REVIEW

Renal cell carcinoma: entering the age of biomarkers

Andrew S. Iskandar, MD, Kevin K. Zarrabi, MD, William J. Tester, MD
Department of Medical Oncology, Sidney Kimmel Cancer Center, Thomas Jefferson University Hospital, Philadelphia, Pennsylvania, USA

ISKANDAR AS, ZARRABI KK, TESTER WJ. Renal cell carcinoma: entering the age of biomarkers. *Can J Urol* 2024;31(4):11921-11930.

Introduction: *Renal cell carcinoma is as the most prevalent form of kidney cancer, with the clear cell subtype comprising approximately 75% of cases. The identification of predictive and prognostic biomarkers has emerged as a crucial area of research within the field. Despite advancements in treatment, metastatic renal cell carcinoma presents formidable challenges, with survival rates heavily dependent upon the optimal choice of treatment.*

Materials and methods: *This review summarizes the current literature regarding the prognostic and predictive value of biomarkers in patients with renal cell carcinoma. We conducted a comprehensive literature search to identify studies that reference biomarkers of interest in this domain.*

We selected studies based on their relevance, publication date, and the quality of the research. Data from these selected papers were compiled and analyzed to provide an overview of the current understanding and advancements in the field. The findings were then synthesized into a concise discussion highlighting the state of biomarker research in renal cell carcinoma today.

Results and conclusions: *While various nucleic acid and protein biomarkers have shown promise in other malignancies, their application in renal cell carcinoma remains limited by the lack of validated predictors. This review aims to highlight the pressing need for robust predictive and prognostic biomarkers in renal cell carcinoma to guide clinicians in tailoring optimal therapeutic strategies. The discussion encompasses the limitations of existing markers and underscores the significance of the most recent advancements within the field. Despite these strides, the clinical application of renal cell carcinoma biomarkers requires further study and validation.*

Key Words: renal cell carcinoma, biomarkers, immunotherapy, tyrosine kinase inhibitors, clear cell, circulating tumor DNA

Introduction

Renal cell carcinoma (RCC) is the most common form of kidney cancer. In 2023, there were 81,800 estimated new cases, accounting for 14,000 deaths in the United States.[1] Approximately 75% of cases are histologically classified as clear cell RCC (ccRCC), while the remainder as non-clear cell RCC.[2] A majority of patients present with localized disease amenable to local management strategies. However, roughly one-third of patients present with distant metastatic disease, either initially or after previous curative intent treatment.[3] Survival statistics depend highly on the initial stage at diagnosis, with estimated 5-year survival for patients with localized and distant disease is 93% and 15.3%, respectively.[4]

Over the last several years, we have an improved understanding of the molecular underpinnings of metastatic renal cell carcinoma (mRCC) and have discerned key features to the biology that has led to many advancements in disease treatment, resulting

Accepted for publication July 2024

Address correspondence to Dr. Andrew S. Iskandar, Department of Medical Oncology, Sidney Kimmel Cancer Center, Thomas Jefferson University Hospital, 1025 Walnut Street, Suite 700, Philadelphia, PA 19107 USA

in a paradigm shift in disease management. As such, Briggs et al described that the treatment of mRCC can be broken into three separate eras.[5]

1. In the 1980s and 1990s, the use of immunotherapy agents, such as interferon-alpha or high-dose interleukin-2, were found to have activity against the disease. Response rates were low, but these agents could achieve long term remission in a minority of patients. Interferon-alpha use was more accepted by the medical oncology community for patients with high risk or metastatic disease, although side effects were limiting. The use of high dose interleukin-2 was limited to few centers because of risk of life-threatening toxicity.
2. During the early 2000s, new knowledge regarding angiogenic growth patterns of renal cancer led to the next era of treatment with agents targeting vascular endothelial growth factor (VEGF) or the VEGF receptor (VEGFR). These agents were significantly better tolerated and more widely accepted by medical oncologists and shown to improve survival of patients with metastatic disease.[6] The use of VEGF inhibitors as adjuvant therapy for patients with high-risk resected tumors was studied in four prospective randomized trials; none showed a survival advantage and only one showed a small benefit in relapse free survival.[7-10] Within these trials, clinical predictors including the Memorial Sloan Kettering Cancer Center (MSKCC) risk tool and the International Metastatic RCC Database Consortium (IMDC) score were correlated with patient outcomes, however, alternative biomarkers were not evaluated.
3. During the past decade potent immuno-oncology (IO) agents directed against PD-1, PD-L1 and CTLA-4 have been developed and studied as single agents or in combination.[11] The combination of PD-1 and CTLA-4 targeted agents (IO/IO) has provided durable responses, with a subset of patients continuing to demonstrate a response for > 60 months.[12] In addition, the combination of IO with a tyrosine kinase inhibitor (TKI) has similarly provided durable responses with long term survivorship. The choice of initial treatment can be difficult because no prospective randomized trial has been conducted to directly compare treatment efficacy or survival in patients treated with IO/IO versus IO/TKI.[13] Outside of the IMDC and MSKCC criteria, which were validated in the era of anti-angiogenesis monotherapies, we presently lack predictive or prognostic biomarkers to aid in the selection of initial therapy for patients with metastatic RCC.

Advances in molecular technology have changed the way that we view the biology of cancer. Biomarkers consist of different molecules that can be measured in tumor specimens or in bodily fluids. These molecules can include proteins and nucleic acids. Potentially useful biomarkers would include DNA, RNA, specific DNA mutations, proteins associated with immune response and angiogenesis. Several nucleic acid and protein biomarkers have been shown to be of strong predictive value in the treatment of other malignancies. Some examples include HER2 for breast, stomach, or esophageal cancers, BRAF for thyroid and skin cancers, and BCR-ABL in leukemia. At this time, clinically useful predictive markers have not yet been discovered to aid in the selection of therapy for patients with renal cell carcinoma. Given their potential ability to change management strategies upon better understanding of their prognostic and predictive nature, it may allow for more personalized treatment for individual patients and improved overall outcomes.

While validated biomarkers for patients with RCC are lacking, several clinical criteria, genomic and transcriptomic based approaches of disease characterization have been explored. Our review will aim to evaluate the advancements in biomarker development for patients with renal cell carcinoma.

Routine clinical parameters as biomarkers

Prior to the International Metastatic RCC Database Consortium (IMDC) score, the most widely utilized prognostic factor model for RCC came from the

TABLE 1. **Risk assessment for metastatic renal cell carcinoma according to the International Metastatic RCC Database Consortium (IMDC)**

IMDC-risk factors (1 point for each)	
Karnofsky performance status < 80%	
Time from initial diagnosis to initiation of systemic therapy < 1 year	
Hemoglobin level < lower limit of normal (LLN)	
Corrected serum calcium > upper limit of normal (ULN)	
Absolute neutrophil count > ULN	
Platelet count > ULN	
Risk profile	
Score: 0	Favorable
Score: 1-2	Intermediate
Score: ≥ 3	Poor

LLN = lower limit of normal; ULN = upper limit of normal

Memorial Sloan-Kettering Cancer Center (MSKCC), which examined 463 patients with mRCC enrolled in clinical trials and treated with interferon.[14] In the era of VEGF-targeted therapies, the IMDC was first proposed in 2009 and is employed in guideline recommendations for treatment of mRCC.[14-16] It includes criteria (as referenced in Table 1) that will stratify patients into either a favorable, intermediate, or poor risk profile. These criteria were developed based on multi-institutional, retrospective case studies involving 645 patients with mRCC treated with sunitinib, sorafenib, or bevacizumab plus interferon.[17] One point is given for variations in the patient's Karnofsky performance status, serum hemoglobin level, corrected serum calcium, neutrophil count, platelet count, and time from initial RCC diagnosis to start of therapy. Survival estimates differ among each risk groups, and thus frontline combination therapy trials have incorporated the criteria as stratification criteria.

The integration of the IMDC criteria into frontline trials, including the CheckMate 214, KEYNOTE-426, and CheckMate 9ER studies, underscores its significance as a prognostic tool in guiding treatment decisions for mRCC. Notably, across these trials, a consistent signal towards the efficacy of combination therapy involving immune checkpoint inhibitors and tyrosine kinase inhibitors (TKIs) emerged in all risk groups as defined by the IMDC criteria.

In CheckMate 214, for instance, favorable-risk patients exhibited a higher objective response rate (ORR) and longer progression-free survival (PFS) when treated with sunitinib compared to those receiving nivolumab plus ipilimumab.[18] Conversely, intermediate- and poor-risk patients demonstrated longer overall survival (OS) and higher ORR with the combination of nivolumab and ipilimumab.

The recognition of IMDC criteria by regulatory bodies such as the Food and Drug Administration (FDA), as evidenced by its integration in approval processes, as well as guideline recommendations employing IMDC criteria to inform treatment algorithms, highlights the value of the criteria as a tool predictive of response.

Interpreting the differential response to therapies based on IMDC risk groups sheds light on underlying tumor biology. Favorable-risk patients may exhibit a more angiogenic phenotype, rendering the tumors more responsive to anti-angiogenic therapies. Conversely, intermediate- and poor-risk patients may be associated with a higher tumor burden due to immune evasion mechanisms, thus benefiting more from an immunotherapy approach involving immune checkpoint inhibition. This dichotomy suggests that IMDC criteria may serve as a surrogate marker for distinct tumor microenvironment characteristics with favorable-risk tumors leaning towards angiogenesis and poor-risk tumors showing a more immunogenic profile.

Attempts to improve the IMDC prognostic model has included addition of commonly used laboratory and clinical factors. The neutrophil-to-lymphocyte ratio (NLR) is an additional tool which may support clinicals in treatment selection. NLR was devised with the biologic rationale that tumor-induced local and systemic inflammation contributes to the development and progression of malignancy.[19,20] Neutrophils release cytokines, chemokines, and other inflammatory mediators may promote cancer progression by directly affecting the tumor's microenvironment by upregulating VEGF and promoting tumor angiogenesis.[21] Presence of lymphocytes are associated with better therapeutic responses from a known dominant role in antitumor effect.[22] Thus, an increased circulating NLR may indicate poorer outcomes in patients, whereas a decreased NLR may reflect impaired cell-mediated immunity and potential for better responses with immunotherapy. The predictive value of NLR and the efficacy of immunotherapy has been explored in several solid tumors, including lung, gastrointestinal, and ovarian tumors.[23-25] In these tumors, it has been described that elevated pre-treatment NLR may be associated with a shorter OS and lower ORR. While there are only a few small studies that have looked into this phenomenon in RCC, Chen et al, summarized that high blood NLR was associated with poor OS and PFS in patients treated with immune checkpoint inhibitors (ICIs), indicating that NLR may have potential as a prognostic and predictive indicator to direct clinical decision-making.[19]

In essence, the concept of IMDC criteria as a biomarker stems from its ability to stratify patients based on underlying tumor biology, guiding therapeutic choices towards personalized medicine approaches in mRCC. As our understanding of the molecular landscape of RCC evolves, the role of IMDC criteria is poised to expand, potentially facilitating the development of novel targeted therapies tailed to specific tumor phenotypes.

Histologic predictors of outcomes

The histologic type of renal carcinoma has been shown to be of prognostic value in several retrospective series. A series of 2,215 metastatic patients collected from the IMDC were treated with VEGF and mTOR

targeted therapies. This study showed worse overall survival for patients with non-clear cell histology, compared to clear cell histology. However, the IMDC model was prognostic for outcomes for the non-clear cell patients, 93% of which were treated with a VEGF targeted therapy.[26]

Certain histologic types of non-clear cell RCC have been associated with worse prognoses. Sarcomatoid and rhabdoid features are associated with aggressive clinical course, resistance to VEGF directed therapy and shorter survival.[27,28] However, these aggressive phenotypes have been associated with improved survival when treated with nivolumab plus ipilimumab compared to sunitinib (Hazard ratio 0.46 [95% CI, 0.29 to 71]; p = .0004).[29]

Immunotherapy-VEGF based combinations for patients with other types of non-clear-cell carcinoma such as papillary, chromophobe and collecting duct histology have not been studied extensively. However, phase II studies report significant activity, especially for patients with papillary histology. The combination of cabozantinib plus nivolumab showed promising efficacy in most non–clear-cell RCC variants, especially for those with papillary features.[30]

A similarly designed phase II trial reported that pembrolizumab plus lenvatinib resulted in significant activity in patients with untreated advanced non-clear-cell renal cell carcinoma. This trial evaluated 158 patients with papillary, chromophobe or unclassified RCC. The overall response rate was 49% (95% CI 41-57), including nine (6%) patients with a confirmed complete response.[31] It is presently clear that the use of histology alone is inadequate for selecting the optimal treatment for patients with advanced stage RCC. Genomic or other biomarkers with stronger predictive value are needed both in clear cell and non–clear-cell RCC to better select treatment.

Immuno-oncology markers

Programmed death receptor ligand 1

The first and most widely biomarkers in the realm of immune checkpoint blockade is programmed death receptor ligand 1 (PD-L1). Analysis of PD-L1 expression on tumor cells and tumor-infiltrating immune cells was originally studied by immunohistochemistry (IHC) to establish a potential correlation between expression and treatment response. Various scoring methods for PD-L1 staining have been developed, including the combined positivity score (CPS), inflammatory cell (IC) score, and tumor proportion score (TPS). High level evidence demonstrating superiority or validation supporting a particular scoring system is lacking in the metastatic and locally advanced RCC setting, making it difficult to compare trials and determine whether PD-L1 is a suitable predictor of a response to immune checkpoint inhibition.

Rates of PD-L1 expression are highly variable across studies involving solid tumor malignancies with enhanced response to ICIs (i.e., NSCLC, melanoma) and reduced sensitivity (i.e., colorectal carcinoma or sarcoma). Patel et al described the variability of PD-L1 expression across different tumor types, which ranged between 12% to 100%, depending upon the tumor type, stage and assay used. With RCC specifically, PD-L1 expression is observed to range from 14% to 54% of tumors.[32]

In the setting of metastatic RCC, several studies have indicated that the expression of PD-L1 on tumor cells signifies a worse prognosis. One retrospective study in particular by Kahlmeyer et al described that patients with PD-L1 expressing tumors often present with higher stage disease, have a lack of response to TKI therapy, and overall have poorer outcomes.[33] Additionally, a recent meta-analysis evaluated over 1,300 patients with all stages of RCC, who received various treatments. 24% of tumors evaluated expressedPD-L1. When the analysis was limited to studies that used IHC as opposed to (ELISA), higher expression of PD-L1 was associated with significantly greater risk of death (HR; 2.05, 95 % CI 1.38–3.05; p < 0.001).[34]

With regards to RCC in the adjuvant setting, the phase III KEYNOTE-564 randomized control trial compared the use of pembrolizumab to placebo for high-risk patients who had previously underwent nephrectomy.[35] This trial showed a significant benefit in DFS at 2 years, 77% versus 68% for the pembrolizumab treated group versus the placebo group, respectively. The only biomarker reported in the analysis was PD-L1. In the subgroup of the patients (n = 748) with PD-L1 combined positive score > 1, treatment with pembrolizumab was associated with statistically significant improvement in DFS [Hazard ratio 0.67 (0.51-0.88)]. In the subgroup of patient tumors with PD-L1 < 1 (n = 237), no benefit in DFS was observed. As nicely exemplified by these results, there may be a benefit to immunotherapy in the adjuvant setting, further study is warranted.

As seen with a variety of malignancies, the presence and quantification of PD-L1 expression has been useful as a predictive biomarker to assess patient's responsiveness to immune checkpoint inhibition. Some examples of this include urothelial, gastrointestinal, and non-small cell lung cancers.[36] However, in metastatic RCC, the potential predictive value remains uncertain as there is a paucity of data any correlation between PD-L1 expression and response to ICIs. Some examples in

the front-line setting included KEYNOTE-426, which on extended follow up study showed that pembrolizumab plus axitinib continues to have superior outcomes in terms of PFS, OS, and ORR over sunitinib.[37] These findings were irrespective of PD-L1 expression status. Five-year data from CheckMate 214 continued to show that dual checkpoint inhibition with nivolumab plus ipilimumab sustained clinical benefit in patients with advanced/metastatic RCC (including those with sarcomatoid features) as they conferred higher response rates and longer survival times compared to sunitinib.[38] The longer OS and higher ORR were observed across tumor PD-L1 expression levels, although the magnitude of the benefits appeared to be greater in patients with PD-L1 expression ≥ 1% compared to those with < 1% (HR; 0.57, 95 % CI 0.40 –0.82; p < 0.01). Interim analysis data from JAVELIN Renal 101 revealed consistent benefits of avelumab and axitinib, compared to sunitinib, across stratifications of IMDC groups and PD-L1 status (HR; 0.643, 95 % CI 0.512–0.806; p < 0.001).[39] In previously treated patients, extended follow-up of CheckMate 025 showed superior OS and ORR in patients with advanced or mRCC treated with nivolumab than those treated with everolimus.[40] This survival benefit was observed regardless of tumor PD-L1 expression level (HR; 0.73, 95 % CI 0.62 –0.85; p < 0.01). Lastly, a negative study in IMmotion 151 trial reported that combination atezolizumab plus bevacizumab showed a PFS benefit versus sunitinib, the final analysis did not demonstrate improved OS for patients with mRCC in either the ITT or PD-L1 positive populations.[41] Results of extended follow up on these trials continue to show that the predictive value of PD-L1 is poor.

LAG-3, TIM-3, and TIGIT

In addition to treatments targeting CTLA-4, PD-1 and PD-L1, more recently discovered inhibitory receptors include lymphocyte activation gene 3 (LAG-3), T-cell immunoglobulin and mucin domain 3 (TIM-3), and T-cell immunoreceptors with immunoglobulin and ITIM domains (TIGIT).[42]

These biomarkers serve as targets for immune checkpoint blockade and clinical trials are actively evaluating the role of LAG-3, TIM-3 and TIGIT blockers.[43] Recently, pre-clinical studies in developmental therapeutics have shown promising antitumor results in RCC in mouse xenograft models treated with an agent targeting TIGIT, either as monotherapy or in combination with PD-1 inhibitors.[44] Cai et al reviewed the data from studies that evaluated the prognostic value of LAG-3 and concluded that higher level of expression is associated with tumor progression and poor prognosis across solid tumor types, including in RCC. Similarly, higher levels of TIM-3 expression were associated with shorter progression-free survival in patients with ccRCC.[45] Giraldo et al performed a retrospective analysis which included a cohort of 135 patients with clear cell RCC. Several markers seen by immunohistochemical quantification were associated with poor prognosis, including LAG-3 and PD-1.[46] These findings are hypothesis-generating and suggest that co-expression of PD-1 and LAG-3 was correlate with outcomes. There is an ongoing phase II trial investigating the combination of nivolumab with relatlimab, a monoclonal antibody targeting LAG-3 underway (NCT05347212).

Genomic biomarkers

Tumor mutation burden (TMB)

TMB has arisen as a predictive biomarker for response to immune checkpoint inhibition, and high TMB tumors hold a tumor-agnostic regulatory approval from the US FDA for pembrolizumab. TMB represents the total number of mutations per coding area of the tumor genome, measured as mutations per mega base (mutations/Mb). These alterations in the genome generate tumor-specific neoantigens that are present on the tumor cell surface cells allowing for T-cell recognition and subsequent anti-tumor immune response.[47] KEYNOTE 158 was a phase 2 study evaluating pembrolizumab monotherapy across solid tumor malignancies which harbored a high TMB (≥ 10 mut/Mb) and demonstrated robust tumor responses, but the study has limited applicability to the RCC population as only 3 patients with RCC were included in the trial.[48]

In general, RCC is a tumor associated with relatively low TMB that can vary across different subtypes (median of 1.1 mut/Mb). With this information, one can hypothesize that the utility of TMB as a predictor of treatment response will likely be poor. Another study by Zhang et al analyzed the somatic mutation patterns of 336 clear cell RCC patients from the Cancer Genome Atlas (TCGA) database. Higher TMB was found to be weakly associated with higher tumor grade, advanced stage and poor overall survival.[49]

Somatic mutations

The Von Hippel Lindau (VHL) gene is located on chromosome 3p, and copy loss occurs in roughly 80% of sporadic cases of ccRCC.[50,51] The VHL protein acts as an E3 ubiquitin ligase that leads to ubiquitination of hypoxia-inducible factor (HIF) and its subsequent proteolysis.[52] Thus, mutations resulting in VHL gene variants reduce VHL protein activity, which results in stabilization of HIF subunits and constitutive activation of HIF transcription factors. These factors

promote vascularization, enhance glucose utilization, and lead to tumorigenesis.[53] In a meta-analysis performed by Kim et al, there was no correlation between the presence of a sporadic VHL alteration and response rate, progression-free survival or overall survival in patients receiving anti-VEGF therapy, thus indicating no prognostic or predictive value of VHL gene alteration in patients with clear cell RCC.[54]

However, germline mutation to VHL observed in Von Hipper-Lindau disease does offer option for targeted therapy. TheMK-6482-004 trial reported positive results for the use of belzutifan for germline VHL RCC patients (ORR 49%, 95% confidence interval, 36 to 62%).[55] While VHL's utility as a biomarker was not assessed in the trial, the knowledge of an effective HIF-2α inhibitor in belzutifan may lead to further studies to see if presence of VHL mutation can have predictive or prognostic ability as a biomarker.

In addition to VHL, loss-of-function mutations to PBRM1, SETD2 and BAP1 may serve as conduits for biomarker-based drug development. According to results from The Cancer Genome Atlas (TCGA), each of these mutations were identified at higher frequency, suggesting significant in pathogenesis of the disease.[56]

Hakimi et al analyzed and evaluated data from gene expression profiles from 609 patients with ccRCC to develop a risk model.[57] BAP1 mutations were associated with worse cancer-specific survival (CSS) in the risk model (HR 7.71, 95% CI 2.08). Median overall survival for BAP1 mutants was 31.2.months versus 78.2 months in wild-type patients. Mutations in PBRM1, as well as VHL, had no impact on CSS.

In addition, recurrent alterations in TP53 and the TERT promoter region were not reported as commonly altered genes in early-stage non-metastatic datasets. It is plausible that these mutations are acquired late in the pathologic process and prior to progression into metastatic disease. TERT promoter mutations are common noncoding and have also been associated with adverse outcomes in several malignancies.[58-60] Wang et al, analyzed a cohort of 109 patients with RCC and found that TERT promoter mutations were only found in 9/96 (9.3%) of clear cell tumors, but were more commonly found in patients with capsular invasion or metastatic disease.[61]

Carbonic anhydrase IX

Carbonic anhydrase IX (CAIX) is a well-described enzyme whose expression is inversely regulated by the wide-type VHL gene.[62] In normal renal tissue, there is minimal detection of CAIX, whereas in RCC, it is abundantly expressed. ZIRCON was an open-label, multicenter trial which utilized 89Zr-DFO-girentuximab (TLX250-CDx) as a radioligand for PET/CT imaging because of its high specificity for CAIX. The study displayed both safety and accuracy in differentiating ccRCC lesions from indeterminate renal anomalies.[63] This data augments the prospect of employing CAIX as a viable therapeutic target.

Circulating tumor DNA

Analyses of circulating tumor cells, cell-free tumor DNA (ctDNA), cell-free DNA (cfDNA), cell-free RNA (cfRNA), exosomes and tumor derived blood proteins have been evaluated in patients with RCC and other cancers. "Liquid biopsies" have advantages over tissue biopsies as they are non-invasive and can be assessed serially to monitor for disease progression or recurrence in a variety of disease settings. Detectable Free DNA in plasma (cfDNA), which is released by necrotic or apoptotic cells, and DNA released by circulating tumor cells in the form of ctDNA is another avenue of biomarker development in RCC. Circulating tumor DNA can only be distinguished from cfDNA based on the presence of tumor-specific genomic alterations. Compared with cfDNA, detectable ctDNA levels are lower and contain smaller fragments. In RCC, a study reported that the fragment size of ctDNA correlates with patient prognosis and has a significant clinical value.[64]

Most studies of RCC patients demonstrated that compared to other tumor types, their level of ctDNA is low and very sensitive methods of detection will be required. It appears that tumor-guided sequencing of selected tumor variants and the use of cell-free methylated DNA immunoprecipitation techniques may be a more sensitive method for ctDNA detection in RCC. One study obtained cfDNA from 40 metastatic RCC patients and performed targeted deep sequencing of mutations found in tumor tissue found ctDNA detection rates of 52%. For 34 of the 40 patients, methylated DNA analysis (cfMeDIP-seq) was also performed. A separate cohort of 38 mRCC patients were used in cfMeDIP-seq analysis to train an RCC classifier. This study showed that cfDNA variant analysis detected 21 candidate variants in 11 of 40 mRCC patients (28%). Among 23 patients with parallel tumor sequencing, cfDNA analysis alone identified variants in 9 patients (39%), while cfDNA analysis focused on tumor sequencing variant findings improved the sensitivity to 52%. In 34 mRCC patients undergoing cfMeDIP-seq, cfDNA variant analysis identified variants in 7 (21%), while cfMeDIP-seq detected all mRCC cases (100% sensitivity) with 88% specificity in 34 control subjects. In 5 patients with cfDNA variants and serial samples, variant

frequency correlated with response to therapy. The authors concluded that cfMeDIP-seq was significantly more sensitive for mRCC detection than cfDNA variant analysis, but that cfDNA variant analysis might be useful for monitoring response to therapy.[65]

The best method ctDNA detection remains unknown. Because ctDNA is often present in minute amounts highly sensitive methods for detection are needed. One study applied three methods to two different RCC patient cohorts: tumor-guided analysis, targeted panel sequencing, and global sequencing of plasma. This study found that tumor-guided analysis had the highest ctDNA detection rate, whereas global sequencing of plasma had the lowest.[66] Another study showed that ctDNA detection in plasma by targeted deep sequencing was feasible in patients with localized or metastatic RCC. The dynamics of ctDNA levels was associated with the therapeutic response of patients with mRCC who were treated with first-line anti-PD1 and anti-CTLA4 combination therapies. This also gave insight into the potential role of ctDNA as an early predictor of treatment responses in mRCC patients receiving first-line immune checkpoint inhibitor.[67]

Few studies have investigated the value of ctDNA in RCC patients. While the level of ctDNA in RCC appears low, patients with metastatic RCC have been found to have greater amounts of ctDNA than patients with localized disease. Studies using multiple methods for ctDNA detection indicate that tumor-guided analysis improves the ctDNA detection rate compared to unguided methods and suggest that cfMeDIP-seq may be a very sensitive method for ctDNA detection. More studies are needed with larger numbers of patients treated in the adjuvant and metastatic settings to establish clear predictive value of ctDNA analysis in RCC. There remains an urgent need to develop predictors for relapse in patients who have undergone nephrectomy and are at risk for recurrence as well as for monitoring the response status of patients receiving treatment for metastatic disease. A reliable "liquid biopsy" could provide valuable predictive value, allowing selection of patients likely to benefit or unlikely to benefit systemic treatment for metastatic disease.

Transcriptomics and gene expression signatures

With advancements in gene expression analysis techniques, we can often detect these alterations and classify these changes. A gene expression signature (GES) is a group of genes that correlates these genetic alterations with specific clinical variables, such as diagnosis and prognosis.[68]

Brannon et al pooled tumor specimens from 48 ccRCC patients collected by the University of North Carolina Tissue Procurement Core Facility from consenting patients undergoing nephrectomy for RCC. They identified two distinct subtypes of ccRCC based on their molecular features: clear cell type A (ccA) and type B (ccB).[69] ccA tumors had improved disease-specific survival (DSS) compared to their ccB counterparts [medial survival of 8.6 years compared with 2.0 years ($p = 0.002$)]. Based on this data, a 34-gene classifier known as ClearCode34 was developed to define these subtypes as good risk (ccA) and poor risk (ccB) disease.[70] ClearCode34 was tested through a TCGA cohort of 157 non-metastatic patients; 69 were classified as ccA and 88 as ccB. Patients with ccB experiences a higher rate of tumor relapse (HR 2.1; 95% CI 1.3 – 3.4), worse cancer-specific mortality (HR 3.0, 95% CI 1.3 – 7), and overall mortality (HR 2.2; 95% CI 1.3 – 3.6) compared with ccA cases.[70] ClearCode34 was further validated through an independent retrospective cohort of 282 patients. Results were consistent in showing worse median overall survival (151 months versus 31 months, $p < 0.001$), median CSS (253 months versus 33 months, $p < 0.001$), and recurrence-free survival (HR 12.20, 95% CI 4.48 – 33.17). Unfortunately, while ClearCode24 has achieved consistent validation through these trials, it has yet to be tested in the prospective trial setting.

Another tissue-based RNA expression signature, known as the cell cycle progression score (CCP), is comprised of 31 genes involved in cell cycle productivity.[71,72] It was originally developed to assess outcomes in prostate cancer, and has also been applied to other tumor types, including bladder and lung. In RCC, it was utilized in a cohort of 64 patients to predict metastasis in those with localized disease treated with nephrectomy. While age, tumor size, and CCP score were all associated with progression to metastatic disease, CCP score showed the highest odds ratio (OR) of the three (OR 3.40; 95% CI 1.24 – 11.27).[73] In a larger study of patients with localized disease following radical nephrectomy, CCP was also an independent predictor of recurrence (HR 1.50; 95% CI 1.07 – 2.09) and disease-specific mortality (HR 2.49; 95% CI 1.53 – 4.04). Further studies looking into the utility of CCP score after diagnostic biopsies and nephrectomies are needed to better understand its potential clinical applications.

The Renal 101 Immuno signature is a 26-gene assay developed from the Javelin Renal 101 trial and was based on the discovery of a cluster of patients with prolonged PFS in the combination avelumab + axitinib arm.[74] Patients with a higher median score of the Renal 101 Immuno signature had longer PFS (HR

0.60; 95% CI 0.439 – 0.834), but this was not matched in the sunitinib arm (HR 0.89; 95% CI 0.670-1.172).[75] When this signature was tested on the data set from the Javelin Renal 100 study[76] and the phase I Javelin Solid Tumor trial[77] of avelumab monotherapy, high expression of the Renal 101 Immuno signature was also able to predict improved PFS. This signature has potential as a predictive biomarker for ICI combination therapy as well as to have prognostic value for survival outcomes.

Exploratory analysis from the IMmotion150 trial, a randomized phase II study of atezolizumab alone or in combination with bevacizumab versus sunitinib, led to the identification of angiogenesis (Angio), T-effector (T_{eff}), and myeloid inflammatory (Myeloid) gene signatures.[74] Investigators found that patients with a higher expression of the Angio gene signature, improved ORR and PFS was seen in the sunitinib group as compared to the atezolizumab + bevacizumab or atezolizumab monotherapy arms. Conversely, improved PFS was seen in the atezolizumab + bevacizumab group in patients with a lower Angio score. In addition, improved ORR and PFS was seen in patients with a higher T-effector score in the atezolizumab + bevacizumab group. Higher Myeloid signatures was associated with worse PFS in the atezolizumab group, but not with sunitinib. The three signatures were not able to differentiate clinical activity of atezolizumab + bevacizumab and atezolizumab monotherapy. When combined, high T_{eff} and Myeloid signatures had improved outcomes in the atezolizumab + bevacizumab arm, compared to those in the atezolizumab monotherapy group, suggesting its use in aiding clinicians in selecting patients who may benefit from combined therapy.[74] This same subgroup of patients with high T_{eff} and Myeloid signatures failed to differentiate PFS in either the avelumab + axitinib arm or the sunitinib arm in the Javelin Renal 101 trial.[75] In the phase III IMmotion151, patients with favorable-risk disease were found to have high Angio expression, while patients intermediate/poor risk disease were found to have higher T_{eff} scores.[40]

More recently, Vano and colleagues utilized a 35-gene expression classifier to describe four distinct clear-cell RCC groups (ccrcc1 to ccrcc4) based on their tumor microenvironment and sensitivity to frontline sunitinib in mRCC. Tumors that were less responsive to sunitinib had either an immune-high (ccrcc4) or an immune-low tumor microenvironment (ccrcc1). Tumors that were most responsive to sunitinib expressed an angiogenic-high and immune-high signature (ccrcc2). The smallest group of patients exhibited a good response to sunitinib and had molecular and pathologic features closest to normal kidney tissue (ccrcc3).[78] A randomized phase II clinical trial involving 202 patients was designed based on their respective molecular profiles. They showed similarly enriched response rates in ccrcc4 tumors classified as immune-high with nivolumab and nivolumab-ipilimumab. Immune-low ccrcc1 tumors had an increased ORR with combination nivolumab-ipilimumab. TKIs were found to be more effective in the ccrcc2 angio-high patient population. This study give promise to the feasibility of molecularly based clinical trials for metastatic ccRCC patients.

A similar and ongoing phase II trial (NCT05361720), OPTIC-RCC further looks into RNA sequencing as a molecular biomarker to predict treatment based on biological drivers.[79] Utilizing RNA sequencing data from the IMmotion 151 patient samples, six distinct patient clusters were forms based on their association to the tumor's biology. Clusters 1 and 2 showed an angiogenic signature and were selected to receive cabozantinib with nivolumab. Clusters 4 and 5 show more of an immune/proliferative signature and were therefore assigned to receive dual checkpoint inhibition with nivolumab and ipilimumab. Clusters 3 and 6 had neither signature and were therefore excluded from further study. Further data analysis on the outcome of this trial is currently pending.

As we continue to make advancements in RCC molecular sequencing, gene expression signatures have the potential to be a useful as prognostic and predictive biomarkers with ongoing development. Evolution of our present understanding with further discovery can aim to leverage RNA sequencing for a more personalized, and tailored treatment approach.

Conclusion

This review highlights the pressing need for robust predictor and prognostic biomarkers in renal cell carcinoma to guide clinicians in tailoring optimal therapeutic strategies. The discussion encompassed the limitations of existing markers and underscored the significance of the most recent advancements within this field. Despite these strides, the landscape of RCC biomarkers remains incomplete. The journey towards a comprehensive understanding and discovery in this area hold immense promise for shaping future advancements within the field. □

References

1. Siegel RL, Miller KD, Wagle NS et al. Cancer statistics, 2023. *CA Cancer J Clin* 2023;73(1):17-48.

2. Ricketts, CJ, De Cubas AA, Fan H et al. The cancer genome atlas comprehensive molecular characterization of renal cell carcinoma. *Cell Rep* 2018;23(1):313-326.e5.
3. Osawa T, Takeuchi A, Kojima T et al. Overview of current and future systemic therapy for metastatic renal cell carcinoma. *Jpn J Clin Oncol* 2019;49(5):395-403.
4. Howlader N, Noone, AM, Krapcho, M et al. SEER Cancer Statistics Review, 1975-2017.
5. Briggs LG, Cone EB, Lee RJ et al. Prognostic and predictive biomarkers for metastatic renal cell carcinoma. *J Cancer Metastasis Treat* 2021;7:46. http://dx.doi.org/10.20517/2394-4722.2021.84.
6. Vachhani P, George S. VEGF inhibitors in renal cell carcinoma. *Clin Adv Hematol Oncol* 2016;14(12):1016-1028.
7. Haas NB, Manola J, Dutcher JP et al. Adjuvant treatment for high-risk clear cell renal cancer: updated results of a high-risk subset of the ASSURE randomized trial. *JAMA Oncol* 2017;3(9):1249-1252.
8. Motzer RJ, Russo P, Haas N et al. PROTECT study investigators. Adjuvant pazopanib versus placebo after nephrectomy in patients with localized or locally advanced renal cell carcinoma: final overall survival analysis of the phase 3 PROTECT trial. *Eur Urol* 2021;79(3):334-338.
9. Gross-Goupil M, Kwon TG, Eto M et al. Axitinib versus placebo as an adjuvant treatment of renal cell carcinoma: results from the phase III, randomized ATLAS trial. *Ann Oncol* 2018;29(12): 2371-2378.
10. Ravaud A, Motzer RJ, Pandha HS et al. Adjuvant sunitinib in high-risk renal-cell carcinoma after nephrectomy. *N Engl J Med* 2016;375(23):2246-2254.
11. Lasorsa F, di Meo NA, Rutigliano M et al. Immune checkpoint inhibitors in renal cell carcinoma: molecular basis and rationale for their use in clinical practice. *Biomedicines* 2023;11(4):1071.
12. Albiges L, Tannir NM, Burotto M et al. Nivolumab plus ipilimumab versus sunitinib for first-line treatment of advanced renal cell carcinoma: extended 4-year follow-up of the phase III CheckMate 214 trial. *ESMO Open* 2020;5(6):e001079.
13. Zarrabi KK, Lanade O, Geynisman DM. Determining front-line therapeutic strategy for metastatic clear cell renal cell carcinoma. *Cancers (Basel)* 2022;14(19):4607.
14. Heng DY, Xie W, Regan MM et al. Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. *J Clin Oncol* 2009;27(34):5794-5799.
15. Heng DY, Xie W, Regan MM et al. External validation and comparison with other models of the International Metastatic Renal-Cell Carcinoma Database Consortium prognostic model: a population-based study. *Lancet Oncol* 2013;14(2):141-148.
16. Guida A, Le Teuff G, Alves C et al. Identification of international metastatic renal cell carcinoma database consortium (IMDC) intermediate-risk subgroups in patients with metastatic clear-cell renal cell carcinoma. *Oncotarget* 2020;11(49):4582-4592.
17. Choueiri TK, Garcia JA, Elson P et al. Clinical factors associated with outcome in patients with metastatic clear cell renal cell carcinoma treated with vascular endothelial growth factor-targeted therapy. *Cancer* 2007;110(3):543-550.
18. Motzer RJ, Tannir NM, McDermott DF et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med* 2018;378(14):1277-1290.
19. Chen X, Meng F, Jiang R. Neutrophil-to-lymphocyte ratio as a prognostic biomarker for patients with metastatic renal cell carcinoma treated with immune checkpoint inhibitors: a systematic review and meta-analysis. *Front Oncol* 2021;11:746976.
20. Nunno VD, Mollica V, Gatto L et al. Prognostic impact of neutrophil-to-lymphocyte ratio in renal cell carcinoma: a systematic review and meta-analysis. *Immunotherapy* 2019; 11(7):631-643.
21. Tazzyman S, Lewis CE, Murdoch C. Neutrophils: key mediators of tumour angiogenesis. *Int J Exp Pathol* 2009;90(3):222-231.
22. Paijens ST, Vledder A, de Bruyn M et al. Tumor-infiltrating lymphocytes in the immunotherapy era. *Cell Mol Immunol* 2021;18(4):842-859.
23. Bagley SJ, Kothari S, Aggarwal C et al. Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell lung cancer. *Lung Cancer* 2017;106:1-7.
24. Wu Y, Li C, Zhao J, Yang L et al. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios predict chemotherapy outcomes and prognosis in patients with colorectal cancer and synchronous liver metastasis. *World J Surg Oncol* 2016;14(1):289.
25. Cho H, Hur HW, Kim SW et al. Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment. *Cancer Immunol Immunother* 2009;58(1):15-23.
26. Kroeger N, Xie W, Lee J-L et al. Metastatic non–clear cell renal cell carcinoma treated with targeted therapy agents: characterization of survival outcome and application of the International mRCC Database Consortium Criteria. *Cancer* 2013;119:2999-3006.
27. Przybycin CG, McKenney JK, Reynolds JP et al. Rhabdoid differentiation is associated with aggressive behavior in renal cell carcinoma: A clinicopathologic analysis of 76 cases with clinical follow-up. *Am J Surg Pathol* 2014;38:1260-1265.
28. Kyriakopoulos CE, Chittoria N, Choueiri TK et al. Outcome of patients with metastatic sarcomatoid renal cell carcinoma: Results from the international metastatic renal cell carcinoma database consortium. *Clin Genitourin Cancer* 2015;13:e79-e85.
29. Rini BI, Signoretti S, Choueiri TK et al. Long-term outcomes with nivolumab plus ipilimumab versus sunitinib in first-line treatment of patients with advanced sarcomatoid renal cell carcinoma. *J Immunother Cancer* 2022;10:e005445.
30. Lee CH, Voss MH, Carlo MI et al. Phase II trial of cabozantinib plus nivolumab in patients with non-clear-cell renal cell carcinoma and genomic correlates. *J Clin Oncol* 2022;40(21):2333-2341.
31. Albiges L, Gurney H, Atduev V et al. Pembrolizumab plus lenvatinib as first-line therapy for advanced non-clear-cell renal cell carcinoma (KEYNOTE-B61): a single-arm, multicentre, phase 2 trial. *Lancet Oncol* 2023;24(8):881-891.
32. Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. *Mol Cancer Ther* 2015;14:847-856.
33. Kahlmeyer A, Stöhr CG, Hartmann A et al. Expression of PD-1 and CTLA-4 are negative prognostic markers in renal cell carcinoma. *J Clin Med* 2019;8:743.
34. Iacovelli R, Nole F, Verri E et al. Prognostic role of PD-L1 expression in renal cell carcinoma. a systematic review and meta-analysis. *Target Oncol* 2016;11:143-148.
35. Powles T, Plimack ER, Soulières D et al. Pembrolizumab plus axitinib versus sunitinib monotherapy as first-line treatment of advanced renal cell carcinoma (KEYNOTE-426): extended follow-up from a randomised, open-label, phase 3 trial. *Lancet Oncol* 2020;21(12):1563-1573. Erratum in: *Lancet Oncol* 2020;21(12): e553.
36. Choueiri TK, Tomczak P, Park SH et al. Adjuvant pembrolizumab after nephrectomy in renal-cell carcinoma. *N Engl J Med* 2021; 385(8):683-694.
37. Choueiri TK, Motzer RJ, Rini BI et al. Updated efficacy results from the JAVELIN Renal 101 trial: first-line avelumab plus axitinib versus sunitinib in patients with advanced renal cell carcinoma. *Ann Oncol* 2020;31(8):1030-1039.
38. Herbst RS, Soria J-C, Kowanetz M et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014;515:563-567.
39. Albiges L, Tannir NM, Burotto M et al. Nivolumab plus ipilimumab versus sunitinib for first-line treatment of advanced renal cell carcinoma: extended 4-year follow-up of the phase III CheckMate 214 trial. *ESMO Open* 2020;5(6):e001079.
40. Motzer RJ, Escudier B, George S et al. Nivolumab versus everolimus in patients with advanced renal cell carcinoma: Updated results

with long-term follow-up of the randomized, open-label, phase 3 CheckMate 025 trial. *Cancer* 2020;126(18):4156-4167.
41. Motzer RJ, Powles T, Atkins MB et al. Final overall survival and molecular analysis in IMmotion151, a phase 3 trial comparing atezolizumab plus bevacizumab vs. sunitinib in patients with previously untreated metastatic renal cell carcinoma. *JAMA Oncol* 2022;8(2):275-280.
42. Takamatsu K, Tanaka N, Hakozaki K et al. Profiling the inhibitory receptors LAG-3, TIM-3, and TIGIT in renal cell carcinoma reveals malignancy. *Nat Commun* 2021;12(1):5547.
43. Cai L, Li Y, Tan J et al. Targeting LAG-3, TIM-3, and TIGIT for cancer immunotherapy. *J Hematol Oncol* 2023;16:101.
44. Hansen K, Kumar S, Logronio K et al. COM902, a novel therapeutic antibody targeting TIGIT augments anti-tumor T cell function in combination with PVRIG or PD-1 pathway blockade. *Cancer Immunol Immunother* 2021;70:3525-3540.
45. Komohara Y, Morita T, Annan DA et al. The coordinated actions of TIM-3 on cancer and myeloid cells in the regulation of tumorigenicity and clinical prognosis in clear cell renal cell carcinomas. *Cancer Immunol Res* 2015;3:999-1007.
46. Giraldo NA, Becht E, Pages F et al. Orchestration and prognostic significance of immune checkpoints in the microenvironment of primary and metastatic renal cell cancer. *Clin Cancer Res* 2015;21(13):3031-3040.
47. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med* 2017;377:2500-2501.
48. Marabelle A, Le DT, Ascierto PA et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. *J Clin Oncol* 2020;38(1):1-10.
49. Zhang C, Li Z, Qi F et al. Exploration of the relationships between tumor mutation burden with immune infiltrates in clear cell renal cell carcinoma. *Ann Transl Med* 2019;7:648.
50. Kammerer-Jacquet SF, Crouzet L, Brunot A et al. Independent association of PD-L1 expression with noninactivated VHL clear cell renal cell carcinoma-A finding with therapeutic potential. *Int J Cancer* 2017;140(1):142-148.
51. Zhang JA-O, Wu TA-O, Simon JA-O et al. VHL substrate transcription factor ZHX2 as an oncogenic driver in clear cell renal cell carcinoma. *Science* 2018;361(6399):290-295.
52. Choueiri TK, Kaelin WG Jr. Targeting the HIF2-VEGF axis in renal cell carcinoma. *Nat Med* 2020;26:1519-1530.
53. Haase VH. The VHL tumor suppressor: master regulator of HIF. *Curr Pharm Des* 2009;15:3895-3903.
54. Kim BJ, Kim JH, Kim HS et al. Prognostic and predictive value of VHL gene alteration in renal cell carcinoma: a meta-analysis and review. *Oncotarget* 2017;8(8):13979-13985.
55. Jonasch E, Donskov F, Iliopoulos O et al. Belzutifan for renal cell carcinoma in von Hippel-Lindau disease. *N Engl J Med* 2021;385(22):2036-2046.
56. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature* 2013;499(7456):43-49.
57. Hakimi AA, Ostrovnaya I, Reva B et al. ccRCC Cancer Genome Atlas (KIRC TCGA) Research Network investigators. 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* 2013;19(12):3259-3267.
58. Vinagre J, Almeida A, Populo H et al. Frequency of TERT promoter mutations in human cancers. *Nat Commun* 2013;4:2185.
59. Bell RJ, Rube HT, Xavier-Magalhaes A et al. Understanding TERT promoter mutations: A common path to immortality. *Mol Cancer Res* 2016;14:315-323.
60. Melo M, da Rocha AG, Vinagre J et al. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. *J Clin Endocrinol Metab* 2014;99:E754-E765.
61. Wang K, Liu T, Liu L et al. TERT promoter mutations in renal cell carcinomas and upper tract urothelial carcinomas. *Oncotarget* 2014;5:1829-1836.
62. Supuran CT. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov* 2008;7:168-181.
63. Shuch BM, Pantuck AJ, Bernhard JC et al. Results from phase 3 study of 89Zr-DFO-girentuximab for PET/CT imaging of clear cell renal cell carcinoma (ZIRCON). *J Clin Oncol* 2023;41(suppl 6):abstr LBA602.
64. Yamamoto Y, Uemura M, Nakano K et al. Increased level and fragmentation of plasma circulating cell-free DNA are diagnostic and prognostic markers for renal cell carcinoma. *Oncotarget* 2018;9(29):20467-20475.
65. Lasseter K, Nassar AH, Hamieh L et al. Plasma cell-free DNA variant analysis compared with methylated DNA analysis in renal cell carcinoma. *Genet Med* 2020;22:1366-1373.
66. Smith CG, Moser T, Mouliere F et al. Comprehensive characterization of cell-free tumor DNA in plasma and urine of patients with renal tumors. *Genome Med* 2020;12:23.
67. Kim YJ, Kang Y, Kim JS et al. Potential of circulating tumor DNA as a predictor of therapeutic responses to immune checkpoint blockades in metastatic renal cell carcinoma. *Sci Rep* 2021;11:5600.
68. Chibon F. Cancer gene expression signatures—The rise and fall? *Eur J Cancer* 2013;49:2000-2009.
69. Brannon AR, Reddy A, Seiler M et al. Molecular stratification of clear cell renal cell carcinoma by consensus clustering reveals distinct subtypes and survival patterns. *Genes Cancer* 2010;1:152-156.
70. Brooks SA, Brannon AR, Parker JS et al. ClearCode34: a prognostic risk predictor for localized clear cell renal cell carcinoma. *Eur Urol* 2014;66:77-84.
71. Cuzick J, Swanson GP, Fisher G et al. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. *Lancet Oncol* 2011;12:245-255.
72. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. *Nat Rev Cancer* 2017;17:93-115.
73. Askeland EJ, Chehval VA, Askeland RW et al. Cell cycle progression score predicts metastatic progression of clear cell renal cell carcinoma after resection. *Cancer Biomark Sect Dis Markers* 2015;15:861-867.
74. McDermott DF, Huseni MA, Atkins MB et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. *Nat Med* 2018;24:749-757.
75. Motzer RJ, Robbins PB, Powles T et al. Avelumab plus axitinib versus sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. *Nat Med* 2020;26:1733-1741.
76. Choueiri TK, Larkin J, Oya M et al. Preliminary results for avelumab plus axitinib as first-line therapy in patients with advanced clear- cell renal-cell carcinoma (JAVELIN Renal 100): an open-label, dose- finding and dose-expansion, phase 1b trial. *Lancet Oncol* 2018;19:451-460.
77. Heery CR, O'Sullivan-Coyne G, Madan RA et al. Avelumab for metastatic or locally advanced previously treated solid tumours (JAVELIN solid tumor): a phase 1a, multicohort, dose-escalation trial. *Lancet Oncol* 2017;18:587-598.
78. Vano YA, Elaidi R, Bennamoun M et al. Nivolumab, nivolumab-ipilimumab, and VEGFR-tyrosine kinase inhibitors as first-line treatment for metastatic clear-cell renal cell carcinoma (BIONIKK): a biomarker-driven, open-label, non-comparative, randomised, phase 2 trial. *Lancet Oncol* 2022;23(5):612-624.
79. Reddy A, Haake SM, Beckermann K et al. Biomarker-driven prospective clinical trial in renal cell carcinoma: Developing machine learning models to allocate patients to treatment arms using RNA sequencing. Poster presented at: American Society of Clinical Oncology annual meeting. June 2-6, 2023. Chicago, IL.