Therapies for genitourinary malignancies have evolved considerably in the past 5 years. Combination treatment targeting biologically relevant immune and angiogenic pathways is improving patient survival in metastatic renal cell carcinoma (RCC), whereas immune checkpoint blockade (ICB), novel targeted therapy, and antibody drug conjugates have changed the landscape of urothelial cancer (UC) treatment. A daily challenge for clinicians is identifying patients who derive a preferential benefit from the available therapeutic options. The completion of large-scale genomics projects has yielded comprehensive descriptions of the molecular heterogeneity present in RCC and UC, although clinical applications of these data continue to evolve. Major molecular subtypes of RCC align well with histology subtype, and although some molecular characteristics appear to carry prognostic information, biomarkers predicting benefit from tyrosine kinase inhibitor (TKI) or immunotherapy are generally lacking. Unexpectedly, similar work has demonstrated that UC can be grouped into “molecular subtypes” that share properties with those found in breast cancer and other solid tumors. Furthermore, this molecular subtype classification is prognostic and potentially predictive of differential benefit from conventional and targeted therapies. This article provides an update on the current state of molecular biomarker development and potential clinical utility in RCC and UC.

INTRODUCTION
Predicting therapeutic benefit and acquired resistance to therapies in any malignancy remains a daily challenge for clinicians. This dilemma is no better exemplified than in RCC and UC, for which biomarkers with a binary actionable outcome are not available. As physicians are inundated with an increase in information from patient characteristics to next-generation sequencing (NGS), utilizing this information to guide treatment decisions can be challenging. We explore the current biomarkers available in RCC and UC and their predictive and prognostic relationships to available therapies.

BIOMARKERS IN METASTATIC CLEAR CELL RENAL CARCINOMA
Although tissue biomarkers continue to evolve, the International Metastatic Renal Cell Cancer Database Consortium (IMDC) risk score or Heng criteria remain a robust set of prognostic criteria that also form the basis of risk stratification for clinical trials. The criteria include low Karnofsky performance status (less than 80%), low serum hemoglobin, high corrected serum calcium, time from initial RCC diagnosis to start of therapy of less than 1 year, increased neutrophil count, and increased platelets. Patients with no risk factors have favorable risk, those with one or two risk factors have intermediate risk, and patients with three or more risk factors have an unfavorable risk profile. Survival estimates differ among risk groups: 43.2 months, 22.5 months, and 7.8 months, respectively. As frontline combination trials stratified patients and defined primary endpoints based on IMDC risk group, this prognostic information also has implications for treatment selection or even the decision to pursue observation. It should be noted that IMDC criteria are based on data collected from TKI therapy, and the applicability of these criteria in the context of combination treatment with immunotherapy will likely need re-evaluation and evolve with time. In particular, the paradox of poor-risk patients who have a remarkable response to immunotherapy and long-term survival remains unresolved under the current criteria.

For patients with favorable-risk disease, surveillance is an important clinical tool, primarily used for those with low-volume or quiescent disease. More accurate risk stratification allows clinicians and patients greater clarity when selecting this as a strategy. One proposed tool is the neutrophil/lymphocyte ratio (NLR), a validated prognostic factor across a multitude of clinical settings in RCC, including the risk of recurrence postnephrectomy, progression post-cytoreductive nephrectomy, and outcome with VEGF-TKI or ICB treatment. As such, it is intuitive to use NLR to refine risk and identify patients with a low risk for progression to undergo surveillance. However, the presence of an unfavorable/favorable NLR overlaps
PRACTICAL APPLICATIONS

- IMDC risk category remains an important prognostic tool that may guide first-line treatment selection.
- Although patients respond to therapies independent of PD-L1 expression, and it is not routinely checked in RCC, it may be useful to define the relative risk of progression versus response with dual-ICB versus ICB/VEGF blockade.
- Other predictive biomarkers for management of RCC are lacking.
- Activating mutations in FGFR select patients with UC for potential treatment with erdafitinib.
- The antibody drug conjugates enfortumab vedotin and sacituzumab govatexan target proteins with near-universal expression in UC; thus, treatment selection based on protein expression is not required.
- Differentiating UC into basal and luminal molecular subtypes shows promise at predicting sensitivity to chemotherapy, but it needs rigorous prospective evaluation.

with a conventional IMDC risk factor (i.e., elevated neutrophil count) that already guides prognostic decisions. As such, the addition of NLR within the context of validated clinical variables and IMDC risk, which largely captures a favorable NLR by categorizing patients as favorable risk, adds little resolution when considering surveillance.

When treatment is chosen, first-line combination treatments utilizing ICB have been approved by the regulatory agencies in metastatic RCC. Nivolumab plus ipilimumab (nivo-ipi) and axitinib plus pembrolizumab confer an overall survival advantage over sunitinib in intermediate-risk and poor-risk clear cell RCC (ccRCC), with the latter offering a survival advantage independent of risk classification. Axitinib plus avelumab offers an improvement in progression-free survival over sunitinib across risk groups. As nivo-ipi did not receive U.S. Food and Drug Administration approval or achieve an advantage in good-risk disease, IMDC risk classification remains an important tool in discussions about frontline systemic therapy. However, although nivo-ipi did not improve response rate or progression-free survival versus sunitinib in good-risk disease, patients with good-risk disease still responded to nivo-ipi, and the complete response (CR) rate approached 10%. Furthermore, there was no detriment in overall survival. Although not endorsed by guidelines or regulatory approvals, given the chance at long-term remission, this remains an attractive option, even in good-risk disease. Furthermore, in updated analyses, the benefit of axitinib-pembrolizumab in good-risk disease was less impressive, suggesting that VEGF monotherapy could still be considered. Further biomarker research is clearly needed to subset good-risk disease and identify the responders who benefit from ICB, with or without VEGF.

In patients with intermediate- or poor-risk disease, for whom all options are approved by regulatory agencies, the likelihood of response and toxicity profile are important variables when considering treatment. If a patient has pending visceral crisis, the improvement in overall response rate (ORR) to near 60% with axitinib-pembrolizumab versus less than 40% with nivo-ipi supports its use. However, other than clinical variables, is there a reliable marker to determine the best therapeutic option? Unlike in some other solid-organ malignancies, such as lung cancer and melanoma, tumor mutational burden has represented a poor correlate for response to ICB in RCC, with responses agnostic to tumor mutational burden. As such, although often reported in NGS reports, it should not drive treatment decisions in metastatic RCC. A strong association exists between expression of PD-L1 via immunohistochemistry and response rate to nivo-ipi. Demonstrated in CheckMate 214, 26% of patients in the nivo-ipi cohort harbor PD-L1+ disease, which is associated with a higher objective response rate than PD-L1− disease, both superior to sunitinib although overall survival was maintained with nivo-ipi independent of PD-L1 status, underscoring the importance of ICB in RCC (Table 1). PD-L1 status should not be used to determine whether ICB should be offered to a patient with RCC. However, when expressing the relative risk of response versus progression, patients with PD-L1+ disease are 4.14 times more likely to have a response to nivo-ipi than to experience disease progression; the relative risk is 1.85 in patients with PD-L1− disease. As such, in patients with PD-L1− disease who risk being unable to receive salvage treatment upon progression, the combination of a TKI with ICB is an attractive option with a more favorable risk-benefit profile, because axitinib-ICB combinations demonstrated high response rates and did not have a differential outcome based on PD-L1 status (despite that being the primary outcome with axitinib-

### Table 1. Relative Risk of Progression Versus Response to Nivo-Ipi in CheckMate 214 IMDC Intermediate/Poor-Risk Cohort Based on PD-L1 Status

<table>
<thead>
<tr>
<th>Response</th>
<th>All Patients</th>
<th>PD-L1 &lt; 1%</th>
<th>PD-L1 ≥ 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>42%</td>
<td>37%</td>
<td>58%</td>
</tr>
<tr>
<td>PD</td>
<td>20%</td>
<td>20%</td>
<td>14%</td>
</tr>
<tr>
<td>PD ORR</td>
<td>1.21</td>
<td>1.185</td>
<td>1.414</td>
</tr>
</tbody>
</table>

Abbreviations: IMDC, International Metastatic Renal Cell Cancer Database Consortium; ORR, overall response rate; PD, progressive disease.
It should be noted that there has not been a significant difference with regard to survival outcome based on PD-L1 expression levels, but trials exclude patients who have critical disease, and immediate response is more highly linked to survival in this context because many patients do not receive second-line treatment.

The presence of sarcomatoid or rhabdoid histology is associated with a more aggressive disease course and a low likelihood of response to TKI monotherapy. A uniformly strong efficacy signal in frontline clinical trials that incorporates checkpoint inhibitors has highlighted the importance of incorporating ICB therapy for these patients. However, a differential response is noted with nivo-ipi; ORR approaches 60% and CR rate approaches 20%, although it continues to carry twice the risk for primary progressive disease compared with axitinib-pembrolizumab (25% vs. 13.7%). Thus, as is the case with RCC without sarcomatoid features, in patients who have limited consequences from progression (e.g., presence of low-volume pulmonary metastases), a greater chance for long-term remission utilizing nivo-ipi may be the favored treatment. For patients in whom progression may compromise subsequent therapy, the lower primary progression rate while on axitinib-ICB treatment is appealing, but it comes at the expense of a reduction in the number of complete responders.

With regard to prognosis, inactivation of TP53 or the CDKN2A tumor suppressors or a CpG island hypermethylation phenotype is associated with poor prognosis across histologic subtypes, whereas inactivating mutations in the BAP1 gene are associated with poor prognosis in ccRCC and type 1 papillary RCC (pRCC). The PBRM1 gene encodes the protein polybromo-1, which acts as a tumor suppressor in RCC. Functional loss is present in 40% of cases of RCC, and it has been associated with increased survival in retrospective trial analyses; recent data from the NIVOREN GETUG-AFU26 trial confirmed its prognostic significance in the largest ICB cohort examined to date, with a modest effect size. Results from retrospective analyses have suggested that PBRM1 mutations confer sensitivity to ICB; in contrast, a study failed to reproduce this observation and instead linked mutations to benefit from sunitinib. Several ongoing analyses examining PBRM1 mutation status will be critical to resolve these contrasting data sets before it can be recommended as a predictive biomarker in the context of patients receiving ICB therapy upfront or in sequence.

The role of NGS in RCC is evolving to consider the mutational landscape of metastatic RCC, ideally to identify predictive markers that inform first-line treatment choice or direct patients to a targeted therapy. ccRCC exhibits enhanced vascular development and immune gene signatures relative to pRCCs or chromophobe RCCs, consistent with the unique role of the VHL-HIF pathway in their development and their relative sensitivity to immunotherapy. News of the 2019 Nobel Prize in Physiology or Medicine being awarded to three investigators who discovered how the VHL-HIF pathway controls the cellular response to hypoxia has focused interest on the development of novel agents targeting this pathway, with a promising small molecule inhibitor of HIF-2 under clinical evaluation and encouraging results to date. Studies in cohorts of patients with ccRCC have identified potentially targetable genomic alterations in 13% of patients, with the clear majority being alterations in TSC1, PIK3A, or MET. Alterations in key components of the PI3K/mTOR pathway have been candidates to confer a response to rapalog therapy, but clinical data have yielded conflicting results on retrospective analyses. In a similar fashion, MET alterations are thought to represent a dominant pathologic pathway actionable through MET-directed therapy. However, because the therapeutic sequence of treatment lines commonly involves exposure to the rapamycin analog everolimus and TKI therapy involving agents that target MET, such as cabozaotinib (a multikinase inhibitor that inhibits VEGF, MET, and AXL), the relevance of these common alterations remains speculative and they do not yet inform treatment choice, although they may play a greater role in the management of patients with variant histology. For a summary of biomarkers related to RCC, refer to Table 2.

Variant histology remains a poor prognostic factor. This is an umbrella category for a heterogeneous group of malignancies including papillary, chromophobe, medullary, collecting duct, TFE3 translocation, and unclassified RCC variants. The Cancer Genome Atlas project largely confirmed that there are strong molecular bases for the histologic heterogeneity observed in variant RCC, and differences in metabolic gene expression and RNA splicing distinguish it from the major RCC histologic variants. MET activating mutations and copy number alterations are a hallmark of pRCCs, which can be segregated into type 1 and type 2 subsets based on messenger RNA expression. The role of activating MET alterations in response to MET inhibitors remains unclear. In one phase II clinical trial of the MET inhibitor foretinib, a correlation was observed between the presence of a germline MET mutation and response. In contrast, in a retrospective real-world study of the effects of cabozaotinib (a multikinase inhibitor incorporating significant activity against MET), somatic alterations were not predictive of benefit. A large collaborative prospective trial evaluating the MET inhibitor...
cabozonib versus sunitinib in pRCC (PAPMET/SWOG 1500) is underway and should help to reconcile these observations.37

Salvolutinib is another small molecule inhibitor of MET that has demonstrated activity in pRCC.38,39 Although closed early to recruitment, the phase III trial result examining salvolutinib versus sunitinib is expected and may shed light on the differential activity against sunitinib.40 However, at this time, treatment continues to be extrapolated from ccRCC, and a phase II trial examining atezolizumab and bevaczumab in combination for variant histology (ORR, 26% vs. 37% in the ccRCC trial IMmotion151) supports this approach, albeit with less efficacy.41,42 More conventional biomarkers of immune infiltration (with the exception of tumor mutational burden or tumor neoantigen burden) appear to more consistently enrich for clinical benefit,22 such as PD-L1 expression, although they are still not robust enough for patient selection.

Finally, the potential value of circulating tumor DNA (ctDNA) in monitoring the molecular evolution of metastatic disease is being evaluated in RCC research projects. Early work suggested that ctDNA may be more difficult to detect in RCC compared with other solid tumors, particularly in patients with earlier-stage disease.43,44 Commercial panels detect ctDNA in about three-fourths of patients with overt metastatic disease45; rates of TP53 and mTOR pathway alterations are higher than those observed in localized tumors. It may also be possible to detect RCC-related mutations in urine,46 although large-scale studies of feasibility must still be performed. Layering the development of convenient noninvasive mutational identification and disease-monitoring methods over tissue-based markers will be important to maximize the utility and validity of new assays, which will increase our understanding of the importance and temporal variation of peripherally detected mutations in RCC.

TABLE 2. Summary of Clinical Utility for Biomarkers in Metastatic Renal Cell Carcinoma

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Study Context</th>
<th>Use in Practice</th>
<th>When to Test</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMDC Risk Score</td>
<td>Retrospective</td>
<td>Yes</td>
<td>Always</td>
<td>Strong</td>
</tr>
<tr>
<td>PD-L1</td>
<td>Prospective</td>
<td>Limited</td>
<td>Critical disease; which ICB combination to use</td>
<td>Weak</td>
</tr>
<tr>
<td>PBRM1</td>
<td>Prospective</td>
<td>No</td>
<td>Not indicated</td>
<td>N/A</td>
</tr>
<tr>
<td>NGS</td>
<td>Retrospective</td>
<td>No</td>
<td>Not indicated</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: IMDC, International Metastatic Renal Cell Cancer Database Consortium; ICB, immune checkpoint blockade; N/A, not applicable; NGS, next-generation sequencing.

Targeting Metastatic Urothelial Cancer

Similar challenges to RCC exist when assessing the treatment landscape of metastatic UC; again, the selection of systemic treatment is not aided to a great extent by readily available biomarkers. The recent approval of five immune checkpoint inhibitors targeting PD-1 and PD-L1 has resulted in much fanfare, primarily as a result of a durability of response that has not been observed in the era of cisplatin-based chemotherapy. However, the proportion of patients who respond remains low, with most trials reporting an approximately 15% to 20% objective response rate in the second-line setting47,48 and slightly higher rates in the frontline setting.49,50 Attempts to use PD-L1 expression for patient selection has yielded little benefit in patients who have received prior treatment. Recent data from IMvigor130, a trial of first-line chemotherapy with or without a checkpoint inhibitor versus single-agent immunotherapy, suggest that patients who are not eligible for treatment with cisplatin and have PD-L1<sup>+</sup> disease benefit from single-agent immunotherapy, and that single-agent immunotherapy should be avoided in favor of systemic chemotherapy in patients with PD-L1<sup>low</sup> tumors.51 A variety of other factors, including tumor mutational burden,52 bladder cancer subtyping by gene-expression profiling,53,54 and the interferon gamma gene signature,55 have been looked at as a means of improving our ability to select patients who may benefit the most from an immune checkpoint inhibitor. Despite these attempts, it still appears that the best way of selecting patients who benefit from an immune checkpoint inhibitor is the patient’s actual response to immune checkpoint inhibition. One landmark analysis of patients receiving second-line nivolumab for more than 1 year suggested that patients with a clinical CR do extraordinarily well, with only two deaths among 17 patients achieving a clinical CR.53 This also raises the question as to whether a depth of response matters. If so, one could envision a future in which clinical trials aim for improvement in CR, in addition to assessing a durability of response, as a potential surrogate for long-term clinical outcomes.

FGFR3-mutated UC recently emerged as a potential marker of resistance to immune checkpoint inhibition based upon its enrichment for an immunologically cold, luminal papillary subtype of UC.54 Erdafitinib is the first-in-class pan–FGFR1–4 inhibitor approved for the treatment of metastatic UC; it is highly effective in the 20% of patients with activating mutations in FGFR.55,56 Early trials of FGFR3 inhibitors used an intermittent schedule because of their toxicity.57,58 As a result, the erdafitinib trial initially assigned patients to an intermittent dose of 10 mg daily (1 week on/1 week off) versus continuous dosing of 6 mg daily. Early results from this randomization suggested that the continuous dose may have better effects on toxicity and clinical activity. Additional pharmacokinetics testing performed during this trial...
suggested an increase in continuous dosing to 8 mg daily, with uptitration to 9 mg daily if the phosphorous level on day 15 was less than 5.5 mg/dL. The use of targeted phosphorus levels reflects on-target inhibition of FGFR3, providing a means of increasing the dose in patients who may benefit from more optimal dosing to enhance response. With a 40% objective response rate and median overall survival of approximately 13.8 months,55 the U.S. Food and Drug Administration granted erdafitinib accelerated approval for the second-line treatment of FGFR3-altered metastatic UC in April 2019.

However, we still do not have an answer to the question of whether FGFR3-altered urothelial tumors derive greater benefit from treatment with an immune checkpoint inhibitor or FGFR3-targeted therapy. In the phase II clinical trial of erdafitinib, 22 patients treated had received prior immune checkpoint inhibition, and one partial response was reported (ORR, 5%).56 Similar results were reported with regorafenib; one in 10 patients had stable disease, which is the best response reported with immunotherapy.59 However, Galsky et al reported a similar objective response rate to an immune checkpoint inhibitor, regardless of whether an FGFR3 mutation was present.50 The phase III trial THOR has the goal of answering this important question. Patients who have received prior chemotherapy, but no prior immune checkpoint inhibitor, for their FGFR3-altered metastatic UC will be randomly assigned to receive erdafitinib or pembrolizumab. Patients who have received a prior immune checkpoint inhibitor will be randomly assigned to receive erdafitinib or taxanes (or vinflunine in Europe). A second clinical trial (NORSE) is also accruing patients to test whether the addition of an immune checkpoint inhibitor to erdafitinib will enhance response or durability of response to combination therapy. However, because this mutation is present in up to 20% of patients, other therapies are needed.

Enfortumab vedotin is another recent addition as the first antibody drug conjugate approved for the treatment of metastatic UC. The antibody targets nectin-4, which is expressed at a high level on 93% of urothelial tumors; therefore, testing for nectin-4 is not required and should not be pursued prior to starting therapy. This antibody uses a protease-cleavable linker to release the antimicrotubule agent auristatin-E following internalization of the antibody. In patients who have received prior chemotherapy and immune checkpoint inhibition, treatment with enfortumab vedotin resulted in a 44% objective response rate, with a median overall survival of approximately 11 months.61 Early results from a small phase II clinical trial of enfortumab vedotin plus pembrolizumab in the frontline treatment of UC in patients who could not receive cisplatin suggest objective response rates as high as 70%.62 Additional studies are in progress to confirm this finding, which could change the treatment paradigm for UC.

Many other targeted therapies show promise in the treatment of UC. Sacituzumab govitecan is an antibody drug conjugate targeting Trop-2, which is widely expressed in multiple tumor types, including UC; it brings the active metabolite of irinotecan directly to tumor cells. Bempedgallesleukin is a pegylated formulation of IL-2 with selective signaling through the IL-2 beta gamma receptor, which results in clonal expansion of lymphocytes associated with an immune response; it has shown evidence of clinical activity, even in PD-L1low tumors, when combined with an immune checkpoint inhibitor.63 VEGF inhibitors, including lenvatinib and sitravatinib,64 which target AXL and MER in addition to VEGF, and many more targeted agents are being studied for the treatment of UC in single-agent trials, as well as in combination with immune checkpoint inhibitors.

The molecular subtyping of tumors into relevant classes based on differential biology may have utility in guiding future clinical decisions. Using whole-transcriptome messenger RNA expression profiling and unsupervised hierarchical clustering, several groups independently concluded that muscle-invasive UC can be grouped into basal and luminal molecular subtypes that resemble the ones that had previously been identified in breast cancers.65-68 Recently, an international team created an open-access single-sample molecular subtyping algorithm that can be used with whole-transcriptome data to assign UC to one of six consensus subtypes.69 Basal cancers are enriched with regard to squamous histopathologic features and stem cell biomarkers, have the most cellular proliferation with high levels of cyclins, and are associated with advanced-stage and metastatic disease at presentation.67,68 Cisplatin-based chemotherapy has remained the mainstay of treatment for UC for more than 30 years as a result of its ability to promote DNA damage resulting in cell death of the most rapidly proliferating cells. Early evidence from patients treated on two clinical trials of chemotherapy with methotrexate-vinblastine-doxorubicincisplatin (MVAC) and dose-dense MVAC suggested that the basal subtype had the best clinical outcomes when treated with neoadjuvant cisplatin-based chemotherapy, a finding that has been replicated in a separate retrospective data set of cisplatin-treated patients.67,70,71 Luminal cancers appear to be more heterogeneous and can be subdivided into as many as four subtypes.69 Luminal papillary tumors are enriched with regard to activating FGFR3 mutations and fusions, are associated with low rates of pathologic upstaging,72 and are associated with the best prognoses.69 Although luminal unstable and luminal infiltrated tumors are more aggressive, they may also be more sensitive to immunotherapy.73,74 Finally, neuroendocrine...
tumors are characterized by combined inactivation of TP53 and RB1 and expression of neuronal differentiation markers. Although they are very clinically aggressive, they may be highly sensitive to immunotherapy. Importantly, these relationships must be prospectively validated before molecular subtyping can inform clinical decision making. One such prospective study examining the relationship between molecular subtype and benefit from neoadjuvant chemotherapy has completed enrollment (Southwest Oncology Group’s S1314 COXEN trial), and the results are currently being analyzed.

Predicting clinical benefit from neoadjuvant chemotherapy is also the purpose of a second class of biomarkers: inactivating mutations in DNA damage and response genes. Using the MSK-IMPACT panel exome-sequencing platform and focusing on extreme responders, one group linked inactivating mutations in ERCC2 to response (i.e., pathologic downstaging) and went on to perform functional studies that demonstrated that these mutations actually cause sensitivity to cisplatin. A second group performed FoundationOne panel sequencing on tumor specimens from a completed trial of dose-dense MVAC and identified mutations in RB1, FANCC, or ATM in all of the responding tumors. They next performed validation studies with samples from an independent clinical trial of dose-dense gemcitabine/cisplatin. Both groups plan to prospectively validate their findings by performing panel exome sequencing on the tumors from the COXEN trial. The investigators have also opened two prospective clinical trials that are designed to test the hypothesis that patients whose tumors contain DNA damage and response mutations can be managed with a thorough transurethral resection of bladder tumor and neoadjuvant chemotherapy without going on to cystectomy. For a summary of biomarkers related to metastatic UC, refer to Table 3.

A third area of major progress in UC is the development of “liquid biopsy” approaches using peripheral blood or urine to monitor minimal residual disease burden and select patients for conventional and targeted therapy.

Although pathologic downstaging in patients treated with neoadjuvant therapies correlates well with survival, it is only a surrogate for the direct effects of systemic therapy on micrometastatic disease. Most of the companies that have panel sequencing products for tumor biopsies also offer products for panel sequencing of ctDNA in plasma, although the relatively modest sequencing depth generated by these platforms makes them unreliable as tools to measure minimal residual disease. However, collaborative efforts involving a university-based academic group and Natera have produced a more personalized test that is an attractive candidate solution for this purpose. Tumor biopsies are first subjected to whole-exome sequencing, and droplet-digital polymerase chain reaction–sequencing assays are designed to measure 16 patient-specific mutations. The assay is then applied to plasma samples that are sequenced to an average of 109,000 × coverage. Applying the assay to longitudinal plasma samples from patients treated with neoadjuvant chemotherapy, the investigators demonstrated that it exhibited extremely high sensitivity and specificity, was highly prognostic at sentinel points in clinical management (after transurethral resection of bladder tumor before neoadjuvant chemotherapy, after neoadjuvant chemotherapy, and before recurrence), and performed better than molecular subtypes or DNA damage and response mutations to predict response.

There is also an unmet need to develop accurate methods for measuring residual disease in the bladder. Although it is possible that plasma ctDNA can also be used for this purpose, a potentially more attractive approach would be to develop assays to measure tumor DNA in urine. Early work using mass spectrometric sequencing approaches established feasibility, and more recent efforts have involved applying panel or personalized NGS approaches to increase sensitivity and specificity.  Although these assays are not ready for clinical implementation, the performance characteristics of the best ones rival blue light cystoscopy (without the need for an experienced urologist). Ongoing work is aimed at determining whether these urine tumor DNA assays can be used to monitor local residual disease burden in patients treated with intravesical therapies and/or neoadjuvant chemotherapy.

**TABLE 3. Summary of Clinical Utility for Biomarkers in Metastatic Urothelial Carcinoma**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Study Context</th>
<th>Use in Practice</th>
<th>When to Test</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR alterations</td>
<td>Prospective</td>
<td>Yes</td>
<td>Second-line treatment</td>
<td>Strong</td>
</tr>
<tr>
<td>PD-L1</td>
<td>Prospective</td>
<td>Sometimes</td>
<td>Cisplatin-ineligible first-line setting</td>
<td>Strong</td>
</tr>
<tr>
<td>Gene-expression profiling</td>
<td>Retrospective</td>
<td>No</td>
<td>Not indicated</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviation: N/A, not applicable.

**CONCLUSIONS**

Biomarker development in RCC and UC has lagged behind the recent advances in systemic treatment. Although VEGF-targeted therapy has been a mainstay in RCC for more than a decade, it has been used without guidance from any biomarker, and emerging antibody drug conjugates in UC look to do the same. Prognostic markers continue to evolve; however, other than FGFR alterations,
there is no predictive biomarker to guide treatment decisions in UC or RCC. Molecular subtyping of disease has generated a greater understanding of the landscape of alterations and their potential impact on treatment response; further improvements in these sequencing and detection methods may define the future landscape for biomarker-directed therapy. To achieve this, successful prospective validation of treatment assignment based on mutation or disease subtyping will be required before routine clinical utilization.

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3 Johns Hopkins Greenberg Bladder Cancer Institute, Baltimore, MD

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AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT
Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI https://doi.org/10.1200/EDBK_279905.


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