Biomarkers for Renal Cell Carcinoma

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Tracy L. Rose and W. Kimryn Rathmell

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T.L. Rose, MD • W.K. Rathmell, MD, PhD (✉)
Division of Hematology/Oncology,
Department of Medicine,
Lineberger Comprehensive Cancer Center,
University of North Carolina at Chapel Hill,
450 West Dr, Chapel Hill, NC 27599, USA
e-mail: Rathmell@med.unc.edu

Key Points

- The report of the Cancer Genome Atlas on the comprehensive profiling of clear cell RCC set the stage for tumor-based biomarker discovery and validation.
- Prognostic biomarkers can establish risk for disease progression and recurrence, but remain to be prospectively validated.
- Predictive biomarkers must focus on available therapeutic options to maximize relevance to clinical practice and immediacy of implementation.
- Diagnostic and early detection biomarkers have the greatest potential to alter the natural history of RCC, but remain distant from clinical realization.

4.1 Definitions and Categories of Cancer Biomarkers

Cancer biomarkers mainly exist as measurable indicators of a carcinogenic process or of a pharmacologic response to a therapeutic maneuver. They are either produced by tumor cells themselves or by the body in response to cancer. Cancer biomarkers, therefore, may be measured not only in tumor tissues but also in normal tissue or bodily fluids. In this chapter, we will break down the current status of tumor tissue-derived

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_4

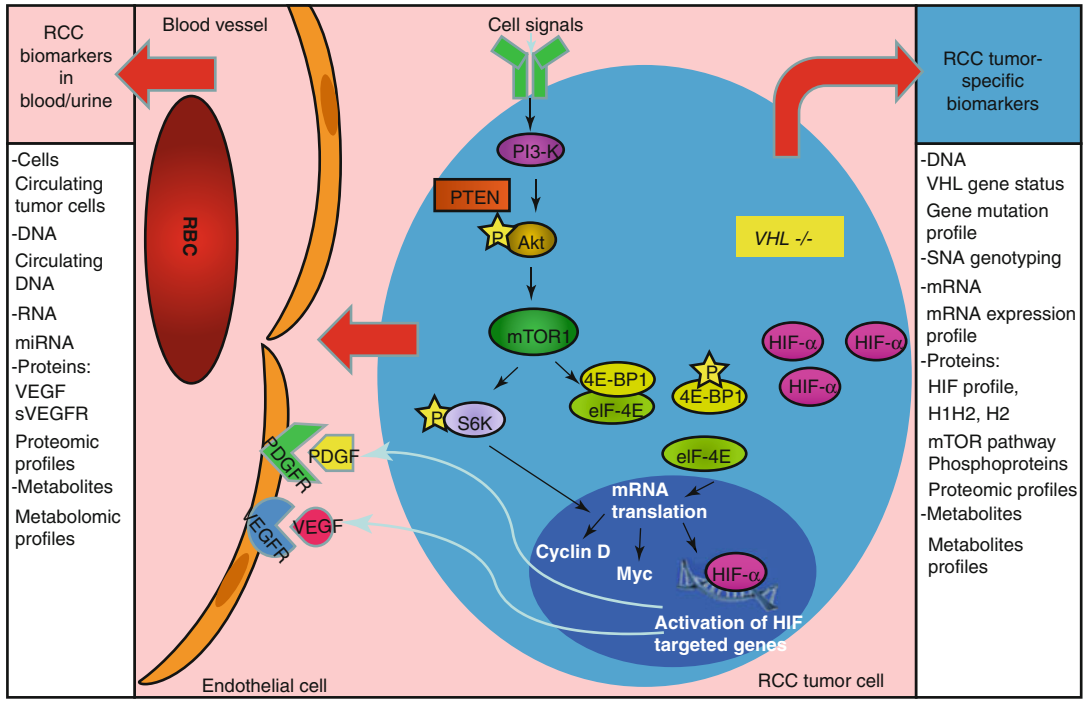

Fig 4.1 Biomarker-relevant biologic pathways in renal cell carcinoma (RCC). In *VHL-/-* tumor cells, the absence of pVHL results in the accumulation of hypoxia-inducible factor alpha (HIF-α). HIF accumulation could also be secondary to the activation of the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway. mTOR phosphorylates and activates pS6K, which leads to increasing translation of downstream target proteins, including cyclin D, Myc, and HIF. Activated mTOR also phosphorylates 4E-BP1, disrupts this complex, and allows eIF-4E to stimulate the mRNA translation as well. Activated HIF translocates into the nucleus and results in the transcription of multiple HIF-target genes, including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). These proteins bind to their receptors and cause cell migration, proliferation, and permeability. RCC biomarkers could be derived from cell components of tumor cell itself, including DNA, RNA, protein, and metabolites. The soluble cell components could also migrate from the cell into the blood vessels and be detected in blood and urine of RCC patients

biomarkers, as well as discuss the emergence of blood- or urine-based biomarkers in renal cell carcinoma (RCC).

Biomarkers can be defined according to the following categories:

1. Early detection biomarkers – used to screen patients for cancer
2. Diagnostic biomarkers – used to assess the presence, absence, or type of cancer
3. Prognostic biomarkers – used to evaluate different phenotypes that correlate with clinical behaviors and/or survival outcomes
4. Predictive biomarkers – used to predict individualized response to therapies, especially targeted therapies

A particular biomarker may satisfy more than one category. For example, a circulating tumor marker may aid in early detection and diagnostic clarification and have prognostic or predictive relevance in the management of cancer.

4.2 The Challenge and Opportunity of Cancer Biomarker Development

Thousands of biomarkers are currently in the developmental pipeline as potential markers for cancer detection, diagnosis, prediction of response, and prognosis. Fewer than 25 biomarkers have been approved by the FDA for monitoring response to treatment or for determining recurrence of cancer [1, 2]. Currently, there is no biomarker that is FDA-approved for RCC screening, staging, monitoring, or prognosis.

4.3 The Importance of RCC Biomarker Development

The early detection and diagnosis of RCC remains a challenge to oncologists and presents a significant barrier to reducing mortality due to this cancer. Roughly 30 % of RCC cases present with metastatic disease at the time of initial diagnosis [3]. Although this percentage has declined in recent years due to increased incidental detection of small renal masses, the mortality rate from RCC has remained steadfastly unchanged [4]. This suggests that RCC with lethal potential are not being identified sufficiently early to prevent metastatic spread, and this presents the single most significant opportunity to reduce death due to RCC. Patients with metastatic RCC have a much poorer prognosis compared with patients with early-stage disease, with a 5-year survival rate of 23 % for stage IV disease as compared to a 5-year survival rate of 96 % for stage I presentation [5].

The use of early detection biomarkers remains in development, but interesting tools are on the horizon. New generations of biomarkers that examine novel substrates such as microRNA (miRNA, miR), proteomic, and metabolomic profiles, with the potential to measure hundreds or more elements simultaneously as a biomarker "profile," are being investigated intensely as tools for RCC early detection and diagnosis. The results have been encouraging [6, 7], but await full clinical validation.

Several early detection serum and urinary biomarkers have been reported as a first step toward a clinically relevant RCC detection assay. Noninvasive detection methods are promising given the increased frequency of detection of RCC from incidental findings on imaging. In one recent study, analysis of RCC cases revealed elevated plasma levels of *N*-methyltransferase (NNMT), L-plastin (LCP1), and nonmetastatic cells 1 protein (NM23A) [8]. A three-marker assay was developed with good positive and negative predictive value for RCC, although results of this study remain unvalidated. Examination of urinary samples from newly diagnosed RCC patients and matched controls identified 86 peptides more frequently found in RCC, most of which were fragments of collagen chains. An assay using these peptides was developed and then validated using an independent set of patients, enabling differentiation of RCC from control with excellent discriminative accuracy (AUC of 0.92) [9]. These assays may help indicate the presence of a kidney primary malignancy, although they need to be further validated and studied in a diagnostic capacity.

Metastatic RCC consists of a heterogeneous group of cancers. This creates incredible challenges to prediction of prognosis and response to different therapeutics. Biomarkers have their most immediate potential in RCC to demystify the heterogeneity and classify RCC into meaningful subgroups. Ultimately, having a rational biological signature from which to draw prognostic or predictive information, yet with low cost and minimal specimens from patients, would be invaluable. In the last decade, the emergence of multiple FDA-approved targeted therapies gives promise to patients with advanced RCC; however, it also adds complexity in the effort of tailoring each agent to different individuals in appropriate sequence. Despite increased understanding of the underlying tumor biology of RCC and its variant histologies (which arguably comprise highly distinct disease entities), the current TNM staging and subtyping of RCC give inadequate insight to refine current algorithms for treatment selection, disease monitoring, and management. The identification and utilization of novel biomarkers for prognosis and prediction of response are important approaches for personalized RCC treatment.

4.4 Understanding the VHL Pathway for RCC Biomarker Development

4.4.1 VHL

Before embarking on an inventory of biomarkers for RCC, it is essential to understand the biology and molecular pathways which are known in this disease and from which the majority of biomarkers are derived (Fig. 4.1). A key event in the pathogenesis of clear cell RCC (ccRCC) appears

to be the inactivation of the von Hippel-Lindau (*VHL*) tumor suppressor gene, which is a biallelic event in over 90 % of sporadic ccRCC [10]. The mechanisms that lead to the loss of *VHL* functionality include large-scale and small-scale deletions, missense mutations, early stop codons, truncations, and silencing of the locus by hypermethylation. The *VHL* gene is located on the short arm of chromosome 3. Large deletions of 3p are commonly identified in ccRCC. This causes a loss of heterozygosity in a majority of ccRCCs, leaving cells susceptible to loss of the remaining allele and full inactivation of *VHL* [11]. Overall, the disengagement of *VHL* in this unique tumor type is likely a critical and common event in ccRCC development.

4.4.2 pVHL

The VHL protein (pVHL) performs a critical cellular function in regulating the cellular response to low oxygen. In the presence of sufficient oxygen, pVHL binds to a family of proteins called the hypoxia-inducible factor alpha (HIF-α) subunits, recruiting them to an E3 ubiquitin ligase complex which polyubiquitinates the HIF-α subunits, thus targeting them for proteasome-mediated proteolysis [12]. The loss of pVHL activity, therefore, permits the constitutive stabilization of HIF-α factors, and high-level expression of HIF-α factors has been a widely recognized feature of ccRCC tumor biology. About 90 % of all ccRCC display HIF-α stabilization apparently as a consequence of *VHL* loss or inactivation [13]. Recent evidence has accrued to indicate that pVHL has functions other than regulation of HIF-related pathways, such as regulation of apoptosis, control of cell senescence, and maintenance of the primary cilium [14].

4.4.3 HIF

HIF is a heterodimeric transcription factor complex consisting of an unstable alpha (α) subunit and a stable beta (β) subunit. Three HIF-α genes (HIF-1α, HIF-2α, and HIF-3α) have been identified in the human genome [15]. Both HIF-1α and HIF-2α function as classical transcription factors, although they can also cooperate with additional factors to maximize activity [16]. The role for HIF-3α, which does not clearly act as a transcriptional regulator and exists with many splice-variant isoforms, is poorly understood [17].

Despite many similarities, HIF-1α and HIF-2α are not fully redundant in function. The global gene expression changes induced by HIF-1α and HIF-2α show that they produce overlapping yet distinct gene expression profiles in both cells and in mice [18].

HIF plays a critical role in tumorigenesis. Indeed, there are several lines of evidence that implicate HIF-α and in particular HIF-2α as playing an active role in *VHL*-/- renal cell carcinogenesis. First, RCC-associated pVHL mutants are at least partially defective with respect to HIF-2α polyubiquitination [19, 20]. Genetic manipulation of HIF expression in human tumor cell line xenografts has clearly demonstrated a growth advantage for cells expressing HIF-2α but not HIF-1α [12, 21]. Examination of human ccRCC tissues provided the ultimate demonstration of a dependence on HIF-2α stabilization, showing that all *VHL*-defective RCCs either stabilize dually both HIF-1α and HIF-2α or solely HIF-2α [13]. This observation provides an alternative way of classifying pVHL-deficient tumors based on this distinction of HIF expression. The *VHL* genotype and the protein expression of HIF-1α and HIF-2α proteins were analyzed in 160 primary tumors. The tumors were examined by immunohistochemistry (IHC) for HIF-1α and HIF-2α and messenger RNA profiling. *VHL*-deficient tumors that exclusively express HIF-2α (H2) tumors displayed greater c-Myc activity and higher rates of proliferation relative to those of *VHL*-deficient tumors expressing both HIF-1α and HIF-2α (H1H2), regardless of tumor stage. H2 tumors also demonstrated increased expression of genes involved in DNA repair, decreased levels of endogenous DNA damage, and fewer genomic copy-number changes. Moreover, those *VHL-deficient* H1H2 tumors and *VHL* wild-type tumors displayed increased activation of AKT/mTOR and ERK/MAPK1 growth factor

signaling pathways and increased expression of glycolytic genes. Thus, there may be two biologically distinct types of *VHL*-deficient ccRCC: those that produce HIF-1α and those that do not. The relevance of this distinction as a biomarker remains to be demonstrated, although consistent with expectations, H2 tumors were of a higher T stage than their H1H2 counterparts.

4.4.4 HIF-Responsive Genes

HIF is a potent transcriptional activator of the cellular hypoxia response and more than 100 direct HIF-responsive genes have been described, with a number of these genes active in carcinogenesis [22]. Although some of these genes and their products are being studied in RCC, two deserve special attention: vascular endothelial growth factor (*VEGF*) and carbonic anhydrase IX (*CAIX*, *CA9*).

4.4.4.1 VEGF

VHL-/- ccRCCs are notoriously angiogenic and overproduce a variety of proangiogenic molecules including the HIF-responsive VEGF. VEGF stimulates endothelial cell proliferation, migration, maturation, and survival and is among the most potent endothelial mitogens. Furthermore, the VEGF receptor, kinase insert domain-containing receptor (KDR), may be present on renal carcinoma cells, suggesting the possibility of an autocrine feedback loop, although receptor activity on tumor cells remains to be demonstrated [23, 24].

VEGF and VEGF receptors (VEGFR) have been thrust into the spotlight as a result of substantial activity of targeted therapies, which engage these factors. Bevacizumab is an antibody that binds circulating VEGF protein and has activity in metastatic RCC [25]. In addition, potent tyrosine kinase inhibitors, such as sunitinib, sorafenib, and pazopanib, target the intracellular signaling pathways of multiple members of the VEGF receptor family of proteins. Multiple phase III trials have demonstrated substantial clinical benefit from blocking VEGFRs with sunitinib, sorafenib, and pazopanib [26, 27, 28]. Below, we will discuss the potential utility of biomarkers of VEGF activity in the context of therapeutics that directly target this signaling pathway, either via tumor cells directly or via supporting cells of the endothelium.

4.4.4.2 CAIX

CAIX is a transmembrane protein that may play a role in the regulation of cell proliferation, oncogenesis, and tumor progression. CAIX is a HIF-responsive, hypoxia-induced protein that accumulates in *VHL*-defective RCCs [29]. A study of CAIX expression in 317 primary and 42 metastatic renal neoplasms showed correlation between CAIX expression with ccRCC histology as well as histologic grade, suggesting that this HIF-dependent protein may provide an effective surrogate for HIF stabilization with the potential to independently serve as a biomarker [30].

4.4.5 AKT/mTOR/HIF Pathway

A better understanding of the molecular biology underlying RCC will lead to the development of biomarkers reflecting aberrant signal transduction pathways within these tumors. Mammalian target of rapamycin (mTOR) is a kinase that activates substrates critical for protein synthesis. It directly phosphorylates the ribosomal subunit S6 kinase (S6K) as well as eukaryotic initiation factor 4E (eIF-4E), which is released from its inhibitory binding partner 4E-BP1 upon its phosphorylation by mTOR. Loss of function mutations of the *PTEN* tumor suppressor gene result in increased mTOR activity via AKT-dependent inactivation of the tuberous sclerosis complex (TSC1 and TSC2), and key members of this pathway have been identified to have non-overlapping mutations in a substantial percentage of tumors [31]. Inhibitors of mTOR decrease global translation of proteins including HIF, cyclin D1, and Myc [32]. There are now two FDA-approved mTOR inhibitors used in the clinic for advanced RCC: temsirolimus [33] and everolimus [34], which have led to both improved progression free survival (PFS) and overall survival. The detection of the effector molecules

(phospho S6, phospho 4EBP1, and phospho AKT) has been linked with response to VEGF-targeted therapy [35] and is both prognostic for overall survival and predictive of response to mTOR therapy [36–38].

4.4.6 Other 3p Genes Involved in RCC

In addition to *VHL*, several other genes located on chromosome 3p have been recently shown via massively parallel sequencing to be commonly mutated in RCC. These genes are important in histone modification and chromatin remodeling. The most commonly mutated gene is *PBRM1* (*polybromo 1*) [39]. Histone methyltransferase *SETD2* (SET domain-containing protein) and the histone deubiquitinase *BAP1* (*BRCA1 associated protein-1*) have also recently been described, in addition to several other less commonly mutated genes [40, 41]. There are both positive and negative genetic interactions among these genes, with *PBRM1* mutations and *SETD2* mutations commonly occurring in the same tumor and *PBRM1* and *BAP1* rarely occurring together [42, 43]. These genes, similar to VHL, are tumor suppressor genes in which one allele is typically inactivated by mutation or hypermethylation and the second is inactivated through a large deletion in chromosome 3p, resulting in loss of heterozygosity [44].

BAP1 is a nuclear deubiquitinase and tumor suppressor gene mutated in about 9–15 % of ccRCCs [41, 45–48] (and commonly in other cancers, most notably metastatic uveal melanoma) [49]. It causes expression of a specific gene expression signature and is associated with increased mTOR activation. BAP1 mediates deubiquitination of histone H2A and binds to host cell factor-1 (HCF-1), a component of the chromatin-remodeling complex, and binding is required for suppression of cell proliferation. Therefore, loss or mutation of *BAP1* is thought to result in loss of tumor suppression [41, 45, 50–52]. A missense variant of *BAP1* has also been found as a germline mutation in familial RCC, although it rarely occurs [53].

SETD2 is a two-hit tumor suppressor gene located in the region of chromosome 3p that is deleted in a majority of ccRCCs. It is present in about 7–16 % of ccRCCs [45, 46]. SETD2 functions as a histone modifier and methyltransferase and is responsible for trimethylation of lysine 36 of histone H3, causing decreased H3K36 levels in some tested ccRCC cell lines [54] and thereby possibly influencing gene expression and transcription activation.

PBRM1 encodes a chromatin/nucleosome remodeling complex protein BAF180 and is mutated in 30–45 % of RCC [39, 46]. It is thought to work as a tumor suppressor gene through regulation of DNA accessibility and gene expression and therefore regulate cell proliferation, although the full mechanism of tumorigenesis due to loss of *PBRM1* is not fully understood [42]. Nearly all *PBRM1*-mutated tumors exhibit a hypoxia signature, suggesting a loss of VHL even though not all cases are associated with a detectable *VHL* mutation [39]. Overall, the recently identified mutations of *BAP1*, *PBRM1*, and *SETD2* represent novel genetic contributors to the pathogenesis of ccRCC, a finding that may reveal important prognostic classification groups and potentially inform therapeutic decisions in the future.

4.5 The Development of RCC Biomarkers in Clinical Decision-Making

While biomarkers for early detection and diagnosis remain at an early stage of development, more advances have been made for prognostic and predictive biomarkers of RCC. Here we will focus our discussion on these markers.

4.5.1 Prognostic Biomarkers

Prognostic biomarkers have been studied in parallel with advances in the tumorigenesis of this cancer. A summary of the potential molecular prognostic biomarkers that have been investigated for RCC is provided (Table 4.1). We will focus the following discussion on the broad spectrum of prognostic biomarkers.

Table 4.1 Potential individual molecular prognostic biomarkers for renal cell carcinoma (RCC)

Biomarker	Type	Source	No. of patients	Reference	Results
Circulating tumor cells (CTCs)	Cells	Blood	154	Bluemke et al. [124]	Detection of CTCs was correlated with poor overall survival (RR 2.3; $p=0.048$)
miRNA-106b	RNA	Tumor	38	Slaby et al. [125]	miR-106b is a potential predictive marker of early metastasis after nephrectomy in RCC patients ($p=0.032$)
miR-23b/27b	RNA	Tumor		Ishihara et al. [126]	Lower expression of miR-23b/27b was associated with shorter OS ($p=0.018$ and $p=0.025$, respectively)
Serum amyloid A protein (SAA)	Protein	Blood	119	Wood et al. [127]	Total SAA protein was of independent prognostic significance ($p=0.017$)
Angiotensin receptor type 2 (AR2)	Protein	Tumor	84	Dolley-Hitze et al. [128]	AR2 was overexpressed in the most aggressive forms of RCC and correlates with PFS ($p=0.006$) and cancer stage ($p<0.001$)
CAIX	Protein	Tumor	357	Klatte et al. [83]	CAIX expression was a strong independent prognostic factor for patients with metastatic cc RCC ($p<0.05$)
C-reactive protein	Protein	Blood	282	Jagdev et al. [129]	C-reactive protein was highly significant for cancer-specific survival ($P<0.0001$) and OS ($p<0.002$)
CXCR4, CXCR7	Protein	Tumor	223	D'Alterio et al. [130]	High CXCR4 expression ($p=0.0061$), high CXCR7 ($p=0.0194$) expression, and the concomitant high expression of CXCR4 and CXCR7 ($p=0.0235$) are independent prognostic factors
Cathepsin D	Protein	Urine	239	Vasudev et al. [131]	Cathepsin D showed evidence of independent prognostic value for OS ($p=0.056$)
E-selectin	Protein	Tumor	559	Tran et al. [107]	Higher baseline E-selectin levels associated with better PFS ($p=0.002$)
EZH2	Protein	Tumor	520	Wagener et al. [132]	High nuclear EZH2 expression was an independent predictor of poor cancer-specific survival (HR 2.72, $p=0.025$)
Global histone acetylation	Protein	Tumor	193	Mosashvilli et al. [133]	Global histone modification level was a universal cancer prognosis marker ($p<0.05$)
HGF	Protein	Blood	559	Tran et al. [107]	High baseline level of HGF was associated with worse PFS ($p=0.013$)
HIF-1α	Protein	Tumor	357	Klatte et al. [83]	Patients with high HIF-1α expression (>35 %) had significantly worse survival than patients with low expression (< or =35 %); median survival, 13.5 vs. 24.4 months, respectively ($p=0.005$)

(continued)

Table 4.1 (continued)

Biomarker	Type	Source	No. of patients	Reference	Results
HuR	Protein	Tumor	152	Ronkainen et al. [134]	HuR expression was associated with reduced RCC-specific survival (HR 2.18; $p=0.015$)
IL-6, IL-8	Protein	Blood	559	Tran et al. [107]	High baseline levels of IL-6 and IL-8 were associated with worse PFS ($p=0.021$ and 0.013, respectively)
IMP3	Protein	Tumor	716	Hoffman et al. [135]	IMP3 expression was associated with a 42 % increase in death from RCC ($p=0.024$)
MMP-9	Protein	Tumor	120	Kawata et al. [136]	MMP-9 was associated with high nuclear grade and was an independent prognostic factor ($p=0.003$)
Osteopontin	Protein	Blood	559	Tran et al. [107]	High baseline levels of osteopontin were associated with worse PFS ($p=0.041$)
p-AKT	Protein	Tumor	40	Jonasch et al. [35]	Higher levels of p-AKT were associated with increased OS (HR 1.15, 95 % CI 1.02–1.29)
PI3K	Protein	Tumor	176	Merseburger et al. [137]	Increased PI3K expression was associated with lower survival ($p=0.030$)
p-mTOR	Protein	Tumor	132	Abou Youssif et al. [138]	Cytoplasmic p-mTOR showed independent prognostic significance ($p=0.029$) and fidelity between primary RCCs and their matched metastases ($p=0.004$)
PAI-1	Protein	Tumor	167	Zubac et al. [139]	PAI-1 was a significant prognostic factor of cancer-specific survival ($p<0.001$)
S100A4	Protein	Tumor	32	Bandiera et al. [140]	Five-year survival was lower in patients with high S100A4 expression than weak expression (41 % vs. 78 %; $p<0.05$)
TIMP-3	Protein	Blood	903	Pena et al. [102]	TIMP-3 was the only biomarker prognostic for overall survival in TARGET trial ($p=0.002$)
TS-1	Protein	Tumor	172	Zubac et al. [107, 141]	Thrombospondin-1 expression was associated with high nuclear grade, advanced stage ($p<0.001$), and tumor progression ($p=0.006$)
VEGF-A	Protein	Blood	559	Tran et al. [107]	High baseline VEGF levels were associated with worse OS (p-0.004)

CAIX indicates carbonic anhydrase IX, *CXCR* chemokine receptor, *EZH2* histone-lysine N-methyltransferase, *HGF* hepatocyte growth factor, *HIF* hypoxia-inducible factor, *HuR* the ubiquitous RNA binding protein, *IL* interleukin, *IMP3* U3 small nuclear ribonucleoprotein protein, *MMP9* matrix metallopeptidase 9, *p-AKT* phosphorylated-AKT, *PI3K* phosphatidylinositol 3-kinases, *p-mTOR* phosphorylated mTOR, *PAI-1* plasminogen activator inhibitor-1, *TIMP-3* metalloproteinase inhibitor 3, *TS-1* thrombospondin-1, *VEGF* vascular endothelial growth factor

4.5.1.1 Clinical Biomarkers

Historically, multiple clinical algorithms were used to estimate prognosis, including the UCLA Integrated Staging System (UISS) to predict risk for disease recurrence or disease-associated death [55] and the Memorial Sloan Kettering Cancer Center (MSKCC) risk criteria for estimating survival for patients with metastatic disease [56]. The UISS incorporates the TNM staging systems, performance status, and the Fuhrman grade of the tumor and is heavily weighted based on tumor stage. While valuable, this staging system does little to risk stratify those patients with non-metastatic but sizeable primary tumors. For patients with metastatic disease, which remains incurable with current therapeutic options, the MSKCC algorithm is a valuable clinical tool to establish prognostic intervals for a disease that can range from indolent to rapidly lethal. This system also takes into account the Karnofsky performance status (which can be highly subjective and variable), time from diagnosis to treatment, and laboratory values of hemoglobin, lactate dehydrogenase, and corrected serum calcium. With the widespread clinical use of targeted therapies in RCC, it is necessary for those criteria, which were validated in the era of cytokine therapies, to recruit new biomarkers to match deregulated pathways with effective inhibitors.

In a recent revision of the model, Motzer et al. developed a nomogram that includes both statistically significant and insignificant factors as biomarkers to create a non-biased prognostic model for patients receiving sunitinib [56]. The additional factors included were the number of metastatic sites ($p<0.01$), the presence of hepatic metastases ($p<0.1$), thrombocytosis ($p<0.01$), prior nephrectomy ($p=0.37$), the presence of lung metastases ($p=0.74$), and serum alkaline phosphatase levels ($p=0.82$) [56].

4.5.1.2 Histological Biomarkers

Tumor stage is widely considered by many clinicians as the most important prognostic factor. Historically, effort has focused on identifying critical features in addition to tumor size, such as extracapsular extension, renal vein invasion, inferior venous cava invasion, lymph node involvement, and presence or absence of adrenal gland metastases. It is only recently that the histologic subtyping of RCC into clear cell, papillary, and chromophobe variants gained its long-deserved attention. Aggregation of data has shown that each tumor subtype is associated with different pathophysiology and clinical behavior. In the largest and most comprehensive retrospective review to date, a group of 3,062 cases was identified between 1970 and 2003, among them 2,466 patients (80.5 %) with clear cell, 438 (14.3 %) with papillary, and 158 (5.2 %) with chromophobe RCC. A significant difference in metastasis-free and cancer-specific survival existed between patients with ccRCC and the two other dominant subtypes. Even after multivariate adjustment, the ccRCC subtype remained a significant predictor of metastasis and cancer-specific death [57].

In an effort to estimate prognosis within the ccRCC group, the Fuhrman grading system has been used to further categorize tumors according to tumor cell morphology and correlates tumor grade to mortality [58]. Other histologic features, including the presence of alveolar features, lymphovascular invasion [59], and sarcomatoid dedifferentiation [60] play pivotal roles in prognosis as well, although the degree to which each of these affect prognosis is uncertain.

4.5.1.3 Genetic Biomarkers

Traditional cytogenetic karyotyping studies have altered the approach used in classifying RCC subtypes. Characteristic karyotypes have been consistently associated with each of the most common subtypes of RCC (clear cell, papillary, and chromophobe) [61–63]. In ccRCC, the most frequently observed cytogenetic abnormalities were loss of 3p (60 %), gain of 5q (33 %), loss of 14q (28 %), trisomy 7 (26 %), loss of 8p (20 %), loss of 6q (17 %), loss of 9p (16 %), loss of 4p (13 %), and loss of chromosome Y in men (55 %) [64]. It is interesting that tumors with loss of 3p typically presented at lower TNM stages. Loss of 4p, 9p, and 14q were all associated with higher TNM stages, higher grade, and greater tumor size. A deletion of 3p was associated with better prognosis, while loss of 4p, 9p, and 14q were

each associated with worse prognosis [64]. With regard to the less common RCC variants, in papillary RCC, trisomies of chromosomes 7 and 17 were found to be specific genetic alterations irrespective of their size, grade, and cellular differentiation [65]. Another study indicated trisomy 16 and chromosome Y were specifically involved in papillary RCC [66]. The rarest subtype of the three, chromophobe RCC, predominantly showed loss of whole chromosomes, such as loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 [67]. A recent evaluation of the somatic mutation spectrum of chromophobe RCC showed these tumors have commonly mutated *TP53* and *PTEN* genes, although less than half of all tumors had one of these mutations [68]. Further analysis revealed frequent *TERT* promoter genomic rearrangements in chromophobe RCC, as well as alterations in mitochondrial DNA including increased mitochondrial genome copy numbers and electron transport gene complex 1 mutations [68].

Karyotyping provides a piece of the genetic puzzle of RCC tumorigenesis by elucidating some chromosomal changes. However, in order to complete the puzzle and identify the stepwise progression of RCC carcinogenesis, we have to rely on genomic or exomic sequencing, array comparative genomic hybridization (a-CGH), or SNP analysis.

Recent advances in sequencing technology have made large-scale genomic sequencing rapid and cost-effective. As above, several genes located on chromosome 3p (*PBRM1*, *SETD2* and *BAP1*) have recently been identified as commonly mutated in ccRCC, along with the frequently mutated *VHL* gene. These results indicate that large-scale gene sequencing is no longer limited by cost and can provide substantial genetic information to identify heterogeneity in ccRCC.

The presence of these genetic mutations has been shown to have prognostic and predictive significance. Patients with *BAP1*-mutated tumors have significantly worse median overall survival with a nearly threefold increased hazard ratio for death than those with *PBRM1* mutations [50]. *BAP1* is also an independent marker of poor prognosis in patients with low-risk disease and may be able to help risk stratify this group of patients [51]. Presence of *BAP1* is also associated with metastatic disease at presentation [45]. The combination of *BAP1*- and *PBRM1*-mutated tumors is rare and has been associated with an even worse overall survival than either mutation alone in most studies, although not in one small study [45, 50]. The *BAP1* mutation was originally described via genetic sequencing [41], but immunohistochemical testing has now been validated and also correlates with poor overall survival and adverse clinicopathological tumor features [52]. *SETD2* mutations are associated with worse cancer-specific survival in a cohort of patients from the Cancer Genome Atlas, but not an MSKCC cohort [46]. The presence of *PBRM1* mutation does not seem to be associated with a change in cancer-specific survival [47], although it has been associated with advanced tumor stage in some earlier studies [69]. It therefore has been suggested to play a more prominent role in tumor initiation instead of disease progression [46].

4.5.1.4 Gene Expression Profiles

Multiple studies have used traditional gene profiling using RT-PCR to quantify RNA expression. In 2001, Takahashi et al. studied the expression profile of 29 ccRCC samples and found 51 genes, which could categorize RCC for prognostic purposes [70]. More recently, an analysis of gene expression profiles using machine learning algorithms refined the notion that more than one type of ccRCC was present and used 49 ccRCC samples to define a panel of 120 genes which can accurately define two groups of ccRCC, designated ccA and ccB [71]. This model was refined for application using a NanoString platform using archival renal tumor tissues, demonstrating the feasibility of the approach and showing an advantage of molecular classification using the ClearCode34 biomarker for ccA and ccB integrated with stage and grade over conventional clinical algorithms [72].

Using an RT-PCR platform adapted for fixed tissue analysis, 931 archival formalin-fixed tumor tissues from patients with localized ccRCC were examined across 732 candidate genes [73]. With a median follow-up of 5.6 years, 448 genes were found to be associated with a longer

recurrence-free interval ($p<0.05$). Sixteen genes had a strong association after consideration of clinical pathologic covariates and false discovery adjustments (HR 0.68–0.80). Among the 16 genes, increased expression of angiogenesis-related genes (*EMCN* and *NOS3*) was associated with lower risk of recurrence, as was increased expression of immune-related genes (*CCL5* and *CXCL9*). This profile provides a feature set readily adaptable to validation studies and has additional promise as a potential predictive biomarker as well. Several of the recently discovered 3p genes commonly mutated in ccRCC also have unique gene expression profiles, but they have been thus far indistinguishable from nonmutant tumors using unsupervised hierarchical clustering algorithms and are therefore not ready for clinical use at this time [42].

4.5.1.5 Hybrid Strategies

The current trend is to incorporate multiple complementary approaches for better identification and understanding of cancer-related genes. Cifola et al. performed the first integrated analysis of DNA and RNA profiles of 27 RCC samples [74]. Seventy-one differentially expressed genes (DEGs) were found in aberrant chromosomal regions and 27 upregulated genes in amplified regions. Among them, the transcripts encoding *LOX* and *CXCR4* were found to be upregulated. Both are implicated for cancer metastasis. Such combinations of genomic and transcriptomic profiling may potentially provide us a more powerful tool for prognostic estimation.

Another trend is to combine epigenetic data with gene expression profiling for better understanding of these interactions. In a preliminary study, an 18-gene promoter methylation panel using quantitative methylation-specific PCR (QMSP) for 85 primarily resected RCC was evaluated [75]. Significant differences in methylation among the four subtypes of RCC were found for *CDH1* ($p=0.0007$), *PTGS2* ($p=0.002$), and *RASSF1A* ($p=0.0001$). *CDH1* and *PTGS2* hypermethylation levels were significantly higher in ccRCC compared to non-ccRCC. *RASSF1A* methylation levels were significantly higher in papillary RCC than in normal tissue ($p=0.035$). Further validation of epigenetic data in larger cohorts is needed to explore the true prognostic value.

4.5.1.6 Copy-Number Analysis

Array comparative genomic hybridization (a-CGH) has been used to identify the specific copy number changes associated with RCC. A comprehensive analysis incorporated a-CGH and gene expression profiles from 90 tumors in order to identify new therapeutic targets in ccRCC [76]. There were 14 regions of nonrandom copy-number change, including seven regions of amplification (1q, 2q, 5q, 7q, 8q, 12p, and 20q) and seven regions of deletion (1p, 3p, 4q, 6q, 8p, 9p, and 14q). An analysis aimed at identifying the relevant genes revealed *VHL* as one of three genes in the 3p deletion peak, *CDKN2A* and *CDKN2B* as the only genes in the 9p deletion peak, and *MYC* as the only gene in the 8q amplification peak. An integrated analysis to identify genes in amplification peaks that are consistently overexpressed among amplified samples confirmed *MYC* as a potential target of 8q amplification and identified candidate oncogenes in the other regions.

a-CGH may also improve the diagnostic accuracy for RCC. A recent study examined a-CGH on ex vivo fine-needle aspiration (FNA) biopsies and tumor fragments of 75 RCC patients. The pattern of genomic changes identified by a-CGH was used blindly to classify the renal tumors and the genetic findings were subsequently compared with the histopathologic diagnosis. a-CGH was successful in 82.7 % of FNA biopsies and in 96 % of tumor fragments. The genetic pattern correctly recognized 93.5 % of ccRCC, 61.5 % of chromophobe RCC, 100 % of papillary RCC, and 14.3 % of oncocytoma, with the negative predictive value being above 90 % [77]. As RCC histology is an independent predictor of prognosis, one could postulate that a-CGH will have powerful prognostic value as well.

4.5.1.7 SNP Genotyping

Single nucleotide polymorphism (SNP) genotyping has been used to detect cytokine gene

polymorphisms in RCC patients to determine its prognostic significance. A panel of 21 SNPs within the promoter regions of 13 cytokine genes were analyzed in a single-center study of 80 metastatic RCC patients [78]. IL4 genotype -589T-33T/-589C-33C was identified as an independent prognostic risk factor in metastatic RCC patients with a median overall survival decreased 3.5-fold (3.78 months, $p<0.05$) compared with patients homozygous for IL4 haplotype -589C-33C (13.44 months). An association was also found between three SNPs (−2578C/A, −1154G/A, and −634C/G) in the VEGF gene and survival of 213 RCC patients [79]. A more recent study found an SNP in IL-8 was associated with survival in patients treated with pazopanib, and these results were validated using data from the COMPARZ trial in sunitinib-treated patients [80, 81]. Multiple VEGF SNPs have also been associated with response and survival as well [80, 82]. These studies contribute evidence that SNP genotyping could be used to develop prognosis algorithms in patients with metastatic RCC.

4.5.1.8 VHL and HIF as Prognostic Biomarkers

Based on the extensive discussion of the derangement of this pathway as a result of *VHL* mutation, it is not surprising then that *VHL* loss or HIF stabilization might provide a prognostic resource. Perhaps owing to the high prevalence of *VHL* mutation among ccRCCs, numerous efforts to demonstrate that *VHL* mutation is a prognostic indicator have been unfruitful. Klatte and colleagues showed preliminary evidence that HIF-1α expression can provide an independent prognostic factor for patients with ccRCC. Patients with high (>35 %) tumor immunostaining of HIF-1α had shorter survival than patients with low (≤35 %) immunostaining of HIF-1α [83]. However, more recent studies have suggested that higher expression of HIF-1α and HIF-2α are associated with improved prognosis [84, 85]. Whether tumor expression of HIF-1α provides substantial prognostic information with respect to the natural history of ccRCC remains to be determined, as does the role of HIF-2α in this setting.

4.5.1.9 Circulating Cells

Levels of circulating endothelial cells and circulating tumor cells have been recently gaining attention as prognostic biomarkers. Several studies have shown that higher levels of circulating endothelial cells or circulating endothelial progenitor cells during the first cycle of VEGF-targeted therapy were associated with improved PFS [86, 87]. However, this technology remains investigational for assessing disease at this time.

4.5.2 Predictive Biomarkers

With the abundance of approved therapies for RCC, oncologists now have the luxury to choose individualized therapy for each patient. Traditional immunotherapy should be retailored to fit selected patients better. Targeted therapies not only have invigorated RCC oncologic practice but also have changed the approaches used to predict response to therapy and to measure clinical outcome. In the next section, we differentiate and discuss biomarkers according to different therapies (Table 4.2).

4.5.2.1 Predictive Biomarkers for Immunotherapy

Despite the advances of targeted therapy, traditional immunotherapy is not obsolete. Immunotherapy offers the possibility of a complete and durable response for a small number of patients with favorable disease factors. However, the toxicities from immunotherapy are significant and the disease factors, which favor immunotherapy, are uncertain. Immunotherapy is therefore often not considered a reasonable option. A reliable biomarker would be ideal to select patients who are likely to have a good response or less toxicity to immunotherapy, as well as to monitor their progress. In addition, the introduction of anti-PD1 (programmed death-1) therapy could also uncover predictive biomarkers for this therapy in the near future.

RCC Subtyping

It is clear that RCC subtyping for clear cell histology is an important predictive biomarker for immunotherapy [88–90]. The Cytokine Working Group performed a retrospective analysis of

Table 4.2 Potential predictive biomarkers of response to targeted therapies for renal cell carcinoma (RCC)

Drug	Biomarker	Reference
Immunotherapy		
IL-2	Clear cell histology	Upton et al. [90]
		McDermott et al. [91]
	CAIX	Bui et al. [142]
	Gene expression profiles	Pantuck et al. [32]
Antiangiogenic therapy		
Sunitinib	Soluble VEGFR	Deprimo et al. [110]
	NGAL, VEGF	Porta et al. [108]
	bFGF	Tsimafeyeu et al. [143]
	HIF-2α	Patel et al. [103]
	TNF-α, MMP-9	Perez-Garcia et al. [144]
	VHL WT	Choueiri et al. [99]
	CXCR4	D'Alterio et al. [112]
Sorafenib	Serum VEGF	Bukowski et al. [106]
		Pena et al. [102]
	TGF-β1 mRNA	Busse et al. [145]
	CAIX	Choueiri et al. [99]
	Osteopontin	Zurita et al. [113]
	VHL loss	Choueiri et al. [99]
Pazopanib	HGF, IL-6, IL-8	Heymach et al. [146]
	IL-6	Tran et al. [107]
Axitinib	*VHL* WT	Choueiri et al. [99]
Bevacizumab	Serum VEGF	Bukowski et al. [147]
	VHL loss	Choueiri et al. [99]
	IL-6, HGF	Nixon et al. [148]
mTOR inhibitors		
Temsirolimus	Non-clear cell histology	Dutcher et al. [114]
	LDH	Armstrong et al. [149]
	p-AKT, pS6K	Cho et al. [92, 122]
Everolimus	LDH	Motzer et al. [150]

IL indicates interleukin, *CAIX* carbonic anhydrase IX, *VEGFR* vascular endothelium growth factor receptor, *NGAL* neutrophil gelatinase-associated lipocalin *bFGF* basic fibroblast growth factor, *HIF* hypoxia-inducible factor, *TNF* tumor necrosis factor, *MMP* matrix metallopeptidase 9, *VHL* von Hippel-Lindau gene, *CXCR* chemokine receptor, *TGF* transforming growth factor, *WT* wild type, *HGF* hepatic growth factor, *LDH* lactate dehydrogenase

tumor tissue from 231 RCC patients treated with interleukin (IL)-2 immunotherapy. The response rate to IL-2 was 21 % in patients with ccRCC, compared with 6 % with non-ccRCC [90]. Similar results were found in the SELECT trial, with zero out of five patients responding [91]. Among the patients with ccRCC, those with >50 % alveolar and no granular or papillary feature had the best response to IL-2 [90].

CAIX

CAIX expression had initially been reported as a predictive biomarker of response to IL-2 [32, 92]. High CAIX expression (>85 % of tumor cells) was observed in 78 % of patients responding to IL-2, compared with only 51 % in nonresponders after examination of 66 RCC patients (27 responders). However, the role of CAIX as a predictive biomarker was further studied in the prospective SELECT trial in combination with histologic features but failed to predict responsiveness to IL-2 [91].

Genetic Studies

Genetic studies as predictive biomarkers have also been explored for immunotherapies. Pantuck

and colleagues reported an expression panel of 73 genes potentially useful to identify complete responders from nonresponders after IL-2 therapy [32]. Interestingly, complete responders to IL-2 possessed unique expression patterns of genes including CAIX, PTEN, and CXCR4. An analysis of a-CGH in ccRCC showed that tumors from complete responders to IL-2 had fewer whole chromosome losses than nonresponders. The loss of chromosome 9p was present in 65 % of nonresponders vs. 0 % of complete responders [93]. Pioneering work using SNP genotyping to predict the response to IFN-α has also been reported [94]. A stepwise logistic regression analysis revealed that the SNPs in signal transducer and activator 3 (STAT3) were significantly associated with better response to IFN-α. All of these findings from exploratory retrospective analyses remain to be validated in prospective studies.

PD1

PD1 (programmed death) is a T-cell immune checkpoint receptor thought to be involved in tumor-mediated immunosuppression. Preliminary data from phase II studies suggest that patients with RCC that expresses PDL1 (the ligand that binds T-cell PD1) on their tumors may benefit from anti-PD1 therapy more than those without, although these results need to be validated in future studies [95].

4.5.2.2 Predictive Biomarkers for VEGF-Targeted Therapy

Clinical Biomarkers

It is intriguing to note that hypertension (HTN), a frequent side effect of VEGF-targeted therapy, has been strongly associated with clinical outcome in the setting of VEGF-directed agents. Rini et al. reported that HTN could be used as a predictive biomarker of efficacy in patients treated with sunitinib [96]. Patients with a maximum systolic blood pressure (SBP) of 140 mmHg or more had a greater improvement in both PFS (12.5 vs. 2.5 months; $p<0.0001$) and OS (30.5 vs. 7.8 months; $p<0.0001$), when compared with patients with lower SBP. Similar results were found in studies of interferon and bevacizumab treatment when patients who developed grade 2 or more HTN had both improved PFS and OS [25, 96–98].

VHL Mutation

VHL gene mutation is a key event of tumorigenesis of ccRCC, a highly vascular neoplasm. Although the incidence of this lesion is >90 %, it has been postulated that *VHL* gene status may serve as a predictive biomarker for ccRCC patients in monitoring of response to VEGF-targeted agents. Recently, Choueiri et al. examined 123 ccRCC patients treated with VEGF-targeted monotherapy with sunitinib, sorafenib, axitinib, or bevacizumab [99]. In multivariate analysis, patients with *VHL* mutational events obtained a significant response rate of 52 % (when missense mutations were excluded) compared to those with wild-type *VHL* who had a response rate of 31 % ($p=0.04$). Interestingly, no responses were noted in patients with wild-type *VHL* receiving sorafenib or bevacizumab. However, *VHL* mutation status did not seem to affect the responses seen in patients treated with potent VEGFR inhibitors sunitinib or axitinib. Other small studies did not provide strong evidence to support the predictive value of *VHL* mutation as a biomarker. In 13 RCC patients treated with axitinib, no correlation was seen between somatic *VHL* mutational status and response [100]. In another study, *VHL* gene status of 78 RCC patients treated with pazopanib was examined, but no association was found between *VHL* gene status and response [101]. *VHL* mutational status did not predict treatment benefit in a large phase III study of sorafenib in advanced RCC, although only a minority of patients had known *VHL* mutation status [102]. Taken together, it remains uncertain whether any correlation exists between *VHL* status and VEGF therapy response, and definitive studies are awaited.

HIF Levels

Patel and colleagues used Western blot to measure HIF expression level in 43 ccRCC specimens prior to sunitinib treatment. Twelve (92 %) of 13 patients with high HIF-2α expression

(>50 % compared to cell line control) responded to sunitinib, whereas only 4 (27 %) of 15 patients with low expression of HIF-2α showed response to sunitinib [103]. A recent abstract reported that both HIF-1α and HIF-2α (H1H2)-positive expressions were correlated with improvement in PFS and OS, as well as response rate to first-line VEGF TKI therapy [84]. This is somewhat contradictory to a previous study by Klatte et al. that showed patients with higher expression levels of HIF-1α had significantly worse overall survival than those with low expression [83]. Studies further establishing the role of classifying tumors according to HIF expression profile have been hindered by technical limitations of antibody nonspecificity, rapid oxidation, and degradation of HIF proteins in improperly handled specimens. In addition, microdeletions in HIF-1α in some cases can lead to nonfunctional protein that retains the domains and features for antigen detection by traditional immunostaining [104].

VEGF/Soluble VEGF Receptor Levels

The value of plasma VEGF levels as a predictive biomarker for antiangiogenesis therapies was addressed in the TARGET trial [105, 106]. High baseline VEGF level was an independent prognostic factor ($p=0.014$) as patients with high baseline VEGF had poorer prognosis. This has been validated in several other trials [107–109]. In another trial, both patients with high VEGF levels and low VEGF levels at baseline benefitted from sorafenib therapy, although those with high VEGF levels had a trend toward more pronounced benefit [102].

A phase 2 trial investigating circulating biomarker changes after sunitinib treatment in cytokine-refractory disease demonstrated significant changes in VEGF, sVEGFR-2, and sVEGFR-3 levels in patients with objective tumor response compared with those with stable disease or disease progression [110, 111]. This finding was similar to findings that lower baseline levels of sVEGFR-3 and VEGF-C were associated with longer PFS and better tumor response in patients receiving sunitinib following disease progression on bevacizumab [109]. Similarly, biomarker studies in a phase 2 trial with pazopanib showed that sVEGFR-2 decrease at day 14 of therapy predicted a better outcome in terms of response and PFS [101].

There has also been some evidence of cross talk between the VEGF pathways and CXCR4 pathway, and one small study has suggested that low CXCR4 expression correlates with improved responsiveness to sunitinib therapy [112].

Cytokines and Angiogenic Factors

Thus far, no single cytokine or angiogenic factor has emerged as reliably predictive of response to VEGF-targeted therapy. However, several studies have explored using clusters of cytokines and angiogenic factors (CAFs) to predict response to therapy. One study found a six-marker baseline signature of factors correlated with improved PFS on sorafenib [113]. However, another study showed no difference in PFS or OS with pazopanib treatment based on CAF signature with similar included factors [107].

4.5.2.3 Predictive Biomarkers for mTOR-Targeted Therapy

RCC Subtyping

RCC subtyping could be an important predictive biomarker for mTOR inhibitors as well. In contrast to immunotherapies, mTOR inhibitors seem more effective in non-ccRCC.

In a subset analysis of a randomized phase 3 trial, median overall survival of patients with non-ccRCC (75 % of whom had the papillary subtype) was 11.6 months in the temsirolimus group vs. 4.3 months in the IFN group [114]. The favorable activity of temsirolimus in non-ccRCC is also different from what was observed with the VEGFR antagonists sorafenib and sunitinib, both of which have demonstrated only limited activity against non-ccRCCs [115]. In the RECORD-3 trial, patients with non-ccRCC had worse PFS than ccRCC when treated with either sunitinib or everolimus as first-line therapy [116]. Patients with non-ccRCC had a longer PFS on first-line sunitinib than everolimus (7.2 vs. 5.1 months), suggesting that perhaps mTOR inhibitors are not more effective than VEGF inhibitors in the non-ccRCC subtypes. A study randomizing patients with non-clear

cell histologies between sunitinib and everolimus showed superiority of sunitinib over everolimus [Ref]. The ongoing phase II ASPEN trial comparing sunitinib and everolimus in non-ccRCC will potentially confirm these findings.

PTEN Loss

The tumor suppressor gene *PTEN* (phosphatase and tensin homologue) encodes a dual specific protein and phospholipid phosphatase that is involved in tumorigenesis and is one of the most commonly lost tumor suppressors in human cancer. It has been reported that *PTEN* loss could be associated with poor prognosis in RCC [117], although interest has focused on *PTEN* deletion as a potential indicator of response to mTOR inhibitor therapy. However, clinical studies have not substantiated either the prognostic role of *PTEN* loss in RCC or any correlation between tumor PTEN expression to either tumor response, OS, or PFS in patients treated with temsirolimus [116–118].

Phospho AKT/Phospho S6K

AKT regulates cell growth and survival mechanisms by phosphorylating a wide spectrum of cellular substrates, including mTOR [119]. Previously, phospho AKT (p-AKT) expression was shown to be correlated with pathologic variables and survival, with higher levels of cytoplasmic p-AKT expression compared with nuclear p-AKT in primary RCC [120]. A recent study found cytoplasmic p-AKT to be significantly correlated to other pathway markers and to nuclear p-AKT in RCC metastases. Unlike primary RCC, p-AKT staining was not prognostic in that cohort of RCC patients [121]. Recent clinical trial data showed that a higher level of p-AKT is associated with both decreased PFS and OS in general in patients with RCC [35].

When mTOR is activated, it phosphorylates two proteins, 4E-BP1 and S6 kinase, to start the cell cycle protein translation process. In primary RCC, phospho S6 kinase (pS6K) expression has been associated with T stage, nuclear grade, incidence of metastasis, and cancer-specific survival [120]. Cho and colleagues investigated *VHL* mutation, p-AKT, and pS6K expression in archival tumor specimens from 20 RCC patients treated with temsirolimus [122]. Although there was no correlation seen between *VHL* mutation and treatment response, protein expression of p-AKT and pS6K, two important proteins indicating activity of the mTOR pathway, was positively associated with response to mTOR-directed treatment. This has been further validated in recent studies with correlation of p-4E-BP1 expression with PFS on mTOR therapy [37]. Another study found that phosphorylation of mTOR and S6RP (the 40S ribosomal protein S6 which increases mRNA transcription in response to mTOR activation) was related to response to mTOR therapy (PFS). However, that study did not show a correlation between expression levels of p-4E-BP1 and efficacy of mTOR therapy [38].

Genetic Biomarkers

A recent case series explored the genetic signatures of several patients who were long-term responders to mTOR inhibitor therapy. Genomic alterations with an activating effect on mTOR signaling were detected in 11 of 14 specimens through alterations in two genes (*TSC1* and *MTOR*) [123].

4.5.2.4 Predictive Biomarkers for Other Targeted Therapies

MET

MET germline mutations have been suggested to play a predictive role in response to new MET inhibitor or multikinase inhibitor therapy in papillary RCC. A recent phase II trial showed up to 50 % partial response rate in papillary RCC with *MET* germline mutations compared to 9 % in those without mutations [167]. These results need to be validated with further studies, but provide one of the most promising rational biomarker/therapy combinations on the horizon.

4.6 Biomarkers on the Horizon

The advent of new technologies and new capacity to bring together these novel methodologies with robust clinical studies heralds a tremendous opportunity for the next generation of biomarkers, reviewed in Table 4.3.

Table 4.3 Potential biomarkers on the horizon for renal cell carcinoma (RCC)

Biomarker	Reference	Mechanism	Potential role in RCC
Proteomic profiles			
Proteomic analysis alone	Xu et al. [151]	High sensitivity and specificity in identifying new proteins or protein amount changes	Early detection, diagnosis
Combined studies	Seliger et al. [7, 152]	Comprehensive analysis of molecular signatures	Early detection, diagnosis
Cytokine and angiogenic factors (CAF)	Zurita et al. [113]	Profile of cytokine and angiogenic factor protein expression levels	Prognostic, potentially predictive for therapy response
Metabolomic profiles			
Tumor specific	Catchpole et al. [153]	Reveals key metabolic features of RCC	Early detection, diagnosis
Body fluids (blood, urine)	Zira et al.; Kim et al. [154, 155]	Easy access, high throughput	Early detection, diagnosis
MicroRNA			
microRNA profile alone	Heinzelmann et al. [156]	miRNA signature may distinguish between metastatic and nonmetastatic ccRCC	Prognosis
Combined RNA studies	Liu et al. [157]	Identify direct mRNA targets of microRNA dysregulated in RCC	Diagnosis
Combined with other studies	Seliger et al. [7]	Comprehensive analysis of molecular signatures	Early detection, diagnosis
DNA			
Circulating cell-free DNA	Feng et al. [158]	Identifies circulating cell-free DNA by PCR, correlates with chance of remission	Response monitoring
Noninvasive imaging biomarkers			
PET imaging			
^{18}F-fluorodeoxyglucose	Minamimoto et al. [159]	Glucose uptake in tumor cells	Staging, response monitoring
^{124}I-cG250	Divgi et al. [160]	cG250 is a monoclonal antibody against CAIX	RCC subtyping, staging
MRI			
Conventional MRI	Spero et al. [161]	Higher sensitivity than renal CT	Staging, subtyping
Modified MRI	Wang et al. [162]	Diffusion-weighted imaging provides images weighted with the local microstructural characteristics of water diffusion	Tumor vascularity assessment and response monitoring
	Hillman et al. [163]	Dynamic contrast-enhanced monitor vascular changes induced by therapeutic agents	
	Pedrosa et al. [164]	Arterial spin labeling uses magnetic fields to label water protons in arterial blood and measures blood flow into tissue	
Magnetic resonance spectroscopy (MRS)	Katz-Brull et al. [165]	Tumor related molecular environment changes cause signal frequency changes	Metabolic portrait of tumors
Ultrasound			
Contrast-enhanced	Lassau et al. [166]	Tumor vascularity	Tumor vascularity assessment and response monitoring

4.7 The Future of RCC Biomarkers Development

Unprecedented progress has been made for RCC biomarker development. However, challenges remain. Most clinical biomarkers need further clinical validations, especially in prospective studies. The bulky panel of potential genetic biomarkers, which we obtained from genomic, proteomic, metabolomic, and microRNA profiling, require further analysis and validation to be useful. Newer biomarkers detectable in serum, urine, and other body fluid need fine-tuning to be isolated from confounding factors. The advancement of therapy for RCC to a new era will undoubtedly involve individualized treatment using biomarkers.

Clinical Vignette

A 66-year-old man underwent a laparoscopic left nephrectomy of a 9-cm renal cell carcinoma (RCC). Three years later, he presents to his oncologist with a 2-month history of nonproductive cough, a 10-lb weight loss, and left hip pain. Further workup reveals multiple pulmonary nodules and a 3×3-cm left sacral lytic lesion. Biopsy of the sacral bony lesion confirmed recurrent RCC. What clinical or biological indicators are needed to determine the most appropriate next step for management of this patient at this time?

This patient has metastatic RCC, and additional information is needed to estimate his prognosis and select the best possible therapy at this time. First, we know that 3 years went by before he developed symptomatic evidence of metastatic disease. To fully assess his prognosis using the Memorial Sloan Kettering risk criteria, we also need to know his performance status and laboratory measures of hemoglobin/hematocrit, corrected serum calcium, and serum lactate dehydrogenase. To refine the risk estimate if the patient is being considered for VEGF receptor-targeted therapy, we also need to know his alkaline phosphatase level and platelet count. This prognostic assessment is invaluable in making plans for treatment and for patients and their families to prepare for the future. The clear cell, papillary, or chromophobe designation becomes essential as therapeutic choices are made between cytokine, VEGF-targeted, and mTOR inhibitor therapies. Other pathologic considerations such as tumor grade, sarcomatoid histology, or alveolar clear cell features also factor into decision-making. To date, none of the molecular markers described above are available as clinical tests to enable earlier detection of this patient's metastatic disease, to further refine his prognosis, or to provide a clear guidepost for therapeutic selection. Patients like the one described above should be encouraged to participate in clinical trials that incorporate biomarker discovery or validation.

References

1. Ludwig JA, Weinstein JN (2005) Biomarkers in cancer staging, prognosis and treatment selection. Nat Rev Cancer 5(11):845–856
2. Fuzery AK et al (2013) Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges. Clin Proteomics 10(1):13
3. King SC et al (2014) Continued increase in incidence of renal cell carcinoma, especially in young patients and high grade disease: United States 2001 to 2010. J Urol 191(6):1665–1670
4. U.S. Cancer Statistics Working Group (2013) United States Cancer Statistics: 1999–2010 incidence and mortality web-based report. [cited 2014 Jul 25]; Available from: www.cdc.gov/uscs
5. Linehan WM RB, Yang JC (2008) Cancer of the kidney. In: DeVita VT HS Jr, Rosenberg SA (eds) Cancer principles and practice of oncology, 8th edn. Lippincott William & Wilkins, Philadelphia, pp 1331–1354
6. Lin L et al (2010) LC-MS based serum metabonomic analysis for renal cell carcinoma diagnosis, staging, and biomarker discovery. J Proteome Res 10(3):1396–1405
7. Seliger B et al (2011) Linkage of microRNA and proteome-based profiling data sets: a perspective for

the priorization of candidate biomarkers in renal cell carcinoma? J Proteome Res 10(1):191–199
8. Su Kim D et al (2013) Composite three-marker assay for early detection of kidney cancer. Cancer Epidemiol Biomarkers Prev 22(3):390–398
9. Frantzi M et al (2014) Discovery and validation of urinary biomarkers for detection of renal cell carcinoma. J Proteomics 98:44–58
10. Nickerson ML et al (2008) Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors. Clin Cancer Res 14(15):4726–4734
11. Zbar B et al (1987) Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature 327(6124):721–724
12. Kaelin WG Jr (2008) The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. Nat Rev Cancer 8(11):865–873
13. Gordan JD et al (2008) HIF-alpha effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. Cancer Cell 14(6):435–446
14. Li M, Kim WY (2010) Two sides to every story: the HIF-dependent and HIF-independent functions of pVHL. J Cell Mol Med. Epub ahead of print
15. Semenza GL (2001) HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol 13(2):167–171
16. Sang N et al (2002) Carboxyl-terminal transactivation activity of hypoxia-inducible factor 1 alpha is governed by a von Hippel-Lindau protein-independent, hydroxylation-regulated association with p300/CBP. Mol Cell Biol 22(9):2984–2992
17. Maynard MA et al (2003) Multiple splice variants of the human HIF-3 alpha locus are targets of the von Hippel-Lindau E3 ubiquitin ligase complex. J Biol Chem 278(13):11032–11040
18. Raval RR et al (2005) Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. Mol Cell Biol 25(13):5675–5686
19. Rathmell WK et al (2004) HIF transcription factor expression and induction of hypoxic response genes in a retroperitoneal angiosarcoma. Anticancer Res 24(1):167–169
20. Hacker KE, Lee CM, Rathmell WK (2008) VHL type 2B mutations retain VBC complex form and function. PLoS ONE 3(11):e3801
21. Iliopoulos O et al (1996) Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. Proc Natl Acad Sci U S A 93(20):10595–10599
22. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3(10):721–732
23. Fox SB et al (2004) Phosphorylated KDR is expressed in the neoplastic and stromal elements of human renal tumours and shuttles from cell membrane to nucleus. J Pathol 202(3):313–320
24. Calvani M et al (2008) Differential involvement of vascular endothelial growth factor in the survival of hypoxic colon cancer cells. Cancer Res 68(1):285–291
25. Rini BI et al (2010) Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. J Clin Oncol 28(13):2137–2143
26. Motzer RJ et al (2007) Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 356(2):115–124
27. Escudier B et al (2007) Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 356(2):125–134
28. Motzer RJ et al (2013) Pazopanib versus sunitinib in metastatic renal-cell carcinoma. N Engl J Med 369(8):722–731
29. Ivanov S et al (2001) Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. Am J Pathol 158(3):905–919
30. Genega EM et al (2010) Carbonic anhydrase IX expression in renal neoplasms: correlation with tumor type and grade. Am J Clin Pathol 134(6):873–879
31. Cancer Genome Atlas Research Network (2013) Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 499(7456):43–49
32. Pantuck AJ, Fang Z, Liu X et al (2005) Gene expression and tissue microarray analysis of interleukin-2 complete responders in patients with metastatic renal cell carcinoma. J Clin Oncol 23(16 suppl pt 1)):Abstract 4535
33. Hudes G et al (2007) Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 356(22):2271–2281
34. Motzer RJ et al (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 372(9637):449–456
35. Jonasch E et al (2010) Upfront, randomized, phase 2 trial of sorafenib versus sorafenib and low-dose interferon alfa in patients with advanced renal cell carcinoma: clinical and biomarker analysis. Cancer 116(1):57–65
36. Atkins MB et al (2009) Treatment selection for patients with metastatic renal cell carcinoma. Cancer 115(10 Suppl):2327–2333
37. Nishikawa M et al (2014) Expression level of phosphorylated-4E-binding protein 1 in radical nephrectomy specimens as a prognostic predictor in patients with metastatic renal cell carcinoma treated with mammalian target of rapamycin inhibitors. Med Oncol 31(1):792
38. Li S et al (2014) Phosphorylation of mTOR and S6RP predicts the efficacy of everolimus in patients with metastatic renal cell carcinoma. BMC Cancer 14(1):376
39. Varela I et al (2011) Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 469(7331):539–542
40. Dalgliesh GL et al (2010) Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature 463(7279):360–363
41. Pena-Llopis S et al (2012) BAP1 loss defines a new class of renal cell carcinoma. Nat Genet 44(7):751–759
42. Brugarolas J (2014) Molecular genetics of clear-cell renal cell carcinoma. J Clin Oncol 32(18):1968–1976

43. Pena-Llopis S et al (2013) Cooperation and antagonism among cancer genes: the renal cancer paradigm. Cancer Res 73(14):4173–4179
44. Toma MI et al (2008) Loss of heterozygosity and copy number abnormality in clear cell renal cell carcinoma discovered by high-density affymetrix 10 K single nucleotide polymorphism mapping array. Neoplasia 10(7):634–642
45. Gossage L et al (2014) Clinical and pathological impact of VHL, PBRM1, BAP1, SETD2, KDM6A, and JARID1c in clear cell renal cell carcinoma. Genes Chromosom Cancer 53(1):38–51
46. Hakimi AA et al (2013) Adverse outcomes in clear cell renal cell carcinoma with mutations of 3p21 epigenetic regulators BAP1 and SETD2: a report by MSKCC and the KIRC TCGA research network. Clin Cancer Res 19(12):3259–3267
47. Hakimi AA et al (2013) Clinical and pathologic impact of select chromatin-modulating tumor suppressors in clear cell renal cell carcinoma. Eur Urol 63(5):848–854
48. Guo G et al (2012) Frequent mutations of genes encoding ubiquitin-mediated proteolysis pathway components in clear cell renal cell carcinoma. Nat Genet 44(1):17–19
49. Harbour JW et al (2010) Frequent mutation of BAP1 in metastasizing uveal melanomas. Science 330(6009):1410–1413
50. Kapur P et al (2013) Effects on survival of BAP1 and PBRM1 mutations in sporadic clear-cell renal-cell carcinoma: a retrospective analysis with independent validation. Lancet Oncol 14(2):159–167
51. Joseph RW et al (2014) Loss of BAP1 protein expression is an independent marker of poor prognosis in patients with low-risk clear cell renal cell carcinoma. Cancer 120(7):1059–1067
52. Kapur P et al (2014) BAP1 immunohistochemistry predicts outcomes in a multi-institutional cohort with clear cell renal cell carcinoma. J Urol 191(3):603–610
53. Farley MN et al (2013) A novel germline mutation in BAP1 predisposes to familial clear-cell renal cell carcinoma. Mol Cancer Res 11(9):1061–1071
54. Duns G et al (2010) Histone methyltransferase gene SETD2 is a novel tumor suppressor gene in clear cell renal cell carcinoma. Cancer Res 70(11):4287–4291
55. Cindolo L et al (2008) Validation by calibration of the UCLA integrated staging system prognostic model for nonmetastatic renal cell carcinoma after nephrectomy. Cancer 113(1):65–71
56. Motzer RJ et al (2008) Prognostic nomogram for sunitinib in patients with metastatic renal cell carcinoma. Cancer 113(7):1552–1558
57. Leibovich BC et al (2010) Histological subtype is an independent predictor of outcome for patients with renal cell carcinoma. J Urol 183(4):1309–1315
58. Frank I et al (2002) An outcome prediction model for patients with clear cell renal cell carcinoma treated with radical nephrectomy based on tumor stage, size, grade and necrosis: the SSIGN score. J Urol 168(6):2395–2400
59. Katz MD et al (2009) The role of lymphovascular space invasion in renal cell carcinoma as a prognostic marker of survival after curative resection. Urol Oncol 29(6):738–744
60. Molina AM et al (2010) Sarcomatoid-variant renal cell carcinoma: treatment outcome and survival in advanced disease. Am J Clin Oncol 34(5):454–459
61. Furge KA et al (2004) Robust classification of renal cell carcinoma based on gene expression data and predicted cytogenetic profiles. Cancer Res 64(12):4117–4121
62. Meloni-Ehrig AM (2002) Renal cancer: cytogenetic and molecular genetic aspects. Am J Med Genet 115(3):164–172
63. Sultmann H et al (2005) Gene expression in kidney cancer is associated with cytogenetic abnormalities, metastasis formation, and patient survival. Clin Cancer Res 11(2 Pt 1):646–655
64. Klatte T et al (2009) Cytogenetic profile predicts prognosis of patients with clear cell renal cell carcinoma. J Clin Oncol 27(5):746–753
65. Balint I et al (2009) Trisomy 7 and 17 mark papillary renal cell tumours irrespectively of variation of the phenotype. J Clin Pathol 62(10):892–895
66. Kovacs G et al (1991) Cytogenetics of papillary renal cell tumors. Genes Chromosom Cancer 3(4):249–255
67. Speicher MR et al (1994) Specific loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 in chromophobe renal cell carcinomas revealed by comparative genomic hybridization. Am J Pathol 145(2):356–364
68. Davis CF, Cherniack AD, Wang M, Yang L, Shen et al (2014) The somatic genomic landscape of chromophobe renal cell carcinoma. Cancer Cell 26(3):319–330
69. Pawlowski R et al (2013) Loss of PBRM1 expression is associated with renal cell carcinoma progression. Int J Cancer 132(2):E11–E17
70. Takahashi M et al (2001) Gene expression profiling of clear cell renal cell carcinoma: gene identification and prognostic classification. Proc Natl Acad Sci U S A 98(17):9754–9759
71. Brannon AR et al (2010) Molecular stratification of clear cell renal cell carcinoma by consensus clustering reveals distinct subtypes and survival patterns. Genes Cancer 1(2):152–163
72. Brooks SA et al (2014) ClearCode34: a prognostic risk predictor for localized clear cell renal cell carcinoma. Eur Urol 66(1):77–84
73. Rini BI ZM, Aydin H et al (2010) Identification of prognostic genomic markers in patients with localized clear cell renal cell carcinoma (ccRCC). J Clin Oncol (Meet Abstr) 28(15 Suppl):4501
74. Cifola I et al (2008) Genome-wide screening of copy number alterations and LOH events in renal cell carcinomas and integration with gene expression profile. Mol Cancer 7:6
75. Costa VL et al (2007) Quantitative promoter methylation analysis of multiple cancer-related genes in renal cell tumors. BMC Cancer 7:133
76. Beroukhim R et al (2009) Patterns of gene expression and copy-number alterations in von-hippel lindau

disease-associated and sporadic clear cell carcinoma of the kidney. Cancer Res 69(11):4674–4681
77. Vieira J et al (2010) Feasibility of differential diagnosis of kidney tumors by comparative genomic hybridization of fine needle aspiration biopsies. Genes Chromosom Cancer 49(10):935–947
78. Kleinrath T et al (2007) Interleukin-4 promoter polymorphisms: a genetic prognostic factor for survival in metastatic renal cell carcinoma. J Clin Oncol 25(7):845–851
79. Kawai Y et al (2007) Associations of single nucleotide polymorphisms in the vascular endothelial growth factor gene with the characteristics and prognosis of renal cell carcinomas. Eur Urol 52(4):1147–1155
80. Xu CF et al (2011) Pazopanib efficacy in renal cell carcinoma: evidence for predictive genetic markers in angiogenesis-related and exposure-related genes. J Clin Oncol 29(18):2557–2564
81. Xu C-F et al (2015) IL8 polymorphisms and overall survival in pazopanib- or sunitinib-treated patients with renal cell carcinoma. Br J Cancer. 112(7):1190–1198
82. Scartozzi M et al (2013) VEGF and VEGFR polymorphisms affect clinical outcome in advanced renal cell carcinoma patients receiving first-line sunitinib. Br J Cancer 108(5):1126–1132
83. Klatte T et al (2007) Hypoxia-inducible factor 1 alpha in clear cell renal cell carcinoma. Clin Cancer Res 13(24):7388–7393
84. Saez MI et al (2012) Hypoxia-inducible factor (HIF) 1{alpha} and 2{alpha} as predictive markers of outcome to VEGFR tyrosine kinase inhibitors (TKI) in renal cell carcinoma (RCC). J Clin Oncol 30(15_Suppl):4630, ASCO Meeting Abstracts
85. Garcia-Donas J et al (2013) Prospective study assessing hypoxia-related proteins as markers for the outcome of treatment with sunitinib in advanced clear-cell renal cell carcinoma. Ann Oncol 24(9): 2409–2414
86. Vroling L et al (2009) Increased numbers of small circulating endothelial cells in renal cell cancer patients treated with sunitinib. Angiogenesis 12(1):69–79
87. Farace F et al (2011) Levels of circulating CD45(dim) CD34(+)VEGFR2(+) progenitor cells correlate with outcome in metastatic renal cell carcinoma patients treated with tyrosine kinase inhibitors. Br J Cancer 104(7):1144–1150
88. Cangiano T et al (1999) Sarcomatoid renal cell carcinoma: biologic behavior, prognosis, and response to combined surgical resection and immunotherapy. J Clin Oncol 17(2):523–528
89. Motzer RJ et al (2002) Treatment outcome and survival associated with metastatic renal cell carcinoma of non-clear-cell histology. J Clin Oncol 20(9): 2376–2381
90. Upton MP et al (2005) Histologic predictors of renal cell carcinoma response to interleukin-2-based therapy. J Immunother 28(5):488–495
91. McDermott DF et al (2010) The high-dose aldesleukin (HD IL-2) "SELECT" trial in patients with metastatic renal cell carcinoma (mRCC). J Clin Oncol 28(15_Suppl):4514, ASCO Meeting Abstracts
92. Atkins M et al (2005) Carbonic anhydrase IX expression predicts outcome of interleukin 2 therapy for renal cancer. Clin Cancer Res 11(10):3714–3721
93. Jaeger E, Waldman F, Roydasgupta R et al. (2008) Array-based comparative genomic hybridization (CGH) identifies chromosomal imbalances between interleukin-2 complete and non-responders. J Clin Oncol 26(15 suppl):Abstract 5043
94. Ito N et al (2007) STAT3 polymorphism predicts interferon-alfa response in patients with metastatic renal cell carcinoma. J Clin Oncol 25(19):2785–2791
95. Topalian SL et al (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366(26):2443–2454
96. Rini BI (2010) Biomarkers: hypertension following anti-angiogenesis therapy. Clin Adv Hematol Oncol 8(6):415–416
97. Rini BI et al (2010) Toxicity of sunitinib plus bevacizumab in renal cell carcinoma. J Clin Oncol 28(17):e284–e285; author reply e286–7
98. Rini BI, Cohen D, Lu D et al. (2010) Hypertension (HTN) as a biomarker of efficacy in patients (pts) with metastatic renal cell carcinoma (mRCC) treated with sunitinib. Presented at the 2010 Genitourinary Cancers Symp [abstr 312]
99. Choueiri TK et al (2008) von Hippel-Lindau gene status and response to vascular endothelial growth factor targeted therapy for metastatic clear cell renal cell carcinoma. J Urol 180(3):860–865; discussion 865–866
100. Gad S, Sultan-Amar V, Meric J et al (2007) Somatic von Hippel-Lindau (VHL) gene analysis and clinical outcome under antiangiogenic treatment in metastatic renal cell carcinoma: preliminary results. Target Oncol 2:3–6
101. Hutson T, Davis I, Macheils JH et al. (2008) Biomarker analysis and final efficacy and safety results of a phase II renal cell carcinoma trial with pazopanib (GW786034), a multikinase angiogenesis inhibitor. J Clin Oncol 26:Abstract 5046
102. Pena C et al (2010) 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 16(19):4853–4863
103. Patel PH, Chadalavada R, Ishill NM et al. (2008) Hypoxia-inducible factor (HIF) 1a and 2a levels in cell lines and human tumor predicts response to sunitinib in renal cell carcinoma (RCC). J Clin Oncol 26:Abstract 5008
104. Shen C et al (2011) Genetic and functional studies implicate HIF1alpha as a 14q kidney cancer suppressor gene. Cancer Discov 1(3):222–235
105. Escudier B et al (2009) 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 27(20): 3312–3318

106. Bukowski RM, Eisen T, Szczylik C et al (2007) Final results of the randomized phase III trial of sorafenib in advanced renal cell carcinoma: survival and biomarker analysis. J Clin Oncol 25:Abstract 5023
107. Tran HT et al (2012) 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 13(8):827–837
108. Porta C et al (2010) Predictive value of baseline serum vascular endothelial growth factor and neutrophil gelatinase-associated lipocalin in advanced kidney cancer patients receiving sunitinib. Kidney Int 77(9):809–815
109. Rini BI et al (2008) Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. J Clin Oncol 26(22):3743–3748
110. DePrimo SE, Bello C (2007) Surrogate biomarkers in evaluating response to anti-angiogenic agents: focus on sunitinib. Ann Oncol 18(Suppl 10):x11–x19
111. Deprimo SE et al (2007) 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 5:32
112. D' Alterio C et al (2012) High CXCR4 expression correlates with sunitinib poor response in metastatic renal cancer. Curr Cancer Drug Targets 12(6):693–702
113. Zurita AJ et al (2012) A cytokine and angiogenic factor (CAF) analysis in plasma for selection of sorafenib therapy in patients with metastatic renal cell carcinoma. Ann Oncol 23(1):46–52
114. Dutcher JP et al (2009) Effect of temsirolimus versus interferon-alpha on outcome of patients with advanced renal cell carcinoma of different tumor histologies. Med Oncol 26(2):202–209
115. Plantade A, C.B., Escudier B et al. (2007) Treatment outcome for metastatic papillary and chromophobe renal cell carcinoma (RCC) patients treated with tyrosine-kinase inhibitors (TKIs) sunitinib and sorafenib. J Clin Oncol 25(18 suppl):Abstract 5037
116. Motzer RJ et al. (2014) 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. J Clin Oncol 32(25):2765–2772
117. Velickovic M et al (2002) Intragenic PTEN/MMAC1 loss of heterozygosity in conventional (clear-cell) renal cell carcinoma is associated with poor patient prognosis. Mod Pathol 15(5):479–485
118. Figlin RA et al (2009) Analysis of PTEN and HIF-1alpha and correlation with efficacy in patients with advanced renal cell carcinoma treated with temsirolimus versus interferon-alpha. Cancer 115(16):3651–3660
119. Sarbassov DD, Ali SM, Sabatini DM (2005) Growing roles for the mTOR pathway. Curr Opin Cell Biol 17(6):596–603
120. Pantuck AJ et al (2007) Prognostic relevance of the mTOR pathway in renal cell carcinoma: implications for molecular patient selection for targeted therapy. Cancer 109(11):2257–2267
121. Youssif TA et al (2010) The mammalian target of rapamycin pathway is widely activated without PTEN deletion in renal cell carcinoma metastases. Cancer 117(2):290–300
122. Cho D et al (2007) Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. Clin Genitourin Cancer 5(6):379–385
123. Voss MH et al (2014) Tumor genetic analyses of patients with metastatic renal cell carcinoma and extended benefit from mTOR inhibitor therapy. Clin Cancer Res 20(7):1955–1964
124. Bluemke K et al (2009) Detection of circulating tumor cells in peripheral blood of patients with renal cell carcinoma correlates with prognosis. Cancer Epidemiol Biomarkers Prev 18(8):2190–2194
125. Slaby O et al (2010) Expression of miRNA-106b in conventional renal cell carcinoma is a potential marker for prediction of early metastasis after nephrectomy. J Exp Clin Cancer Res 29:90
126. Ishihara T et al. (2014) Expression of the tumor suppressive microRNA-23b/27b cluster is a good prognostic marker in clear cell renal cell carcinoma. J Urol 192(6):1822–1830
127. Wood SL et al (2010) Association of serum amyloid A protein and peptide fragments with prognosis in renal cancer. Br J Cancer 103(1):101–111
128. Dolley-Hitze T et al (2010) Angiotensin-2 receptors (AT1-R and AT2-R), new prognostic factors for renal clear-cell carcinoma? Br J Cancer 103(11): 1698–1705
129. Jagdev SP et al (2010) Improving the accuracy of pre-operative survival prediction in renal cell carcinoma with C-reactive protein. Br J Cancer 103(11): 1649–1656
130. D'Alterio C et al (2010) Concomitant CXCR4 and CXCR7 expression predicts poor prognosis in renal cancer. Curr Cancer Drug Targets 10(7):772–781
131. Vasudev NS et al (2009) Pre-operative urinary cathepsin D is associated with survival in patients with renal cell carcinoma. Br J Cancer 101(7):1175–1182
132. Wagener N et al (2010) Enhancer of zeste homolog 2 (EZH2) expression is an independent prognostic factor in renal cell carcinoma. BMC Cancer 10:524
133. Mosashvilli D et al (2010) Global histone acetylation levels: prognostic relevance in patients with renal cell carcinoma. Cancer Sci 101(12):2664–2669
134. Ronkainen H et al (2010) HuR expression is a marker of poor prognosis in renal cell carcinoma. Tumour Biol 32(3):481–487
135. Hoffmann NE et al (2008) External validation of IMP3 expression as an independent prognostic marker for metastatic progression and death for patients with clear cell renal cell carcinoma. Cancer 112(7):1471–1479

136. Kawata N et al (2007) Significant relationship of matrix metalloproteinase 9 with nuclear grade and prognostic impact of tissue inhibitor of metalloproteinase 2 for incidental clear cell renal cell carcinoma. Urology 69(6):1049–1053
137. Merseburger AS et al (2008) Activation of PI3K is associated with reduced survival in renal cell carcinoma. Urol Int 80(4):372–377
138. Abou Youssif T et al (2011) The mammalian target of rapamycin pathway is widely activated without PTEN deletion in renal cell carcinoma metastases. Cancer 117(2):290–300
139. Zubac DP et al (2010) Type 1 plasminogen activator inhibitor (PAI-1) in clear cell renal cell carcinoma (CCRCC) and its impact on angiogenesis, progression and patient survival after radical nephrectomy. BMC Urol 10:20
140. Bandiera A et al (2009) Prognostic factors and analysis of S100a4 protein in resected pulmonary metastases from renal cell carcinoma. World J Surg 33(7):1414–1420
141. Zubac DP et al (2009) The expression of thrombospondin-1 and p53 in clear cell renal cell carcinoma: its relationship to angiogenesis, cell proliferation and cancer specific survival. J Urol 182(5):2144–2149
142. Bui MH et al (2003) Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin Cancer Res 9(2):802–811
143. Tsimafeyeu I, Demidov L, Ta H et al (2010) Fibroblast growth factor pathway in renal cell carcinoma. J Clin Oncol (Meet Abstr) 28(15 Suppl): 4621
144. Perez-Gracia JL et al (2009) Identification of TNF-alpha and MMP-9 as potential baseline predictive serum markers of sunitinib activity in patients with renal cell carcinoma using a human cytokine array. Br J Cancer 101(11):1876–1883
145. Busse A et al (2011) Systemic immune tuning in renal cell carcinoma: favorable prognostic impact of TGF-beta1 mRNA expression in peripheral blood mononuclear cells. J Immunother 34(1):113–119
146. Heymach J, Tran H, Fritsche HA et al. (2009) Lower baseline levels of plasma hepatocyte growth factor (HGF), IL-6, IL-8 are correlated with tumor shrinkage in renal cell carcinoma patients treated with pazopanib. Presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics. Abstract A11
147. Bukowski RM et al (2007) Randomized phase II study of erlotinib combined with bevacizumab compared with bevacizumab alone in metastatic renal cell cancer. J Clin Oncol 25(29):4536–4541
148. Nixon AB et al. (2013) Identification of predictive biomarkers of overall survival (OS) in patients (pts) with advanced renal cell carcinoma (RCC) treated with interferon alpha (I) with or without bevacizumab (B): results from CALGB 90206 (Alliance). J Clin Oncol 31(15_suppl):4520 ASCO Meeting Abstracts
149. Armstrong AJ, George D, Halabi S et al (2010) Serum lactate dehydrogenase (LDH) as a biomarker for survival with mTOR inhibition in patients with metastatic renal cell carcinoma (RCC). J Clin Oncol (Meet Abstr) 28(15 Suppl):4631
150. Motzer RJ et al (2010) Phase 3 trial of everolimus for metastatic renal cell carcinoma : final results and analysis of prognostic factors. Cancer 116(18):4256–4265
151. Xu G et al (2009) Application of SELDI-TOF-MS to identify serum biomarkers for renal cell carcinoma. Cancer Lett 282(2):205–213
152. Seliger B et al (2009) Combined analysis of transcriptome and proteome data as a tool for the identification of candidate biomarkers in renal cell carcinoma. Proteomics 9(6):1567–1581
153. Catchpole G et al. (2009) Metabolic profiling reveals key metabolic features of renal cell carcinoma. J Cell Mol Med 15(1):109–118
154. Kim K et al (2009) Urine metabolomics analysis for kidney cancer detection and biomarker discovery. Mol Cell Proteomics 8(3):558–570
155. Zira AN et al (2010) (1)H NMR metabonomic analysis in renal cell carcinoma: a possible diagnostic tool. J Proteome Res 9(8):4038–4044
156. Heinzelmann J et al (2011) Specific miRNA signatures are associated with metastasis and poor prognosis in clear cell renal cell carcinoma. World J Urol 29(3):367–373
157. Liu H et al (2010) Identifying mRNA targets of microRNA dysregulated in cancer: with application to clear cell renal cell carcinoma. BMC Syst Biol 4:51
158. Feng G et al (2013) Quantification of plasma cell-free DNA in predicting therapeutic efficacy of sorafenib on metastatic clear cell renal cell carcinoma. Dis Markers 34(2):105–111
159. Minamimoto R et al (2011) Evaluation of response to multikinase inhibitor in metastatic renal cell carcinoma by FDG PET/contrast-enhanced CT. Clin Nucl Med 35(12):918–923
160. Divgi CR et al (2007) Preoperative characterisation of clear-cell renal carcinoma using iodine-124-labelled antibody chimeric G250 (124I-cG250) and PET in patients with renal masses: a phase I trial. Lancet Oncol 8(4):304–310
161. Spero M et al (2010) Preoperative staging of renal cell carcinoma using magnetic resonance imaging: comparison with pathological staging. Clin Imaging 34(6):441–447
162. Wang H et al (2010) Renal cell carcinoma: diffusion-weighted MR imaging for subtype differentiation at 3.0T. Radiology 257(1):135–143
163. Hillman GG et al (2010) Dynamic contrast-enhanced magnetic resonance imaging of sunitinib-induced vascular changes to schedule chemotherapy in renal cell carcinoma xenograft tumors. Transl Oncol 3(5):293–306
164. Pedrosa I, Alsop DC, Rofsky NM (2009) Magnetic resonance imaging as a biomarker in renal cell carcinoma. Cancer 115(10 Suppl):2334–2345

165. Katz-Brull R et al (2005) Decreases in free cholesterol and fatty acid unsaturation in renal cell carcinoma demonstrated by breath-hold magnetic resonance spectroscopy. Am J Physiol Renal Physiol 288(4):F637–F641
166. Lassau N et al (2010) Dynamic contrast-enhanced ultrasonography (DCE-US): a new tool for the early evaluation of antiangiogenic treatment. Target Oncol 5(1):53–58
167. Choueiri et al (2013) Phase II and biomarker study of the dual MET/VEGFR2 inhibitor foretinib in patients with papillary renal cell carcinoma. J. Clin Oncol. 31(2):181–186