Current and Proposed Molecular Diagnostics in a Genitourinary Service Line Laboratory at a Tertiary Clinical Institution

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Abstract: The idea that detailed knowledge of molecular oncogenesis will drive diagnostic, prognostic, and therapeutic clinical decision making in an increasingly multidisciplinary practice of oncologic care has been anticipated for many years. With the recent rapid advancement in our understanding of the molecular underpinnings of genitourinary malignancies, this concept is now starting to take shape in the fields of prostate, kidney, bladder, testicular, and penile cancer. Such breakthroughs necessitate the development of robust clinical-grade assays that can be quickly made available for patients to facilitate diagnosis in challenging cases, risk-stratify patients for subsequent clinical management, select the appropriate targeted therapy from among increasingly diverse and numerous options, and enroll patients in advanced clinical trials. This rapid translation of basic and clinical cancer research requires a streamlined, multidisciplinary approach to clinical assay development, termed here the molecular diagnostics service line laboratory. In this review, we summarize the current state and explore the future of molecular diagnostics in genitourinary oncology to conceptualize a genitourinary service line laboratory at a tertiary clinical institution.

Key Words: Prostate cancer, kidney cancer, bladder cancer, testicular cancer, penile cancer, immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), in situ hybridization (ISH), whole-genome sequencing

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The genomic era is rapidly revolutionizing the practice of health care, and in almost no area is this more apparent than oncology, where molecular data are increasingly driving patient care in terms of diagnosis, prognosis, and therapeutics. The promise of personalized medicine, wherein the therapeutic options for an individual patient are tailored to his/her specific tumor genetics and biology, requires robust clinical assays for the biomarker(s) of interest. Our evolving understanding of the molecular underpinnings of urologic malignancies provides an emerging role for molecular testing in the treatment of these common neoplasms. Here, we envision the concept of a genitourinary service line laboratory at a tertiary clinical institution, and using an organ-based approach, we review the current state and explore the future of molecular diagnostics in genitourinary oncology.

Genitourinary Service Line Laboratory: Concept and Services

With the advent of widespread whole-genome sequencing of tumors, going forward, we expect the pace of molecular discoveries to quicken rather than abate. This trend will undoubtedly be associated with the need to bring advances made in the laboratory rapidly into the realm of routine clinical practice. We believe there are several ways this is already happening and will further develop in the future. As the cost of sequencing decreases, one such possibility is routine whole-genome sequencing of clinical tumor specimens, with the selection of therapeutics based on the prevalent targetable molecular alterations. A clinical sequencing pilot project, termed MI-ONCOSEQ, has already been established at the University of Michigan Health System (UMHS) for patients with advanced tumors that are resistant to conventional histology-based therapies.[1] Based on the results of this novel project, it seems clear to us that there are tumors that would benefit from this whole-genome sequencing approach. It is also evident that cancers arising in different organs—as well as different cancer subtypes within the same organ system—are not uniformly similar but instead house different genetic drivers. In this age of translational medicine, it is imperative that discoveries from technologies such as clinical sequencing or traditional clinical cancer research be quickly incorporated into the development of clinical-grade assays in a CLIA (Clinical Laboratory Improvement Amendments)–certified environment. Broader availability of such assays will facilitate patient enrollment in a new generation of clinical trials that incorporate these rapid molecular advances and will expand the available pool of clinical sites beyond specialized academic centers.

Hence, we propose the concept of dedicated molecular diagnostic service line laboratories, initially purposed for and centered around specific organ systems (i.e., genitourinary, pulmonary, etc.) (Fig. 1). These laboratories would serve as dynamic platforms for the translation of novel but clinically important molecular oncology results into CLIA-certified diagnostic, prognostic, or therapeutic molecular and/or immunohistochemical assays. A team of clinicians, including, but not limited to, surgical and molecular pathologists, cytogeneticists, medical oncologists, surgical oncologists, computational biologists, and radiation oncologists, would jointly oversee the selection of molecular assays and liaison with basic clinical scientists—in both academic institutions and industry. As the number of assays expand and common molecular aberrations are identified, tests could be shared between service line laboratories (i.e., *ALK* FISH in inflammatory myofibroblastic tumors (IMTs) and non–small

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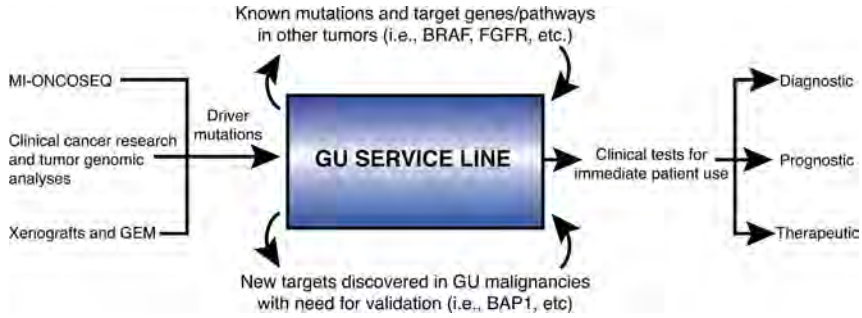

FIGURE 1. Illustration of genitourinary (GU) service line laboratory concept. Potential driver mutations in GU malignancies are identified from a number of sources, including clinical sequencing projects (i.e., MI-ONCOSEQ), clinical cancer research and tumor genomic analyses, and xenograft and/or genetically engineered mouse (GEM) models. These mutations are evaluated by the GU service line personnel for potential clinical utility and divided into at least 2 groups: known mutations in other tumors and novel targets discovered in GU malignancies. Known mutations are screened for available or existing testing modalities and offered for immediate patient use in a CLIA-certified laboratory setting. Novel mutations are validated in independent clinical cohorts, and appropriate CLIA-certified testing modalities are developed by the GU service line before patient use. These tests may have diagnostic, prognostic, or therapeutic value.

cell lung cancer, etc.), similar to the way that clinical immunohistochemistry (IHC) laboratories function today. In the following sections, we describe our current concepts of the molecular underpinnings of genitourinary cancers, including their current clinical applications (Table 1), as well as future assays of promise.

Prostate Cancer

Prostate cancer is the most common noncutaneous malignancy in men and has a variable course, with a subset of patients developing aggressive, treatment-resistant, and eventually lethal tumors. Major advances in the molecular oncology of prostate cancer over the past 15 years have begun to shed light on the clinicopathologic behavior of these tumors.

Gene Fusions in Prostate Cancer

Computational analysis of gene expression profiles and follow-up genomic characterization identified recurrent gene fusions involving the ETS family of transcription factors in nearly 50% of human prostate cancers, thus marking a paradigm shift in our understanding of gene rearrangements that drive

TABLE 1. Selected Targeted Clinical Trials for Genitourinary Tumors With Molecular Inclusion Criteria (Where Applicable)

Target	Cohort	Therapy (Class)	Phase	ClinicalTrials.gov Identifier	Molecular Inclusion Criteria
Prostate					
PARP1	CRPC	ABT-888 (PI)	II	NCT01576172	ETS rearrangement stratification
MET/VEGF	CRPC	Cabozantinib (TKI)	III	NCT01522443	
BRAF	Any solid tumor	MEK162 (MI)	II	NCT01885195	*BRAF* mutation/rearrangement
PTEN	Any solid tumor	GSK2636771 (PI3KI)	I	NCT01458067	*PTEN* deletion by IHC
AURKA	NEPC	MLN8237 (AKI)	II	NCT01799278	NE markers by IHC
Kidney					
EGFR/VEGF	HLRCC and sporadic PRCC	Bevacizumab (Ab) and erlotinib (TKI)	II	NCT01130519	*FH* mutation (for HLRCC)
MET/VEGF	RCC	Cabozantinib (TKI)	III	NCT01865747	
MET	Metastatic type I PRCC	Crizotinib (TKI)	II	NCT01524926	*MET* mutation
PD1	RCC	Nivolumab (Ab)	III	NCT01668784	
Bladder					
FGFR3	Early-stage urothelial carcinoma	Dovitinib (TKI)	II	NCT01732107	*FGFR3* mutation or overexpression
HER2	Stage IV urothelial carcinoma	Lapatinib (TKI)	II/III	NCT00949455	HER2 overexpression
ALK	IMT	Crizotinib (TKI)	II	NCT01524926	*ALK* mutation/rearrangement
		AP26113 (TKI)	I/II	NCT01449461	
Testis					
CD30	Treatment-resistant germ cell tumor	Brentuximab (Ab)	II	NCT01851200	CD30 expression

CRPC indicates castration-resistant prostate cancer; EGFR, epidermal growth factor receptor; PI, PARP1 inhibitor; PI3KI, PI3 kinase inhibitor; AKI, aurora kinase A inhibitor; NE, neuroendocrine; TKI, tyrosine kinase inhibitor; Ab, antibody; MI, MEK inhibitor.

common epithelial cancers.[2–4] *TMPRSS2-ERG*, the dominant ETS gene fusion in prostate cancer, is produced by genomic rearrangements involving chromosome 21, which bring *ERG* expression under androgen control via androgen receptor-mediated *TMPRSS2* transcriptional regulation. These gene fusions have been implicated in the pathogenesis of prostate cancer[5,6] and help explain the multifocal nature and clonal metastatic dissemination of prostate cancer.[7,8] ETS fusions are prime candidates for development of new diagnostic assays, including urine-based noninvasive assays, and molecular subtyping of prostate cancer for selection of targeted therapeutics.

While a variety of gene fusions involving *ERG* and other ETS family members have been described,[3] the vast majority of ETS prostate cancer rearrangements can be detected by a set of fluorescent in situ hybridization (FISH) assays.[5,9] Immunohistochemistry with an anti-ERG antibody detects the *ERG* fusion gene product and also demonstrates high sensitivity and specificity for *ERG* aberrations.[10] Because *ERG* rearrangement is an early, clonal, and specific event in the evolution of prostate cancer, ERG IHC and/or *ERG* FISH are useful in some commonly encountered pathologic dilemmas: diagnostically challenging atypical small acinar proliferation in needle biopsies, suspected prostate cancer in biopsy specimens showing metastatic poorly differentiated carcinoma of unknown primary,[9,10] and high-grade prostatic adenocarcinoma versus urothelial carcinoma (with or without neuroendocrine features) in transurethral resections. The majority of benign mimics of prostate cancer, for example adenosis and atrophy (including partial atrophy), demonstrate a small acinar growth pattern. ERG IHC can be helpful in this scenario as *ERG* aberrations (and corresponding ERG protein overexpression) have been demonstrated only in prostate cancer; thus, ERG expression by IHC essentially rules out a benign process.[11] Because of dysregulation of androgen signaling pathways in high-grade prostate cancer, common prostate biomarkers such as prostate-specific antigen, prostate-specific membrane antigen, and androgen receptor are not reliably expressed in these tumors. These cases, however, might be positive for an ETS rearrangement, which can be detected by ERG IHC or, more reliably, *ERG* FISH. Thus, during pathologic evaluation of a poorly differentiated carcinoma detected during a transurethral resection or a metastatic cancer diagnosed as carcinoma of unknown primary (where a prostatic primary is suspected), evidence of an ETS rearrangement, either by ERG IHC or *ERG* FISH, would be strong evidence of prostate cancer. However, careful evaluation of ERG IHC needs to be done in this context; *ERG* expression is androgen regulated, and thus especially in the castration-resistant state, it is important to realize that ERG protein may not be overexpressed (Fig. 2). In such cases, *ERG* FISH may be the optimal strategy to detect the presence of *ERG* aberrations. This is especially true of small cell carcinomas of the prostate, the majority of which are characterized by an *ERG* aberration at the genomic level (and hence detectable by FISH), but only a subset of which demonstrate detectable ERG protein expression.[12] Of course, the utility of ERG IHC and/or *ERG* FISH in either of these clinicopathologic scenarios is limited to ERG rearrangement–positive tumors, and a negative result does not exclude a diagnosis of prostate cancer.

In addition to diagnostic utility, there is potential therapeutic benefit for molecular testing in patients with locally advanced or metastatic prostate cancer, as many of these patients have failed (or eventually will fail) conventional treatments. For example, PARP1, a recently identified ERG cofactor, is required for ERG-mediated transcription, and a recent study demonstrated that pharmacologic PARP1 inhibition in mouse xenograft prostate cancer models specifically reduces proliferation of tumor cell lines harboring ETS rearrangements—but did not affect those cell lines without ETS rearrangements.[13] These results suggest that ETS FISH or ERG IHC may be useful for identifying prostate cancer patients who would potentially benefit from PARP1 inhibitor therapy. Currently, clinical trials with PARP1 inhibitors in metastatic, hormone-refractory prostate cancer patients are underway and will hopefully provide an answer to the clinical utility of PARP1 inhibitors in prostate cancer. Evaluation of ETS rearrangement status by IHC and FISH is currently being performed as a stratification factor in one such clinical trial (NCT01576172; Table 1).

Transcriptomic sequencing in ETS rearrangement–negative prostate cancers has uncovered recurrent gene rearrangements in RAF pathway members.[14] Gene fusions involving the RAF pathway members *BRAF* and *RAF1* are relatively rare (approximately

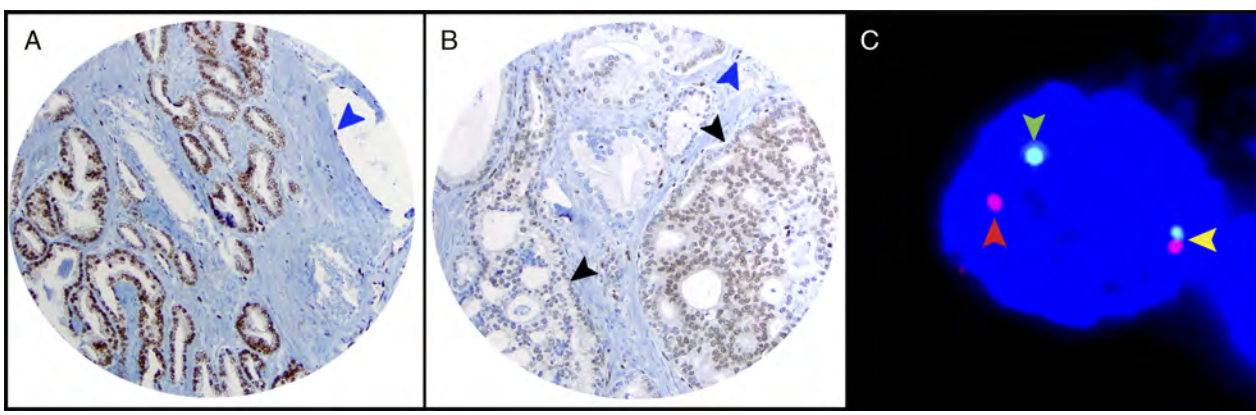

FIGURE 2. ERG IHC and *ERG* FISH in *ERG* rearrangement positive (ERG^{pos}) prostate cancer. A and B, ERG IHC and (C) *ERG* FISH. In ERG^{pos} prostate cancer, ERG IHC staining intensity varies from (A) strong in low-grade prostate cancers to (B) weak in a subset of high-grade prostate cancers. Both of these patients had an *ERG* rearrangement detectable at the genomic level by *ERG* FISH, as demonstrated in C. Black arrowhead indicates weak ERG IHC staining in ERG^{pos} tumor cells (B), and blue arrowheads indicate strong ERG endothelial cell expression (A, B; internal positive control). Break-apart *ERG* FISH strategy: yellow signal demonstrates wild-type *ERG* allele with colocalized signals (yellow arrowhead), and *ERG* rearrangement yields a single green signal (green arrow) and single red signal (red arrow). Because of androgen-signaling dysregulation in a subset of high-grade ERG^{pos} prostate cancers, ERG protein may not be detectable by IHC despite *ERG* rearrangement. In these scenarios, *ERG* FISH may be a more reliable tool to confirm the diagnosis of prostate cancer.

1%–4% of all prostate cancers); however, these rearrangements have been associated with more aggressive/advanced disease and can be detected by next-generation sequencing, as well as FISH. Drugs targeting the RAF pathway are currently under investigation in clinical trials for tumors with *BRAF* or *RAF1* aberrations (NCT01885195; Table 1).

Other Genomic Alterations in Prostate Cancer

In addition to gene fusion events, other types of genomic alterations have been identified in prostate cancer. Expression profiling in ETS rearrangement–negative prostate cancers revealed outlier expression of *SPINK1*.[15] SPINK1 overexpression, which can be assessed by IHC with an anti-SPINK1 antibody, is found in approximately 10% of all prostate cancers and is correlated with clinically and biologically aggressive tumors. The tumor suppressor gene *PTEN* is frequently mutated in human prostate cancer, and both hemizygous and homozygous deletions are associated with clinically aggressive disease.[16] Fluorescent in situ hybridization utilizing a locus-specific *PTEN* probe can detect both hemizygous and homozygous *PTEN* deletions[16] (Fig. 3), and in some studies, loss of PTEN expression by IHC with an anti-PTEN antibody has been correlated with genomic *PTEN* alterations.[17] Whether PTEN IHC or *PTEN* FISH status correlates better with prostate cancer clinical outcome still needs to be determined. However, of promise, several PIK3C clinical inhibitors are currently in clinical trials and can potentially target prostate cancers where PTEN levels are altered (NCT01458067; Table 1). Prostate cancer with neuroendocrine features (NEPC) and small cell carcinoma of the prostate is generally associated with low PSA, presence of visceral metastasis, and poor clinical outcome.[18] Prostate cancer with neuroendocrine feature has been an area of intense investigation recently, and coamplification of genes encoding the cell cycle kinase Aurora kinase A (*AURKA*) and transcription factor N-myc (*MYCN*) have been implicated in up to 40% of NEPC.[19] In these tumors, *AURKA* and *MYCN* amplification could be detected by FISH, among other methods. Based on these promising findings, an ongoing clinical trial aims to evaluate the efficacy of aurora kinase inhibitors in patients with metastatic NEPC (NCT01799278; Table 1).

More recently, genomic aberrations involving *SPOP*, *HOXB13*, *CHD1*, and *ADRB2* have been implicated as crucial events contributing to the pathogenesis of prostate cancer[20–23]; however, the clinical utility of these mutations is not fully understood. Another exciting development is the identification of abundant long noncoding RNA (lncRNA) as critical components of cancer biology; roles for lncRNA as tumor suppressors and/or oncogenic drivers have been reported in a number of common cancers, including breast and prostate.[24] In the future, in situ hybridization (ISH) for lncRNA such as SChLAP1 may aid the detection of high-risk prostate cancers (Fig. 4).[25]

Early and Accurate Detection of Prostate Cancer

Finally, a particularly exciting area of development is the use of more specific biomarkers to improve screening for prostate cancer. The current standard for prostate cancer screening is serum PSA; whereas an elevated serum PSA level is sensitive for prostate cancer, a major criticism of this screening program is that it identifies too many low-grade, indolent tumors, resulting in overtreatment in some cases. In addition, some benign conditions, such as prostatitis, can cause elevated serum PSA levels, leading to unnecessary prostate biopsies. With this in mind, several potential new biomarkers have been proposed for the early detection of prostate cancer, including *DD3/PCA3* and *TMPRSS2-ERG*.[26,27] These assays are based on the detection of secreted transcripts in urine after digital rectal examination and prostatic massage, and for patients with low serum PSA levels (<10 ng/mL), they are more specific for the subsequent diagnosis of prostate cancer. A recent prospective study from our group reported improved prediction of prostate cancer on biopsy in a multicenter cohort of patients using an algorithm integrating results from all 3 of these biomarkers (*DD3/PCA3*, PSA, and *TMPRSS2-ERG*).[28,29] A test incorporating these 3 biomarkers, termed Mi-Prostate Score, has recently become available at UMHS and provides a likelihood of a patient having prostate cancer, as well as the chance of that cancer being aggressive.

Emerging Assays and Technologies

From these discoveries, it is evident that a genitourinary service line laboratory needs to be able to facilitate detection of such biomarkers in robust, CLIA-certified clinical assays. At UMHS, currently validated assays include ERG IHC for the detection of prostate cancer in biopsy material and Mi-Prostate Score for prostate cancer detection. Future clinical-grade assays may include FISH for ETS family members (especially *ERG* and *ETV1*) and *BRAF* rearrangements, *AURKA* and *MYCN* amplification, and *PTEN* deletions, as well as PTEN and SPINK1 IHC. Availability of such assays may be helpful in providing a second layer of confirmation for discoveries made from clinical sequencing;

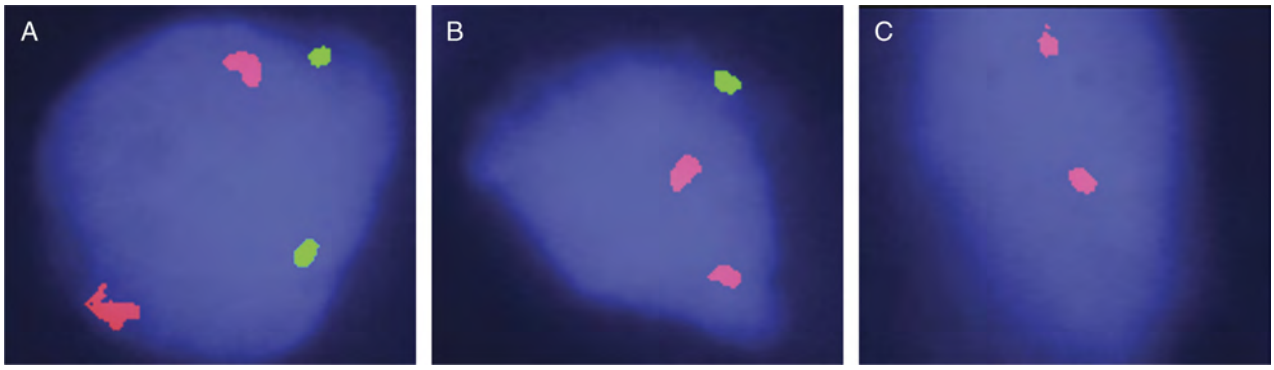

FIGURE 3. Locus-specific *PTEN* FISH in prostate cancer. A, Wild-type, (B) heterozygous *PTEN* locus deletion, and (C) homozygous *PTEN* locus deletion in prostate cancer. The green signal indicates an intact *PTEN* locus: 2 green signals = wild type, 1 green signal = heterozygous deletion, and no green signal = homozygous deletion. Dual red signals indicate the presence of 2 total chromosomes (centromeric probes; internal positive control).

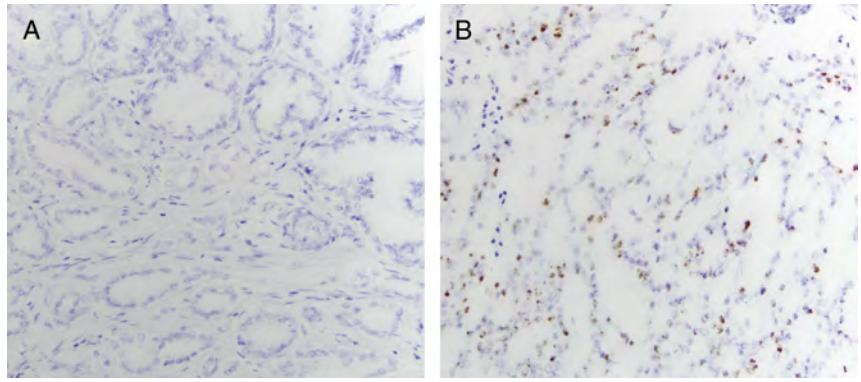

FIGURE 4. SChLAP1 lncRNA ISH in prostate cancer. A, Low Gleason score (GS) prostate cancer shows no detectable SChLAP1 lncRNA staining by ISH, whereas (B) high GS prostate cancer with a poor outcome demonstrates abundant accumulation of SChLAP1 lncRNA.

for example, FISH validation of a *BRAF* fusion in a patient with advanced metastatic prostate cancer might make the patient eligible for an ongoing clinical trial of RAF pathway inhibitors.

Kidney Cancer

Although kidney cancer is far less common than prostate cancer, its mortality rate is almost twice as high, and advanced, metastatic tumors have a particularly poor prognosis. Similar to prostate cancer, our understanding of the molecular oncogenesis of kidney tumors—both sporadic and hereditary—has advanced dramatically over the past 15 years.[30]

Biomarkers in Sporadic Kidney Cancer

The overwhelming majority of kidney tumors are renal cell carcinomas (RCC), and most of these are sporadic. The 2 most common histologic subtypes of sporadic RCC are clear cell (CCRCC) and papillary (PRCC). Although less is known about the molecular mechanisms underlying sporadic PRCCs, most sporadic CCRCCs are associated with genomic deletions and loss of heterozygosity on chromosome 3p, which contains the *VHL* (von Hippel-Lindau) tumor suppressor gene.[30] *VHL* loss of heterozygosity in these tumors leads to dysregulation of the hypoxia-inducible factor pathway, which stimulates tumor growth and angiogenesis via "pseudohypoxic drive."[30] While the diagnosis of CCRCC—particularly at resection—is usually based on morphology and/or routine IHC, FISH for chromosome 3p deletions may be useful in diagnostically challenging cases (e.g., small biopsies with limited material).[31] In contrast, PRCCs are frequently associated with gain of chromosomes 7 and/or 17, which can be detected by FISH.[32]

The hypoxia-inducible factor target CAIX can also be detected by IHC, and in CCRCC, strong CAIX IHC staining has been demonstrated to be predictive of therapeutic response to interferon α, although it is used only to a limited extent clinically.[33] CAIX IHC can also be useful in differentiating CCRCC, PRCC, and clear cell papillary RCC (CCPRCC), a recently described entity with overlapping gene expression patterns of CCRCC and PRCC but without loss of chromosome 3p25 or chromosomal gain of 7 and/or 17.[34,35] The majority of PRCC do not express CAIX, whereas CCRCC and CCPRCC show characteristic membranous expression patterns (Fig. 5). Finally, PRCC and mucinous tubular and spindle cell carcinoma (MTSCC) can demonstrate considerable histomorphologic overlap, in which case FISH to detect gain of chromosomes 7 and/or 17 can be useful as a PRCC biomarker (Fig. 6).[36]

Translocation-Associated RCC

Translocation-associated RCC (tRCC) comprises a unique clinicopathologic subtype of kidney tumors with clear molecular diagnostic implications[37–39]; these tumors tend to occur in children or younger adults and are associated with a worse overall prognosis (especially in adults). Two main classes of tRCC have been defined: translocations involving the *TFE3* locus on chromosome Xp11 and multiple gene fusion partners and t(6;11) translocations generating the *Alpha-TFEB* gene fusion. Because of the morphologic similarity to other RCC types, these renal tumors can be diagnostically challenging without the aid of ancillary studies. Whereas IHC for the transcription factors TFE3 or TFEB, as well as their common transcriptional target cathepsin K, is highly specific for Xp11 and t(6;11) tRCC, the sensitivity is not high enough to exclude the possibility of false-negative results.[39–41] More recently, FISH has been effectively used for the diagnosis of these tumors and demonstrates a high degree of sensitivity and specificity (Fig. 7).[41–43] Fluorescent in situ hybridization for *TFE3* and *TFEB* gene aberrations is currently offered as one of the clinical assays of the genitourinary service line laboratory at UMHS.

Other Genomic Alterations in Kidney Cancer

Recent sequencing results have identified recurrent mutations in the chromatin modulator *BAP1* in a subset of CCRCC; these mutations are associated with a worse overall prognosis and correlate with loss of BAP1 expression, which can be assayed by IHC.[44–46] In addition, frequent mutations in the chromatin modulators *SETD2* and *PBRM1* have been identified in CCRCC and are associated with advanced clinical and pathologic stage.[47] The clinical application of these findings is an important area of future research.

Familial Cancer Syndromes

Although much less common than sporadic RCC, renal tumors associated with hereditary cancer syndromes have important diagnostic, prognostic, and therapeutic implications, and biologic investigation of these unique tumors has been essential to the molecular understanding of dysregulated pathways in sporadic kidney cancer. Inherited renal neoplasms can demonstrate specific morphologic features, which, when recognized by the pathologist, can trigger genetic testing referral for specific familial cancer syndromes (Fig. 8).[30,48] Germline mutations in *VHL* are found in patients with the VHL hereditary cancer syndrome, and VHL patients have an increased risk of

developing CCRCC. Patients with hereditary leiomyomatosis and renal cell cancer (HLRCC) frequently have aggressive renal tumors, as well as cutaneous and uterine leiomyomata; HLRCC is caused by germline mutations in *FH*, which encodes a key protein in the Krebs (citric acid) cycle.[49,50] Hereditary leiomyomatosis and renal cell cancer–associated kidney tumors

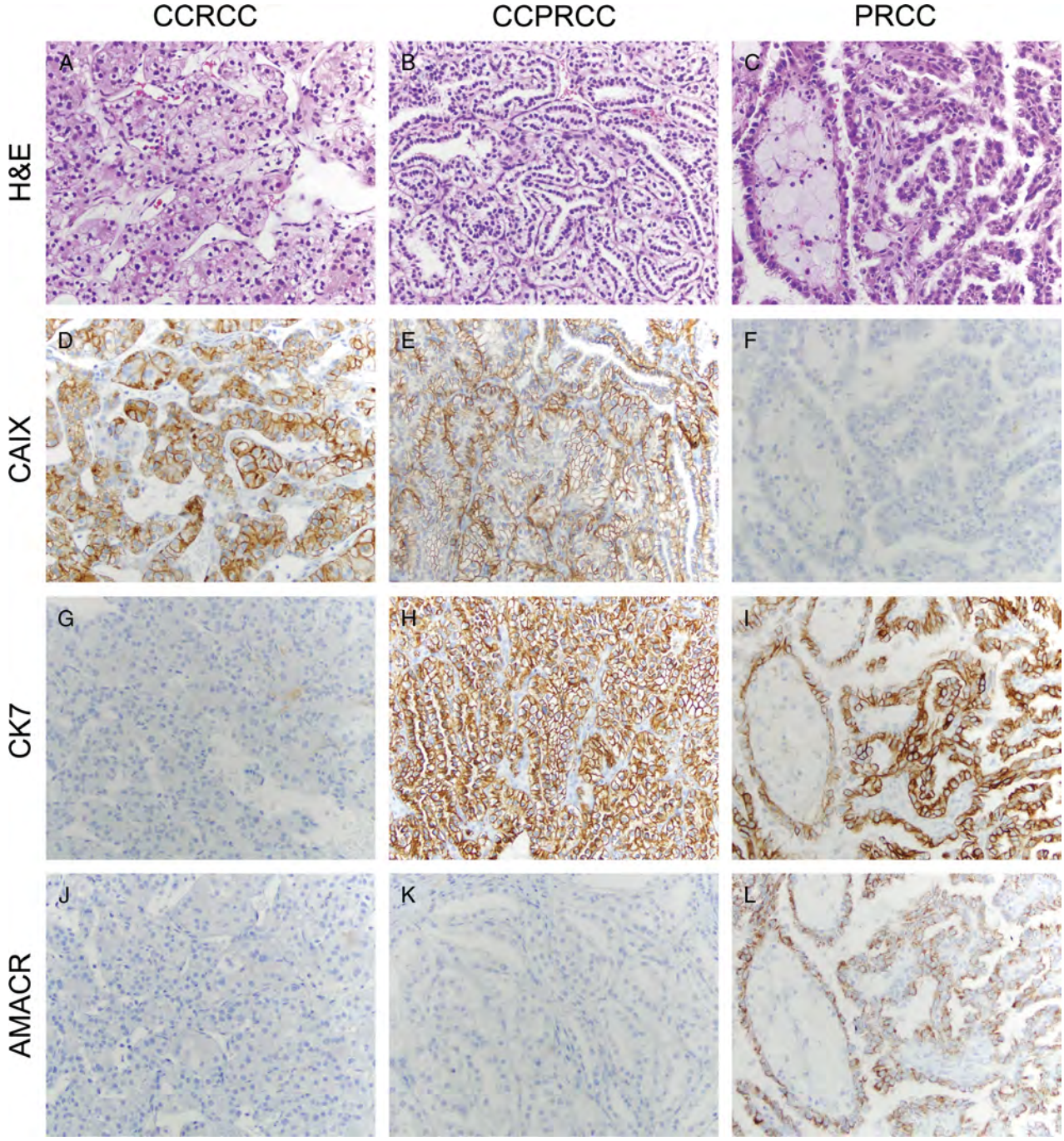

FIGURE 5. Immunohistochemistry in renal tumors with clear cell and/or papillary features. A–C, Hematoxylin-eosin (H&E) images, (D–F) CAIX IHC, (G–I) cytokeratin 7 (CK7) IHC, and (J–L) α-methylacyl-CoA racemase (AMACR) IHC in (A, D, G, J) clear cell RCC (CCRCC), (B, E, H, K) clear cell papillary RCC (CCPRCC), and (C, F, I, L) papillary RCC (PRCC). A high-grade CCRCC with cytoplasmic eosinophilia (A); these tumor cells demonstrate diffuse, strong, membranous CAIX expression (D) and are negative for CK7 (G) and AMACR (J) expression. A CCPRCC demonstrates clear cells with linear arrangement of nuclei away from basal aspect of cells (B); these tumor cells demonstrate a ''cup-like'' pattern of strong, membranous CAIX expression (E) and are diffusely positive for CK7 (H) but negative for AMACR (K) expression. A PRCC exhibits a papillary architecture with abundant foamy macrophages (C); these tumors cells are negative for CAIX (F) expression but positive for CK7 (I) and AMACR (L) expression. This panel of IHC markers (CAIX, CK7, and AMACR) can be helpful to differentiate between CCRCC, CCPRCC, and PRCC in difficult cases with limited material (e.g., core needle biopsies).

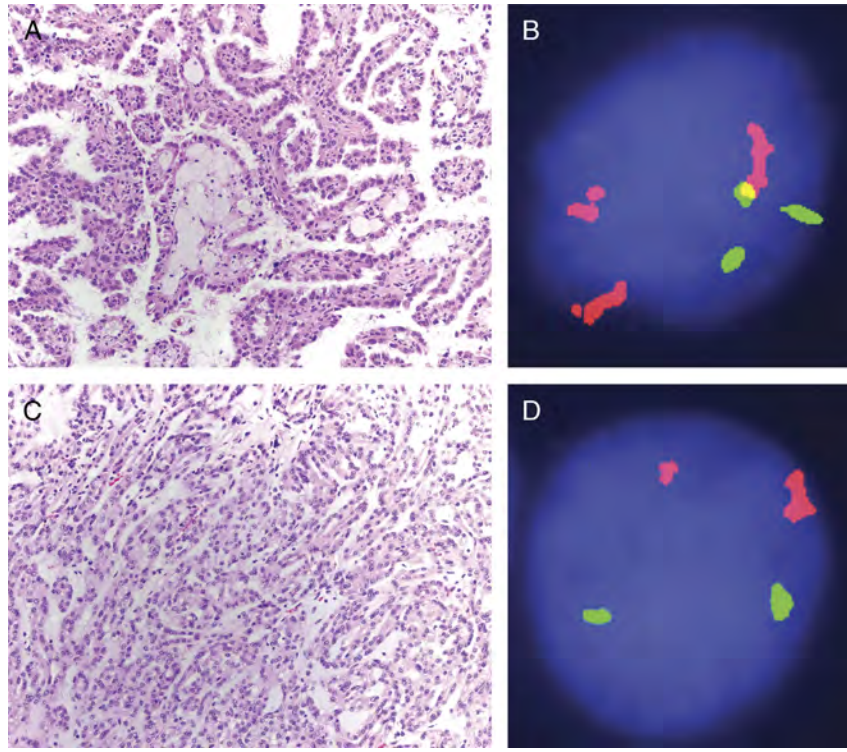

FIGURE 6. Chromosomes 7 and 17 FISH as an ancillary study for the differential diagnosis of PRCC and MTSCC. (A,C) H&E images and (B,D) chromosomes 7 and 17 FISH in (A,B) PRCC and (C,D) MTSCC. PRCC demonstrates trisomy for chromosomes 7 and 17, which distinguishes it from MTSCC, which does not demonstrate this chromosomal abnormality. Red signal highlights chromosome 7 centromeric probe, and green signal indicates chromosome 17 centromeric probe.

can have a papillary architecture and have characteristic morphologic features; reduced FH enzymatic activity in these tumors results in aberrant succination of protein cysteine residues, which can be assayed with high sensitivity and specificity by 2SC IHC.[51] Hereditary PRCC (HPRCC) syndrome is caused by germline mutations in the *MET* proto-oncogene, and HPRCC patients often present with multiple, bilateral PRCC.[52] Patients with Birt-Hogg-Dubé (BHD) syndrome have germline mutations in *FLCN* and develop multiple, usually bilateral kidney tumors—including characteristic hybrid oncocytic tumors.[53] A patient with a suspected inherited renal neoplasms can be evaluated for possible by directed sequencing of the causative gene. Recognition of these familial cancer states is important, as these patients have specific underlying genetic mechanisms of disease, which is potentially susceptible to targeted therapy (e.g., NCT01130519 and NCT01524926; Tables 1 and 2).

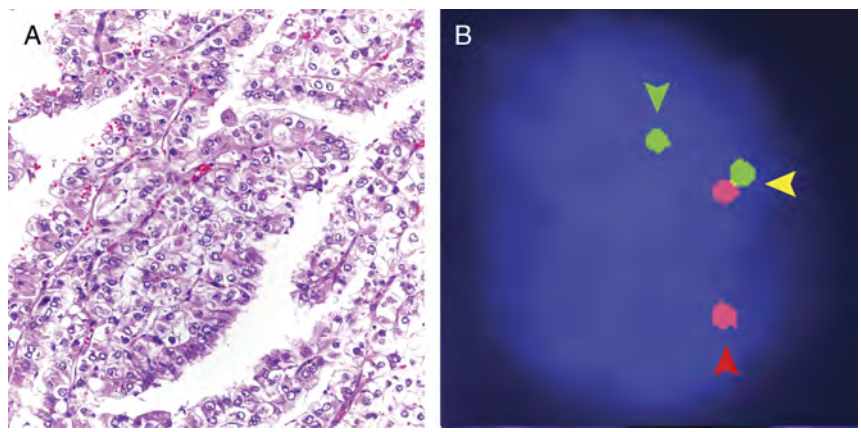

FIGURE 7. *TFE3* FISH in tRCC. A, H&E image of tRCC demonstrates a prominent papillary architecture and cells with voluminous eosinophilic and clear cytoplasm. B, *TFE3* FISH using *TFE3* break-apart probes demonstrates translocation at the *TFE3* locus. Break-apart *TFE3* FISH strategy: yellow signal demonstrates wild-type *TFE3* allele with colocalized signals (yellow arrowhead), and *TFE3* translocation yields a single green signal (green arrow) and single red signal (red arrow).

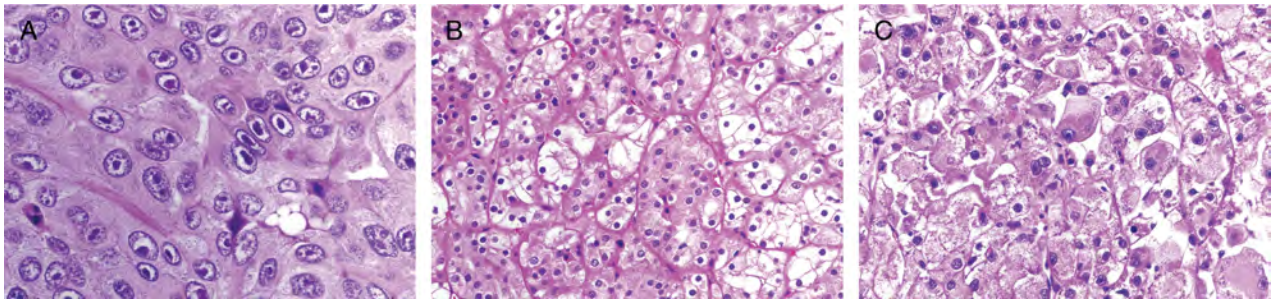

FIGURE 8. Histomorphology of renal tumors with syndromic associations. H&E images of renal tumors in (A) HLRCC, (B) BHD, and (C) tuberous sclerosis complex (TSC). HLRCC-associated RCCs have large nuclei with prominent eosinophilic (''inclusion-like'') nucleoli and prominent perinucleolar clearing (A). Hybrid oncocytic tumors, which are oncocytic tumors with scattered clusters of cells with clear cytoplasm, are characteristic of BHD (B). A subset of TSC-associated RCC (as demonstrated in C) has cells with high-grade nuclei and abundant granular eosinophilic cytoplasm with cytoplasmic vacuolation. Recognition of morphologic features (like described in this figure) might trigger referral for genetic testing for specific inherited cancer syndromes.

Emerging Biomarkers and Targeted Therapeutics

An important role for immunologic checkpoints in the antitumor immunologic response (or lack thereof) has recently been described and clinically validated in multiple pioneering clinical trials.[54,55] One such group of immunologic molecules is PD1 and its cognate ligand PDL1, which attenuate T cell–mediated antitumor responses.[56] Although the clinical value of these biomarkers is still under investigation, several cancer types, including RCC, overexpress PDL1 (which can be detected by IHC), positing a role for targeted therapy with PD1 inhibitors in these tumors. Indeed, a stage III clinical trial is currently examining salvage monotherapy with the anti-PD1 antibody nivolumab in patients with metastatic CCRCC (NCT01668784; Table 1).

Insight into the molecular pathology of kidney cancers has led to the development of new therapeutic drug classes. Vascular endothelial growth factor inhibitors (i.e., bevacizumab, sunitinib, cabozantinib, etc.) target HIF pathway–stimulated "pseudohypoxic drive" and are either currently approved or in trial for the treatment of CCRCC (NCT01865747; Table 1).[30] mTOR inhibitors, including temsirolimus and everolimus, have also gained regulatory approval for CCRCC[57]; these drugs block mTOR-mediated protein synthesis and thereby inhibit tumor cell proliferation and production of proangiogenesis factors. The identification of robust molecular biomarkers that predict response to mTOR inhibitors for patients with advanced or metastatic CCRCC is an active area of research.

Urinary Bladder Cancer

Similar to kidney cancer, bladder cancer is less common but more often lethal than prostate cancer. Urothelial carcinomas, which constitute the vast majority of bladder cancers, demonstrate a wide spectrum of clinical behavior, ranging from indolent, noninvasive papillary cancers to aggressive, locally invasive tumors with a penchant for distant metastasis.

Molecular Oncogenesis of Bladder Cancer

The molecular basis for these divergent outcomes is only recently beginning to emerge.[58] Early activation of oncogenes, such as *FGFR3* or *RAS*, in low-grade, noninvasive tumors is followed, in a minority of cases, by mutation of 1 or more tumor suppressor genes, including *TP53*, *RB1*, and *PTEN*, resulting in progression to invasive disease. This accounts for the relatively high rate of recurrence but low rate of progression for low-grade, noninvasive papillary urothelial carcinoma. Alternatively, mutation of tumor suppressor genes can occur without preexisting or concurrent activation of oncogenes; such lesions, including flat urothelial carcinoma in-situ, are inherently genomically unstable and will more often progress to invasive carcinoma. Once a bladder tumor has become invasive, there is a similar dichotomy in clinical behavior between superficial (pT1) and deeper (pT2 or greater) invasion, with pT1 tumors generally having a relatively good prognosis compared with more invasive cancers. However, even within this subset of superficially invasive (pT1) urothelial carcinomas, there is a risk of progression and subsequent metastasis.

There are few molecular diagnostic studies available for the prognostication of urothelial carcinomas, and none of them is widely used in current clinical practice. In a recent large, multi-institutional study, the presence of aberrant p53 expression by IHC in superficially invasive (pT1) urothelial carcinomas was associated with increased risk of recurrence and progression.[59] Similarly, in another recent study, diffuse Ki-67 expression by IHC was associated with increased risk of progression of high-grade urothelial carcinoma.[60] FGFR family mutations and fusions have been recently described in urothelial carcinomas, and FGFR overexpression holds promise as a prognostic and predictive biomarker (Fig. 9).[60–62] An ongoing clinical trial is evaluating targeted therapy with the FGFR3 inhibitor dovitinib in patients with treatment-resistant early urothelial carcinoma (NCT01732107; Table 1). Finally, a recent study identified a subset of urothelial carcinomas with coinfection of high-risk human papillomavirus (HPV) strains.[63] These tumors had a unique histomorphology, with prominent basaloid squamous-type differentiation, and commonly occurred in patients with neurogenic bladder. In situ hybridization for high-risk HPV strains can confirm coinfection in suspected cases (Fig. 10).

Other Genomic Alterations in Bladder Cancer

HER2/neu overexpression, which is found in many types of cancer including breast carcinoma, can be assayed directly by IHC or indirectly by FISH for *ERBB2* amplification (Fig. 11), and a recent study found that HER2/neu overexpression in noninvasive or superficially invasive urothelial carcinoma was associated with increased risk of progression.[64] This suggests the possibility of targeted therapy for HER2 overexpressing tumors. Indeed, a feasibility/safety study of the HER2 inhibitor trastuzumab in urothelial carcinoma has been reported,[65] and at least 1 clinical trial is currently evaluating treatment with the HER2 inhibitor lapatinib in patients with advanced urothelial carcinoma (NCT00949455; Table 1). Results of a recent

TABLE 2. Renal Tumors With Syndromic Associations: Morphologic and Molecular Features

Syndrome	Gene(s) Implicated	Chromosome	Renal Manifestations	Target/Pathway	Drugs Approved or In Trials[a]	Detection Method
BHD	*BHD/FLCN*	17p11.2	Bilateral and multiple tumors Hybrid oncocytic tumor Chromophobe RCC Clear cell RCC Papillary RCC Oncocytoma Background oncocytosis	mTOR	Temsirolimus Everolimus	Direct sequencing*
Germline succinate dehydrogenase (SDH) mutation	*SDHB*	1p35-36.1	SDHB: intracytoplasmic inclusions	HIF/VEGF	Pazopanib Sunitinb Axitinib Bevacizumab	Direct sequencing* SDHB IHC
	SDHC	1q23.3	SDHC/SDHD: clear cell morphology			
	SDHD	11q23				
Hereditary leiomyomatosis and RCC (HLRCC)	*FH*	1q42.1	Unilateral and solitary tumors Large nuclei with prominent eosinophilic nucleoli and perinucleolar clearing	HIF/VEGF	Pazopanib Sunitinb Axitinib Bevacizumab (+ erlotinib[a])	Direct sequencing* 2 SC IHC
HPRCC	*MET*	7q31	Bilateral and multiple tumors Type I PRCC Papillary adenomatosis	MET	Crizotinib[a] Tivantinib +/− erlotinib Foretinib[a]	Direct sequencing*
Tuberous sclerosis	*TSC1*	9q34	Bilateral and multiple AML	mTOR	Everolimus†	Direct sequencing*
	TSC2	16p13.3	Clear cell RCC RCC with eosinophilic cytoplasm and high grade nuclei Background AML tumorlets		Temsirolimus‡ Pazopanib‡ Sunitinb‡ Axitinib‡ Bevacizumab‡	
VHL	*VHL*	3p25.3	Bilateral and multiple tumors Clear cell RCC Cysts lined by clear cells Clear cell RCC tumorlets	HIF/VEGF	Pazopanib Sunitinb Axitinib Bevacizumab	Direct sequencing*

*Direct Sanger sequencing is available in reference laboratories across the country including academic centers.

†AML only.

‡AML and RCC.

HIF indicates hypoxia-inducible factor; VEGF, vascular endothelial growth factor; 2SC, S-(2-succinyl) cysteine; AML, angiomyolipoma.

sequencing study with a large cohort of high-grade urothelial carcinomas demonstrated frequent, nonoverlapping mutations in the RAF and mTOR pathways, suggesting the possibility of new targeted therapies for clinical trials.[66] The pending release of the bladder cancer cohort data from The Cancer Genome Atlas project should reveal additional details about the molecular underpinnings of urothelial carcinoma and provide more potential actionable targets for drug therapy. Finally, an emerging area of focus in the prognostication of urothelial carcinoma is the utility of mRNA gene sets. While these assays are still in development, they have shown promise in predicting tumors at risk for progression[67] and patients who might benefit from chemotherapy.[68]

Urine-Based Screening for Bladder Cancer

In addition to biopsy and urine cytology, FISH for detection of aneuploidy of chromosomes 3, 7, and 17 and loss of chromosome 9p21 in exfoliated urothelial cells from urine specimens has been implemented in bladder cancer screening programs[69]; this assay, which is marketed by Vysis, Inc, as UroVysion, has relatively high sensitivity and specificity for the presence of urothelial carcinoma. As such, UroVysion is currently used as a companion diagnostic for the initial diagnosis or subsequent monitoring of urothelial carcinoma.

ALK Aberrations in Inflammatory Myofibroblastic Tumors

Despite accounting for only a small fraction of bladder tumors, IMTs have important molecular diagnostic and therapeutic implications. Inflammatory myofibroblastic tumors are mass-forming spindle cell neoplasms with a predominantly benign clinical course,[70] and approximately half of these tumors are associated with rearrangements at the *ALK* locus.[71] Given their morphologic similarity to sarcomas and sarcomatoid carcinomas, IMT can be diagnostically difficult for pathologists—particularly in limited biopsy specimens. The presence of ALK expression by IHC or *ALK* rearrangement by FISH is helpful in making the diagnosis of IMT, as *ALK* rearrangements can be demonstrated by IHC or FISH in more than 60% of cases of IMT.[72,73] Current clinical trials are investigating a variety of tyrosine kinase inhibitors (i.e., the ALK inhibitor crizotinib) in the treatment of *ALK* rearranged tumors (NCT01524926 and NCT01449461; Table 1).

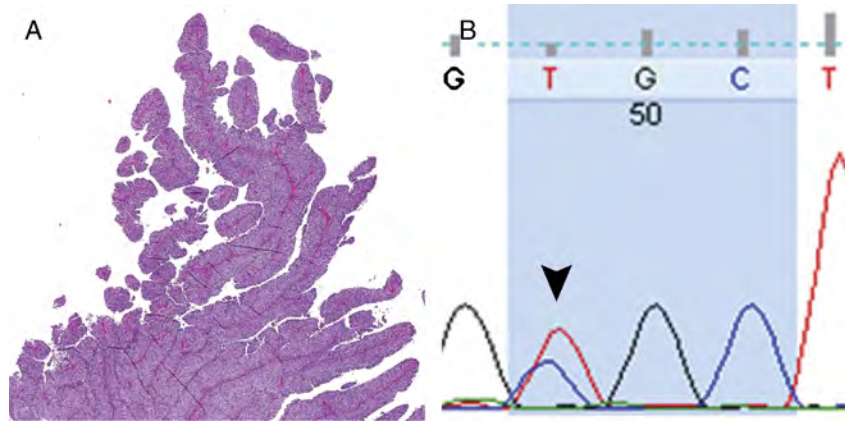

FIGURE 9. *FGFR3* mutations in bladder cancer. A, H&E image of papillary urothelial carcinoma with a bulky, exophytic component and branching papillary architecture, as well as irregular nuclei; these morphologic features have been associated with the presence of *FGFR3* mutations. B, Sanger sequencing of the *FGFR3* gene locus in this case demonstrates an exonic nucleotide transition (cytosine to thymine; black arrowhead) that produces an arginine to cysteine missense mutation at amino acid position 248 (courtesy of Dr. Hikmat A. Al-Ahmadie, Memorial Sloan-Kettering Cancer Center).

Interestingly, a recent report of a patient with an aggressive, *ALK* rearranged IMT documented partial response to crizotinib,[74] suggesting the potential for targeted therapy of *ALK* rearranged IMT in the future.

Testicular Cancer

In contrast to other genitourinary tumors, our understanding of the molecular oncogenesis of testicular cancer is relatively limited.[75] The vast majority of testicular tumors are of germ cell origin and occur predominantly in younger men. The morphology of these tumors is diverse, and they are generally divided into pure seminoma and nonseminomatous/mixed types. Single-gene mutations are uncommon in testicular tumors, but when present involve *CKIT*, *TP53*, *KRAS/NRAS*, or *BRAF*.[76] The majority (approximately 80%) of germ cell tumors also share a common, unique molecular feature: chromosome 12p copy number gain, classically seen as isochromosome 12p [i(12p)].[77] Fluorescent in situ hybridization for chromosome 12p is a sensitive and specific assay for testicular germ cell tumors.[78] Although the diagnosis of a germ cell tumor is primarily based on morphologic and (if needed) immunohistochemical evaluation of orchiectomy specimens, chromosome 12p FISH may be useful for early detection of testicular cancer from semen samples[79] or as a diagnostic aid for metastatic germ cell tumors.[78] This may be particularly useful in cases of secondary somatic transformation (SST) of a germ cell tumor, a phenomenon seen in about 5% of testicular tumors in which a somatic teratomatous component becomes morphologically malignant and is associated with aggressive growth. This SST can manifest in the form of sarcoma, adenocarcinoma, primitive neuroectodermal tumor (PNET) or leukemia and can present a diagnostic dilemma at metastatic sites. The presence of isochromosome 12p, however, has been demonstrated in these SST (including leukemias), indicating a clonal relationship to the original germ cell tumor—which also serves as a useful diagnostic adjunct in this scenario.[80–82] Next-generation sequencing can also reliably detect chromosome 12p gain/aberration when present in cases of testicular germ cell tumors with SST (Fig. 12).

Overall, the prognosis for testicular germ cell tumors is good, with a complete cure possible in greater than 90% of cases. If adjuvant therapy is required, seminomas are susceptible to and treated with radiation, while nonseminomatous/mixed germ cell tumors (with the notable exception of mature teratomatous elements) are sensitive to platinum-based chemotherapy. Interestingly, a recent study of treatment resistance in nonseminomatous/mixed germ cell tumors reported that resistant tumors showed increased microsatellite instability, BRAF V600E mutation, and *MLH1* promoter hypermethylation (although the clinical value of routine microsatellite instability testing in these tumors is

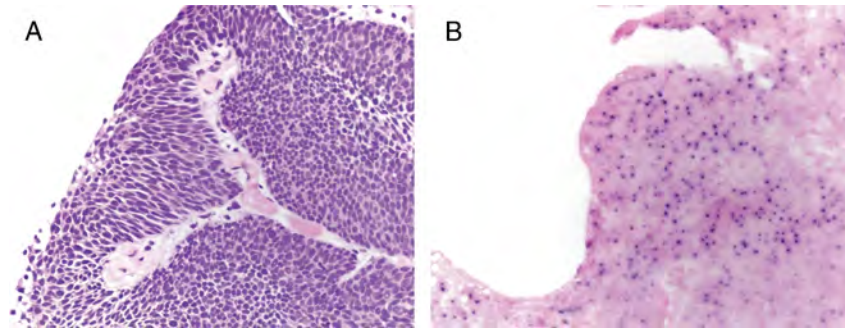

FIGURE 10. High-risk *HPV* ISH in a subset of bladder cancer. A, H&E image of urothelial carcinoma with squamous differentiation and prominent basaloid features. B, *HPV* ISH demonstrates infection of these neoplastic cells by high-risk HPV strains.

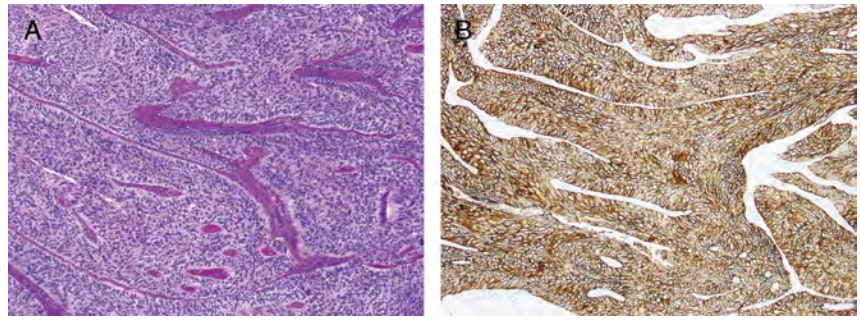

FIGURE 11. HER2 IHC in bladder cancer. A, H&E image and (B) IHC for HER2 expression in high-grade urothelial carcinoma. Strong membranous HER2 IHC staining indicates *HER2* amplification/overexpression (courtesy of Dr. Hikmat A. Al-Ahmadie, Memorial Sloan-Kettering Cancer Center).

unclear).[83] Finally, the propensity for certain germ cell tumor histologic subtypes to express the cell surface marker CD30 and the emerging availability of anti–CD30-based targeted therapy (i.e., brentuximab) have spurred at least 1 clinical trial to evaluate brentuximab salvage therapy for treatment-resistant, metastatic germ cell tumors that express CD30 by IHC (NCT01851200; Table 1).

Penile Cancer

Penile cancer is rare, particularly in developed countries such as the United States, but despite its low incidence, the mortality rate is relatively high (and has not significantly improved over the past 20 years).[84] The vast majority of penile tumors are squamous cell carcinoma (SCC), which can be roughly stratified into HPV-associated and non–HPV-associated groups.[85] Human papillomavirus–associated SCC frequently has a basaloid-type histomorphology and a propensity to metastasize, whereas non–HPV-associated SCC is more likely to be keratinizing type and less likely to metastasize. Human papillomavirus–associated SCCs usually demonstrate coinfection by high-risk HPV strains (which can be detected by ISH in diagnostically challenging cases) and p16 overexpression by IHC; alternatively, non–HPV-associated SCCs demonstrate p53 overexpression by IHC and are negative for p16 expression.[86] Lymph node metastasis is a poor clinical indicator in penile SCC, and currently, routine pathologic examination is the only reliable predictor of locally aggressive disease.[87] As such, penile cancers are an important area for future molecular investigation. Interestingly, a recent study detected amplification of the transcription factor SOX2 gene (*SOX2*) by FISH and overexpression of SOX2

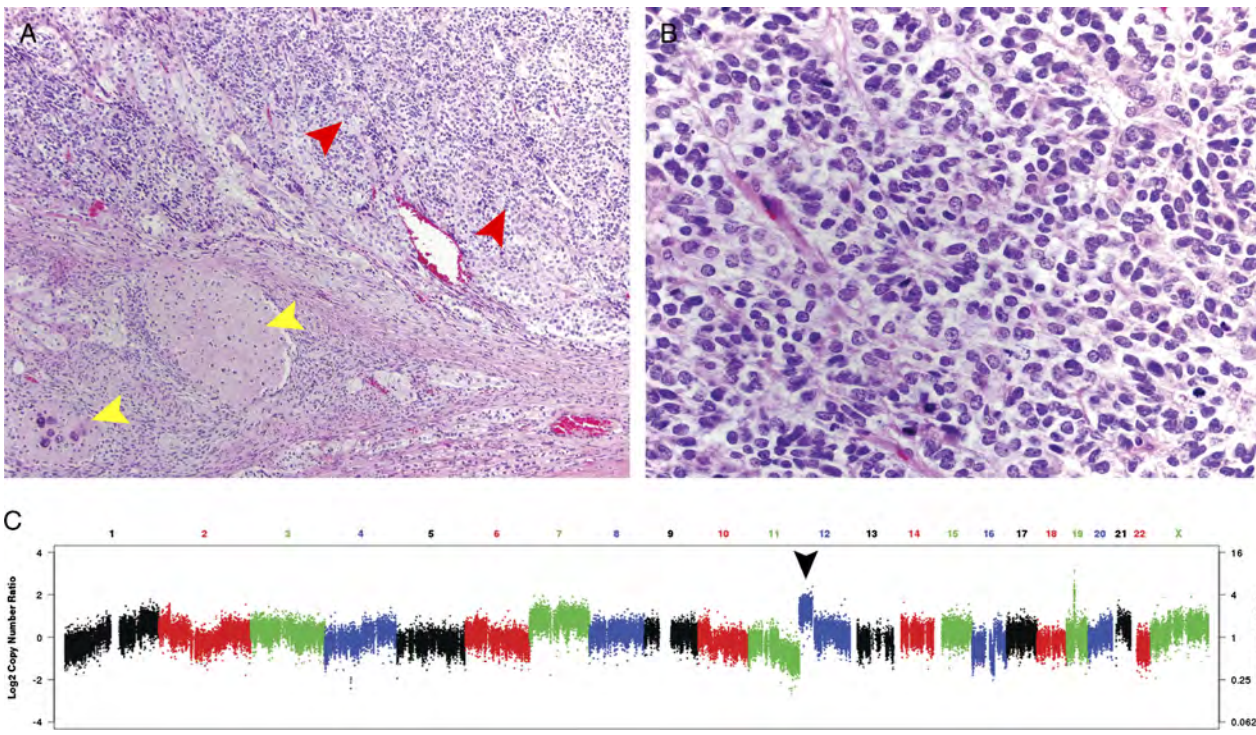

FIGURE 12. Copy number analysis in a metastatic germ cell tumor with SST. A, H&E image demonstrates the classic histomorphologic appearance of a PNET (red arrowheads) along with teratoma (yellow arrowheads), as seen in a patient with SST. B, High-power H&E image of PNET. C, A copy number plot derived from whole-genome clinical sequencing of a metastatic germ cell tumor with SST demonstrates gain of the short arm of chromosome 12 (black arrowhead). Chromosome 12p FISH can also be used to detect chromosome 12p gains.

TABLE 3. Summary of Current and Proposed Molecular Diagnostics in Genitourinary Oncology

	Prostate	Kidney	Bladder	Testicular	Penile
Current	ERG IHC and *ERG* FISH	Chromosome 3p FISH	HPV ISH	Chromosome 12p FISH	HPV ISH
	T2-ERG (urine)	Trisomy 7/17 FISH	HER2 IHC and *HER2* FISH	CD30 IHC	p16/p53 IHC
	PCA3 (urine)	CAIX IHC	UroVysion (urine)		
		TFE3/TFEB FISH	*ALK* FISH and ALK IHC		
		TFE3, TFEB, and cathepsin K IHC			
		2SC IHC			
		FH, *MET*, and *FLCN* sequencing			
Proposed	*ETV1* FISH	BAP1 IHC	*FGFR3* sequencing		
	SPINK1 IHC	PD1/PDL1 IHC	mRNA gene sets		
	PTEN IHC and *PTEN* FISH				
	BRAF FISH				
	AURKA FISH				
	MYCN FISH				
	lncRNA ISH				

T2-ERG indicates *TMPRSS2-ERG*.

protein by IHC in approximately one-third of penile SCC[88]; however, the clinical utility of this potential biomarker remains to be explored.

CONCLUSIONS

In summary, the expansion of knowledge regarding the molecular oncogenesis of tumors has created new and exciting prospects for molecular diagnostics to improve the clinical practice of oncology and, ultimately, patients' lives. For genitourinary malignancies, these advances are translating into clinical-grade assays that increasingly inform diagnostic, prognostic, and therapeutic clinical decision making (Table 3). The emerging molecular era also provides a unique opportunity to restructure and formalize the manner in which basic science and clinical cancer research results are translated into clinical assays—a concept that we refer to as the molecular diagnostics service line laboratory.

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