relatively few publications related to genetic markers for TKI response. Garcia-Donas and colleagues studied 101 patients receiving Sunitinib for advanced ccRCC with the aim of characterizing single-nucleotide polymorphisms on relevant genetic targets of Sunitinib, as well as predicting treatment tolerability and response [37]. Two polymorphisms of the VEGFR3 gene, rs307826 and rs307821, were identified in 8–9 % of participants and were associated with decreased progression-free survival in multivariate analysis (respective hazard ratio (HR) per polymorphism 1.75–7.30; p=0·0079 and 1.64–6.68; p=0·014). One allele of the CYP3A5*1 gene, rs776746, was identified in 6 % of participants and was associated with drug toxicity related to high metabolism (HR 1·67–8·41; p=0·022).

Choueri et al correlated VHL gene status with treatment response in patients receiving a TKI for advanced ccRCC [38]. In brief, an analysis of VHL gene status in paraffin-embedded RCC tissues was correlated with treatment response, and evaluated by RECIST. The authors identified an improved response in univariate analysis among those with loss-of-function mutations in the VHL gene vs. the wild-type form (52 % vs. 31 % p = 0.04). Patients in the study received one of three TKIs (i.e., Sunitinib, Sorafenib or Axitinib) or Bevacizumab. Patients with wild-type VHL did not respond to Bevacizumab or Sorafenib. However, a significant response to Sunitinib and Axitinib was noted, regardless of VHL status, in 63 and 14 patients, respectively. VHL status did not correlate with progression-free survival or overall survival, which brings the utility of this marker into question. Further studies are necessary regarding this target as a marker for treatment response.

Enzymes related to drug metabolism and genetic features of TKI targets along the VEGF and HIF pathway may serve as potential markers for treatment response, progression, resistance, etc. Several medications exist within the TKI class, with different targets and efficacy. Therefore, potential markers of efficacy or progression are unlikely to be extrapolated across medications within the same class. Moreover, TKIs are used to treat various tumor types, including for cancers of the breast and lung. However, tumor biology varies widely, and a successful marker for TKI treatment prognosis in lung cancer may not necessarily have efficacy in RCC. Similar to TKIs for RCC, headway has been made in biomarkers for antitumor activity and in the pharmacogenetics of TKIs used in other cancers, such as lung and esophageal [39, 40]. Xu and colleagues studied polymorphisms related to Pazopanib's mechanism of action (multi-target TKI for VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α/β, and c-kit), metabolism, and involvement with angiogenesis in 397 patients with advanced RCC. Eight polymorphisms related to cytokine metabolism were identified as related to IL-8, HIF1α, NR1|2, and VEGFA that were also significantly associated with progression-free survival (PFS) and response rate. The phenotypes IL-8 2767TT and HIF1α 1790AG were significantly associated with decreased progression-free survival compared to their respective wild types; (IL-8 276TT 27 vs. 48 weeks; HIF1α: 20 vs. 44 weeks). The authors hypothesize that since IL-8 signaling is an alternative pathway for angiogenesis, and HIF1α is a transcription factor for downstream targets that promote angiogenesis, both IL-8 and HIF1α negatively correlate with PFS. Further, patients with a more active form of these genes will experience an inferior anti-angiogenesis effect from Pazopanib. Similar studies have not been published for the other TKIs that have been approved for ccRCC. Further research in this field is warranted. Ideally, a nomogram would predict optimal survival and response to known TKIs based on genetic makeup.

Circulating endothelial cells (CECs)

One of the hallmarks of RCC is the extreme neovascularization associated with tumors, stimulated by VHL and its downstream targets. Mature endothelial cells from these new vessels, or CECs, can be found in the blood in abnormal levels in cancers, including RCC, and other abnormalities of vasculature [41, 42]. In patients with localized RCC, circulating endothelial progenitors are elevated and decrease after surgery [43]. This was confirmed in a murine xenograft study where CEC (*CD31+CD45-*) and CEP (*CD31+CD45 intermediate CD117+*) were significantly elevated in the xenograft model compared to controls [44]. It would follow that a marker of angiogenesis would be practical in the setting of advanced RCC, as targeted therapies are anti-angiogenic in nature. Indeed, this has been found in patients treated with Sunitinib for mRCC. The pretreatment levels of $CD45^{dim}$CD34+VEGFR2+7AAD- progenitor cells were associated with poor PFS and overall survival (OS). Poor PFS was more likely if the levels remained stable or decreased after starting treatment as opposed to increased levels[45]. A second group investigated CD146+ circulating progenitor cells in patients receiving Sunitinib for advanced RCC, of

which 80 % were ccRCC cases [46]. Again, baseline CEC levels were noted to be higher than normal controls. Patients with improved PFS exhibited increased CEC levels after 28 days, whereas PFS was notably poor compared to the mean when no significant change in CEC occurred at 1 month. Ultimately, PFS could not be elucidated based on pre-treatment CEC levels.

Serum Markers for Targeted Therapy

Molecules detected in serum satisfy several qualities of the ideal biomarkers. They are minimally invasive, generally cost effective, easy to assay, and hence, convenient for the patient and the clinician. Markers in general can be used to predict the aggressiveness of a disease (prognostic factors) or response to a specific therapy (predictive factors). They can also be used to monitor response to treatment. ccRCC responds in a heterogeneous fashion to the different types of targeted therapy without a clear way to predict this response. For this reason, there is a strong interest in discovering serum markers of prognosis and response to the current available medications. Below, we will review the currently available serum biomarkers for response to treatment and prognosis in advanced RCC.

TKI—The role of the VHL gene in the development of ccRCC has been extensively described. As detailed above, inactivation of the signaling pathways regulated by this gene leads to overexpression of pro-angiogenic elements, such as VEGF, which play a critical role in RCC tumor development. TKIs, which target this pathway, are the mainstay of treatment for advanced ccRCC, and therefore the most widely studied. Several targets of the angiogenic cascade have been evaluated as predictors of prognosis and response to TKI.

VHL—Rini and colleagues retrospectively studied 43 patients with mRCC, treated with either TKIs (i.e., Sunitinib or Axitinib) or IFNα + bevacizumab, and found an increase in time to tumor progression (TTP) between patients with methylation or frame-shift mutations of the VHL gene. (13.3 vs. 7.4 months, p=0.06) [47]. This group also found that lower baseline levels of VEGFR-3, and VEGF-C, an isoform of VEGF, were associated with longer PFS and overall response rates in patients receiving Sunitinib or Axitinib in Bevacizumab-refractory disease [48].

VEGF—VEGF is one of the most important products overexpressed in ccRCC. Therapy that targets VEGF and its receptors, VEGF2 and 3, have shown remarkable results in the treatment of RCC. VEGF as a prognostic biomarker was studied within the scope of the TARGET trial; phase 3, randomized, placebo-controlled trial for Sorafenib treatment in patients with advanced ccRCC who were previously treated with standard therapy [49]. In this study, baseline serum VEGF levels in 712 patients were shown to correlate inversely with PFS and OS. High baseline levels of VEGF were also associated with higher MSKCC score and ECOG performance score, respectively, along with markers of poor prognosis and performance [50, 51]. Multivariate analysis revealed baseline VEGF to be an independent factor for PFS in placebo patients. VEGF was also evaluated as a predictive factor, however both high- and low-VEGF groups derived benefit from Sorafenib treatment and no significant difference was seen.

A follow-up paper by Pena et al. confirmed that patients with baseline VEGF levels higher than the median had a shorter OS interval than those with low baseline VEGF levels (12.7 v 18 mo., HR 1.645, P=0.0027) in the placebo group. They also demonstrated improved PFS in patients who had VEGF levels greater than 75 percentile when treated with Sorafenib compared to placebo (HR 0.33 v 069 P=0.023). VEGF increased in the Sorafenib-treated group in subsequent measurements performed at treatment weeks 3 and 12. These changes

were not seen in the placebo group. However, the magnitude of change did not correlate with PFS or OS.[52]

VEGF has also shown promise as a marker in patients treated with other medications. Porta and colleagues evaluated baseline levels of VEGF in patients treated with Sunitinib and found a relative risk for progression of 2.14 in patients with levels above the normal threshold [53]. De Primo and colleagues measured levels of VEGF, VEGFR-2, VEGFR-3 and Placental Growth Factor (PlGF) at day 1 and day 28 of each treatment cycle with Sunitinib (4 weeks treatment, 2 weeks off). VEGF decreased and VEGFR-2 and VEGFR-3 increased during treatment, and then returned to baseline levels between cycles, strongly suggesting a drug-induced response. Mean fold change was significantly larger in patients exhibiting partial response compared to those with stable or progressive disease [54].

Serum Amyloid Alpha (SAA)

SAAs are a group of apolipoproteins associated with the inflammatory response. SAA proteins have been shown to be a prognostic indicator for OS in mRCC [55]. Baseline SAA was measured by conventional antibody-directed enumeration assays in a cohort of 114 patients with mRCC, mostly treated with interferon-based therapy. Elevated SAA was found to be an independent prognostic factor for decreased PFS and OS. These findings were then confirmed in a validation cohort of 151 patients treated with Sunitinib or Sorafenib. SAA alone had a similar accuracy in determining OS when compared to the MSKCC model, and improved this model's accuracy when it was included as a risk factor. In a follow-up study, Vermaat and colleagues re-measured SAA at 6–8 weeks after initiation of treatment. Persistently low SAA was correlated with improved PFS when compared with increasing SAA, declining SAA and persistently elevated SAA [56].

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

NGAL is a 25 kD protein produced by innate immunity cells, as well as tumor cells, and forms complexes with MMP-9 to protect it from degradation—thus enhancing tumor growth [57]. NGAL levels were measured in 85 patients prior to the initiation of Sunitinib treatment by immunohistochemistry [57]. Patients were classified as favorable (n=46) and intermediate (n=3) according to MSKCC criteria. There were no poor-risk patients. Patients with baseline NGAL levels above normal had an increased risk of progression (RR 1.86, 95% CI 1.142–3.019) when compared to patients with normal NGAL levels.

Erythrocyte Sedimentation Rate (ESR)

Inflammatory marker ESR has been previously associated with poor survival in RCC [58]. Zhang and Colleagues studied ESR as a predictive factor in 83 patients who failed immunotherapy with interferon and were treated with Sorafenib [59]. ESR was measured by Westergren method before initiation of treatment and every 4 weeks thereafter. Patients were divided into three groups according to ESR kinetics (increased, decreased, and stable). Patients with decreasing ESR levels had longer PFS than the stable and increasing ESR groups (27, 12 and 6 mos., respectively). Median OS was 37 months in the stable ESR group and 13 months in the increasing ESR group. Median survival in the decreasing ESR group was not reached. ESR kinetics remained an independent predictor of PFS in a multivariate analysis where Karnofsky Performance Status (KPS), method of nephrectomy, time from nephrectomy to first administration of Sorafenib, lung metastasis, anemia, LDH and baseline ESR were also evaluated. Of note, baseline ESR was not a predictor of response to Sorafenib.

MMP-9 and TNFα

MMP-9 is a member of the metalloproteinase family shown to be involved in angiogenesis and metastatic growth [60]. Tumor cells secrete TNFα, acting as an autocrine growth factor. Elevated levels of TNFα have also been shown to correlate with poor prognosis (Harrison). Serum samples of 31 patients with mRCC who were treated with Sunitinib were screened for 174 cytokines as potential markers; MMP-9 and TNFα were elevated in patients who progressed ($p<0.05$). Furthermore, lower baseline levels of MMP-9 and TNFα were associated with longer TTP and OS. MMP-9 decreased with Sunitinib treatment, regardless of response [61].

Markers for mTOR inhibitors

LDH—LDH is a validated prognostic marker for several malignancies, including RCC [62, 63]. Armstrong and colleagues compared serum LDH samples in 404 subjects with MSKCC, poor-risk, advanced RCC treated with IFNα or Temsirolimus. Among patients with LDH levels above the upper limit of normal, OS was improved in the group treated with Temsirolimus when compared to the IFNα-treated group. This OS benefit was not seen in patients with normal LDH levels. LDH levels during treatment were also evaluated but yielded conflicting results. LDH decline was associated with longer OS in the IFNα group and with worse prognosis in the Temsirolimus group [64].

Cholesterol—A recent study by Lee and colleagues evaluated changes in cholesterol levels during treatment as a marker of response. A total of 416 subjects with intermediate- to poor-risk, advanced RCC were randomized to receive either IFNα or Temsirolimus. Baseline cholesterol, triglyceride and fasting glucose levels were repeated every 2 weeks. Serum cholesterol had a mean increase of 37 mg/dL in the Temsirolimus group but no significant change was observed in the IFNα group. The reduction in risk of death was calculated via univariate analysis to be 18 % for every 39 mg/dL (1mmol/L) of increase in serum cholesterol. This association remained significant in multivariate analysis. No association was seen between OS and triglyceride or fasting glucose levels [65].

Marker combination—In an effort to improve the prognostic and predictive significance of serum markers, combinations have been studied. Tran and colleagues evaluated 17 cytokine and angiogenic factors for response in 344 subjects with Pazopanib versus placebo. Low baseline levels of four of these cytokine and angiogenic factors (Hepatocyte growth factor, IL-8, osteopontin, TIMP-1) were associated with prolonged PFS in patients treated with Pazopanib. In the placebo group, IL-6, IL-8 and OPN were prognostic for PFS. Patients with an elevated IL-6 derived a greater relative benefit than those who received Pazopanib versus placebo [66].

Zurita and colleagues measured the levels of 52 cytokine/angiogenic factors in plasma samples of 69 mRCC patients treated with Sorafenib or Sorafenib + IFNα combination. A “signature” pattern of six factors (OPN, VEGF, TNF-related apoptosis-inducing ligand (TRAIL), ColIV, and sVEGFR2) was found correlate with PFS. “Signature positive” subjects had a longer PFS when treated with Sorafenib when compared with the combination group, whereas, the opposite was true for the “signature negative” group [67].

Conclusion

Research strongly points toward the molecular characterization of individual tumors as a promising method of predicting treatment response and toxicity to targeted therapy in metastatic RCC. However, individual markers have yet to be validated. Large scale, multi-centered prospective trials are necessary to confirm marker validity, making them clinically

practical in everyday patient treatment. Eventually, markers can be combined to form a panel of markers that will facilitate individualized patient treatment. Moreover, effective markers for monitoring disease recurrence would allow for earlier intervention, before tumors are visible on CT scan or MRI.

Abbreviations

CA-9	Carbonic anhydrase-9
cRCC	Clear cell renal cell carcinoma
CRCs	Circulating endothelial cells
ESR	Erythrocyte sedimentation rate
HIF	Hypoxia inducible factor
IFN	interferon
IL	Interleukin
LDH	Lactate dehydrogenase
MMP-9	Matrix metalloproteinase-9
mTOR	Mammalian Target of Rapamycin
mRCC	Metastatic renal cell carcinoma
NGAL	Neutrophil Gelatinase Associated Lipocalin
OS	Overall survival
PDGF	Platelet-derived growth factor
PFS	Progression-free survival
RCC	Renal cell carcinoma
RECIS	Response Evaluation Criteria in Solid Tumors
SAA	Serum amyloid alpha
TKI	Tyrosine kinase inhibitor(s)
TNF	Tumor necrosis factor
TTP	Tumor progression rate
VEGF	Vascular endothelial growth factor
VHL	Von Hipple Lindau

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Table 1

Summary of studies on biomarkers for predicting response to targeted therapies for mRCC

Marker	Author	Sensitivity/ specificity	Method	Medication	Applicability
Carbonic anhydrase IX	Bui et al. 2003	NC	Immunohistoc hemistry, Rt-PCR, ELISA	None	Prognostic factor
VEGFR3 polymorphism: rs307826, rs307821	Garcia-Donas, et al. 2011	NC	Single nucleotide polymorphism	Sunitinib	Predictive factor
IL-8 2767TT, HIF1α 1790AG phenotypes	Xu et al. 2011	NC	Sequencing	Pazopanib	Predictive factor
CD45dimCD34+VE GFR2+7AAD-	Farace et al. 2011	NC	Flow cytometry	Sunitinib	Clinical efficacy
CD146+	Gruenwald et al. 2010	NC	Flow cytometry	Sunitinib	Clinical efficacy
VHL mutation/methylation	Rini et al. 2006	NC	PCR, DNA sequencing	Interferon-α + Bevacizumab	Predictive factor
VEGFR-3	Rini et al. 2008 Escudier et al, 2009 DePrimo et al, 2007	NC	ELISA ELISA	Sunitinib Sorafenib Sunitinib	Clinical efficacy Prognostic factor
VEGF-C	Rini et al. 2008	NC	ELISA	Sunitinib	Clinical efficacy
VEGFR-2	Escudier et al, 2009 DePrimo et al, 2007	NC	ELISA ELISA	Sunitinib Sunitinib	Prognostic factor
VEGF	Escudier et al, 2009 Pena et al, 2010 Porta et al, 2010 DePrimo et al, 2007	NC	ELISA ELISA ELISA ELISA	Sunitinib Sorafenib Sunitinib Sunitinib	Prognostic factor Prognostic/Predictive factor Prognostic factors
SAA	Vermaat et al, 2012	59% / 77%	ELISA	Sunitinib Sorafenib	Prognostic factor
NGAL	Perrin et al, 2011	NC	ELISA	Sunitinib	Prognostic factor
ESR	Zhang et al, 2011	NC	Westegren	Sorafenib	Predictive factor
MMP-9	Perez-Garcia et al, 2009	55.6% / 75%	ELISA	Sunitinib	Prognostic factor
TNFα	Perez-Garcia et al, 2009	88% / 75%	ELISA	Sunitinib	Prognostic factor
Hepatocyte growth factor, IL-8, OPN, TIMP-1)	Tran et al, 2012	NC	ELISA	Pazopanib	Prognostic factor
OPN, VEGF, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), ColIV and sVEGFR2	Zurita et al, 2012	NC	Multiplex bead array, ELISA	Sorafenib	Predictive factor
LDH	Armstronge et al, 2012	NC		Temsirolimus	Predictive factor
Cholesterol	Lee et al, 2012	NC		Temsirolimus	Predictive factor