Renal Cell Carcinoma in the Era of Precision Medicine: From Molecular Pathology to Tissue-Based Biomarkers

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ABSTRACT

Renal cell carcinoma (RCC) is not a single entity but includes various tumor subtypes that have been identified on the basis of either characteristic pathologic features or distinctive molecular changes. Clear cell RCC is the most common type of RCC and is characterized by dysregulation of the von Hippel Lindau/hypoxia-inducible factor pathway. Non-clear cell RCC represents a more heterogeneous group of tumors with diverse histopathologic and molecular features. In the past two decades, the improved understanding of the molecular landscape of RCC has led to the development of more effective therapies for metastatic RCC, which include both targeted agents and immune checkpoint inhibitors. Because only subsets of patients with metastatic RCC respond to a given treatment, predictive biomarkers are needed to guide treatment selection and sequence. In this review, we describe the key histologic features and molecular alterations of RCC subtypes and discuss emerging tissue-based biomarkers of response to currently available therapies for metastatic disease.

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PATHOLOGY OF RENAL CELL CARCINOMA

The histologic classification of renal cell carcinoma (RCC) has changed dramatically during the last few decades. Compared with the categories of clear cell carcinoma and granular cell carcinoma of the 1970s, contemporary classification has evolved from the 1997 Heidelberg classification, which first attempted to incorporate molecular genetics into the classification of RCC.¹⁻³ In recent years, a number of new entities were added on the basis of either characteristic pathologic features or distinctive molecular alterations³ (Table 1). In general, the histologic subtyping of RCC not only guides management and conveys prognostic information but also may possess predictive value for treatment. Here we discuss the key pathologic features and molecular alterations of sporadic (Table 2) and familial (Table 3) RCC subtypes.

Clear Cell RCC

Clear cell RCC (ccRCC) is the most common type of RCC and comprises approximately 60% of all renal cell tumors and 75% to 80% of RCCs that metastasize. The characteristic histologic findings include clear cell cytology, acinar growth patterns, and a rich vascular network. However, it is not

uncommon to find tumor cells displaying granular and eosinophilic cytoplasm or areas of alveolar, cystic, solid, or pseudopapillary architecture. Rhabdoid cytologic features and sarcomatoid differentiation are associated with a worse prognosis. The presence of focal areas with the classic appearance of ccRCC in an otherwise poorly differentiated rhabdoid or sarcomatoid tumor would strongly suggest this diagnosis. Diffuse membranous staining pattern of carbonic anhydrase IX in the nonnecrotic area is a helpful adjunctive marker with high specificity when used in combination with other ancillary markers.⁴

Common molecular alterations identified in ccRCC are 3p loss and inactivation of the von Hippel Lindau (*VHL*) gene, a tumor suppressor residing at the 3p25 locus. Patients with germ line *VHL* mutation (ie, *VHL* syndrome) are predisposed to develop multiple bilateral ccRCCs in a background of renal cystic lesions. In sporadic ccRCC, *VHL* inactivation has been reported in 60% to 90% of cases.⁵⁻⁸ The inactivation of its protein product (pVHL) results in aberrant stabilization of hypoxia-inducible factor (HIF), which drives the transcription of numerous genes involved in tumor formation.⁹ Large-scale genomic efforts in recent years have identified other prevalent somatic mutations in ccRCC, which most frequently involve genes such as *PBRM1*,

Table 1. Histologic Subtypes of Renal Cell Tumors

WHO 2016 classification of renal cell tumors
Clear cell renal cell carcinoma
Papillary renal cell carcinoma
Chromophobe renal cell carcinoma
Collecting duct carcinoma
Renal medullary carcinoma
Microphthalmia-associated transcription family translocation carcinomas
Hereditary leiomyomatosis renal cell carcinoma–associated renal cell carcinoma
Succinate dehydrogenase–deficient renal cell carcinoma
Mucinous tubular spindle cell carcinoma
Tubulocystic renal cell carcinoma
Acquired cystic disease–associated renal cell carcinoma
Clear cell papillary renal cell carcinoma
Unclassified renal cell carcinoma
Multilocular cystic renal neoplasm of low malignant potential
Oncocytoma
Papillary adenoma
Additional hereditary or emerging renal cell tumors with distinct histologic features
Tuberous sclerosis complex–associated renal cell neoplasms
Birt-Hogg-Dubé syndrome–associated renal cell neoplasms
Oncocytic renal cell carcinoma developed postchemotherapy
Thyroid-like follicular renal cell carcinoma
Anaplastic lymphoma kinase rearrangement–associated renal cell carcinoma
Renal cell carcinoma with (angio)leiomyomatous stroma

SETD2, *BAP1*, and *KDM5C*.^{5,6,10,11} Interestingly, a significant portion of these mutated genes are chromatin modifiers, although their exact roles in tumorigenesis are still under investigation. Furthermore, *PBRM1*, *SETD2*, and *BAP1* are all located at 3p21 and near the *VHL* gene, indicating an important role for 3p loss in the oncogenesis of ccRCC. At the chromosomal level, besides 3p

loss (> 90%), 5q gain (67%) and loss of 14q (45%) are relatively frequent in sporadic tumors.⁵ *BAP1* and *SETD2* mutations or protein/function loss have been associated with worse outcomes in ccRCC.¹²⁻¹⁶

The molecular characterization of ccRCC also coincidentally revealed a subtype of RCC with wild-type *VHL* gene and intact 3p

Table 2. Sporadic Renal Cell Tumors and Associated Molecular Alterations

Tumor Type	Copy Number Variations or Translocations	Somatic Mutations or Alterations
Clear cell RCC	–3p (3p14.2, 3p21, 3p25) Additional: +5q, –14q, –9p, –6q, –8p	<i>VHL</i> , <i>PBRM1</i> , <i>SETD2</i> , <i>BAP1</i> , <i>KDM5C</i> , <i>TERT</i> promoter, <i>MTOR</i>
Papillary RCC, type 1	+7, +17 (trisomy or gain)	<i>MET</i>
Chromophobe RCC	–1, –2, –6, –10, v13, –17, –21 (monosomy or loss)	<i>PTEN</i> , <i>TP53</i> , mitochondrial DNA mutations, structural rearrangement of <i>TERT</i> promoter
Mucinous tubular and spindle cell carcinoma	–1, –4, –6, –8, –9, –11, –13, –14, –15, –18, –22	<i>PTPN14</i> , <i>NF2</i> , and other genes implicated in Hippo pathway
Oncocytoma	–1, v14, –Y Rearrangement involving 11q13 (<i>CCND1</i>) Normal karyotype	Mitochondrial DNA mutations
MiT family (TFE3/TFEB) translocation RCC	t(X;1)(p11.2;q21.2): <i>PRCC-TFE3</i> t(X;1)(p11.2;p34): <i>SFPQ-TFE3</i> t(X;17)(p11.2;q25): <i>ASPSCR1(ASPL)-TFE3</i> t(X;17)(p11.2;q23): <i>CLTC-TFE3</i> t(X;17)(p11.2;p23): <i>DVL2-TFE3</i> t(X;17)(p11.2;q21): <i>LUC7L3-TFE3</i> t(X;3)(p11.2;q21): <i>PARP14-TFE3</i> t(X;19)(p11.2;p13.3): <i>KHSRP-TFE3</i> inv(X)(p11.2;q12): <i>NONO-TFE3</i> inv(X)(p11.2;p11.23): <i>RMB10-TFE3</i> t(6;11)(p21;q12): <i>MALAT1 (Alpa)-TFEB</i>	Uncertain
Clear cell papillary RCC	None	None
ALK translocation RCC	t(2;10)(p23;q22): <i>VCL-ALK</i> t(1;2)(p21;p23): <i>TPM3-ALK</i> inv(2)(p21p23): <i>EML4-ALK</i>	Unknown
Unclassified RCC	Variable	<i>NF2</i> -loss subset: <i>NF2</i> , <i>SETD2</i> mTOR subset: <i>TSC1</i> , <i>TSC2</i> , <i>MTOR</i>

Abbreviations: ALK, anaplastic lymphoma kinase; MiT, microphthalmia-associated transcription; mTOR, mammalian target of rapamycin; RCC, renal cell carcinoma.

Table 3. Selected Hereditary RCC Syndromes and Associated Molecular Alterations

Syndrome	Clinical Manifestations	Gene (chromosome)	Protein
von Hippel-Lindau	Clear cell RCC, pheochromocytoma, pancreatic endocrine tumors, CNS and retinal hemangioblastomas	<i>VHL</i> (3p25)	pVHL
Hereditary papillary RCC	Type 1 papillary RCC	<i>MET</i> (7q31)	MET
Birt-Hogg-Dubé	Renal tumors (hybrid oncocyctic and other types), fibrofolliculomas, pulmonary cysts	<i>BHD/FLCN</i> (17p11.2)	Folliculin
Hereditary leiomyomatosis and RCC	RCC, leiomyomas of skin and uterus (leiomyosarcoma), malignant pheochromocytoma/paraganglioma	<i>FH</i> (1q42.1)	Fumarate hydratase
Tuberous sclerosis complex	Multiple renal AML, cardiac rhabdomyomas, hamartomas, neurologic disorders and seizures, RCCs (morphologic spectrum)	<i>TSC1</i> (9q34) <i>TSC2</i> (16p13.3)	Hamartin, tuberlin
Succinate dehydrogenase B-associated pheochromocytoma/paraganglioma syndrome Type 4	Bilateral and extra-adrenal pheochromocytoma/paraganglioma, RCC, and other malignancies	<i>SDHB</i> (1p36.1-p35)	Succinate dehydrogenase
BAP1 hereditary cancer predisposition syndrome	Uveal melanoma, cutaneous melanoma, mesothelioma, RCC, lung adenocarcinoma	<i>BAP1</i> (3p21)	<i>BRCA1</i> -associated protein-1

Abbreviations: AML, angiomyolipoma; BAP1, *BRCA*-associated protein-1; pVHL, von Hippel Lindau tumor suppressor protein; RCC, renal cell carcinoma.

that was not previously recognized. *TCEB1* (*ELOC*)-mutated RCC harbors recurrent hotspot mutations of *TCEB1* (8q21), a component of the VHL E3 ligase complex, and chromosome 8 loss of heterozygosity, providing another mechanism of activating the HIF pathway.^{6,17}

Non–Clear Cell RCC

Non–clear cell RCC (nccRCC) constitutes a heterogeneous group of tumors. The current diagnostic criteria for some of these tumors rely more on histologic features, such as those for papillary, chromophobe, mucinous tubular, spindle cell, and tubulocystic carcinoma, whereas others emphasize anatomic locations (eg, collecting duct carcinoma [CDC]) or clinical associations (eg, renal medullary carcinoma in patients with sickle cell trait or other hemoglobinopathy). However, a few newly recognized subtypes are distinguished by their specific molecular alterations, such as microphthalmia-associated transcription (MiT) family translocation RCC and hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome that associates with *FH* germ line mutations, and succinate dehydrogenase–deficient RCC.^{18–22} When a tumor does not fit into one of the established histologic subtypes, it is categorized as unclassified RCC (uRCC).

Papillary RCCs (pRCCs) represent about 10% to 15% of all renal cell tumors and are the second most common renal neoplasms. Histologically, they have been divided into two types: type 1 has papillae covered by tumor cells arranged in a single layer and typically low-grade nuclei; type 2 is characterized by pseudostriated and often large tumor cells with higher nuclear grade. However, the histologically defined type 2 pRCC exhibits a rather diverse morphologic spectrum, whereas other subtypes of nccRCCs may show prominent papillary architecture and mimic type 2 pRCC, including HLRCC, MiT translocation RCC, CDC, and newly described entities such as acquired cystic disease–associated RCC, often leading to inconsistent diagnosis among pathologists. Recent genomic characterization also supports that type 2 pRCC is a heterogeneous group and can be further divided into at least three subtypes.²³ In comparison, sporadic type 1 pRCC has nearly universal gains or trisomy of chromosomes 7 and 17 and

less frequent gain of chromosomes 2, 3, 12, 16, and 20.²³ Mutations of the *MET* gene (7q31), identified from hereditary pRCC patients, has been reported in 10% to 13% of sporadic type 1 cases.^{23,24}

Chromophobe RCC (chRCC) makes up 6% to 11% of renal cell neoplasms, and stage-by-stage, it has a significantly better prognosis than ccRCC. The eosinophilic variant of chRCC consists of cells with densely eosinophilic cytoplasm and may closely mimic other oncocyctic renal tumors, including benign oncocyctoma. Cytogenetically, most chRCCs are characterized by combined losses involving most or all of chromosomes 1, 2, 6, 10, 13, 17, and 21.^{25–27} Recent comprehensive genomic analysis identified mutations in *TP53* (32%) and *PTEN* (6%), along with defects in mitochondrial DNA and structural rearrangement involving the *TERT* promoter region in subsets of cases.²⁸

MiT family translocation RCC, or *TFE3* (Xp11.2) and *TFEB* [t(6;11)] translocation RCCs, accounts for less than 5% of all renal neoplasms in children, but it is the most common type of RCC in this age group. Approximately 10% to 15% of cases in children have a history of prior exposure to chemotherapy.²⁹ An increasing number of cases are being recognized in adults.³⁰ The *TFE3* or *TFEB* gene rearrangement in these tumors (Table 2) results in an overexpression of the corresponding protein that can be detected by immunohistochemistry, a relatively specific assay that is difficult to standardize in a routine laboratory setting.³¹ Fluorescent in situ hybridization assays for *TFE3* and *TFEB* translocations have also been developed.^{32,33} With the increased use of these ancillary tools in daily pathologic diagnosis, we are recognizing an expanding morphologic spectrum for this entity.¹⁸

CDC is a rare and aggressive subtype of RCC that likely originates from cells of the collecting ducts of renal medulla.³⁴ The histologic spectrum and diagnostic features of this entity are not entirely specific, which also leads to difficulty in molecular characterization. It is noteworthy that HLRCC-associated RCCs not infrequently show the histology that fulfills the morphologic criteria of CDC.^{20,35}

Renal medullary carcinoma is a rare and distinctive entity occurring almost exclusively in young patients with sickle cell trait or rarely other hemoglobinopathies.³⁶ The loss of nuclear expression of SMARCB1 (INI1/BAF47) protein is a consistent finding in renal medullary carcinoma.^{37–39} Translocations disrupting

SMARCB1 gene and homozygous deletion have recently been reported.^{40,41}

Mucinous tubular spindle cell carcinoma, tubulocystic carcinoma, and acquired cystic disease–associated RCC are rare entities with distinctive morphologic features. Among these, the molecular features of mucinous tubular spindle cell carcinoma have been better elucidated.^{42,43}

Diagnostic Challenges

There are some challenging areas for practicing pathologists to apply the current histologic classification of RCC. One frequently encountered issue is the pink/oncocyctic tumor conundrum. Among the established common entities, benign oncocytoma and eosinophilic variants of chRCC are the two main differential diagnoses to be considered. However, a significant portion of oncocytic tumors seen in surgical specimens do not meet the standard morphologic criteria of either. Even with the recognition of patients with Birt-Hogg-Dubé syndrome,^{44,45} renal oncocytosis,⁴⁶ clinical suspicion of tuberous sclerosis complex,^{47,48} and succinate dehydrogenase–deficient RCC or TFEB tumors resembling oncocytoma, we still routinely face renal oncocytic tumors with relatively low-grade nuclei that do not fit into any of the established classifications. Although the term hybrid tumor has been used by some, its meaning in a sporadic setting remains unclear. Because of these cases, many pathologists refrain from giving a definitive diagnosis, particularly when biopsy material is limited. Efforts to identify characteristic morphologic and molecular features, establish their clinical behavior, and develop ancillary tests are urgently needed for these tumors.

For nccRCCs with high-grade histologic features and aggressive clinical behaviors, we have made some progress in recent years, such as the development of reliable diagnostic markers for MiT family translocation RCC and HLRCC-associated RCC and the recognition of their wide morphologic spectrum.^{18,20,49-51} However, the distinction of and definition of high-grade pRCC and CDC remain controversial and frequently lead to confusion and inconsistent reporting between institutions. Given the poor outcomes and lack of standard therapy for these patients, identifying important oncogenic pathways and predictive markers to stratify patients for management or identifying those that may lead to novel treatment options should be the priority. Because of these concerns, we favor assigning high-grade nccRCCs that do not show the typical clinicopathologic presentations of the established subtypes to the uRCC category instead of forcing them into an existing subtype. A recent molecular analysis of 62 high-grade uRCC primary tumors identified recurrent somatic mutations in 29 genes including *NF2* (18%), *SETD2* (18%), *BAP1* (13%), *KMT2C* (10%), and *MTOR* (8%), revealing distinct molecular subsets with diagnostic and therapeutic implications.⁵²

PREDICTIVE BIOMARKERS FOR RCC: A PRESSING NEED

Our improved understanding of the molecular pathology of RCC over the past few decades has led to the discovery of new treatments for advanced kidney cancer. The identification of the central role played by the dysregulation of the VHL/HIF pathway and the

activation of mammalian target of rapamycin (mTOR) signaling in ccRCC^{5,9,53-55} provided a scientific rationale for developing vascular endothelial growth factor (VEGF)–targeted therapies (bevacizumab, sorafenib, sunitinib, pazopanib, axitinib, and cabozantinib) and mTOR inhibitors (temsirolimus and everolimus) in this tumor type. Recently, the demonstration that tumors can exploit immune-inhibitory signals (immune checkpoints) to evade the immune system⁵⁶ has led to the development of novel immunotherapeutic agents targeting the cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death protein 1 (PD-1) pathways.^{57,58} In patients with previously treated metastatic ccRCC (mccRCC), the anti-PD-1 antibody nivolumab produced improved overall survival (OS) and fewer serious adverse events than everolimus, leading to its approval by the US Food and Drug Administration.⁵⁹ Moreover, the combination of nivolumab plus ipilimumab (anti-CTLA-4) recently showed improved clinical benefit compared with sunitinib in treatment-naïve patients with mccRCC and is now approved by Food and Drug Administration for the treatment of intermediate or poor risk previously untreated patients.⁶⁰

Despite these important advances, only a minority of patients with metastatic RCC (mRCC) who receive either targeted agents or immune checkpoint inhibitors respond to a given treatment. The availability of multiple therapeutic approaches that target different molecular pathways and possibly benefit different subgroups of patients requires the identification of robust predictive biomarkers that can help clinicians select optimal treatment for individual patients. Here, we discuss potential tissue-based biomarkers of response to VEGF-targeted therapies and checkpoint inhibitors in RCC. It should be noted that, to date, most biomarkers discovery efforts have focused on ccRCC, and nccRCC subtypes remain largely understudied.

Tissue Biomarkers for VEGF-Targeted Therapies

Because *VHL* inactivation leads to the upregulation of several proangiogenic HIF targets (eg, VEGF, platelet-derived growth factor [PDGF]), various studies have investigated the role of members of the pVHL-HIF pathway as potential biomarkers of response to VEGF-targeted therapy in ccRCC. These included *VHL* gene mutations, expression of HIF-1 α and HIF-2 α , expression of HIF targets (eg CA-IX), and angiogenesis-related markers.^{7,61-65} Overall, such analyses have produced either negative or largely conflicting results, possibly because of the limited size of the analyzed patient cohorts, differences in experimental protocols, interobserver variability, or other technical issues. Thus, larger independent and more controlled studies are needed to further clarify the significance of these findings.

Although these older studies mostly relied on single-gene sequencing and single-marker immunohistochemical stains, the recent implementation of new sequencing technologies has provided a platform for large-scale biomarker discovery. Hsieh and colleagues⁶⁶ evaluated the association between somatic gene mutations and treatment outcomes in a randomized trial comparing first-line sunitinib with everolimus in patients with mRCC (RECORD-3; Efficacy and Safety Comparison of RAD001 Versus Sunitinib in the First-line and Second-line Treatment of Patients With Metastatic Renal Cell Carcinoma). In the everolimus arm, tumor *PBRM1* mutations were shown to be associated with

a longer progression-free survival (PFS), whereas in the sunitinib arm, longer PFS correlated with *KDM5C* mutations. Later, a post hoc analysis from the phase III COMPARZ (Pazopanib Versus Sunitinib in the Treatment of Locally Advanced and/or Metastatic Renal Cell Carcinoma) trial in patients with treatment-naïve advanced or mcrRCC found that those with *PBRM1* mutant tumors had significantly improved OS and PFS compared with the *PBRM1* nonmutant group.⁶⁷ In line with these findings, in the phase II IMmotion 150 (A Study of Atezolizumab [an Engineered Anti-Programmed Death-Ligand 1 (PD-L1) Antibody] as Monotherapy or in Combination With Bevacizumab [Avastin] Compared to Sunitinib [Sutent] in Participants With Untreated Advanced Renal Cell Carcinoma) trial in treatment-naïve mcrRCC patients, *PBRM1* mutations were associated with improved PFS in the sunitinib-treated group but not in the other treatment groups.⁶⁸ The potential role of *PBRM1* mutations as a predictive biomarker for VEGF-targeted therapy is intriguing because results from two recent independent investigations have shown that inactivation of *PBRM1*, which codes for BAF180 (a subunit of the PBAF subtype of the switch-sucrose nonfermentable chromatin remodeling complex), enhances HIF signaling in human and murine *VHL*-deficient ccRCC cells and tumors.^{69,70} Because *PBRM1* mutations tend to co-occur with *VHL* alterations in human ccRCC,^{5,11} it is conceivable that tumors characterized by inactivation of both genes display high dependency on HIF signaling and are therefore more sensitive to antiangiogenic therapies directed against the HIF target VEGF.

Large-scale transcriptome profiling of pretreatment ccRCC tissues has contributed to the recent identification of transcriptional signatures that might be useful in predicting clinical benefit from VEGF-targeted agents. In an exploratory analysis of the IMmotion 150 trial, the expression of six angiogenesis-associated genes was identified as a potential predictive marker of response to sunitinib.⁶⁸ A different angiogenesis gene signature was found to be associated with longer PFS and OS in patients treated with first-line sunitinib or pazopanib as part of the COMPARZ trial.⁶⁷ The predictive value of *PBRM1* mutation and angiogenic gene signatures needs to be independently validated in the context of large controlled clinical trials.

Tissue Biomarkers for Immune Checkpoint Inhibitors

In mRCC, the PD-1 inhibitor nivolumab has been recently approved by the Food and Drug Administration for treating patients for whom previous therapy has failed. Other immune checkpoint inhibitors, including the anti-PD-1 pembrolizumab and the anti-programmed death-ligand 1 (anti-PD-L1) atezolizumab are still under development.

Compared with everolimus, nivolumab improves OS in mcrRCC, but only a subset of patients (20% to 25%) respond to this agent. The expression of PD-L1 in tumor cells (TCs) assessed by immunohistochemistry increases the likelihood of benefit with nivolumab in nonsquamous non-small-cell lung cancer⁷¹ but fails to identify responders reliably enough to guide clinical decision making in other cancers, including RCC.^{59,72,73} In the phase II dose-finding study of nivolumab in patients with mcrRCC (CA209-010; BMS-936558 [MDX-1106] in Subjects With Advanced/Metastatic Clear-Cell Renal Cell Carcinoma), improvements in median PFS and overall response rate were observed in patients with TC surface PD-L1 expression but they failed to reach

statistical significance.⁷⁴ This result was promising, especially because TC PD-L1 expression is a well-established marker of poor clinical outcome in ccRCC.⁷⁵ Nonetheless, in the phase III trial comparing nivolumab to everolimus (CheckMate 025; Study of Nivolumab [BMS-936558] vs. Everolimus in Pre-Treated Advanced or Metastatic Clear-cell Renal Cell Carcinoma), no association between PD-L1 expression and improved OS on nivolumab was found after a minimum follow-up of 14 months.⁵⁹

There are several possible biologic explanations for the lack of a strong association between TC PD-L1 expression and benefit from single-agent nivolumab in ccRCC. Some ccRCCs with no detectable PD-L1 expression in TCs could respond to PD-1 blockade because in these tumors, PD-1-mediated inhibitory signals might be driven by the expression of PD-L1 in immune cells or by the expression of the second PD-1 ligand, PD-L2, rather than PD-L1. Alternatively, some PD-L1-positive ccRCCs could fail to respond to PD-1 inhibitors because their microenvironment might contain highly exhausted tumor infiltrating lymphocytes that express multiple immune checkpoint receptors (eg, PD-1, TIM-3, LAG-3) that are resistant to single checkpoint blockade. Such hypotheses are being tested in preclinical experiments and by analyzing pretreatment patient samples.⁷⁶

The limited predictive value of TC PD-L1 for nivolumab in ccRCC might also be partly explained by the high level of intra-tumoral heterogeneity that characterizes this tumor type.⁷⁷ Callea and colleagues⁷⁸ recently studied a set of paired primary ccRCCs and metastases and found that in a substantial percentage of cases (approximately 20%), there was discordant TC PD-L1 expression between the primary tumor and the corresponding metastatic lesion(s). Because in RCC PD-L1 levels are commonly assessed in primary tumors, the results of these analyses might not accurately estimate PD-L1 expression in metastases, which are the target of systemic therapy. Consequently, a more accurate assessment of TC PD-L1 as a predictive biomarker for PD-1 blockade in mcrRCC may require the study of metastatic lesions.

In the recent phase III trial comparing nivolumab plus ipilimumab with sunitinib (CheckMate 214; Nivolumab Combined With Ipilimumab Versus Sunitinib in Previously Untreated Advanced or Metastatic Renal Cell Carcinoma), longer PFS was observed with nivolumab plus ipilimumab than with sunitinib among patients with positive TC PD-L1 expression but not among those with negative TC PD-L1 expression.⁶⁰ Validation studies are needed to confirm that TC PD-L1 expression represents a valuable predictive biomarker for the nivolumab-ipilimumab combination in RCC.

To identify potential genomic determinants of response to nivolumab, Miao et al⁷⁹ recently analyzed whole exome sequencing of pretreatment tumors from patients with ccRCC treated with anti-PD-1/PD-L1 therapy alone or in combination with anti-CTLA-4. Loss-of-function somatic mutations in *PBRM1* were found to be significantly more common in patients who experienced clinical benefit from checkpoint inhibitors compared with patients who had no clinical benefit. The investigators also showed that PBAF-deficient ccRCC cell lines had distinct immune-related gene expression profiles compared with PBAF-proficient ones, suggesting that *PBRM1* loss in ccRCC may alter global TC expression in a way that affects tumor immune interactions and response to immunotherapy.

In conclusion, the histologic classification of RCC will likely evolve further as our understanding of the underlying molecular

mechanisms continues to improve. Recent investigations have identified several emerging tissue-based predictive biomarkers in mcrRCC. The clinical utility of these candidate biomarkers now needs to be validated either within prospective biomarker-driven clinical trials or through the analysis of banked specimens from large randomized efficacy trials. The collection of high-quality tumor specimens that are representative of the disease treated in patients will be key to the success of the validation process.

Future efforts should be directed toward discovering potential biomarkers of response for metastatic nccRCC. The main challenge is represented by the highly heterogeneous nature of these tumors. Correlative studies performed within ongoing clinical trials for patients with nccRCC represent a unique opportunity to advance our knowledge in this challenging area.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Renal Cell Carcinoma in the Era of Precision Medicine: From Molecular Pathology to Tissue-Based Biomarkers

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