Report From the International Society of Urological Pathology (ISUP) Consultation Conference on Molecular Pathology of Urogenital Cancers. I. Molecular Biomarkers in Prostate Cancer

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Abstract: The combined clinical and molecular heterogeneity of prostate cancer necessitates the use of prognostic, predictive, and diagnostic biomarkers to assist the clinician with treatment selection. The pathologist plays a critical role in guiding molecular biomarker testing in prostate cancer and requires a thorough knowledge of the current testing options. In the setting of clinically localized prostate cancer, prognostic biomarkers such as Ki-67 labeling, PTEN loss or mRNA-based genomic signatures can be useful to help determine whether definitive therapy is required. In the setting of advanced disease, predictive biomarkers, such as the presence of DNA repair deficiency mediated by *BRCA2* loss or mismatch repair gene defects, may suggest the utility of poly-ADP ribosylase inhibition or immune checkpoint blockade. Finally, androgen receptor–related biomarkers or diagnostic biomarkers indicating the presence of small cell neuroendocrine prostate cancer may help guide the use of androgen receptor signaling inhibitors and chemotherapy. In this review, we examine the current evidence for several prognostic, predictive and diagnostic tissue-based molecular biomarkers in prostate cancer management. For each assay, we summarize a recent survey of the International Society of Urology Pathology (ISUP) members on current testing practices and include recommendations for testing that emerged from the ISUP Working Group on Molecular Pathology of Prostate Cancer and the 2019 Consultation Conference on Molecular Pathology of Urogenital Cancers.

Key Words: prostate cancer, biomarkers, PTEN, Ki-67, AR, neuroendocrine, homologous recombination, mismatch repair

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Prostate cancer is a clinically and molecularly heterogenous disease with a wide spectrum of management strategies tailored to its highly variable clinical outcomes. Once diagnosed, these features make it essential to have accurate prognostic, predictive and diagnostic biomarkers to assist the clinician in treatment selection. Beyond accurate diagnosis, grading and staging, the pathologist plays a key role in guiding subsequent tissue-based molecular biomarker testing in prostate cancer and requires a thorough knowledge of the available assays, their application in specific disease states and the evidence for their clinical utility. In the localized setting, prostate cancer clinical management options include active surveillance (AS), radical prostatectomy and radiation therapy, and prognostic biomarkers can assist in determining whether definitive therapy is required. Historically, treatment of metastatic prostate cancer has largely been independent of histology or biomarkers, limiting most pathologists' experience with histologic assessment of metastatic

prostate cancer or guidance of appropriate tissue-based biomarker testing. More recently, recognition of both AR signaling–dependent and AR signaling–independent castration-resistant prostate cancer (CRPC) (the latter often representing small cell neuroendocrine [NE] carcinoma), potentially targetable subtypes of CRPC (eg, homologous recombination-deficient and mismatch repair [MMR]-deficient), as well as increasing therapeutic options (eg, chemotherapy, AR signaling inhibitors, targeted therapies and immunotherapy) have driven intense investigation of diagnostic and predictive biomarkers that can be used to guide CRPC management.[1]

In this review, we examine the current evidence for several prognostic, predictive and diagnostic tissue-based molecular biomarkers in prostate cancer management. For each assay, we summarize a recent survey of the International Society of Urology Pathology (ISUP) members on current testing practices. This survey included data from 256 ISUP members (all pathologists), 75% of whom have been in practice for >10 years (including residency), with the majority (62%) working in university/academic settings. In addition, for each assay, we include recommendations for testing that emerged from the ISUP Working Group on Molecular Pathology of Prostate Cancer and the 2019 Consultation Conference on Molecular Pathology of Urogenital Cancers.

PROGNOSTIC BIOMARKERS IN LOCALIZED PROSTATE CANCER

Prognostic biomarkers estimate the overall likelihood of an adverse clinical outcome, regardless of the specific therapeutic setting. These assays may be used to guide clinical management of localized prostate cancer, where AS may be a reasonable option, or as decision support tools after radical prostatectomy where adjuvant radiation and hormonal therapy may be efficacious in aggressive disease. Countless single markers have been claimed to have prognostic utility in prostate cancer during the past decades, yet none has survived the valley of death on the way to the routine clinical application until now.[2] The proliferation marker Ki-67 and the tumor-suppressor protein, PTEN have emerged as the most promising single immunohistochemical (IHC) markers based on accumulated published evidence at this point of time. In addition, several RNA-based commercial genomic assays can provide reproducible prognostic information by expression profiling and are listed by the guidelines of the National Comprehensive Cancer Network (NCCN) as an option for better patient stratification. Despite considerable progress in biomarker validation, systematic use of prognostic biomarkers in prostate cancer is currently not recommended by urological societies. Thus, urologists and/or oncologists may drive the selection and use of the tests depending on institutional policy.

Ki-67

Background

Ki-67 is a protein encoded by the *MKI67* gene and was first described in Hodgkin lymphoma as an antigen that is highly expressed in cycling cells but absent in resting G0-phase cells.[3] Although the biological function of Ki-67 remains poorly understood, Ki-67 has been shown to regulate cell cycle progression by different mechanisms.[4,5] Ki-67 has become the prototype IHC "cell proliferation marker" and has extensively been used in pathology for diagnostic or prognostic purposes across different tumor types including breast cancer, NE tumors, lymphomas, and sarcomas. The fraction of Ki-67 positive tumor cell nuclei is commonly referred to as the Ki-67 labeling index (LI) or Ki-67 score. In breast cancer, Ki-67 LI is used to help stratifying patients for neoadjuvant chemotherapy and it serves as a key diagnostic criterion for grading gastrointestinal NE tumors according to the criteria of the World Health Organization (WHO).[6,7]

Evidence

Accumulated published evidence have also consistently shown a prognostic role of Ki-67 in localized prostate cancer, as recently summarized in a meta-analysis of 21 selected studies between 1996 and 2014 including 5419 patients with localized prostate cancer.[8] A prognostic effect was observed across all endpoints including biochemical recurrence (BCR)-free survival, disease-specific survival, metastasis-free survival, and overall survival. An independent prognostic role of Ki-67 LI has also been emphasized in a subsequent large multi-institutional study on over 1000 radical prostatectomy specimens.[9] The authors used tissue microarrays (TMA) with each surgical specimen being represented by 3 TMA cores at a diameter of 1 mm, thus mimicking transrectal ultrasound–guided core biopsies. Although TMA studies are practical for efficient biomarker studies, analysis of Ki-67 LI on core biopsies is more relevant since they better reflect clinical practice in routine pathology laboratories. In fact, several studies showed that the independent prognostic value of Ki-67 also holds true in core biopsy specimens.[10–12] Prospectively assessed Ki-67 LI in preoperative biopsies with low-volume or low-grade (Gleason score <7) prostate cancer in routine clinical practice was shown to be an independent prognostic factor in terms of postoperative biochemical-free recurrence.[13] In a recent retrospective analysis of 756 conservatively managed patients, Ki-67 LI on biopsy grade group 1 and 2 prostate cancer also emerged as an independent prognostic marker of tumor-specific survival.[12] These studies suggest that Ki-67 LI has high promise in helping to better select patients for AS. In fact, high Ki-67 LI has been shown to be independently associated with a switch to active treatment after AS when tested on TMA made from pre-AS prostate biopsies from 60 patients.[14]

Aside from the lack of prospective clinical validation, there are several postanalytical hurdles explaining why Ki-67 testing has not yet widely entered clinical practice. These include lack of a consensus on the optimal threshold to define low and high proliferation, different scoring procedures and interobserver variability in reading IHC slides. Important lessons can be learned from the breast cancer field in this respect. Despite major efforts in harmonizing Ki-67 analysis in breast cancer and recommendations on how to evaluate Ki-67 LI, interlaboratory variability in Ki-67 evaluation

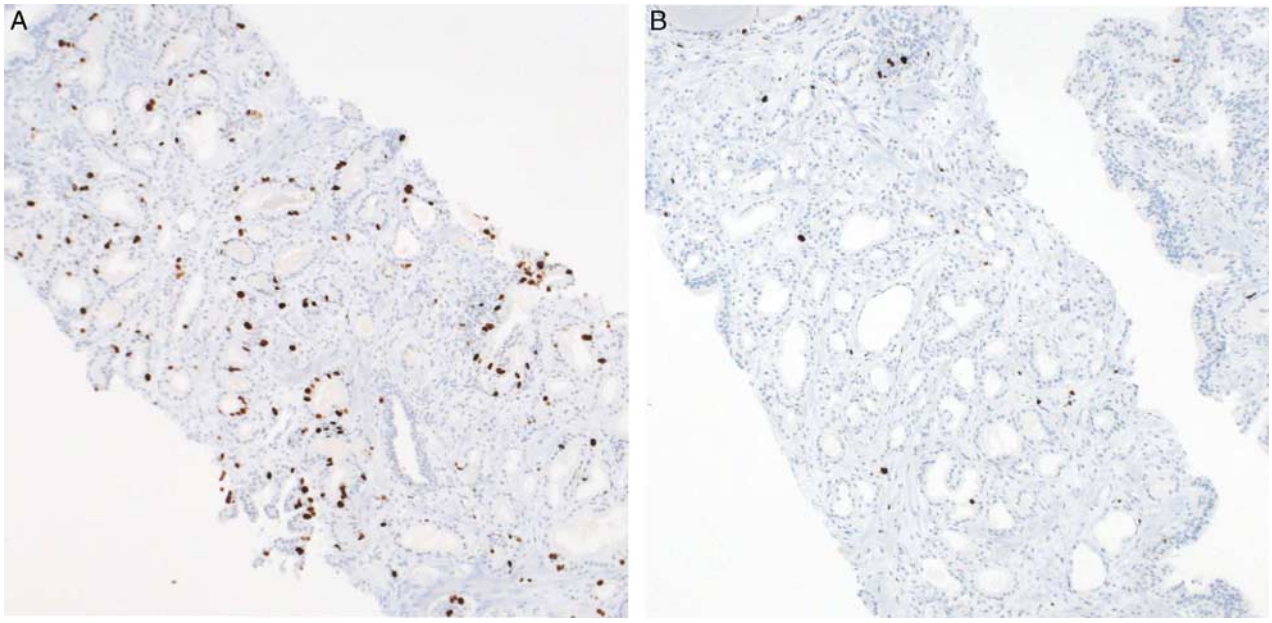

FIGURE 1. Ki67 LI in Grade Group 1 prostate cancer core biopsies. A, High LI (~15%). B, low Ki-67 LI (< 5%).

has remained substantial even in the most experienced laboratories.[15–17] Nevertheless, a large meta-analysis showed the independent prognostic value of Ki-67 LI for overall survival, and a cutoff of ≥25% was proposed as most appropriate for prognostic stratification in breast cancer.[18] Because of its established clinical value, wide availability and low costs relative to commercial genomic signatures, Ki-67 testing of breast cancer has been endorsed in European practice guidelines.[19,20] However, given the evident variability between laboratories, it was noted that scores should be interpreted in the light of local laboratory values to define Ki-67 LI as clearly low (eg, <10%) or clearly high (eg, > 30%).[19,20] According to meta-analyses, the reported median Ki-67 LIs in prostate cancer are lower than in breast cancer (6.1% vs. 14%).[8,18] Thus, the most appropriate threshold for Ki-67 LI in prostate cancer appears to lie between 5% and 10%.[8,9,12] Since Ki-67 LI is a continuous variable, there will be an inevitable intermediate gray zone between a clearly low (eg, <5%) and a clearly high Ki-67 LI (eg, > 15%) (Fig. 1). Other technical details remain to be clarified for Ki-67 scoring of prostate cancer, including the required minimum number of tumor cells and the mode of evaluation (eg, whole area vs. hotspots and counting vs. visual estimates). Automated image analysis for Ki-67 scoring is feasible and might play an increasing role in standardizing and facilitating Ki-67 scoring, although it has not yet been proven to be superior to visual evaluation.[9,12,21,22]

Discussion for Ki-67

In the ISUP survey preceding the Consultation Conference, the majority of respondents indicated that they did not currently recommend (58%) or use (67%) any in situ prognostic biomarkers in radical prostatectomy samples. Given that the final Grade Group, pathologic stage and other clinical-pathologic parameters in the radical prostatectomy sample are highly prognostic, this seems a reasonable approach. In addition, none of these biomarkers have been directly tested for prognostic value in the setting of adjuvant or salvage radiation therapy, which is the clinical context in which they would be used postsurgery. In the context of prostate core biopsies, survey respondents were more evenly divided about whether prognostic markers such as Ki-67 and PTEN could be recommended. In this setting, 46% of respondents answered that prognostic biomarkers could not be currently recommended in routine practice, while 45% felt that they could be helpful in decision-making regarding AS versus definitive therapy. Among in situ biomarkers, Ki-67 and PTEN were the most commonly used in core biopsies, however <10% of respondents reported using either biomarker overall.

According to the accumulated evidence, Ki-67 LI appears as a robust prognostic marker in prostate cancer that can be advocated for use in clinical practice. Ki-67 LI might be particularly useful in patients considered for AS. However, further efforts are needed to improve the standardization of Ki-67 scoring and to validate the prognostic value in prospective studies. In addition, Ki-67 LI testing, which is inexpensive and globally established in pathology laboratories, needs to be compared against the more expensive and centrally tested commercial genomic assays that measure proliferation signatures, as described below. For the formal recommendations of the Working Group for Ki-67 and PTEN, see discussion after the PTEN section below.

PTEN

Background

Among the common molecular alterations observed in primary prostate cancer, PTEN loss has emerged as one

of the more robust prognostic biomarkers. The PTEN protein functions primarily as a lipid phosphatase, opposing the oncogenic PI3K/AKT signaling cascade. In primary prostate cancer, PTEN is lost most commonly by genomic deletion, though genomic rearrangements and more rarely, truncation mutations leading to PTEN inactivation have been described.[23] Accordingly, fluorescence in situ hybridization (FISH) assays to detect *PTEN* deletions or rearrangements, as well as IHC assays to detect PTEN protein loss, are the most common assays utilized to ascertain PTEN status, and demonstrate a high level of concordance in numerous studies[23–26] (Fig. 2). As with many prognostic biomarkers including Ki-67, the frequency of PTEN loss increases with increasing Grade Group as well as pathologic stage,[27] thus the prevalence of PTEN loss varies significantly between different cohorts. Among surgically treated patients, most studies have identified PTEN loss in ~20% of prostate tumors, with the prevalence

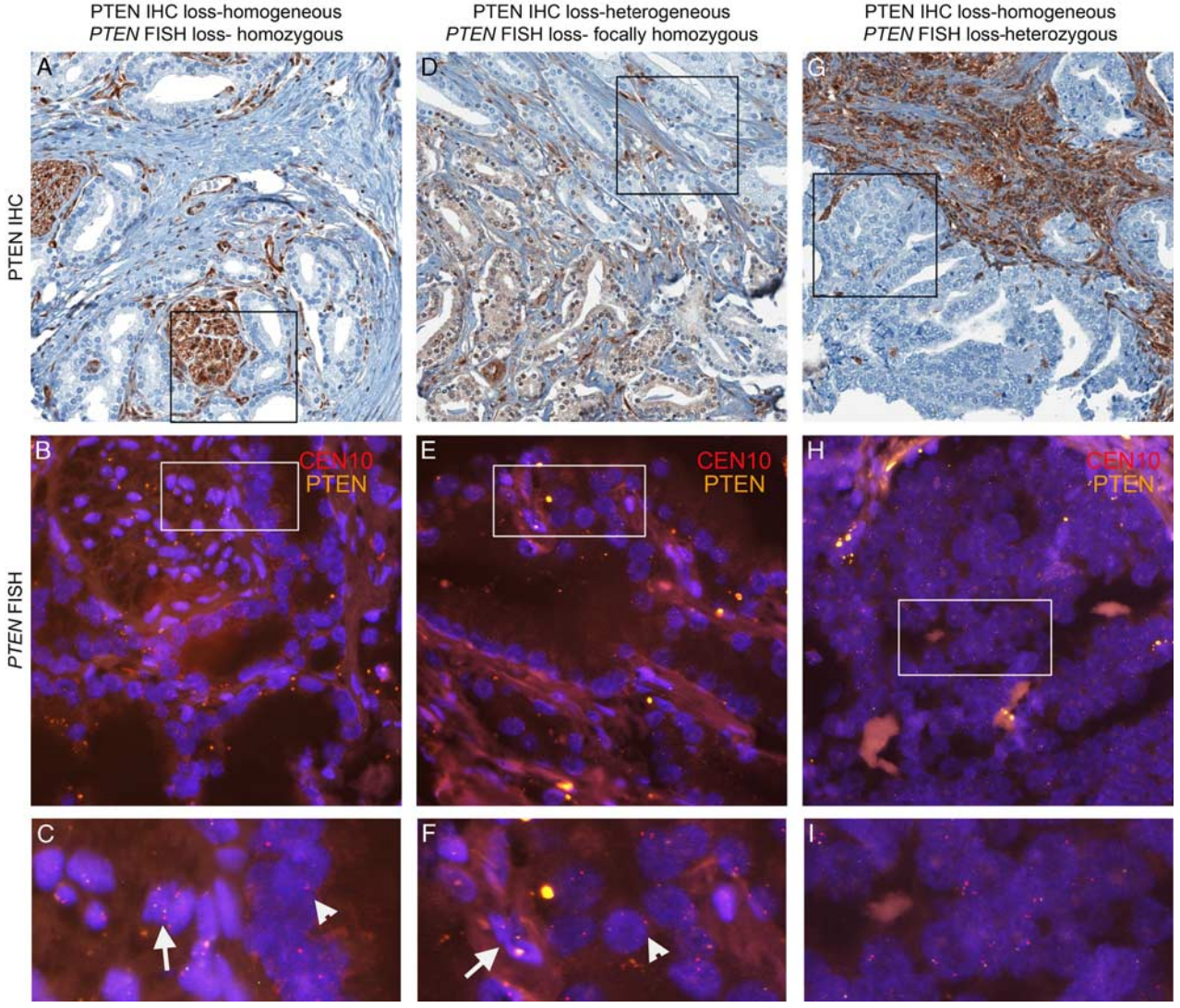

FIGURE 2. PTEN IHC and *PTEN* FISH in primary prostate cancer samples. A, PTEN IHC loss is homogeneous in the sampled tumor, with nerves providing an internal positive control (box depicts area examined by FISH in B). B, *PTEN* gene loss by FISH is assessed by examining the *PTEN* probe (orange) and centromere 10 (CEN10) probe counts (red) in tumor cells. In this sample, *PTEN* loss is homozygous in tumor cells and *PTEN* is intact in nearby Schwann cell nuclei (box depicts area examined at higher magnification in C). C, Schwann cell nuclei show intact *PTEN* (arrow) while adjacent tumor nuclei (arrowhead) have homozygous *PTEN* loss. D, PTEN IHC loss is heterogenous in the sampled tumor, with stromal cells providing an internal positive control (box depicts area examined by FISH in E). E, In the region of PTEN protein loss, *PTEN* loss is homozygous in tumor cells and PTEN is intact in nearby stromal cells (box depicts area examined at higher magnification in F). F, Stromal cell nuclei show intact PTEN (arrow) while adjacent tumor nuclei (arrowhead) have homozygous *PTEN* loss. G, PTEN IHC loss is homogeneous in the sampled tumor, with stromal cells providing an internal positive control (box depicts area examined by FISH in H). H, *PTEN* loss is hemizygous in tumor cells (box depicts area examined at higher magnification in I). I, Tumor nuclei have hemizygous *PTEN* loss.

rising to 40% among metastatic tumors.[23] Though PTEN loss most commonly occurs in the primary tumor with identical PTEN status across metastases within a given patient,[28] *PTEN* deletion occurs subsequent to some other alterations, such as *ERG* gene rearrangement[29,30] and is subclonal or heterogenous in up to 40% of primaries with underlying PTEN loss.[23,31]

Evidence

Most studies examining PTEN as a prognostic biomarker have focused on surgically treated patients and examined the clinical outcome of BCR (rising prostate-specific antigen [PSA] serum levels). In the majority of these studies, tumors with PTEN loss (either by FISH or IHC) have had a statistically significant increase in the hazard ratio (HR) of BCR compared with tumors with intact PTEN (with the HR typically around 1.2 to 1.5 after adjusting for Grade Group and stage).[32–35] Cases with hemizygous genomic deletion generally have outcomes somewhat intermediate between cases with intact *PTEN* and homozygous deletion.[32] Similarly, cases with heterogenous or subclonal protein loss typically have worse outcomes than those with intact PTEN but somewhat better outcomes than patients with clonal or homogenous PTEN protein loss.[24,26] Though studies of patients treated by radiation therapy have been fewer in number, PTEN loss is generally similarly prognostic in these cohorts as well.[36,37]

Despite the abundance of studies using this outcome measure, it is widely accepted that BCR is a suboptimal surrogate for metastasis and death. Thus most prognostic biomarkers must be tested for their association with metastatic or lethal prostate cancer.[38] Given that the duration of follow-up must be quite extensive to detect lethal events in surgically treated prostate cancer, many early studies of PTEN and lethal prostate cancer were performed in nonsurgical cohorts, including transurethral resection of the prostate or needle biopsy samples from patients who were conservatively managed. In these cohorts, PTEN has remained highly significant as a prognostic biomarker.[39,40] To date, there have only been 2 large studies of PTEN in surgical cohorts with metastasis or death as an outcome measure, including 1 population-based cohort and 1 single-institution cohort.[41,42] In both of these, PTEN loss remained significantly associated with the risk of lethal prostate cancer, even after adjusting for clinical-pathologic variables, with HR between 2 and 5.

While it is clear that PTEN is prognostic on its own, there has also been great interest in discerning whether PTEN loss may be more clinically significant in specific molecular contexts. Rearrangements involving the *ERG* gene are among most common structural variants in prostate cancer[43] and while not associated with poor outcomes by themselves in surgically treated cohorts,[44–46] *ERG*-rearranged prostate tumors are more likely to have PTEN loss than those that lack this rearrangement.[30,31,47–52] Accordingly, animal studies have suggested that there may be a synergistic effect of ERG expression and PTEN loss on prostate cancer progression.[48,49,53] In humans, using the endpoint of BCR in surgically treated cohorts, most studies examining the potential interaction between PTEN and ERG have had mixed results, with the largest studies indicating there is no interaction between the 2 alterations.[47,52] However, using the outcome of prostate cancer-specific mortality, several studies have found that PTEN loss in the *absence* of *ERG* gene rearrangement has worse outcomes compared with PTEN loss occurring with *ERG* gene rearrangement. In both conservatively managed,[40,51] as well as surgical cohorts,[40,41] *PTEN* deletion or loss was strongly associated with an increased risk of lethal prostate cancer among ERG-negative, but not among ERG-positive tumors, though not all studies have formally tested the statistical interaction. To date, it remains unclear why these data in humans differ as a function of the clinical outcome analyzed, and why they diverge so dramatically from murine models.

Because of its strong correlation with the pathologic grade, PTEN loss is perhaps most useful as a prognostic biomarker in the setting of prostate transrectal ultrasound–guided core biopsies. In this context, the pathologic stage is unknown and Grade Group may be underestimated due to insufficient sampling. Thus, patients with PTEN loss in Grade Group 1 tumors at biopsy have a nearly 3-fold increase in the likelihood of upgrading to Grade Group 2 or higher at radical prostatectomy.[54] Similarly, PTEN loss in Grade Group 2 biopsies is associated with an increased risk of nonorgan confined disease and BCR after radical prostatectomy.[55] Finally, at least one study has demonstrated that PTEN loss in core biopsies is associated with the development of metastasis, prostate cancer–specific mortality and CRPC in surgically treated patients.[56] While these studies suggest that PTEN loss may be useful in identifying patients who should avoid AS only 2 studies have directly tested this biomarker within AS cohorts. In the largest study to date, PTEN loss was associated with a 2.6-fold increase in upgrading on repeat biopsy.[57] In another case-control study, PTEN loss was less common among patients who remained on AS after 8 years of follow-up compared with patients who had rapid or significant upgrading, though this difference did not reach statistical significance in this relatively small case-control study.[58]

As PTEN and other prognostic biomarkers undergo extensive validation, it is essential to do comparison studies between markers to nominate only the best and most economical assays for clinical use. To date, only a few studies performing head-to-head comparisons of PTEN and RNA-based biomarkers (described in more detail below) have been published. Two of these studies have focused on surgical cohorts, with BCR as the outcome and have demonstrated that when PTEN and Oncotype Dx Genomic Prostate Score (GPS) or the Prolaris Cell Cycle Proliferation (CCP) score are both included in multivariable models, PTEN is no longer significantly associated with risk of recurrence.[59,60] However, PTEN and the Prolaris CCP assay performed comparably when metastasis and death were used as clinical outcomes and the concordance indices were formally compared for association with this outcome.[42] Of note, none of these studies included a cost-benefit analysis, which is essential given the difference in cost between IHC assays (a few hundred dollars) and the RNA-based assays (a few thousand

dollars) (Table 1). In addition, none of these studies were performed in the context of biopsy samples, which is the clinical setting where the biomarker testing would likely take place. It is clear that additional direct comparison studies are required to aid in the selection of the optimal tissue-based prognostic biomarker(s).

Discussion and Recommendations for PTEN and Ki-67

Taken together, the Working Group felt that current evidence supports the following recommendations:

- Ki-67 LI and PTEN are potentially useful prognostic biomarkers in the subset of Grade Group 1 (and/or Grade Group 2) prostate cancer biopsies where the clinician is seeking to determine whether the patient is eligible for AS.
- In this context, high Ki-67 LI or PTEN loss would be one factor (among several) suggesting that the patient should seek definitive treatment, while intact PTEN would not be an informative result in this regard.
- Testing could be recommended for the single highest grade/highest tumor volume core, with the option to test additional cores, based on results of recent studies suggesting that this may better capture overall heterogeneity and cases with subclonal PTEN loss.[61]
- This testing could be done by IHC (for Ki-67 and PTEN) and/or FISH (for PTEN) as determined by the pathologist.

However, while Ki-67 and PTEN remain among the most promising prognostic molecular biomarkers studied to date, the Committee agreed that additional dedicated studies of AS populations are warranted before widespread adoption.

mRNA-BASED GENOMIC SIGNATURES

Background

mRNA-based signatures have been gaining traction in recent years as new methods to aid in prognostication and potential therapy response in patients with localized prostate cancer. In the context of newly diagnosed prostate cancer, such tests may add to current clinical-pathologic prognosticators and may be useful for further refining risk stratification.[62–64] More recently, the applicability of such tests has also been extended to AS cohorts.[65,66] In the context of surgically treated patients, some of these assays aim to answer questions related to complex postsurgical management, such as whether patients with adverse pathology on radical prostatectomy require salvage radiotherapy or may safely avoid adjuvant radiation.[67,68]

Evidence

The best-studied RNA-based tests available commercially for clinical use include Prolaris (Myriad Genetics), Oncotype Dx (Genomic Health), and Decipher (GenomeDX Biosciences). Prolaris measures expression levels of a panel of 31 CCP genes by reverse transcriptase-polymerase

TABLE 1. Common Example of Commercial mRNA-based Tests Available Clinically

Test	Indication	Science	Results	Cost
Oncotype Dx Genomic Health	Biopsy-based NCCN very low, low and intermediate-risk Provides personalized risk assessment	Assay looks at the expression of 17 genes by RT-PCR within 4 pathways (androgen signaling, stromal response, cellular organization, proliferation) to assess tumor aggressiveness	GPS from 0 to 100 Likelihood of freedom from Dominant 4 or high Gleason score and/or nonorgan confined disease GPS is reflective of the biology of the tumor at the time of biopsy	$3820 Medicare = No ABN required* Other insurance: If estimated out-of-pocket cost > $100, the company will contact the patient to offer a financial assistance program
Prolaris (CCP) Myriad Genetics	Biopsy tissue–based test for patients who are AS candidates -or- Postprostatectomy tissue–based test to determine the relative risk of BCR	46-gene expression signature by RT-PCR includes cell cycle progression genes (CCP) selected based upon correlation with prostate tumor cell proliferation	Prolaris score Biopsy is < or = or > AUA risk group and estimates 10 y mortality risk Postsurgical is similar but 10 y risk for BCR	$3400 Medicare = No ABN required* Other insurance: If estimated out-of-pocket cost > $375, the company will contact the patient to make arrangements … they have a financial assistance program
Decipher GenomeDx Biosciences	Postprostatectomy tissue–based test used for patients who are candidates for secondary therapy postsurgery (pT2 with positive margins or any pT3 or BCR) More recent studies in needle biopsy cohorts to predict adverse pathology and oncologic outcomes after radical prostatectomy	Analyzes the expression of 22 genes in multiple pathways using Affymetrix microarrays to measure the tumor's biological potential for metastasis	Decipher reports the probability of metastasis at 5 y after surgery and 3 y after PSA recurrence	$4250 Medicare = No ABN required*; Centers for Medicare & Medicaid Services (CMS) draft coverage policy published Other insurance: Company contacts all patients to offer a financial assistance program Majority of patients have out-of-pocket cost no > $395

*Without an ABN (Advanced Beneficiary Notification) the patient is not held responsible for any unreimbursed expenses.
AUA indicates American Urological Association.

chain reaction (RT-PCR) (Table 1). It was initially validated to determine the risk of death from prostate cancer in a cohort of conservatively managed patients using core biopsy specimens.[62] However subsequent studies expanded its use to cohorts of surgically treated and radiation-treated patients, where the CCP score is associated with BCR when measured in the core biopsy.[69,70] Finally, a few studies have validated this assay using radical prostatectomy specimens, where higher CCP is associated with risk of BCR and metastasis[71] and could potentially support decision-making for use of adjuvant therapies in this setting. To date, CCP has not been studied in an AS population.

Similar to CCP, Oncotype Dx GPS is an RT-PCR based assay that measures expression levels across a 17 gene panel including stromal response, androgen signaling, cellular organization, and proliferation genes.[63] Oncotype Dx GPS has predominantly been validated in biopsy samples from patients who were subsequently surgically treated, where GPS is associated with increased risk of adverse pathology at radical prostatectomy that adds to the concordance index of the Cancer of the Prostate Risk Assessment (CAPRA) clinical-pathologic risk classifier.[72] In addition, Oncotype Dx GPS has more recently been evaluated for utility in biopsies from patients on AS who underwent radical prostatectomy, where it was associated with a higher risk of adverse pathology and BCR.[66] Finally, the Decipher genomic classifier includes 22 genes where expression is measured by high-density Affymetrix microarrays.[64] This test has been most extensively validated using radical prostatectomy tissue for the purpose of predicting the risk of metastasis and assisting with clinical decision-making surrounding the use of adjuvant radiation and hormonal therapy.[73–75] More recent studies have examined the Decipher classifier in the context of core biopsies.[76] Though not yet reported in AS populations, at least one study in men who were candidates for AS found that Decipher improved on CAPRA risk assessment if the conventional cutoff point was lowered.[63,65]

Although considerable progress has been made to date, moving forward, it is clear that prospective studies testing these RNA-based assays in actual AS cohorts will be required. In addition, future studies should focus on validating them in conjunction with updated pathologic Grade Groups and newer grading initiatives, such as reporting of percentages of Gleason pattern 4 tumor and/or the presence of cribriform Gleason 4 pattern, which may add additional prognostic value.[77] Though a few cost-effectiveness studies have emerged recently, additional studies are required before widespread adoption of these costly tests can be recommended.[78]

Given that prostate cancer is a heterogenous disease that is often multifocal in nature, studies evaluating the reliability of RNA-based tests to inform on unsampled or adjacent higher Grade Group tumor foci are needed.[79] Unsurprisingly, in a recent study, derived RNA-based tests failed to predict the presence of unsampled higher grade foci in the context of multifocal disease when they are performed on lower Grade Group foci.[80] This highlights a global issue for all molecular tests performed on biopsy tissue in prostate cancer, and additional studies are necessary to determine the best ways to mitigate the risks of undersampling for tissue-based biomarker assays. Certainly the advent of multiparametric magnetic resonance imaging–guided biopsies has the potential to improve sampling in prostate cancer and prospective studies combining molecular testing with image-guided biopsies are needed.

Discussion and Recommendations for mRNA-based Genomic Signatures

The ISUP survey documented that close to 60% of practicing pathologists have never used RNA-based tests on core biopsy specimens and the majority of such tests are performed after clinician requests. This may be due to the fact that there is commercial marketing of these tests directly to the urologists. In addition, $>70\%$ of pathologists reported that they do not perform such tests in the setting of radical prostatectomy.

The working group felt that RNA-based genomic signatures are of potential benefit in the biopsy and radical prostatectomy settings, with careful attention to what test is ordered for which indication based on previous validation studies for specific tests.[81] However, the potential for undersampling remains a major concern for all tissue-based tests and future clinician input may be useful to shape any additional definitive recommendations given that clinicians order the majority of these assays.

- Genomic signatures are of potential benefit in providing additional information regarding progression risk for prostate cancer in the AS and postradical prostatectomy settings. However, they would do so only if the focus harboring the disease was adequately sampled and not missed completely.
- The improvement in such signatures should be compared with implementing robust pathologic assessment and potential use of IHC biomarkers which needs further validation
- Studies are needed to assess signatures performance in relation to heterogeneity in needle biopsy samples and to confirm the proper way of performing such tests (ie, which biopsy to use vs. using multiple biopsies from different foci, etc.)

Predictive Biomarkers

Predictive biomarkers estimate the chances of response to specific therapy and cannot be distinguished from prognostic biomarkers unless 2 different therapies are compared for biomarker-positive and biomarker-negative patients.[82] Although there is interest in developing predictive biomarkers for the localized setting in prostate cancer (eg, to determine whether patients benefit more from radiation or surgical therapy), to date, predictive biomarkers have largely been studied in the context of metastatic disease. Despite their importance for precision medicine, relatively few tissue-based predictive biomarkers have been validated.

DNA REPAIR DEFICIENCY

Background

One area of particular excitement in this area in recent years has been in the field of DNA repair deficiency.[83] Sequencing efforts in metastatic prostate cancer demonstrated a surprisingly high rate of genomic alterations in genes involved in the homologous recombination DNA repair pathway—approaching 20% of cases of advanced CRPC and including alterations in the *BRCA2*, *BRCA1*, and *ATM* genes most commonly.[84] Perhaps even more surprising was the fact that nearly half of these advanced cases with homologous recombination defects (HRD) had germline mutations in these genes, comprising close to 10% of men with CRPC.[84,85] The nearly 2-fold enrichment in somatic HRD mutations in metastatic compared with the primary disease[86] strongly suggested that these alterations could be associated with the development of aggressive disease and a number of subsequent studies have confirmed this hypothesis for germline HRD.[87–90] Germline alterations in *BRCA2* and *ATM* are significantly more common in lethal compared with indolent primary prostate cancer[91] and associated with grade reclassification in AS populations.[92] Further, in aggressive histologic subsets of primary prostate cancer (including ductal and intraductal prostate cancer and primary Gleason pattern 5 disease) the prevalence of HRD mutations (both germline and somatic) reaches or even exceeds that seen in metastatic disease in recent studies.[93–98]

Evidence

Given the large number of studies of HRD as a predictive biomarker in breast and ovarian cancer over the last 2 decades, it is perhaps unsurprising that HRD mutations are predictive for therapy response in metastatic prostate cancer as well, at least in initial studies. As in breast and ovarian carcinomas, CRPC cases with germline HRD alterations have improved responses to platinum chemotherapy in retrospective studies.[99,100] Even more exciting is the apparent specific response to poly-ADP ribosylase (PARP) inhibitors among CRPC cases with HRD mutations. In the phase II TOPARP-A trial, 88% (12/14) of men with HRD responded to the PARP inhibitor olaparib.[101] Based in large part on these results, both olaparib and rucaparib have received "Breakthrough Therapy" designation by the Food and Drug Administration (FDA) for priority review in CRPC. Recent reports from the Triton2[102] and GALAHAD[103] trials have also reported responses among patients with *BRCA2* mutations and the biomarker-selected PROfound trial[104] suggested that biomarker-selected HRD patients derive more benefit from olaparib than enzalutamide or abiraterone in the setting of CRPC. Notably, however, these trials and some retrospective studies[105] have failed to confirm responses among many cases with *ATM* mutations, suggesting that not all HRD mutations are similarly predictive for therapy response and highlighting the need for a functional biomarker of HRD in prostate cancer.

Defects in the MMR pathway are also enriched in metastatic compared with primary prostate cancer. Pathogenic mutations in MMR genes are seen in up to 10% of CRPC cases, compared with <3% of primary tumors if all Grade Groups are considered together.[106–108] However, similar to HRD, MMR mutations are highly enriched among primary tumors with aggressive histology, including both ductal adenocarcinomas and primary Gleason pattern 5 tumors as recently reported.[93,109,110] Relative to HRD alterations, fewer of the MMR mutations (~20%) in prostate cancer are germline, though prostate cancer is now understood to be enriched among patients with Lynch syndrome.[111,112] Though prostate cancer has shown only rare responses to immunotherapy in most trials (with programmed death-ligand 1 expression in only 8% of primary tumors and 32% of CRPC[113]), recent trials have demonstrated anecdotal responses among patients with underlying MMR defects[114] and the FDA has approved pembrolizumab for use in all progressing tumors with MMR defects or microsatellite instability (MSI) based largely on data in colorectal carcinoma.[115] Indeed, 2 small retrospective series in CRPC patients documented responses to checkpoint blockade among 2 of 4 patients in one series[116] and 6 of 11 patients in a second series,[108] though whether responses will be as durable as those observed in colorectal cancers with MMR defects remains unclear.

Discussion and Recommendations for DNA Repair Deficiency Markers

In the ISUP survey preceding the conference, close to 20% of respondents had tested BRCA1/2 mutation status in tissue specimens of advanced prostate cancer in the past year, while close to 30% had tested MMR protein status. Currently, the NCCN guidelines for prostate cancer recommend germline testing in high-risk subsets of patients with clinically localized prostate cancer, including all patients with Grade Group 4 or higher tumors or patients with PSA of ≥ 20 ng/mL.[117] In addition, germline testing should be performed in all patients with the metastatic disease if clinically indicated, with appropriate genetic counseling. Currently, all metastatic patients should also be offered somatic genomic testing of tumor tissue for HRD and MMR defects if clinically indicated. The panel agreed with these current recommendations and additionally addressed the important questions of which tissues should be tested and which genomic tests should be performed as pathologists frequently face these issues. The majority of DNA repair defects appear to be early driver events based on studies to date,[108,109] thus though the sequencing of metastatic tissues is preferred, where this tissue is not available, testing of the primary tumor may be performed. Defective MMR assessment may be performed via IHC for MMR proteins (MSH2, MSH6, MLH1, and PMS2), which appears specific for underlying genomic alterations, though formal testing of sensitivity has not been performed in the setting of prostate cancer. Importantly, polymerase chain reaction–based MSI testing has been demonstrated to be insensitive in prostate cancer compared with IHC and

more extended MSI testing by next-generation sequencing assays.[109,118] Thus, polymerase chain reaction–based MSI testing should be performed in concert with IHC and/or sequencing wherever feasible. Targeted sequencing assays may not detect inactivating rearrangements in MMR genes, and thus should be paired with IHC assays where there is suspicion for MMR defects based on the high mutational burden or MSI.

Recommendations of the Working Group were the following:

- In combination with appropriate genetic counseling, germline panel testing for DNA repair gene alterations should be offered (if clinically indicated) to patients with:

  Localized Grade Group ≥ 4 tumors.
  Any Grade Group with PSA ≥ 20.
  Known metastatic disease.

- Somatic tumor DNA testing should be offered to all patients with the known metastatic disease if clinically indicated. It can be performed on metastatic tissue or, if unavailable, primary tissue. Testing should include:

  Defective MMR assessment via MMR IHC for MSH2, MSH6, MLH1, PMS2 with or without MSI testing and/or sequencing of MMR genes (and tumor mutation burden estimate).

  AND

  Defective HR assessment via sequencing for *BRCA1*, *BRCA2* at a minimum, with the ability to detect copy number alterations.

ANDROGEN RECEPTOR-RELATED MARKERS

Background

Metastatic prostate cancer is frequently treated initially with anti-androgen therapy (either alone or in combination with other agents), with most patients responding. Subsequent progressive disease despite castrate levels of serum testosterone is termed CRPC. Additional therapies directly or indirectly targeting the androgen receptor (AR) signaling are frequently used in CRPC and include agents designed to block androgen synthesis (abiraterone) or direct AR antagonists (enzalutamide and apalutamide). Although initially thought to be independent of AR signaling, CRPC usually remains dependent on AR signaling, and *AR* is the most frequently altered gene in CRPC.[1,119] In addition to point mutations and amplifications of *AR*, amplification of enhancers of *AR* signaling are also frequent, along with the expression of *AR* splice variants that lack the ligand-binding domain and result in constitutive activation.[28,120–124]

Evidence

Building from gene expression profiling studies in cell lines showing increased *AR* expression in castration-resistant models over 15 years ago,[125] large scale genomic studies have shown that at least 50% of CRPC harbor *AR* mutations (hotspots) or amplifications, in addition to amplifications of *AR* enhancers[28,120,121,123,124] (Fig. 3A). Such genomic *AR* alterations can also be detected in cell-free DNA (cfDNA),[126] and importantly are essentially absent in untreated prostate cancer.[121,125] In addition, again based on discoveries from cell line models,

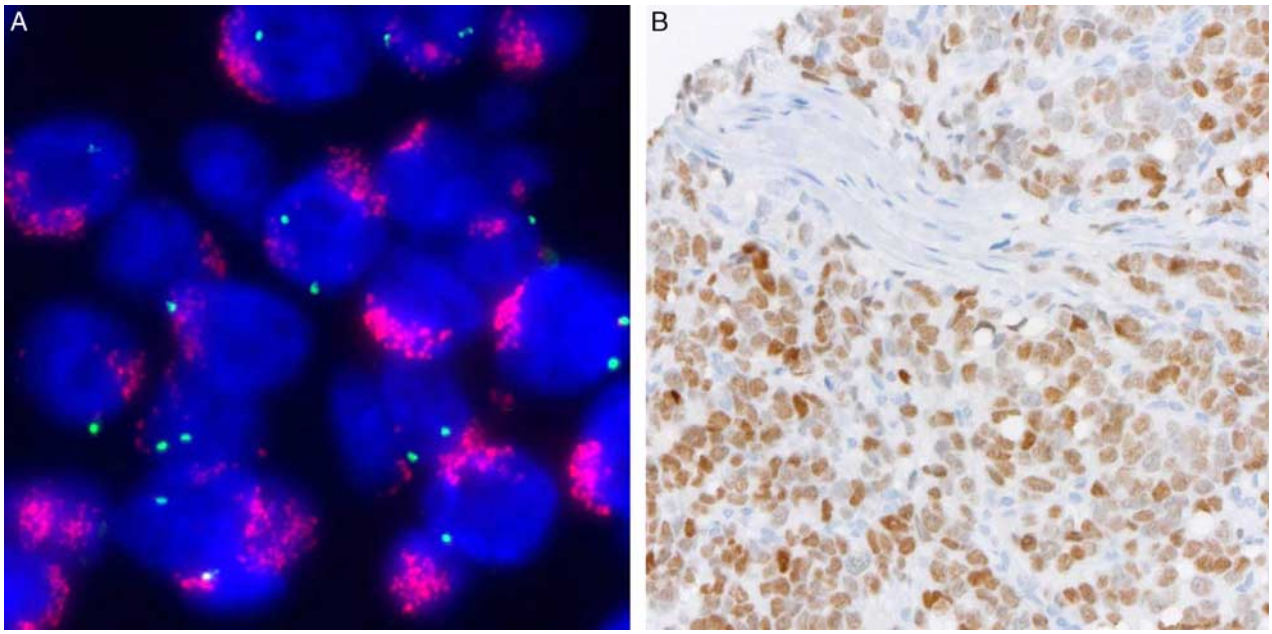

FIGURE 3. *AR* amplification and ARV7 expression in CRPC. A, FISH showing high-level *AR* amplification with tight clusters of red *AR* signals and few green CEPX reference signals. B, IHC showing homogeneous nuclear ARV7 expression (rabbit monoclonal antibody clone RM7; Dianova).

expression of *AR* splice variants, most commonly *ARv7*, have been frequently detected in CRPC.[127] *ARv7* and other *AR* splice variant expression rates vary across detection methodology (eg, RT-PCR, RNAseq, or IHC), biospecimen (eg, tissue samples vs. circulating tumor cells [CTCs]), and line of therapy[1,120–122,127–131] (Fig. 3B). Importantly *ARv7* and other splice variants have also been detected at least in low levels in both untreated prostate cancer as well as benign prostate tissue.[121,122]

To date, tissue-based assessment of *AR* alterations, including *ARv7* expression, have not been shown to be strongly prognostic or, more importantly, predictive in CRPC.[120,121,123] Of note, by a variety of methods, many men with CRPC harbor clonal populations with distinct *AR* alterations and varying AR signaling output, and *ARv7* expression is very common in tumors after castration.[28,122,132] Hence, in part due to the infrequency of routine biopsy of CRPC metastases, *ARv7* detection in CTCs and *AR* amplifications in cfDNA are the most clinically advanced *AR*-based molecular biomarkers.

In both retrospective and prospective studies, *ARv7* detection in CTCs by both RT-PCR and IHC has been shown to be prognostic with respect to AR signaling inhibitors in CRPC.[130,131,133] ARv7 detection in CTCs by IHC has also been shown to be predictive in a retrospective study, with patients who harbor ARv7-positive CTCs showing benefit from taxane chemotherapy versus AR signaling inhibitors.[129] This IHC-based CTC test, Oncotype Dx ARV7 Nucleus Detect Test, has received a positive local coverage determination in the United States for men with CRPC. *AR* amplifications by cfDNA have also been shown to be strongly prognostic in multiple retrospective clinical trials and prospective/retrospective nontrial cohorts.[126,132,134–139] Importantly, for both *ARv7* in CTCs and *AR* amplifications in cfDNA, prospective randomized trials incorporating these biomarkers demonstrating their prognostic or predictive ability have not been reported.[128]

Discussion and Recommendations for AR-related Markers

The ISUP survey demonstrated that the vast majority of urologic pathologists have limited experience with *AR* as a tissue or blood-based biomarker in advanced prostate cancer. More than 80% of responding pathologists have not used *AR* mutations, *AR* amplifications, *ARv7* expression or AR expression by IHC as a tissue-based biomarker in CRPC in the past year. More than half have never been asked by a clinician to order these biomarkers, with 20% of responding pathologists reporting being asked in <1% of cases. Likewise, >90% report their institution does not provide *ARv7* testing from liquid biopsies to predict response to AR signaling inhibitors.

On the basis of the above, the following recommendations were made by the Working Group:

- At present, tissue-based *AR* alteration assessment (amplifications, mutations, expression, splice variant expression) has no clear clinical utility.

AR amplification and *ARv7* expression are prognostic in CRPC; emerging evidence suggests that ARv7 and AR amplification may be predictive, however, the evidence is not yet sufficient to justify systematic ARv7 or *AR* amplification testing.

Diagnostic Biomarkers

Diagnostic biomarkers are the most familiar to pathologists and are most commonly deployed to assist in the initial diagnosis of prostate cancer on core biopsy samples. Tissue-based diagnostic biomarkers utilized in this context include IHC stains for basal-cell markers and have been reviewed elsewhere.[140] In the last decade, several newer tissue-based and urine-based markers have also been introduced to assist with risk stratification in the setting of a negative biopsy.[141] Here, we will focus exclusively on molecular markers to assist in the histologic classification of prostate tumors at diagnosis, specifically in the setting of NE differentiation which may function as a predictive biomarker of response to AR-targeted therapy in the primary and, more commonly, metastatic settings.

NEUROENDOCRINE PROSTATE CANCER

Background

The diagnosis of small cell or neuroendocrine prostate cancer (NEPC) is rare in clinically localized disease but frequently associated with poor outcomes.[142] In advanced CRPC, small cell or NE morphology is observed in around 10% of cases that have undergone biopsy[120,143] and may develop as a form of linage plasticity enabling resistance to AR-targeted therapies. The clinical median survival for men with metastatic NEPC is poor with a median 7 months survival.[144] Though no large trials have been conducted, in their pure form, these tumors generally respond poorly to AR-targeted therapies leaving clinicians with limited treatment options that include platinum chemotherapy or, in the setting of clinical trials, newer targeted therapies. Since this diagnosis will dramatically change treatment strategies in the CRPC setting, accurate pathologic diagnosis is critical.

A major challenge for practicing pathologists is the accurate classification of CRPC given the lack of experience by most pathologists with this disease state and the lack of clarity regarding how to integrate new molecular findings. In its most classical form, small cell NEPC may be recognized by classic morphologic features, including sparse cytoplasm, small and often overlapping nuclei, and the absence of prominent nucleoli. The chromatin is described as "salt and pepper" in appearance. However there is increasing recognition of a wide spectrum of NE differentiation present in primary tumors, and particularly in CRPC, this may present a diagnostic dilemma for the practicing pathologist and necessitate the use of molecular biomarkers[145] (Fig. 4).

Evidence

The most recent 2016 WHO classification delineates 5 manifestations of NE differentiation in prostate cancer

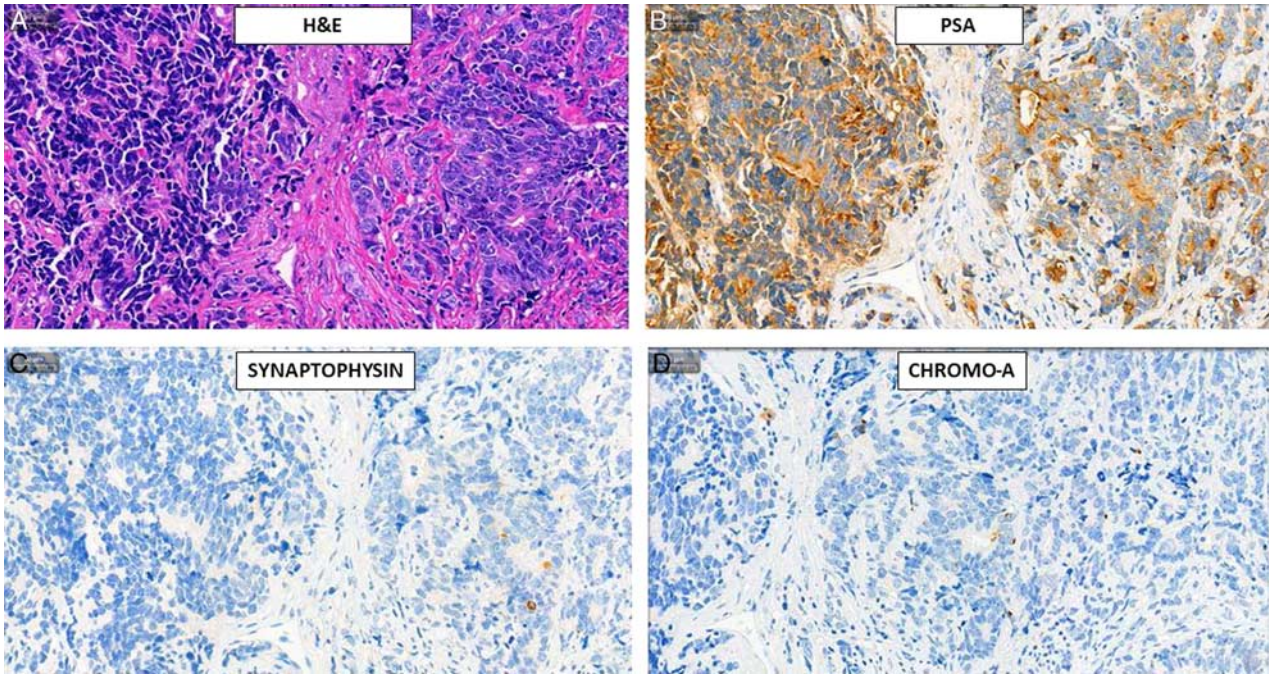

FIGURE 4. Brain metastasis with overlapping features between small cell NE carcinoma and conventional acinar adenocarcinoma. A, Two distinct adjacent areas with NE features on left and adenocarcinoma on the right. B, PSA positivity in both components. C and D, Lack of expression of NE markers synaptophysin and chromogranin-A, respectively. H&E indicates hematoxylin and eosin.

(Table 2).[146] To better represent the observed spectrum, other authors have recently added "mixed adenocarcinoma and neuroendocrine carcinoma" and "amphicrine carcinoma".[145,147] Morphologic classification of small cell NEPC has historically been assisted by application of NE IHC markers, such as chromogranin, synaptophysin, and CD56, however, it is recognized that these markers individually are not highly sensitive for small cell NEPC.[142] More importantly, NE markers are not specific for small cell NEPC and may be expressed, at least focally, in a large proportion of usual-type adenocarcinomas and have limited prognostic value in the primary setting.[148,149] Genomically, similar to its lung counterpart, NEPC has an increased frequency of *RB1* and *TP53* inactivation.[120,150,151] However, these alterations are not specific to NEPC and can be observed in usual-type adenocarcinoma, particularly in the CRPC setting.[120] Finally, AR expression and signaling may be absent or low based on IHC or gene expression in small cell NEPC, however, this finding is also not specific for small cell NEPC (can be seen in AR-independent, non-NEPC) and is also insensitive for small cell NEPC as some subsets of cases may express AR.[152]

TABLE 2. 2016 WHO Genitourinary Neuroendocrine Tumor Classification

2016 WHO Genitourinary Tumor Classification[146]
NE cells in usual prostate cancer
Adenocarcinoma with Paneth cell-like NE differentiation
Well-differentiated NE tumor (carcinoid)
Small cell NE carcinoma
Large cell NE carcinoma

Recently, precision oncology studies have focused on molecular and pathology alterations seen during the course of androgen deprivation therapy.[120,143] One recent study looking at 444 CPRC cases treated with standard-of-care AR signaling-targeted therapies demonstrated a range of histologies, including 10% with NE features.[120] Even with more extensive genomic profiling, the overlap between morphologic NE features and molecular alterations at the gene transcript or DNA level was good but remained imperfect. CRPC tumors, which would be classified as adenocarcinoma without NE features, may display NE molecular features in some instances. This observation may reflect a type of resistance also seen in epidermal growth factor receptor-mutant non–small lung cancers that undergo lineage plasticity towards small cell carcinoma during the course of targeted therapy.[153] Similar to lung tumors, this change from adenocarcinoma to small cell NEPC roughly parallels the lack of response to AR signaling inhibitors. From a clinical perspective, accurate classification of these tumors is extremely important given the variety of therapies available today for men with advanced prostate cancer. Ultimately, dedicated, biomarker-driven clinical trials must occur to better define the most clinically relevant biomarkers of NEPC.

Discussion and Recommendations for NEPC Markers

For clinically localized prostate cancer, focal NE differentiation does not clearly impact biological behavior. For advanced CRPC, we still need to define the molecular and morphologic features that are predictive of a lack of response to AR signaling-targeted therapy. Though mo-

lecular markers such as *TP53* and *RB1* inactivation or AR expression may be helpful, they are not sensitive or specific on their own and <10% of survey respondents endorsed using these markers.

The current recommendations of the Working Group are the following:

- For clinically localized prostate cancer, unless there are clear morphologic NE features, immunostaining for NE expression (eg, synaptophysin, chromogranin, or CD56) is not recommended.
- Given its clinical implications, the term NE differentiation is best reserved for high-grade cancers and not usual-type adenocarcinomas or well-differentiated NE tumors.
- Advanced metastatic CRPC may manifest a range of morphologic features of NE differentiation and a combination of molecular evaluation and morphologic features may be required in future definitions of CRPC, guided by biomarker-driven clinical trials.

CONCLUSIONS

Only a few pathologists are applying molecular markers in prostate cancer in routine practice due to insufficient evidence, lack of standardization, and the complexity of the diverse disease stages, treatment modalities and clinical endpoints. However, Ki-67 and PTEN are emerging as potentially useful and widely available prognostic markers to support treatment decisions at an early stage. Similarly, mRNA-based commercial genomic signatures can help stratifying the risk of progression in individual patients, although more studies are needed before the widespread use of prognostic markers can be recommended. There has been considerable progress of predictive marker analysis in poorly differentiated and/or clinically advanced and CRPC, where markers of DNA repair deficiency (eg, *BRCA1/2* mutation and MMR defects) provide new opportunities for personalized treatment with PARP inhibitors and/or immunotherapy. Moreover, a better understanding of enhanced AR signaling (eg, *AR* amplification and ARV7 expression) and NE transdifferentiation as mechanisms of castration resistance can impact patient management.

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