Review

Biomarkers Towards New Era of Therapeutics for Metastatic Renal Cell Carcinoma

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Abstract. With the improved knowledge of molecular oncology and the introduction of targeted therapies as well as immunotherapies, there has been significant progress in the treatment of patients with metastatic renal cell carcinoma (mRCC). At present, treatment decisions are still made mainly based on clinical factors because no validated prognostic and predictive biomarkers for mRCC exist. Currently, inflammatory markers, genetic markers, and immune checkpoint molecules are candidate biomarkers for more personalized treatment of mRCC. RCC has been considered to be an inflammatory tumor and its underlying inflammatory mechanism would play some roles in forming resistance to systemic therapy. The von Hippel-Lindau (*VHL*) gene is inactivated by either mutation or methylation in over 80% of clear cell RCC (ccRCC). Thus, most, if not all, ccRCC may have deregulation of the VHL pathway. For some reason, *VHL* status is difficult to use as a prognostic marker. Polybromo 1 (*PBRM1*) is the second most frequently mutated gene in ccRCC and loss of function mutations in the *PBRM1* gene have been shown to be associated with improved survival in patients with mRCC treated with systemic therapies. The expression of programmed death ligand 1 (PD-L1) on tumor cells in RCC seems to be associated with a higher tumor stage, a worse response to tyrosine kinase inhibitor (TKI) therapy, and a worse prognosis. Future challenges are required to develop and validate predictive biomarkers in order to establish a more personalized treatment for mRCC.

Keywords: Carcinoma, renal cell, biomarkers, C-reactive protein, programmed cell death 1 receptor

INTRODUCTION

Localized renal cell carcinoma (RCC) can potentially be treated with curative intent surgically by partial or radical removal of the involved kidney. However, up to 30% of the patients present with metastatic disease at the time of diagnosis and a further 30% will eventually develop metastases during the course of the disease [1]. Within the last decade, the approval of targeted agents and immune checkpoint inhibitors (ICIs) has dramatically changed the scenario of systemic treatment of metastatic renal cell carcinoma (mRCC) to achieve improved survival [2–6]. At first, an improved understanding of functional loss of von Hippel Lindau (*VHL*) protein, which upregulates vascular endothelial growth factor (VEGF)-dependent angiogenesis in clear cell RCC (ccRCC), has led to the development of tyrosine kinase inhibitors (TKIs) targeting mainly VEGF receptors (VEGFR). Next, the findings that dysregulation of the phosphatidylinositol-3 kinase (PI3K)-Akt-mammalian Target Of Rapamycin (mTOR) pathway, activated at different levels of the signaling cascade, drives ccRCC progression

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has led to the development of mTOR inhibitors [3]. Recently, ICIs targeting natural immune homeostasis pathways to drive anti-tumor immune responses have also been developed in the treatment of mRCC [5, 6].

Understanding the biology and possible molecular mechanisms of ccRCC has aided in the identification of candidate biomarkers. However, the transfer of biomarkers from the discovery stage to clinical practice is difficult for many reasons, such as a lack of specificity and/or sensitivity, or lower reproducibly. Currently, no validated predictive biomarkers that could help clinicians identify patients who are more likely to respond to a given therapy are available. Thus, there still remains the need to discover potential prognostic and predictive biomarkers that could help to identify more personalized treatments for mRCC.

In this review, we will focus on: 1. Risk stratification model; 2. Clinical biomarkers; 3. Prognostic inflammatory markers; 4. Gene expression as predictive biomarkers; 5. Mismatch repair deficiency (dMMR) and mutational load; 6. Expression of immune checkpoint proteins as a predictor of systemic therapy; and 7. Gut microbiota composition. Finally, we briefly highlight likely future perspectives of predictive biomarkers of systemic therapy for mRCC.

Risk stratification model

Despite the advances in treatment options for mRCC, treatment decisions are mainly based on clinical factors [7]. Motzer et al. developed a prognostic model to predict the prognosis of mRCC patients by combining five clinical factors, namely low Karnofsky performance status, high lactate dehydrogenase, low serum hemoglobin, high corrected serum calcium, and time from initial RCC diagnosis to start of interferon-alpha therapy of less than one year [8]. In the era of molecular-targeted therapy, Heng et al. developed a prognostic model with six clinical variables, namely low Karnofsky performance status, low serum hemoglobin, high corrected serum calcium, neutrophils greater than the ULN, platelets greater than the ULN, and time from initial RCC diagnosis to start of systemic therapy of less than one year [9]. These clinical prognostic model could stratify the prognosis of each patient with mRCC into favorable (with zero risk factors), intermediate (with one or two risk factors) and poor risk groups (with three or more risk factors). These models are important tools for treatment decisions. For example, temsirolimus is approved only for patients with poor risk, and the combination of ipilimumab plus nivolumab as a first line setting is approved for patients with IMDC intermediate/poor risk.

Clinical biomarkers

During targeted therapy, treatment-related adverse events (AEs) are frequently observed, which indicate on-target effects of a targeted agent. Hypertension developing during treatment with VEGFR TKIs was significantly correlated with improved progression free survival (PFS) and overall survival (OS) [10]. Hypothyroidism is another frequent but generally mild AE known to be caused by VEGFR TKIs. Conflicting results exist about the utility of hypothyroidism as a predictive marker. In one study, hypothyroidism was associated with a better outcome in patients receiving VEGFR TKIs. Subclinical hypothyroidism diagnosed during the first 2 months of treatment has also been reported to be associated with survival [11]. On the other hand, a meta-analysis paper evaluating 11 studies failed to identify any predictive value of hypothyroidism [12]. Hand foot syndrome (HFS) is frequently observed in patients receiving VEGFR TKIs, and some patients discontinue treatment when it is severe, because HFS impacts the patient's quality of life. A possible explanation for this adverse event is dermal endothelial cell apoptosis due to inhibition of VEGFR and platelet-derived growth factor receptor (PDGFR). It has been reported that HFS during sunitinib treatment was associated with longer PFS in patients with mRCC [13]. Thus, treatment-related adverse events might be well-known predictive markers of a response, however, these are not evaluable prior to treatment initiation.

Inflammatory markers

RCC is considered to be an inflammatory tumor. With recent advancements in the understanding of cancer pathogenesis, it has become well established that the host inflammatory response plays an integral role in cancer progression [14]. C-reactive protein (CRP), which belongs to an acute phase protein, is mainly synthesized by hepatocytes, and has been widely used as an unspecific marker of systemic inflammation. Elevation in CRP is often observed in advanced cancers. Furthermore, patients with advanced cancer, including mRCC, and with elevated baseline CRP often show primarily poor responses to systemic treatment [15]. The exact

mechanisms for CRP elevation in cancer patients are not clearly understood, however, several possible mechanisms have been suggested to explain the association between RCC and increased CRP levels. One possible explanation is that inflammatory cytokines, such as interleukin (IL) -1, tumor necrosis factor, and IL-6, produced by RCCs, can induce systemic inflammation [16]. Experimental studies have showed that some renal cancer cell lines could produce IL-6, which promotes autocrine tumor growth, to induce the production of acute phase proteins, including CRP, in hepatocytes. These results imply that the presence of a systemic inflammatory response would reflect tumor aggressiveness. Additionally, in other types of cancers, CRP has been shown to promote cancer cell growth and metastasis by directly inhibiting apoptosis of cancer cells [17]. For mRCC, several studies have already revealed a prognostic role for CRP in patients treated with systemic therapy. Fujita et al. reported that a normal level of CRP at baseline was an independent predictive marker of a response to targeted therapy by multivariate analysis [18]. Teishima et al. demonstrated that an increase in the serum CRP level during targeted therapy indicated the progression of mRCC [19]. Mizuno et al revealed that an elevated baseline of high sensitivity CRP, which can detect low grade inflammation, would predict resistance to sunitinib [20].

Another marker of systemic inflammation, the neutrophil-to-lymphocyte ratio (NLR), was also found to add prognostic information in patients with mRCC [21, 22]. More recently, the NLR has also emerged as a predictive marker of a response to sunitinib [23]. Another study demonstrated that a baseline NLR <3 and duration of prior anti VEGF therapy of <6 months, independently predicted longer PFS as well as longer OS with ICI therapy in mRCC [24]. Changes in the NLR during ICI therapy are also associated with prognosis. Lalani et al demonstrated that an early decline (decrease ≥25%) of NLR at 6 weeks was associated with an improved PFS and significantly better OS, whereas a relative increase by ≥25% was associated with poorer PFS and OS [25]. These results imply that an underlying inflammatory mechanism would play some roles in forming resistance to systemic therapy.

Distinct genetic features

Genetic and epigenetic inactivation of *VHL*, which is found in more than 70% of cases of ccRCC, has been identified as the earliest and fundamental major driving event in the pathogenesis of RCC [26]. Functional loss of *VHL* results in constitutive activation of hypoxia inducible factors (HIFs), which act as transcription factors for various pro tumorigenic target genes including VEGF [27]. VEGF is the major factor responsible for tumor angiogenesis, and many therapeutic approaches to target this molecular pathway have been established for the treatment of mRCC. Thus, *VHL* gene alteration plays a key role in RCC pathogenesis and provides a therapeutic target, and its impact on prognosis has been studied in a variety of case series. However, the findings from these studies demonstrated conflicting results, therefore, the clinical significance of *VHL* gene alteration in RCC has not been clearly ascertained. *VHL* status is difficult to use as a prognostic marker for several reasons. One possible explanation is that its genomic classification is complicated because *VHL* gene inactivation is caused by mutation, loss of heterozygosity, and promoter methylation [28]. Additionally, various features of the *VHL* locus itself have posed technical challenges for sequencing, including its heavy guanine-cytosine content, small coding region, and frequent occurrences of large insertion and deletions. Some studies focused on single nucleotide polymorphisms (SNPs) in the *VHL* gene. The minor alleles of 2 *VHL* SNPs, rs1642742 and rs1642743, which would be responsible for a change in the microenvironment of ccRCC, are candidate biomarkers for poor OS in mRCC patients receiving first-line VEGFR-TKI [29].

The chromosome 3p region, which contains the *VHL* gene, also harbors genes encoding chromatin regulatory factors. Next generation sequencing (NGS) of ccRCC revealed novel, frequent mutations of chromatin modifying tumor suppressor genes, namely *PBRM1*, *BAP1*, *SETD2*, and *KDM5C* [30]. After *VHL*, *PBRM1* is the second most frequently mutated gene in ccRCC, and it encodes the SWI/SNF chromatin remodeling complex component BAF180 protein. The *BRCA1* associated protein-1 (*BAP1*) gene, which locates on chromosome 3p between the *VHL* and *PBRM1* genes, is mutated in approximately 15% of patients with ccRCC [31]. In most of the cases with ccRCC, *BAP1* and *PBRM1* mutations are mutually exclusive, whereas the molecular basis of this relation is still unknown [32]. Whereas *BAP1*-mutant tumors tend to exhibit a high Fuhrman grade, sarcomatoid transformation, and may show coagulative necrosis, *PBRM1*-mutant tumors may be of high or low grade and less frequently exhibit necrosis [33–35]. A growing number of analyses in a

non-metastatic setting have suggested that the protein expression statuses of *BAP1* and *PBRM1* are prognostic for cancer-specific survival [36, 37]. In the retrospective cohort study utilizing samples from the COMPARZ and RECORD-3 trials, the mutation statuses of *BAP1*, *PBRM1*, and *TP53* have been independently demonstrated to be prognostic indices in patients with mRCC treated with first-line TKIs [38]. Recently, loss of function mutations in the *PBRM1* gene have been shown to be associated with improved survival in patients with mRCC treated with ICIs [39], however, several limitations restrict the clinical application of a *PBRM1* mutation as a biomarker in mRCC treatment. Those limitations include a modest effect of *PBRM1* mutation on prognosis [39, 40], a lack of evidence for a PBRM1 mutation effect on ICIs in the front line setting [41], and a possible association between mutations and benefit from prior antiangiogenic treatment [41].

In a recent study, molecular features which differentiate therapy-specific outcomes and might inform personalized therapy strategies have been proposed. The phase II IMmotion150 trial compared atezolizumab plus bevacizumab with sunitinib in first line treatment and validated predictive gene signatures [41]. A T effector cell signature and PD-L1 expression were associated with favorable outcomes for atezolizumab plus bevacizumab and were similar across risk groups. An angiogenic gene signature was associated with favorable outcomes with sunitinib, and was higher in good risk patients. Patients with sarcomatoid tumors responded poorly to sunitinib and had lower angiogenic signatures, but higher T effector cell signatures and PD-L1 expression. The phase III JAVELIN Renal 101 trial compared avelumab plus axitinib with sunitinib in first line treatment [42]. In this study, gene expression analysis was performed for 720 baseline tumor samples and identified the top 26 genes associated with immune-related function and PFS, creating a JAVELIN Renal 101 signature composed of five groups of genes associated with T-cell receptor signaling; T-cell activation, proliferation, and differentiation; natural killer cell-mediated cytotoxicity; chemokines; and other immune response genes [43]. The PFS according to the signature showed that high expression in the avelumab plus axitinib arm led to a PFS benefit (HR 0.60, 95%CI 0.439–0.834, $p = 0.0019$), but no difference in the sunitinib arm (HR 0.89, 95%CI 0.670–1.172, $p = 0.3973$). The PFS according to mutations and polymorphisms demonstrated that mutations in *CD163L1* and *DNMT1* and wild type in *PTEN* were associated with PFS benefit in the avelumab plus axitinib arm.

Tumor mutational burden and microsatellite instability

Tumor mutational burden (TMB) is a measurement of mutations carried by tumor cells. Previous studies demonstrated that a high TMB was predictive for the response to ICIs in melanoma and non-small cell lung cancer (NSCLC). However, RCCs display intermediate levels of mutational load [44]. Instead, it was demonstrated that RCCs have the highest proportion and number of insertion and deletion mutations across the pan-cancer cohort [45]. Recently, the U.S. Food and Drug Administration (FDA) has granted an accelerated approval to the PD-1 antibody pembrolizumab for the treatment of patients with unresectable or metastatic solid tumors, which have progressed following prior treatment and that have been identified as having high microsatellite instability (MSI-High) or mismatch repair deficiency (dMMR). A previous report indicated that complete MSI is uncommon in RCC [46].

PD-1/PD-L1 expression

The major functions of immune checkpoint proteins are to regulate overall immune homeostasis by modulating T cell responses. Cancer cells often escape from T cell antitumor immune responses when these immune checkpoint proteins are up regulated in the tumor micro environment [47]. The prognostic significance of expression patterns of immune checkpoint proteins in patients with various types of malignant tumors has been investigated [48]. The programmed cell death protein-1 (PD-1; CD279) is an immune inhibitory receptor that interacts with its ligand, programmed death ligand 1 (PD-L1; CD274, B7–H1). They comprise one of the main immune checkpoint pathways that downregulates immune activity. PD-1, which plays a pivotal role in immune resistance in the tumor environment, is expressed at high levels on activated T cells, B cells, natural killer T cells, monocytes and myeloid dendritic cells. Meanwhile, PD-L1 is widely expressed on several malignant tumor cells as well as antigen-presenting cells and other immune cells. With the introduction of ICIs, including antibodies directed against PD-1/PD-L1, the landscape of cancer treatment has been

Table 1
Selected trials on the role of PD-L1 expression as a predictive biomarker of response to systemic therapy for metastatic renal cell carcinoma

Trials	PD-L1 cut offs	PD-L1 status	RR%	survival	Regimen
Check Mate 025	≥1% tumor cells	+	25	OS 21.8 (16.5 to 28.1)	nivolumab
	membrane staining	−	75	OS 27.4 (21.4 to NE)	nivolumab
	≥5% tumor cells	+	11	OS 21.9 (14.0 to NE)	nivolumab
	membrane staining	−	89	OS 24.6 (21.4 to NE)	nivolumab
Check Mate 214	≥1% tumor cells	+		OS NR (NE to NE)	ipilimumab/nivolumab
	membrane staining	−		OS NR (28.2 to NE)	ipilimumab/nivolumab
	≥1% tumor cells	+		OS 19.6 (14.8 to NE)	sunitinib
	membrane staining	−		OS NR (24.0 to NE)	sunitinib
JAVELIN Renal 101	≥1% immune cells	+	55	PFS 13.8 (11.1 to NE)	axitinib/avelumab
	staining positive	ITT	51	PFS 13.8 (11.1 to NE)	axitinib/avelumab
	≥1% immune cells	+	25	PFS 7.2 (5.7 to 9.7)	sunitinib
	staining positive	ITT	26	PFS 8.4 (6.9 to 11.1)	sunitinib
IMmotion 151	≥1% tumor infiltrating	+	43	PFS 11.2 (8.9 to 15.0)	bevacizumab/atezolizumab
	immune cells	ITT	37	PFS 11.2 (9.6 to 13.3)	bevacizumab/atezolizumab
	≥1% tumor infiltrating	+	35	PFS 7.7 (6.8 to 9.7)	sunitinib
	immune cells	ITT	33	PFS 8.4 (7.5 to 9.7)	sunitinib
COMPARZ	>55 H score	+		OS 15.1 (9.4 to 45.1)	pazopanib
	membrane staining	−		OS 35.6 (27.2 to 40.8)	pazopanib
	>55 H score	+		OS 15.3 (11.2 to 30.5)	sunitinib
	membrane staining	−		OS 27.8 (23.7 to 32.9)	sunitinib
	>125 H score	+		OS 5.1 (4.2 to NE)	pazopanib
	membrane staining	−		OS 33.1 (26.7 to 40.4)	pazopanib
	>125 H score	+		OS 8.9 (2.6 to 38.1)	sunitinib
	membrane staining	−		OS 27.4 (21.4 to 32.0)	sunitinib
CABOSUN	≥1% tumor cells	+		PFS 8.4 (1.1 to 16.6)	cabozantinib
	membrane staining	−		PFS 11.0 (6.8 to 15.6)	cabozantinib
	≥1% tumor cells	+		PFS 3.1 (1.6 to 10.1)	sunitinib
	membrane staining	−		PFS 5.0 (3.0 to 12.9)	sunitinib
	≥1% tumor cells	+		OS 18.1 (1.1 to 35.0)	cabozantinib
	membrane staining	−		OS 30.3 (18.8 to NE)	cabozantinib
	≥1% tumor cells	+		OS 21.0 (6.4 to 30.8)	sunitinib
	membrane staining	−		OS 22.4 (7.6 to NE)	sunitinib

RR: response rate; OS: overall survival; PFS: progression free survival; NR: not reached; NE: not estimable; ITT: intent to treat.

shifting. ICIs have been expected to hold promise as a treatment for various cancer types, including RCC. Nivolumab, a monoclonal antibody targeting PD-1, was approved for previously treated patients with mRCC, and based on the CheckMate 025 study demonstrated OS benefit over everolimus [5]. More recently, in the CheckMate 214 study, the combination of nivolumab and ipilimumab, a CTLA-4 antibidy, demonstrated a statistically superior median OS and higher objective response rate compared with sunitinib in previously untreated patients with mRCC [6].

The expression of PD-L1 on cancer cells or tumor-infiltrating immune cells by immunohistochemistry (IHC) has been used as a biomarker to predict which patients might benefit from ICI therapy in several cancer types. A phase I study of nivolumab in patients with solid tumors demonstrated objective responses for some cancer types. A relationship between PD-L1 expression on tumor cells and an objective response was demonstrated [49]. Currently, some assays for PD-L1 expression have been validated as companion diagnostic tests, however, several problems concerning using PD-L1 as a biomarker exist, such as the use of different IHC assays, different cut-offs, intra-tumor heterogeneity, and dynamic changes of PD-L1 expression. Attempts to standardize IHC assays are warranted. In mRCC, positive PD-L1 expression indicates aggressive features and a poor prognosis (Table 1). Recent reports from the COMPARZ trial, a non-inferiority trial which compared pazopanib to sunitinib as first line treatment in mRCC patients, demonstrated that positive expression of PD-L1 at baseline could predict a poor prognosis [50]. On the other hand, in the CheckMate 025 study, subsequent nivolumab treatment demonstrated a survival benefit over everolimus in patients regardless of the extent of PD-L1 expression in their tumor specimen [5]. These

results suggest that to determine subsequent treatment with nivolumab, the PD-L1 expression status before first line treatment alone is insufficient, since PD-L1 status might change dynamically with first line treatment. In the CheckMate 214 study, patients who were treated with a combination of nivolumab and ipilimumab as a first line setting demonstrated OS benefit over the sunitinib group regardless of their PD-L1 expression, however, the benefit was much more pronounced in patients with PD-L1 positive status, although patients with PD-L1 negative status could still benefit from combination therapy [6]. On the other hand, the cancer-specific survival with sunitinib therapy was worse in patients with PD-L1 positive status. From these results, PD-L1 expression status did not seem to be a useful marker to make decisions between these 2 regimens based on the World Health Organization (WHO) tumor response criteria (RECIST) [51, 52]. Recent studies demonstrated that immune-related response criteria (irRECIST) may more accurately predict ORR or prognosis in cancer patients treated with ICIs when compared with RECIST [53, 54]. In the CheckMate-010 trial, a dose-finding study in patients with mRCC, tumor cell PD-L1 expression was not significantly associated with improved PFS, however, median irPFS was significantly longer in the tumor cell PD-L1 expression $\geq 1\%$ group compared with the tumor cell PD-L1 $<1\%$ group [55]. Thus, we must take into consideration that PD-L1 expression is more useful when utilized in different response criteria. In fact, some PD-L1 negative patients also benefited from ICI treatment, and there was still a large proportion of PD-L1 positive patients who did not respond to the treatment. Explanations with respect to the discrepancy between the trial results and expectations involve many possibilities. Among them, the heterogeneity of the tumor may play an important role, in particular the heterogeneity between the primary and metastatic tumors [56].

Recently, in the phase III IMmotion 151 study, patients who were treated with a combination of atezolizumab, an anti PD-L1 antibody, and bevacizumab as a first line setting demonstrated longer PFS over sunitinib in the PD-L1 positive population [57]. In the JAVELIN Renal 101 study, patients who were treated with a combination of avelumab, an anti PD-L1 antibody, and VEGFR-TKI axitinib as a first line setting demonstrated longer PFS over sunitinib in the PD-L1 positive and overall population [42]. In the phase 2 CABOSUN study, patients in the IMDC intermediate/poor risk group who were treated with cabozantinib, an oral inhibitor of MET, AXL, and VEGFR2, as a first line setting demonstrated longer PFS over sunitinib irrespective of PD-L1 expression on tumor cells [58, 59].

Gut microbiota composition

Accumulating evidence indicates that intestinal microbiota can play a role in the maturation of host immunity. The unexpected link between the gut microbiota and the response to immune checkpoint inhibitors has recently been studied in several cancers, including mRCC. The baseline gut flora composition is believed to stimulate or inhibit the host immune response [60]. Modification of the gut flora composition by use of antibiotics before or shortly after ICI was shown to be associated with a poorer response and worse OS for patients with non small cell lung cancer (NSCLC), urothelial carcinoma (UC), and RCC [61]. In the same study, the baseline gut microbiota compositions of 100 NSCLC and RCC patients were analyzed and Akkermansia muciniphila was found to be present in 69% of the responders compared to 34% of the non responders ($p = 0.007$). Thus, gut microbiota composition has the potential to be a novel ICI biomarker and some prospective studies are ongoing to evaluate the prognostic or predictive effect of gut microbiota on ICI outcome.

SUMMARY

With the rapid development of therapeutic agents and sequential treatments over the past decade, significant clinical benefit has been achieved in patients with mRCC. More recently, combination therapy including ICIs has prolonged survival and has become a mainstay of upfront settings. Although there are several potential biomarker candidates, their predictive values have not yet been validated by prospective clinical trials. Therefore, the optimal selection and sequencing of agents still remain a challenge. Future attempts to develop and validate predictive biomarkers to establish a more personalized treatment for mRCC are needed.

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AUTHOR CONTRIBUTIONS

RM: Literature Research, Drafting the manuscript; MO: Critical revision of the manuscript, Supervision.

CONFLICT OF INTEREST

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