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## Renal Tumors:

### Diagnostic and Prognostic Biomarkers

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### Abstract

The International Society of Urological Pathology convened a consensus conference on renal cancer, preceded by an online survey, to address issues relating to the diagnosis and reporting of renal neoplasia. In this report, the role of biomarkers in the diagnosis and assessment of prognosis of renal tumors is addressed. In particular we focused upon the use of immunohistochemical markers and the approach to specific differential diagnostic scenarios. We enquired whether cytogenetic and molecular tools were applied in practice and asked for views on the perceived prognostic role of biomarkers. Both the survey and conference voting results demonstrated a high degree of consensus in participants' responses regarding prognostic/predictive markers and molecular techniques, whereas it was apparent that biomarkers for these purposes remained outside the diagnostic realm pending clinical validation. Although no individual antibody or panel of antibodies reached consensus for classifying renal tumors, or for confirming renal metastatic disease, it was noted from the online survey that 87% of respondents used immunohistochemistry to subtype renal tumors sometimes or occasionally, and a majority (87%) used immunohistochemical markers (Pax 2 or Pax 8, renal cell carcinoma [RCC] marker, panel of pan-

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CK, CK7, vimentin, and CD10) in confirming the diagnosis of metastatic RCC. There was consensus that immunohistochemistry should be used for histologic subtyping and applied before reaching a diagnosis of unclassified RCC. At the conference, there was consensus that TFE3 and TFEB analysis ought to be requested when RCC was diagnosed in a young patient or when histologic appearances were suggestive of the translocation subtype; whereas Pax 2 and/or Pax 8 were considered to be the most useful markers in the diagnosis of a renal primary.

### Keywords

renal cell carcinoma; immunohistochemistry; CK7; Pax 2; Pax 8; translocation carcinoma; International Society of Urological Pathology

The role of biomarkers in renal cell carcinoma (RCC) is expansive and can range from aiding pathologic diagnosis, understanding the histogenesis of a renal tumor, classifying new entities, and choosing appropriate therapy in patients who present with advanced disease, to the more investigative arena of elucidating predictive and prognostic behavior of renal neoplasms. Among platforms used in determining the presence of biological markers in surgical pathology specimens, immunohistochemistry is perhaps the most commonly available tool in the routine diagnostic laboratory. Immunohistochemistry allows detection of antigens expressed on tumor cells, hence permitting characterization of the tumor. Less commonly used methods in the context of renal neoplasms are conventional karyotyping, fluorescence in situ hybridization (FISH), and molecular cytogenetics. More novel molecular analyses such as expression profiling, comparative genomic hybridization, single nucleotide polymorphism array, methylation status, and mutational analysis are currently being used more experimentally to identify specific molecular pathways involved in various tumor types and to identify potential therapeutic targets.

In this report, we document the results of an online survey conducted by the International Society of Urological Pathology (ISUP) in which 206 members participated. The rationale, organization, and processes of the premeeting survey and consensus conference are detailed elsewhere.<sup>1</sup> Working group 4 interrogated the use of immunohistochemical markers among participating ISUP members in routine practice and their approach in handling diagnostic situations that might require application of ancillary assays. Questions relating to respondents' use of cytogenetics or other molecular platforms were included. In addition, participants were asked whether they relied on any markers for prognostication or whether there was clinical interest in pathologic provision of predictive biomarkers at their institutions. Additional questions asked during the consensus conference held in Vancouver on March 17, 2012 were related to the use of tests for confirming translocation RCC, redefining markers for confirming a renal primary, distinguishing clear cell from chromophobe RCC, and in the workup of sarcomatoid RCC. Table 1 summarizes the online survey and consensus conference results.

## USE OF IMMUNOHISTOCHEMISTRY

Diagnosis and subtyping of RCC can usually be accomplished through a thorough morphologic appraisal of the resected tumor, which in itself offers valuable prognostic

information.<sup>2-4</sup> Occasionally, however, there is a need to use ancillary markers to verify the histologic subtype<sup>5</sup> or to distinguish primary RCC from benign mimics and other tumor types that can occur in the kidney or from the rare metastasis to the kidney.<sup>2</sup> Metastases of RCC to distant sites also usually need to be confirmed with the use of a panel of markers.<sup>2,6</sup> The classification of the tumor type on limited material, such as core biopsies, may warrant immunohistochemical assessment.<sup>7</sup>

Abundant literature exists with regard to the spectrum of antibodies that are useful in various diagnostic settings.<sup>6,8-16</sup> A wide panel of antibodies has been applied as an adjunct to the assessment of renal tumors, and these include cytokeratins, vimentin,  $\alpha$ -methylacyl CoA racemase (AMACR), carbonic anhydrase IX (CAIX), Pax 2, Pax 8, RCC marker, CD10, E-cadherin, kidney-specific cadherin, parvalbumin, claudin-7, claudin-8, S100A1, CD82, CD117, TFE3, TFEB, thrombomodulin, uroplakin III, p63, and S100P.<sup>2</sup>

The main subtypes of RCC are clear cell, papillary, chromophobe, collecting duct, and unclassified.<sup>2,4</sup> Apart from the unclassified group, each subtype has typical immunohistochemical staining profiles that can assist in corroborating correct classification. It is relevant to note that qualitative staining characteristics and subcellular localization are also important, aside from mere positive or negative immunohistochemical reactivity (Fig. 1). Clear cell RCC is usually positive for vimentin, keratin, EMA, CD10, Pax 2, RCC marker, and CAIX and negative for kidney-specific cadherin and parvalbumin. It has been shown that CD10 and Pax 2 upregulation is due to *VHL* inactivation in clear cell RCC,<sup>17</sup> whereas CAIX is also consistently expressed because of its regulation by the *VHL* protein.<sup>18,19</sup> Papillary RCC type 1 is positive for vimentin, broad-spectrum keratins, CK7, AMACR, and RCC marker, and negative for CD117, kidney-specific cadherin, and parvalbumin. Papillary RCC type 2 has variable staining patterns, consistent with the fact that this is likely a heterogeneous category rather than a distinct entity. Immunohistochemical analysis of chromophobe RCC shows diffuse reactivity for E-cadherin, kidney-specific cadherin, parvalbumin, CD117, EMA, broad-spectrum keratins, and CK7 and no expression of vimentin, CAIX, and AMACR. Collecting duct carcinoma is often positive for EMA, CK7, high-molecular weight keratin, Pax 2, and Pax 8 and negative for CD10 and CK20.<sup>2,3,20</sup>

The advent of radiologically guided percutaneous needle biopsy and aspiration procedures to assess renal masses has challenged the pathologist to maximize the use of small amounts of tissue and cellular material for diagnosis. In such circumstances, ancillary immunohistochemistry may help to secure a firm conclusion.<sup>21,22</sup> In an *ex vivo* study of the role of immunohistochemistry in evaluating core biopsies of renal masses, Al-Ahmadie et al<sup>22</sup> found that 81% of cases could be correctly classified by routine light microscopy, with accuracy that was improved to 90% when immunohistochemical analysis was added.

Oncocytoma, angiomyolipoma, and metanephric adenoma are benign mimics of RCC. Morphologic distinction can be problematic on occasion, and immunohistochemistry may then be required to assist in confirming the diagnosis. Differentiation of oncocytoma from chromophobe RCC, specifically the eosinophilic variant, is addressed below. For angiomyolipoma, the epithelioid variety can closely resemble RCC,<sup>23</sup> although positive

immunohistochemical reactivity for HMB45, melan-A, and SMA and negative expression of keratins support a diagnosis of angiomyolipoma.<sup>4</sup> Metanephric adenoma, which may be mistaken for type 1 papillary RCC, shows positive immunostaining for S100,<sup>24</sup> WT1, and CD57 and negative reactivity for AMACR,<sup>11</sup> in contrast to the latter tumor. AMACR, CK7, WT1, and CD57 form a recommended panel to distinguish metanephric adenoma from papillary RCC.<sup>11</sup>

The majority (56%) of survey respondents used immunohistochemistry, when considered necessary, to assist in histologic subtyping of RCC. Of the remainder, 16% applied immunohistochemistry in the workup of a core biopsy of a renal mass, 14% used it for distinguishing a nonrenal tumor from RCC, and 11% for evaluating metastatic lesions wherein a renal primary was considered a possibility. Two participants stated that they used immunohistochemistry for all of the aforementioned reasons. The distribution of responses reflects a lack of consensus among participants in deciding the commonest reasons for using immunohistochemistry and underlies the broad spectrum of scenarios to which this tool may be applied.

Regarding the frequency of use of immunohistochemistry for histologic subtyping, 45% of respondents reported that they utilized it “occasionally,” 42% reported that they “sometimes” applied it, whereas 13% “rarely” used it. These results translate to a consensus of 87% of respondents who would occasionally or sometimes use immunohistochemistry in subtyping renal neoplasms.

## DIAGNOSIS OF RENAL CELL NEOPLASIA

With the large armamentarium of immunohistochemical markers available for use in assessing renal tumors, it is perhaps difficult to achieve unanimity regarding any particular marker that is used most frequently, as the choice of specific markers will depend on the diagnostic dilemma that is being addressed. A panel approach is often adopted, which means that there is usually no single marker that is used in isolation and that there will be at least 2 to 3 markers used to resolve a diagnostic conundrum.

Although the majority of tumors arising in the kidney will be of primary renal origin, there are rare circumstances in which tumors of nonrenal origin can masquerade as kidney neoplasms, such as retroperitoneal masses that encase or involve the kidney, adrenal neoplasms, and metastasis to the kidney (Fig. 2). When these lesions are investigated with needle biopsy or aspiration, the limited material available compounds the interpretive challenge. A comprehensive panel incorporating epithelial (AE1/3, Cam5.2, and EMA), RCC-related (RCC antigen, CAIX, and CD10), and adrenocortical (calretinin, melan-A, and neuroendocrine) markers has been advocated for differentiating clear cell RCC from histologic mimics of adrenocortical neoplasms and paragangliomas.<sup>12</sup> Caution, however, has been expressed with regard to the use of CD10, as it may also react with adrenocortical tumors and is therefore not a specific marker of clear cell RCC.<sup>25</sup> Another study found antisteroidogenic factor-1, calretinin, inhibin, and melan-A to be indicative of an adrenocortical origin, whereas anti-human kidney injury molecule-1 (hKIM-1), Pax 8,

hepatocyte nuclear factor-1b, EMA, and CAIX were valuable in establishing a tumor as a clear cell RCC.<sup>8</sup>

Although clear cell RCC is the main diagnostic contender when a kidney tumor with a predominantly clear cell population is encountered, the occurrence of clear cells in other subtypes of renal carcinoma, as well as in tumors arising from the adjacent adrenal gland and liver, warrants not only thorough histologic study but also immunohistochemical arbitration in many instances (Fig. 3).<sup>13,26</sup>

Infrequently, metastases to the kidney from other primary tumor sites such as the thyroid, breast (Fig. 4), and lung, as well as hematolymphoid neoplasms,<sup>27-29</sup> will require confirmation with immunohistochemistry. Sarcomatoid RCC is a priority differential diagnosis when a tumor with mesenchymal appearances occurs in the kidney.<sup>4</sup> Nevertheless, retroperitoneal sarcomas that invade the kidney or primary renal sarcomas should be excluded through a combined morphologic and immunohistochemical assessment.

Among the options of CK7, Pax 2 and/or Pax 8, CD10, vimentin, and AMACR (P504S) as immunohistochemical markers for the diagnosis of a renal cell neoplasm, 49% of survey participants reported that CK7 was most frequently used, followed by CD10, which was used by 23%, Pax 2 and/or Pax 8 by 16%, vimentin by 7%, and AMACR by 5%. As these results were somewhat surprising in view of the relatively low proportion selecting Pax 2 and/or Pax 8 as the favored choice, the question as to which was the most preferred marker for confirming a renal neoplasm was posed again during the consensus conference in Vancouver, with 71% of attendees opting for Pax 2 and/or Pax 8, indicating consensus. Interestingly, CK7 was chosen by only 3% of conference participants, diverging considerably from the online survey result. This anomaly may be because of the manner in which the question was interpreted during the online survey, as voting at the consensus conference was conducted only after an overview of the subject was presented to the group, which may have influenced the attendees' final selection.

## DIAGNOSIS OF METASTASIS OF RENAL ORIGIN

Investigative workup for a metastasis often includes a needle biopsy of the metastatic lesion for histologic confirmation of tumor type and origin. In the presence of a history of RCC, a diagnosis of metastasis may be less problematic (Fig. 5). However, histologic appearances of the metastasis may vary from the original primary tumor, or there may be no history of renal neoplasm, and the patient can present with metastases of an "unknown" origin for which a detailed radiologic and pathologic workup becomes necessary.

The immunohistochemical panel applied to such lesions depends on the diagnoses under consideration. When a papillary lesion is seen in a metastasis and when papillary RCC is a possibility, RCC marker and Pax 2 have been reported to be 100% sensitive for diagnostic purposes.<sup>9</sup> Other authors have suggested Pax 8, Pax 2, human kidney injury molecule-1, RCC marker, CD10, and antiphosphorylated H2AX as being potentially informative in confirming a metastasis of renal origin.<sup>6,10,30</sup> One clinical scenario unique to patients with von Hippel-Lindau disease is the propensity to develop clear cell RCC and capillary hemangioblastoma of the central nervous system.<sup>4</sup> As capillary hemangioblastoma shares

cytoarchitectural similarities with clear cell RCC, the question often arises as to whether the lesion found in the central nervous system could be in reality metastatic clear cell RCC. Reports show aquaporin1 with cytokeratin AE1/3,<sup>31</sup> RCC marker,<sup>32</sup> CD10 and inhibin  $\alpha$ ,<sup>33</sup> and Pax 2 and inhibin  $\alpha$ <sup>34</sup> to be useful discriminants. Clear cell papillary cystadenoma of the epididymis is also another lesion that can be encountered as part of von Hippel-Lindau disease and may be mistaken for metastatic clear cell RCC.<sup>35</sup> Negative staining for RCC marker and CD10 and positive staining for CK7 help distinguish this from clear cell RCC, which is RCC marker and CD10 positive but CK7 negative or only focally CK7 positive.<sup>36</sup>

For the diagnostic workup of a metastasis of possible renal origin, 30% of survey respondents used Pax 2 and/or Pax 8, 24% used RCC marker, 19% used a panel of pan-CK, CK7, and vimentin, and 15% used CD10, when given these choices for selection in the questionnaire. This translated to 87% of pathologists utilizing immunohistochemistry for this purpose. Twenty-six (13%) participants offered their own panels, with the broad range of preferred markers reflecting the wide spectrum of markers available that are differentially expressed in different tumor types, as well as the diversity of lesions with which metastatic RCC may be histologically confused.

## DISTINGUISHING CLEAR CELL FROM CHROMOPHOBE RCC

Clear cell RCC consists of alveolar tumor nests permeated by a fine arborizing vascularity. The tumor cells contain lipid and glycogen, which when dissolved during histologic processing display characteristic cytoplasmic clarity that typifies the microscopic appearance of these tumors.<sup>4</sup> Occasionally, the tumor cells harbor granular to pink eosinophilic cytoplasm and may resemble chromophobe RCC, which more typically contains polygonal cells with transparent to reticulated cytoplasm rimmed by thickened cell membranes.<sup>4,37</sup> In an investigation of concordance between pathologists for the diagnosis of chromophobe RCC using a set of 32 renal tumors with predominantly eosinophilic cytoplasm, it was found that total agreement was achieved in only 59% of cases on the basis of histology alone. It was concluded that a small but significant number of renal tumors composed of cells with eosinophilic cytoplasm cannot be correctly classified without resorting to immunohistochemistry, utilizing a panel of markers of known sensitivity and specificity.<sup>37</sup>

The most useful markers, according to Zhou et al,<sup>20</sup> in the separation of clear cell from chromophobe RCC are CK7, RCC marker, CD10, vimentin, CD117, parvalbumin, and E-cadherin. The most common profile for chromophobe RCC is CK7 positive, RCC marker negative, CD10 negative, vimentin negative, CD117 positive, parvalbumin positive, E-cadherin positive, EMA positive, MUC1 positive, CK20 negative, and AMACR negative; this is in contrast to the profile of clear cell RCC, which is often CK7 negative, RCC marker positive, CD10 positive, vimentin positive, CD117 negative, parvalbumin negative, E-cadherin negative, EMA positive, MUC1 positive, CK20 negative, and AMACR negative.<sup>38</sup> Some cases of clear cell and chromophobe RCC, however, can show reverse staining for CK7, being unusually positive and negative for this marker, respectively.<sup>39</sup> A combined panel of CD117 and RCC marker was reported as effective in differentiating chromophobe



RCC from clear cell RCC with eosinophilic/granular cytoplasm.<sup>14</sup> Table 2 summarizes the comparative staining results of these 2 tumor subtypes.

When asked to select the most commonly used marker/s in their laboratories to discriminate clear cell from chromophobe RCC, 50% of survey participants responded that they used a combination panel of CK7 and CK20, 27% used CD10, 12% used E-cadherin or Ksp-cadherin, 8% used RCC marker, and 3% used parvalbumin. As there was no consensus in the online survey and important options of CD117 and CAIX were not provided to survey respondents, this question was reasked during the consensus conference, with these markers being included in the options. Although there was again no consensus, it was noteworthy that 41% of attendees used a combined panel of CD117 and CAIX to distinguish clear cell from chromophobe RCC, with 31% selecting CK7, 17% selecting CD117, 10% selecting Hale colloidal iron, and a single (1%) participant opting for CAIX alone.

## **DISTINGUISHING EOSINOPHILIC CHROMOPHOBE RCC FROM ONCOCYTOMA**

The distinction of eosinophilic chromophobe RCC from benign oncocytoma is important and may be potentially challenging on light microscopy. There are well-documented differences in histologic appearances of these 2 tumors; chromophobe RCC displays pale cytoplasm with crenated nuclei, perinuclear haloes, and thickened cell membranes, whereas oncocytoma has scanty granular pink cytoplasm and dark nuclei. However, the eosinophilic variant of chromophobe RCC can closely mimic an oncocytoma,<sup>3,4</sup> which is not surprising given their similar histogenesis from the intercalated cell of the cortical part of the distal collecting duct.<sup>3</sup> Hale colloidal iron is used in some laboratories to corroborate a light microscopic diagnosis of chromophobe RCC (Fig. 6). In their initial description of this tumor, Thoenes et al<sup>40</sup> referred to “slightly opaque or finely reticular cytoplasm” in chromophobe cells on hematoxylin and eosin-stained sections, which could be distinguished from clear cell RCC through a strong positive reaction with the Hale colloidal iron method. However, Hale colloidal iron reacts with a variety of renal neoplasms including oncocytoma, albeit with different staining patterns.<sup>41</sup> Chromophobe RCC shows a diffuse and strong reticular, microvacuolated appearance, whereas for oncocytoma there is also a fine dust-like or apical positivity with colloidal iron stains.<sup>41,42</sup> Because of overlapping staining results compounded by difficulties in executing the stain and the need to recognize subtle differences in the staining pattern and distribution,<sup>16</sup> Hale colloidal iron is often not solely relied upon to make that critical distinction between chromophobe RCC and oncocytoma.

Immunohistochemically, both chromophobe RCC and oncocytoma express parvalbumin, Ksp-cadherin, and CD117. CK7 shows a differential staining pattern in the 2 tumors, with the majority of chromophobe RCC diffusely expressing membranous CK7 and oncocytoma being typically negative or, at most, focally positive in scattered cells.<sup>3,43,44</sup> Other markers described as being potentially helpful include epithelial marker MOC31 and EpCam (positive in chromophobe RCC, negative in oncocytoma),<sup>3</sup> caveolin-1 being expressed in chromophobe RCC but diminished in oncocytoma,<sup>45</sup> endogenous avidinbinding activity, which is positive in oncocytoma but infrequently expressed in RCC.<sup>46</sup> Combinations of



markers used as panels are also advocated as having discriminatory ability—vimentin, GST- $\alpha$ , and EpCam for separating chromophobe RCC from oncocytoma and clear cell RCC<sup>39</sup>; CK7 and parvalbumin for differentiating chromophobe RCC from oncocytoma<sup>47</sup>; and CK7, vimentin, S100A1, and CD117 for differentiating oncocytoma from its mimics.<sup>48</sup> Table 3 documents the differential staining patterns of these tumors. Use of these immunohistochemical antibodies depends on pathologist familiarity as well as availability, in addition to requiring their validation in terms of specificity and sensitivity.<sup>3</sup>

For the distinction between eosinophilic chromophobe RCC and oncocytoma, 47% of survey participants responded that they used CK7, 23% contributed their preferred panel, 15% responded that they did not use any markers, and a further 15% used colloidal iron stains; 1% used CD82 or S100A1.

## DIAGNOSIS OF UNCLASSIFIED RCC

Unclassified RCC constitutes about 4% to 5% of renal cancers. It is diagnosed when the histologic appearances of the tumor do not fit any specific defined category of renal parenchymal malignancy.<sup>4</sup> This subset includes tumors with variable microscopic features such as sarcomatoid changes devoid of epithelial elements or other unusual cell types. Thorough sampling of the resected tumor is needed, although immunohistochemistry is often called upon to further delineate such tumors.

When the question was asked as to whether it was necessary to use selective immunostains before the diagnosis of unclassified RCC, 48% agreed that a selective panel of markers would be needed, 33% noted that they always used a panel with multiple markers, whereas 20% thought that it was unnecessary to apply markers before rendering a diagnosis of unclassified RCC. Combining both groups that would utilize immunohistochemistry, a clear majority and consensus of 81% of respondents was achieved.

At the consensus conference, there was no clear agreement among participants regarding which marker was most often used in the workup of a sarcomatoid RCC, with 46% using broad-spectrum keratins, 30% stating that they did not use any markers, and 20% relying on Pax 2 and Pax 8.

## MARKERS FOR PROGNOSTICATION

Biomarkers for potential prognostication of RCC have been well reviewed.<sup>49</sup> They include molecules in intracellular pathways and a variety of tumor markers. Some show promise, although their roles have not entered clinical practice. Given the level of interest this topic has engendered recently as well as the work being done by the Cancer Genome Atlas, the International Cancer Genome Consortium, and several academic centers, it is very likely that significant breakthroughs will be seen in the near future. A relatively new discovery is polybromo-1 (*PBRM1*) as the second most frequently mutated gene after *VHL*.<sup>50</sup> Importantly, loss of the *PBRM1* protein expression product BAF180 was recently shown to be associated with advanced tumor stage and worse patient outcome.<sup>51</sup>

An overwhelming response of 94% of survey respondents confirmed that they did not use any markers for prognostication of RCC. Of those who did use prognostic immunomarkers, CAIX was the marker most frequently mentioned, followed by Ki-67.

## CYTOGENETICS FOR RENAL CARCINOMA DIAGNOSIS AND PROGNOSIS

RCC subtypes are associated with typical and defining chromosomal abnormalities. Clear cell RCC shows chromosome 3p aberrations, most commonly loss of 3p and mutations of the *VHL* gene, whereas papillary RCC shows a variety of abnormalities, most often trisomies of chromosomes 7 and 17.<sup>52</sup> A myriad of karyotypic changes have been described in chromophobe and collecting duct cancers. Chromophobe RCC is known to harbor multiple numerical losses of chromosomes 1, 2, 6, 10, and 17,<sup>53</sup> whereas for collecting duct carcinomas there are many numerical and structural aberrations, with involvement of chromosomes 1 and X or Y, either as translocations, deletions, or monosomies. Abnormalities of chromosomes 22 and 13 are also infrequently detected.<sup>54</sup> Translocation RCCs are defined by translocations involving chromosome Xp11.2, resulting in *TFE3* gene fusions.<sup>4,55,56</sup> Histologically, these tumors can resemble various renal carcinomas, most commonly clear cell RCC, and the presence of chromosomal translocation and/or strong and diffuse nuclear expression of TFE protein in tumor cells confirms the diagnosis (Fig. 7). Another variant of translocation-associated RCC is characterized by fusion of the *TFEB* gene on chromosome 6p to the *alpha* gene on 11q12, which leads to expression of the TFEB protein.<sup>57</sup> An antibody to this protein exists, but the assay is difficult to perform so it is currently undertaken only in few academic laboratories.

At the conference, there was consensus agreement by 80% of participants that TFE3 and TFEB analysis, using either immunohistochemistry or FISH, should be requested when an RCC is diagnosed in a patient under 30 years of age and/or when the morphology suggests translocation carcinoma. Some authors have used 40 years as the threshold age below which translocation carcinoma should be ruled out. Only 6% stated that they did not request these tests, and this may possibly be related to access issues. Interestingly, when specifically asked whether FISH for TFE3 or TFEB should be requested when translocation-associated RCC is suspected, there was no consensus among conference attendees, with 35% believing that it should be requested only for cases with histologic features of translocation RCC but with negative or equivocal TFE3/TFEB immunostaining, and 33% agreeing with the aforementioned indication in addition to confirmation of cases with positive TFE3/TFEB immunostaining.

The overlapping morphologic and immunohistochemical profiles of mucinous tubular and spindle cell carcinoma and the papillary RCC with spindle cell areas may make diagnostic distinction difficult,<sup>58</sup> with some authors suggesting that they are related tumors.<sup>59</sup> Despite this, at least 1 study suggests that they can be distinguished through the presence of chromosomal 7 and 17 trisomies in papillary RCC.<sup>58</sup>

In clear cell papillary RCC that may arise in the background of end-stage kidneys, lack of chromosomal 7 gains or chromosomal Y losses, together with absence of deletion of chromosomal 3p, suggest that it is a unique clinicopathologic entity that is distinct from

either conventional clear cell or papillary RCC.<sup>60–62</sup> Some chromosomal abnormalities may have prognostic value, such as loss of chromosome 9p in clear cell RCC, which is associated with a significantly poorer cancer-specific survival.<sup>63</sup>

Regarding the role of cytogenetics in renal carcinoma diagnosis, 50% of survey participants replied that they occasionally required cytogenetics, 48% replied that they never used cytogenetics, whereas 3% responded that they routinely incorporated cytogenetics into their diagnostic algorithm for renal cancers.

Almost all survey participants (98%) noted that they did not use cytogenetic information for prognostic purposes.

## MOLECULAR TECHNIQUES FOR CLINICAL MANAGEMENT OF PATIENTS WITH RCC

Molecular cytogenetics to detect specific chromosomal aberrations can be of diagnostic value. For instance, numerical abnormalities of chromosomes 7 and 17 in oncocyctic papillary RCC cases observed on FISH analysis confirm the utility of molecular cytogenetics in differentiating this from oncocytoma.<sup>64</sup> Other diagnostic roles of molecular cytogenetics are alluded to above.

From the list of choices provided in the questionnaire, 66% of participants responded that FISH was the molecular method most often used in their practice, whereas 28% used conventional cytogenetics. For options of array-based comparative genomic hybridization, polymerase chain reaction-based mutation analysis, single nucleotide polymorphism-based array analysis, and RNA expression arrays, the returns were 1%, 3%, 1%, and 1%, respectively. Importantly, 27% survey participants did not provide a response to this question, which may reflect the unavailability of, or unfamiliarity with, these methods in their practice.

Regarding molecular analyses, 59.9% of survey participants reported that they never used these tools, whereas 38.6% stated that they occasionally did. Only 2% of participants reported that they routinely used molecular analyses for diagnostic purposes.

*VHL* mutations are seen in up to 61% of sporadic clear cell RCC.<sup>65</sup> These mutations can impact the hypoxia-inducible factor (HIF) pathway and provide insights into HIF-targeted treatment strategies for clear cell RCC.<sup>66</sup> Dysregulation of HIF fosters upregulation of multiple downstream molecules, including CAIX, which explains why this molecule is preferentially expressed in a membranous distribution in clear cell RCC.

When asked whether *VHL* mutation or loss of heterozygosity analysis was performed in their diagnostic practice, a majority of 83% said they never sought these tests, 16.4% responded that they occasionally did, and only 1 participant noted that such analysis was part of routine practice.

## PREDICTIVE MARKERS

Predictive markers are those that can provide information on whether or not there will be response by a cancer to specific types of therapy.<sup>67</sup> Although novel oncologic options are rapidly becoming available for patients with RCC, especially in the setting of metastatic disease, the use of predictive biomarkers for clinical stratification and management planning has yet to enter routine practice and still awaits validation studies.<sup>49,68,69</sup>

Currently, the majority of patients with advanced clear cell RCC receive vascular endothelial growth factor (VEGF) pathway antagonists as first-line therapy for metastatic disease, on the basis of the demonstration that these agents prolong progression-free survival (PFS) when compared with interferon- $\alpha$  treatment or placebo. Despite the availability of a number of agents, the most effective second-line therapy, as well as the optimal sequencing of these agents, remains unclear. Both sorafenib and pazopanib are associated with an improved PFS when compared with placebo in patients who have previously received cytokine therapy. In patients who have progressed on first-line therapy with a VEGF pathway antagonist, everolimus was hitherto the only agent shown to offer clinical benefit (modest prolongation of PFS compared with placebo in a randomized phase III study).<sup>70,71</sup> Axitinib, a potent, selective, second-generation VEGF receptor (VEGFR 1, 2, 3) inhibitor, was recently shown to offer a superior PFS compared with sorafenib, a first-generation VEGFR and RAF inhibitor, in the second-line setting in a phase III AXIS trial.<sup>72</sup>

Analysis of *VHL* mutation status and that of plasma CAIX, VEGF, sVEGFR2, tissue inhibitor of metalloproteinase 1 (TIMP-1), and Ras p21 were performed in the TARGET trial of sorafenib versus placebo in advanced RCC.<sup>73</sup> On multivariate analysis that included ECOG performance status, MSKCC score, and the biomarkers assayed, only baseline TIMP-1 levels were prognostic for survival, whereas no predictive markers were identified.<sup>74</sup> Choueiri et al<sup>75</sup> evaluated tumor CAIX expression using immunohistochemistry in 94 patients treated with antiangiogenic therapies. CAIX expression was neither prognostic nor predictive of response to sunitinib, for sorafenib-treated patients, although elevated CAIX expression (>85%) was associated with decreased tumor size in response to treatment.

Other targeted agents being pursued in advanced clear cell RCC include Temozolomide (CC-779), a selective mTOR inhibitor. Partial responses were noted in 7% of patients and minor responses in 26%. The median survival rate was 15 months. The notable activity of the drug in patients with poor prognostic features prompted a phase 3 trial.<sup>76,77</sup> Cho and Chung<sup>78</sup> examined expression of CAIX, phosS6, phosAkt, and PTEN in 20 patients with advanced clear cell RCC treated with temsirolimus in a phase II clinical trial. These investigators found a positive significant association between phosS6 expression and objective response to temsirolimus. A similar trend was associated with positive expression of phosAkt.

When questioned regarding the use of predictive markers for renal cancer, most survey participants (84%) replied that their clinicians had never asked for any biomarkers. Among the markers requested, CAIX was most frequently mentioned. CAIX is a transmembrane

protein that enzymatically catalyses the reversible hydration of carbon dioxide into bicarbonate and a proton, allowing cellular maintenance of a neutral pH.<sup>79</sup> One study reported an association between CAIX expression and grade of clear cell RCC.<sup>80</sup> CAIX expression has been noted to have prognostic significance with apparent improved survival and sensitivity to IL-2 therapy.<sup>79</sup> Diminished CAIX expression is independently correlated with poor survival in advanced renal cell cancer patients.<sup>81</sup>

When asked about identification of CAIX expression in tumor tissue, a majority of 66% of respondents felt that this was unnecessary.

## CONCLUSIONS

The spectrum of antibodies that pathologists have access to in their routine practice differs, hence influencing familiarity, usage, and choice. What is important is the need for laboratories to develop immunohistochemical quality assurance and control programs, to be knowledgeable about the range of specificities and sensitivities of antibodies in their diagnostic service menus, and to be judicious in applying discriminatory immunohistochemical panels for appropriate diagnostic situations. Table 4 represents a summary of helpful markers that may be used to facilitate the diagnosis of renal tumors.

The ISUP survey demonstrated a low degree of consensus in participants' responses to questions on prognostic/predictive markers and molecular techniques, underscoring the fact that biomarkers for these purposes remain outside the diagnostic realm while awaiting clinical validation. No individual antibody or panel of antibodies reached consensus for the classification of renal tumors or for ruling out metastatic disease during the online survey, apart from a consensus that immunohistochemistry was used for histologic subtyping and should be applied before confirming a diagnosis of unclassified RCC. At the conference, however, there was consensus that TFE3 and TFE8 analysis should be requested when RCC is diagnosed in a young patient or when histologic appearances were suggestive of the translocation subtype, whereas Pax 2 and/or Pax 8 were considered to be the most useful markers in the diagnosis of a renal primary malignancy.

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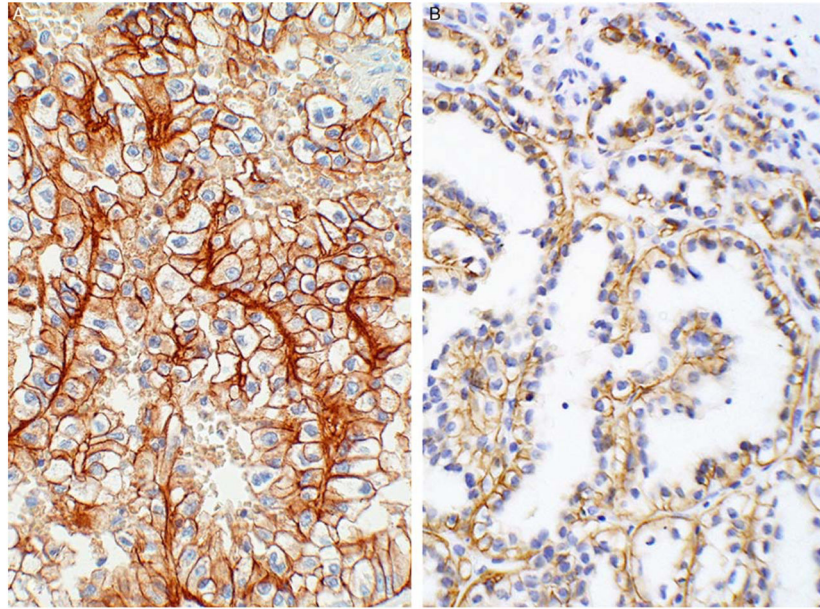
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## APPENDIX

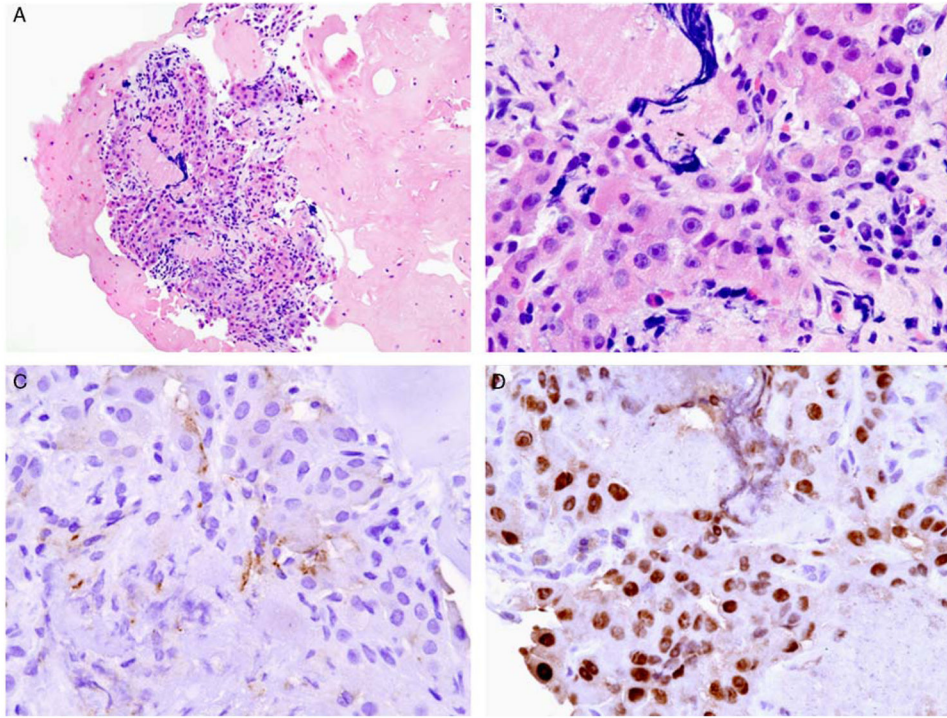
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Anila Abraham, Adebawale Adeniran, Khalid Ahmed, Hikmat Al Ahmadi, Ferran Algaba, Robert Allan, Mahul Amin, Pedram Argani, Ulrika Axcrone, Marc Barry, Dilek Baydar, Louis Bégin, Dan Berney, Peter Bethwaite, Athanase Billis, Ruth Birbe, Stephen Bonsib, David Bostwick, Fadi Brimo, Helen Cathro, Ying-Bei Chen, Liang Cheng, John Cheville, Yong Mee Cho, Ai-Ying Chuang, Cynthia Cohen, Henry Crist, Brett Delahunt, Warick Delprado, Fang-Ming Deng, Lars Egevad, Jonathan Epstein, Andrew Evans, Oluwole Fadare, Daniel Fajardo, Sara Falzarano, Samson Fine, Stewart Fleming, Eddie Fridman, Bungo Furusato, Masoud Ganji, Masoumeh Ghayouri, Giovanna Giannico, Neriman Gokden, David Griffiths, David Grignon, Nilesh Gupta, Omar Hameed, Ondrej Hes, Michelle Hirsch, Jiaoti Huang, Wei Huang, Christina Hulsbergen-van de Kaa, Peter Humphrey, Sundus Hussein, Kenneth Iczkowski, Rafael Jimenez, Edward Jones, Laura Irene Jufe, James Kench, Masatoshi Kida, Glen Kristiansen, Lakshmi Priya Kunju, Zhaoli Lane, Mathieu Latour, Claudio Lewin, Kathrine Lie, Josep Lloreta, Barbara Loftus, Antonio Lopez-Beltran, Fiona Maclean, Cristina Magi-Galluzzi, Guido Martignoni, Teresa McHale, Jesse McKenney, Maria Merino, Rose Miller, Hiroshi Miyamoto, Holger Moch, Rodolfo Montironi, Hedwig Murphy, John Nacey, Tipu Nazeer, Gabriella Nesi, George Netto, Peter Nichols, Marie O'Donnell, Semra Olgac, Roberto Orozco, Adeboye Osunkoya, Aysim Ozagari, Chin-Chen Pan, Anil Parwani, Joanna Perry-Keene, Constantina Petraki, Maria Picken, Maria Pyda-Karwicka, Victor Reuter, Katayoon Rezaei, Nathalie Rioux- Leclercq, Brian Robinson, Stephen Rohan, Ruben Ronchetti, Laurie Russell, Hemamali Samaratunga, Marina Scarpelli, Ahmed Shabaik, Rajal Shah, Jonathan Shanks, Steven Shen, Maria Shevchuk, Mathilde Sibony, John Srigley, Bhuvana Srinivasan, Martin Susani, Sueli Suzigan, Joan Sweet, Hiroyuki Takahashi, Pheroze Tamboli, Puay Hoon Tan, Satish Tickoo, Isabel Trias, Kiril Trpkov, Larry True, Toyonori Tsuzuki, Funda Vakar- Lopez, Theo Van der Kwast, Cheng Wang, Anne Warren, Jorge Yao, Asli Yilmaz, Jin Zhao, Ming Zhou, Debra Zynger.

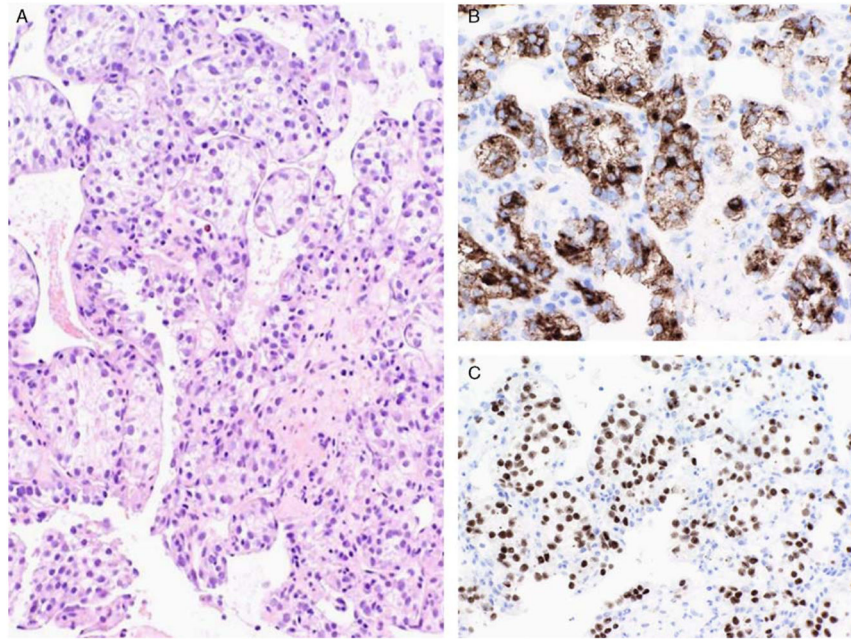


**FIGURE 1.** CAIX immunohistochemistry in RCC. A, Circumferential membrane staining of tumor cells in a clear cell RCC. B, Basolateral delineation of clear cell papillary renal carcinoma cells, with sparing of the apical surfaces.



**FIGURE 2.**

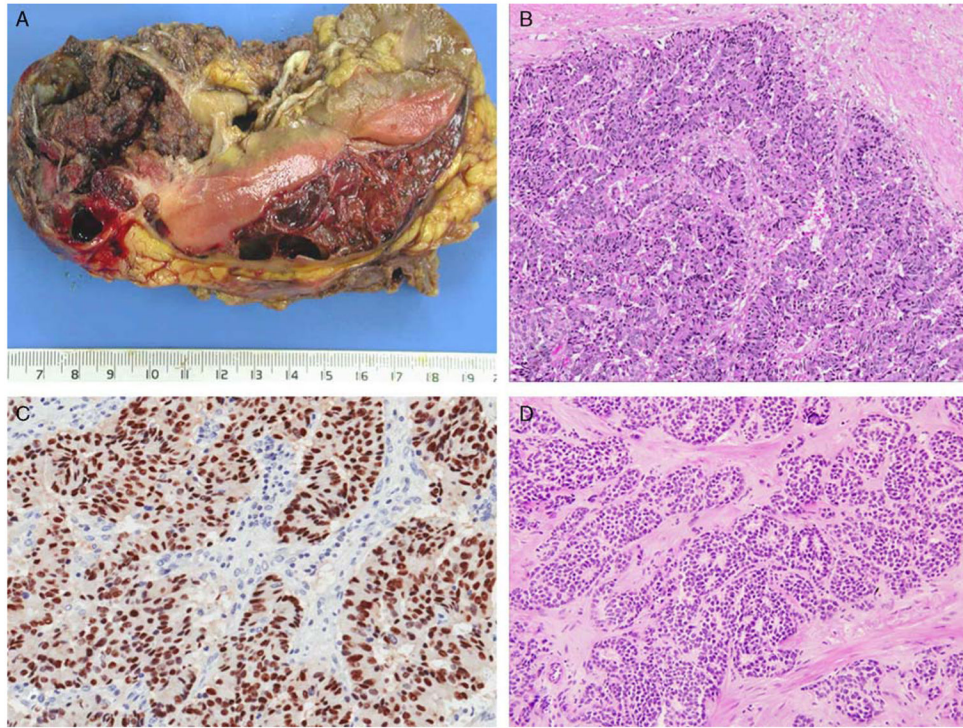
Needle aspiration of a retroperitoneal mass in a man with a history of RCC and pancreatic neuroendocrine tumor. A, Low magnification of the cell block preparation shows groups of eosinophilic tumor cells. B, Higher magnification shows variably sized vesicular nuclei with distinct nucleoli and ample pink cytoplasm. C, Immunohistochemical analysis with RCC marker shows a few cells with cytoplasmic membrane reactivity. D, Pax 8 immunohistochemistry reveals distinct nuclear reactivity confirming a primary renal origin of the retroperitoneal recurrence.



**FIGURE 3.**

A, Needle biopsy of an adrenal mass with alveolar nests of clear cells.

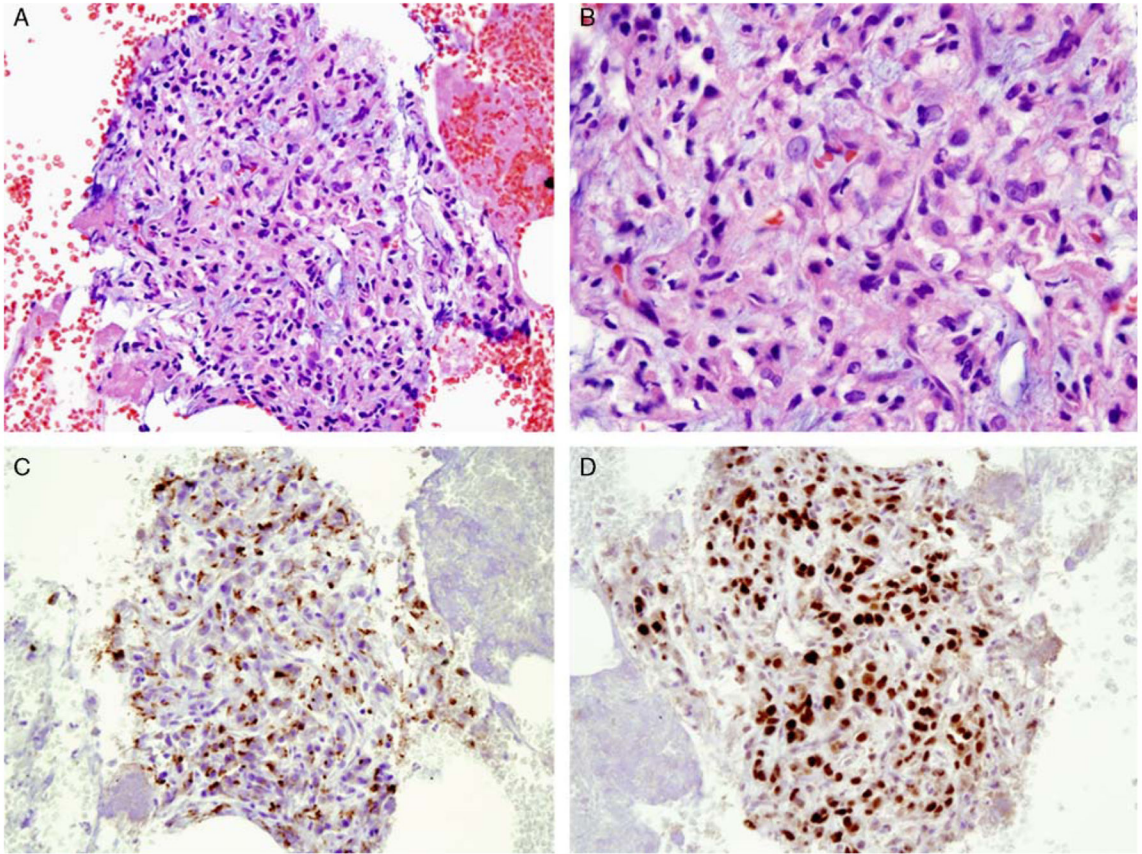
Immunohistochemical analysis shows diffuse reactivity of the cells for CD10 (B) and Pax 2 (C).



**FIGURE 4.**

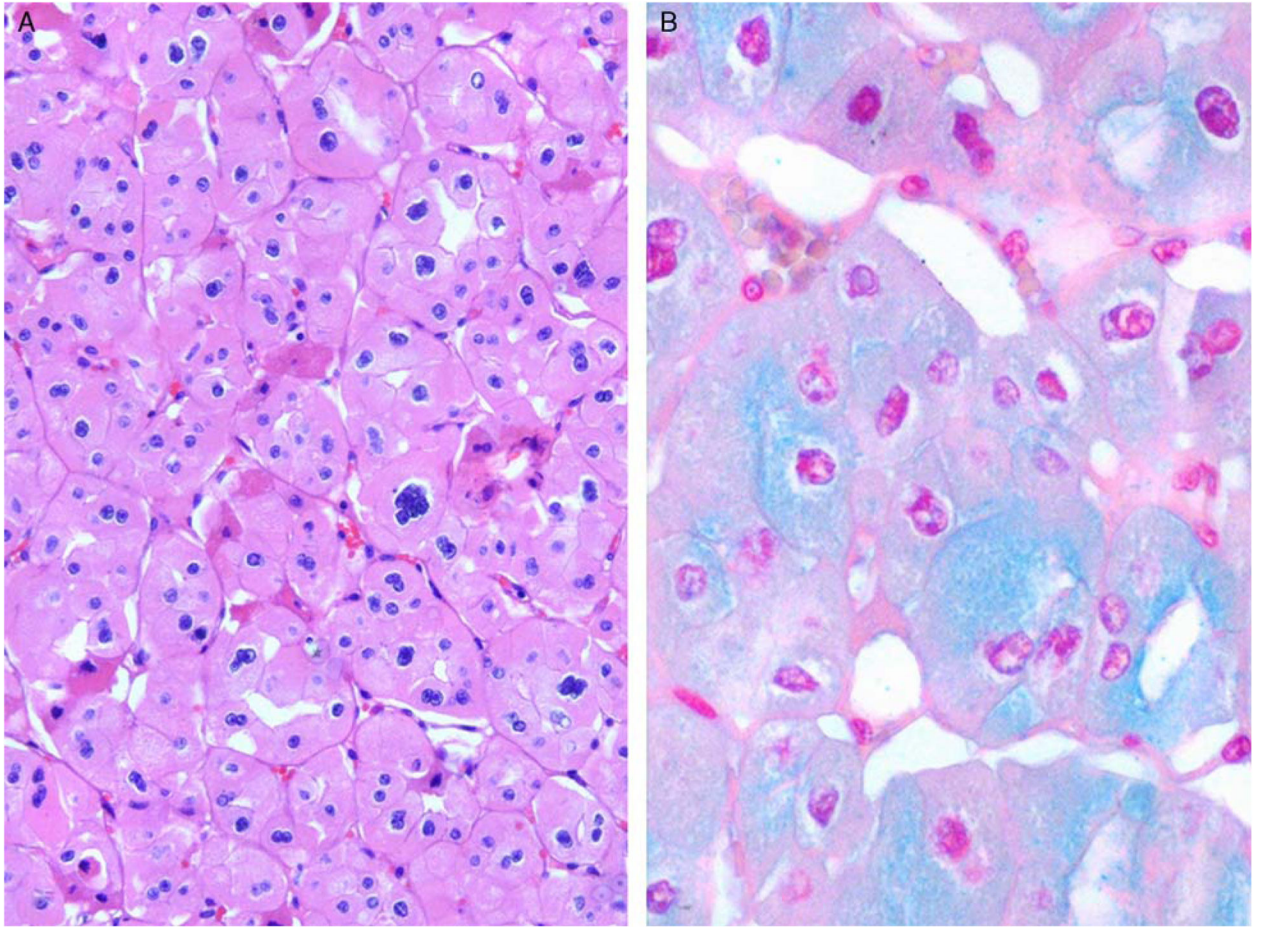
A, Nephrectomy specimen from a woman with a history of breast cancer, containing a hemorrhagic friable tumor mass extending from one renal pole along the subcapsular renal cortex to the opposite renal pole. B, Light microscopy of the tumor in the kidney reveals anastomosing trabeculae and tubules. C, ER immunohistochemistry shows diffuse nuclear reactivity. D, Light microscopy from the original primary breast carcinoma shows fused tubular and cribriform structures.





**FIGURE 5.**

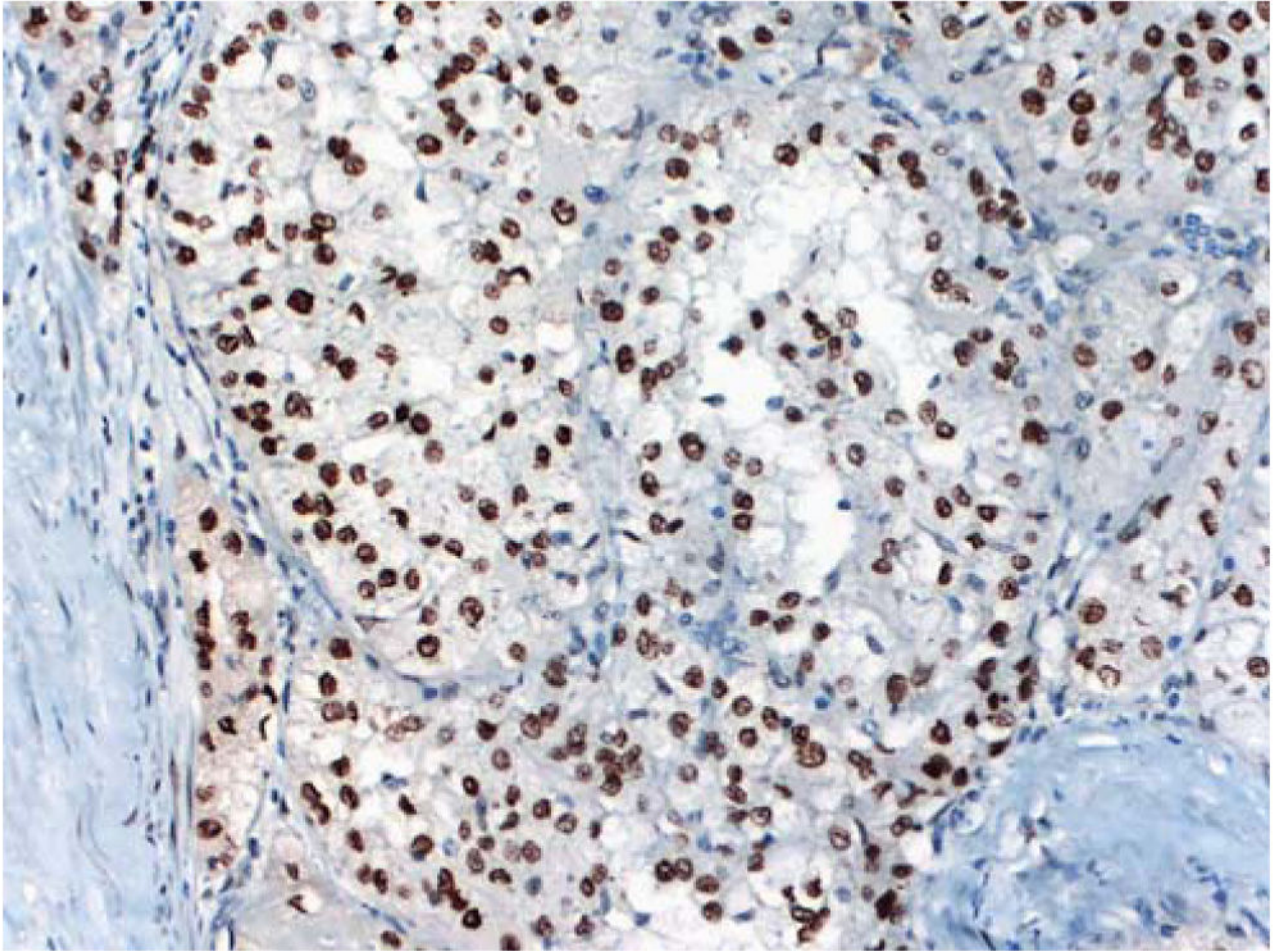
A 69-year-old man, with a history of renal cancer 11 years ago, presented with a 1cm right lung nodule, which was investigated with fine-needle aspiration under computed tomography guidance. A, Cell block preparation shows tumor cells with pink cytoplasm. B, Higher magnification of tumor cells with pink cytoplasm and nuclei that are vesicular and hyperchromatic. C, Immunohistochemical analysis with RCC marker shows strong cytoplasmic reactivity. D, Pax 2 immunohistochemistry reveals strong nuclear staining, confirming a metastasis to the lung from a primary renal tumor.



**FIGURE 6.**

A, Chromophobe RCC consists of nests of cells with abundant pink cytoplasm and irregular hyperchromatic nuclei with irregular nuclear contours. B, Hale colloidal iron stain shows fine microvacuolated positive staining within the cytoplasm of the tumor cells.





**FIGURE 7.**  
TFE immunohistochemistry shows positive nuclear staining in a case of translocation RCC.

TABLE 1

## Summary of Online Survey and Conference Results on Biomarkers in RCC

	% of Responses
Questions with consensus online responses	
Immunohistochemistry is occasionally/sometimes used for histologic subtyping	86.9
Immunohistochemistry is used before diagnosing unclassified RCC	80.5
Biomarkers and cytogenetics are currently not used for prognostication	94, 98
VHL mutations/LOH analyses are not performed in diagnostic practice	83.1
Predictive markers are not required by clinical colleagues	83.8
FISH is the most commonly used molecular platform	66.2
CAIX does not need to be identified in tumor tissue	66.1
Questions with consensus conference responses	
TFE3 and TFEB analysis (immunohistochemistry and/or FISH) should be requested when RCC is diagnosed in a patient under 30 years of age, and/or when the morphologic appearances are suggestive	79.6
Pax 2 and/or Pax 8 are the most useful markers in confirming a renal primary	70.9
Questions without consensus online responses	
	Highest percentage response obtained
Reason for most frequent use of immunohistochemistry	56.2
Immunohistochemical marker that is most frequently used in participant's laboratory in the diagnosis of a renal neoplasm	48.5
Markers used most frequently to support the diagnosis of metastasis of renal primary	29.6
Most commonly used marker in participant's laboratory to differentiate clear cell from chromophobe RCC	50.3
Use of markers to differentiate eosinophilic chromophobe RCC from oncocytoma	47
Use of cytogenetics for renal cancer diagnosis	50
Use of molecular analyses for renal cancer diagnosis	59.9
Questions without consensus conference responses	
Should FISH for TFE3 or TFEB be requested when a translocation carcinoma is suspected	34.9
Marker most frequently used in the workup of sarcomatoid RCC	46.3
Markers used for distinguishing clear cell from chromophobe RCC	40.9

Consensus is defined as 65% agreement on responses.

LOH indicates loss of heterozygosity.

**TABLE 2**

Comparison of Biomarkers in Clear Cell and Chromophobe RCC

<b>Biomarker</b>	<b>Clear Cell RCC</b>	<b>Chromophobe RCC</b>
CK7	-	+
RCC marker	+	-
CD10	+	-
Vimentin	+	-
CD117	-	+
Parvalbumin	-	+
E-cadherin	-	+
EMA	+	+
MUC1	+	+
CK20	-	-
AMACR	-	-

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**TABLE 3**

Comparison of Biomarkers in Chromophobe RCC and Oncocytoma

<b>Biomarker</b>	<b>Chromophobe RCC</b>	<b>Oncocytoma</b>
CK7	+	-/focal +
MOC31	+	-
EpCam	+	-
Caveolin-1	+	-
EABA	-	+
CD82	+	-
S100A1	-	+
Parvalbumin	+	+
Ksp-cadherin	+	+
CD117	+	+

EABA indicates endogenous avidin-binding activity.

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**TABLE 4**

## Summary Table Delineating Helpful Markers in Differential Diagnosis of Renal Tumors

	<b>Positive Markers</b>	<b>Negative Markers</b>
Clear cell RCC	vim, keratin, EMA, CD10, RCCm Pax 2/8, CAIX	CK7, Ksp-cadherin, parvalbumin
Papillary RCC	Keratin, CK7, AMACR, RCCm	CD117, Ksp-cadherin, parvalbumin, WT1
Chromophobe RCC	E-cadherin, Ksp-cadherin, CD117, EMA, CK, CK7	vim, CAIX, and AMACR
Collecting duct	EMA, p63, CK7, HMWCK, Pax 2, Pax 8	CD10, RCCm, and CK20
Clear cell papillary RCC	CK7, Pax 2, Pax 8	AMACR, RCCm
Translocation RCC	TFE3, TFEB, CD10, RCCm	CK (or weak)
Oncocytoma	Ksp-cadherin, CD117, parvalbumin, S100A1	CK7, Moc31, EpCam, EABA, CD82
Metanephric adenoma	S100, WT1, CD57	AMACR, RCCm
RCC with sarcomatoid features	CK7, Pax 2/Pax 8, CD10, vim, and AMACR	
Angiomyolipoma	HMB45, melan-A, and SMA	CK, CD10, RCCm, Pax 2, Pax 8
Urothelial carcinoma	CK, CK7, CK20, p63, thrombomodulin, uroplakin III	RCCm, CD10, Pax 2, Pax 8

EABA indicates endogenous avidin-binding activity; vim, vimentin.