

Molecular Genetics of Prostate Cancer and Role of Genomic Testing



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KEYWORDS

- Prostate cancer • Molecular profiling • Homologous recombination deficiency
- Microsatellite instability • Targeted treatment • PARP inhibitors • Genomic classifiers
- Germline testing

Key points

- PCa molecular landscape is profoundly heterogeneous and a classification into 7 molecular subgroups has been proposed based on molecular profiling of localized PCa (TCGA cohort).
- For localized PCa, several genomic classifiers are available to predict risk of distant metastasis, or/and PCa-related mortality. These assays can support clinical decision making regarding active surveillance or indication of adjuvant therapy following radical prostatectomy.
- In metastatic disease, main therapeutically actionable alterations are HRD, MSI-H, and CDK12 deficiency. Alterations in DNA damage response genes are present in 19% of localized and up to 31% of metastatic PCas. Genomic instability assays assessing HRD may predict sensitivity to PARP inhibitors.
- MSI-H (1–5%) sensitizes to immune checkpoint inhibitors (ICIs) and can be identified by immunohistochemistry (IHC), microsatellite PCR, or NGS, as well as sequencing of mismatch repair (MMR) genes. CDK12-deficient PCas (1%–5%) present variable degrees of sensitivities to ICIs.
- Germline testing is recommended in patients with personal history of high or very-high-risk localized PCa, regional or metastatic PCa, as well as family history of PCa.

ABSTRACT

Prostate cancer (PCa) is characterized by profound genomic heterogeneity. Recent advances in personalized treatment entail an increasing need of genomic profiling. For localized PCa, gene expression assays can support clinical decisions regarding active surveillance and adjuvant treatment. In metastatic PCa,

homologous recombination deficiency, microsatellite instability-high (MSI-H), and CDK12 deficiency constitute main actionable alterations. Alterations in DNA repair genes confer variable sensitivities to poly(ADP-ribose)polymerase inhibitors, and the use of genomic instability assays as predictive biomarker is still incipient. MSI can be assessed by immunohistochemistry. To date there is a lack of consensus as to testing standards.

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OVERVIEW

Comprehensive molecular profiling of large cohorts of primary prostate cancer (PCa) and metastatic PCa (mPCa), using genome-wide next-generation sequencing (NGS) approaches, has significantly contributed to the characterization of the profoundly heterogeneous molecular landscape of PCa.^{1–5} These studies have identified main therapeutically actionable molecular subtypes of PCa, such as homologous recombination-deficient (HRD), defined as harboring alterations in the homologous recombination repair pathway (HRR)⁶ and more strictly an HRD mutational signature^{7,8}; microsatellite instability-high (MSI-H); or CDK12-deficient tumors.^{1–5} In localized disease, multiple studies have shown that gene expression assays performed on PCa biopsies or prostatectomy samples can predict risk of metastatic progression and PCa-specific mortality,^{9–13} and current clinical guidelines integrate these assays as a useful tool to support clinical decision making regarding active surveillance and indication of intensification of therapy following radical prostatectomy (RP).¹⁴

In the metastatic disease scenario, recent clinical studies have demonstrated efficacy of targeted treatment of specific molecular subtypes of PCa, such as poly(ADP-ribose)polymerase (PARP) inhibitors for HRD PCas,^{15–19} and immune checkpoint inhibitors (ICIs) for MSI-H^{20,21} and a subset of CDK12-deficient²² PCas. These advances in PCa precision oncology treatment have motivated an increasing demand on genomic testing for patients with mPCa, starting from early treatment lines.²³ In this article, the molecular alterations reported in localized and advanced PCa are summarized, and main molecular diagnostic assays are reviewed, with focus on gene expression assays for localized PCa and genomic instability and MSI assays for advanced disease.

MOLECULAR LANDSCAPE OF LOCALIZED AND METASTATIC PROSTATE CANCER

Most frequent genomic alterations in localized PCa and mPCa are fusions implicating members of the E26 transformation-specific (*ETS*) transcription factors family. Concretely, the *TMPRSS2-ERG* fusion is the overall most frequent molecular alteration, found in 40% to 50% of PCas.^{1,2,4,24,25} Following the *ETS* fusions, most frequent genomic alterations in localized PCa are found in *PTEN* (17%), most frequently homozygous deletions; *SPOP* (11%); *TP53* (8%); and *FOXA1* (4%).¹ Based on whole exome sequencing (WES) data from 333

primary PCas, The Cancer Genome Atlas (TCGA) Research Network proposed PCa classification into the following 7 molecular subtypes: PCas with *ERG* (46%), *ETV1* (8%), *ETV4* (4%), and *FLI1* (1%) fusions and *SPOP*- (11%), *FOXA1*- (3%), and *IDH1*-mutated (1%) PCas.¹ *SPOP* mutations are the most frequent mutations in localized PCa and are mutually exclusive with the *ETS* fusions.¹ This molecular classification could cluster 74% of the analyzed tumors. The remaining “not-clusterable” group of PCa tumors (26%) was enriched in mutations in *TP53*, *KDM6A*, and *KMT2D*; deletions in chromosomes 6 and 16; as well as *MYC* and *CCND1* amplifications (Fig. 1).

In addition, molecular profiling data from several cohorts of mPCa are available. Whole genome sequencing (WGS) of the CPCT-02 cohort, consisting of 197 metastatic castration-resistant PCas (mCRPC), revealed that 68% of the cases could be clustered into the 7 subtypes proposed by the TCGA classification, and that the therapeutically actionable subtypes HRD, MSI-H, and *CDK12*^{-/-} (tandem duplication genotype) did not show correlation with the TCGA subgroups.⁴ Other studies have shown that *CDK12* alterations are relatively mutually exclusive with *SPOP*, *ETS* fusions, *TP53*, and *PTEN/PIK3CA* alterations.^{22,26} In the CPCT-02 cohort, mutations in *AR*, *TP53*, *ZMYM3*, *APC*, *RB1*, *CDK12*, *ERF*, and *ZFP36L2* were significantly enriched in mCRPC when compared with the TCGA cohort.⁴ WES and transcriptomics profiling of the SU2C-PCF cohort including 150 patients with mCRPC showed that most frequently altered genes in mCRPC are *AR* (62.7%), most frequently amplifications; *ETS*-family members (56.7%); *TP53* (53.3%); and *PTEN* (40.7%). Also in this cohort, *AR* and *TP53* alterations were enriched in mCRPC when compared with primary PCas, with *AR* and *GNAS* mutations being uniquely found in mCRPC.² In addition to the *ETS* fusions, other fusions uncovered involved *BRAF*, *RAF1*, *PIK3CA/B*, and *RSPO2*.² From a therapeutic point of view, whereas *SPOP* mutations are associated with longer response to androgen receptor signaling inhibitors (ARSI), shorter responses have been associated with alterations in *RB1*, *TP53*, and *AR*. Moreover, alterations in *RB1* have been correlated with shorter overall survival (OS)³ (see Fig. 1).

ALTERATIONS IN DNA REPAIR

Alterations in Homologous Recombination, Fanconi Anemia Pathway, and CDK12

Alterations in DNA damage response (DDR) genes have been reported in 19% of the 333 primary PCa tumors from the TCGA cohort, including alteration

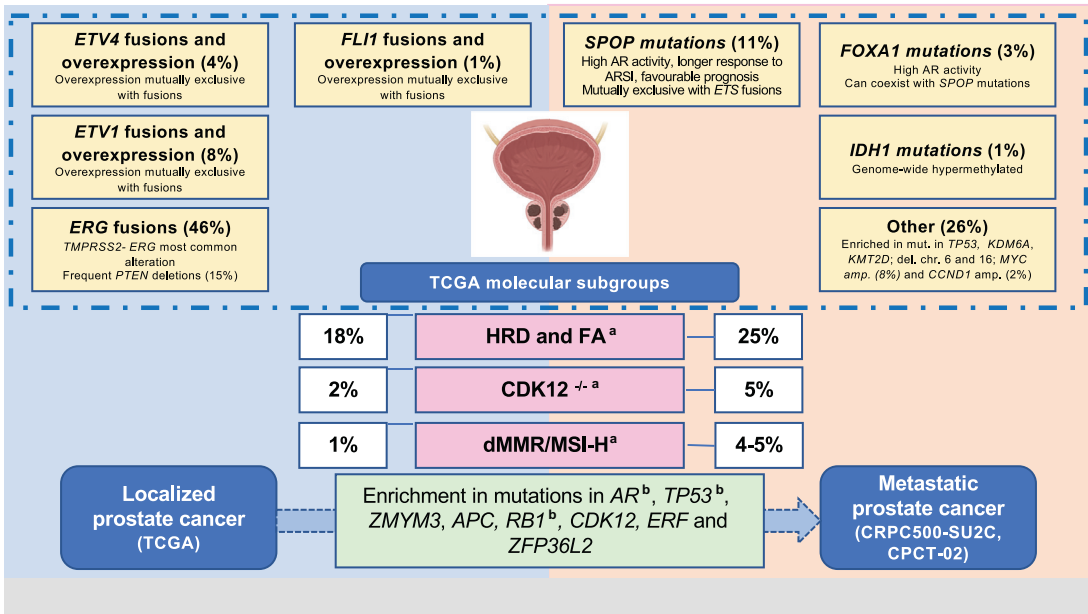


Fig. 1. Molecular landscape of localized and metastatic prostate cancer. Genomic alterations reported from the analysis of the TCGA, CRPC500-SU2C, and CPCT-02 cohorts are illustrated. amp., amplification; ARSI, androgen receptor signaling inhibitors; chr., chromosome; del., deletions; dMMR/MSI-H, mismatch repair deficient, microsatellite instable-high; FA, Fanconi anemia pathway; mut., mutations; OS, overall survival. ^aActionable alterations; ^bAlterations in *RB1*, *AR*, and *TP53* associated with shorter response to ARSI and *RB1* additionally with shorter OS.

in the distinct genes involved in the HRR⁶⁻⁸ and Fanconi anemia (FA) pathways, as well as *CDK12*.^{1,27} Similarly, the SU2C-PCF cohort reported alterations in DDR genes in 23% of the cases.^{2,3} A cohort of 3476 PCas (1660 samples from localized stage and 1816 mPCa samples) has been molecularly profiled using the FoundationOneCDx assay.⁵ Alterations in the HRR and FA pathways have been uncovered in 24.4% of the cases, with most frequent alterations found in *BRCA2* (9.8%) and *ATM* (5.2%).⁵ In addition, 5.6% of the cases had alterations in *CDK12*.⁵ In the TCGA cohort of localized PCas, the most frequent alterations were found in *FANCD2* (7%), *ATM* (4%), *BRCA2* (3%), and *RAD51 C* (3%),¹ and, in the mCRPC SU2C-PCF cohort, in *BRCA2* (13%) and *ATM* (7.3%).^{2,27}

Alterations in Mismatch Repair Genes

MSI-H or mismatch repair deficiency (dMMR) has been reported in 1% to 5% of PCas.^{1,2,28,29} In the SU2C-PCF, 3 cases of *MSH2* (2%) and 1 case of *MLH1* (0.7%) mutations have been reported, corresponding to hypermutated tumors with high tumor mutational burden (TMB).² In the PCa cohort analyzed by FoundationOneCDx assay, 4% of the cases harbored alterations in MMR genes (most frequently in *MSH2* and *MSH6*, followed by *MLH1* and *PMS2*) and 0.1% in *POLE* (V411 E).⁵ In a cohort of 433 patients

with mPCa, who underwent liquid biopsy with targeted cell-free DNA (cfDNA) sequencing, pathogenic mutations in *MSH2* or *MSH6* were uncovered in 2.3% of the cases.²⁸

GERMLINE ALTERATIONS

Main germline alterations reported in PCa involve DDR and MMR genes. In the SU2C-PCF cohort, germline alterations in DDR genes were found in 8% of the patients, most frequently *BRCA2* mutations (5.3%), followed by *ATM* (1.3%) and *BRCA1* (0.7%).² In a study including 3607 men diagnosed with PCa and who received germline testing, germline variants associated with PCa were identified in 17.2% of patients,³⁰ with most frequent alterations found in *BRCA2* (4.7%), *CHEK2* (2.9%), *MUTYH* (2.4%), and *ATM* (2.0%). In this study distinct assays had been used, covering between 2 and 80 genes. Alterations in *HOXB13* were reported in 1.1%, and in MMR genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*), in 1.7% of the patients. Screening for germline alterations across 20 DDR genes in a cohort of 692 unselected patients with mPCa uncovered pathogenic mutations involving 16 genes in 11.8% of the studied cohort, with most frequent alterations in *BRCA2* (5.3%), *ATM* (1.6%), *CHEK2* (1.9%), and *BRCA1* (0.9%).³¹ When compared with metastatic cohorts, the frequency of germline alterations in localized PCa (TCGA) was 4.6%.¹

THE ROLE OF GENOMIC TESTING IN LOCALIZED AND ADVANCED PROSTATE CANCER

GENE EXPRESSION ASSAYS FOR RISK STRATIFICATION IN LOCALIZED PROSTATE CANCER

For localized PCa, active surveillance is recommended for patients with very-low-risk and most patients with low-risk PCa.¹⁴ Several studies have shown that in addition to clinical and pathologic features, gene expression assays can improve risk stratification for localized PCa.^{11–13,32,33} Based on this, these assays have been integrated into the routine assessment and therapeutic decision making for localized PCa.³⁴ Five gene expression assays are commercially available: Decipher, Decipher PORTOS, Oncotype Dx Genomic Prostate Score (GPS), Prolaris, and ProMark.³⁴

Decipher is a 22 gene-expression assay suitable for formalin-fixed paraffin-embedded (FFPE), both PCa biopsy and prostatectomy material,⁹ which was developed based on an originally whole-transcriptome panel.³⁵ In a prospective registry of 855 patients receiving PCa biopsy, high-risk scores on the Decipher Biopsy test predicted shorter time to treatment in patients undergoing active surveillance and shorter time to treatment failure in patients receiving local treatment.³⁶ In the post-RP setting, the prognostic value of Decipher has been assessed within the NRG/RTOG 9601 trial, which randomized patients with PCa with biochemical recurrence and pT3N0 or pT2N0 disease with positive margins, to receive salvage radiotherapy with placebo versus salvage radiotherapy with antiandrogen therapy (bicalutamide for 2 years).^{9,37} The analysis of 352 RP samples from this study using the Decipher classifier showed that the test was independently prognostic for distant metastasis, PCa-specific mortality, and OS, when used as a continuous score (0 to 1.0), or following a risk category classification.⁹ In addition, this study suggested that patients with lower Decipher scores derived little or no benefit from the addition of antiandrogen therapy to salvage radiotherapy, whereas those patients with higher Decipher scores obtained much more benefit from the antiandrogen therapy. Moreover, a meta-analysis including 5 retrospective studies and a total of 855 patients evaluated the prognostic role of Decipher post-RP, and confirmed that the test can successfully predict the 10-year metastasis risk.¹⁰ As a consequence of these studies, the National Comprehensive Cancer Network (NCCN) guidelines now recommend

consideration of Decipher testing to aid decision making in the postoperative setting.¹⁴ Another complementary test, the Decipher PORTOS score, covers 24 genes and was validated in a matched retrospective study, which demonstrated that high PORTOS scores were significantly associated with decreased 10-year metastasis risk in patients who received postoperative radiotherapy compared with those who did not; conversely, low PORTOS scores were not associated with any difference in metastases rates based on treatment with postoperative radiotherapy.³⁸ Therefore, Decipher PORTOS is the only genomic classifier with predictive value regarding response to adjuvant or salvage radiotherapy.³⁴

Oncotype DX GPS is another gene-expression panel consisting of 12 PCa-related and 5 housekeeping genes (score 0 to 100), suitable for formalin-fixed biopsy material. This assay has been assessed in a cohort of 431 low- to intermediate-risk PCa biopsies, showing correlation with adverse pathologic features (Grade Group ≥ 3 or extraprostatic extension), biochemical recurrence, and risk of metastasis.¹¹ However, a more recent study in a large prospective cohort of 432 patients treated with active surveillance failed to validate the GPS test, and suggested that adding GPS to a model containing Prostate-specific antigen (PSA) kinetics and diagnostic Gleason grading did not significantly improve stratification of risk for adverse pathology over the clinical variables alone.³⁹

Prolaris is a broader gene-expression panel including 31 cell cycle-related and 5 housekeeping genes, which can be performed on FFPE material and has shown prognostic value when applied to biopsies and RP samples, being able to predict 10-year metastatic risk after RP and PCa-specific mortality after conservative treatment.^{12,13,34} The NCCN guidelines propose the use of Decipher or Prolaris to support risk assessment in patients with unfavorable intermediate- to high-risk localized PCa and a life expectancy of at least 10 years, and allow the use of any of the 3 tests (Decipher, Prolaris, or Oncotype DX Prostate) for patients with low to favorable intermediate risk.¹⁴ ProMark is an 8 protein-based assay, which showed ability to predict adverse pathologic features (Grade Group ≥ 2 or T $\geq 3b$) when applied to PCa biopsies.⁴⁰

GENOMIC INSTABILITY ASSAYS TO ASSESS HOMOLOGOUS RECOMBINATION DEFICIENCY

To date a limited number of studies have evaluated the role of HRD scores in PCa as a predictive

biomarker of response to PARP inhibitors or platin-based chemotherapy. For ovarian cancer, Myriad Genetics MyChoice CDx is the only US Food and Drug Administration (FDA)-approved test developed to assess HRD. This assay calculates a genomic instability score (GIS) taking into account genomewide loss of heterozygosity (LOH), telomeric allelic imbalance, and large-scale state transitions, with a score greater than or equal to 42 classified as high. In addition, the assay detects variants and large rearrangements in *BRCA1* and *BRCA2*.⁴¹ GIS analysis has been performed in a cohort of 557 localized PCas, and showed that patients with *BRCA2* alterations and higher HRD scores had longer progression-free survival (PFS) on olaparib.⁴² Interestingly, tumors with alterations in *ATM* and *CHEK2* had lower scores when compared with *BRCA2*-altered samples.⁴²

TruSight Oncology 500 HRD test is a recently developed assay, combining targeted NGS (Illumina TruSight Oncology 500) with Myriad HRD assay. Illumina TruSight Oncology 500 interrogates for single nucleotide variants, deletions, insertions, and copy number variants in a total of 523 genes, as well as for fusions in 55 genes, providing also information on MSI status and TMB. The combined assay showed high agreement with the results of Myriad MyChoice CDx Plus regarding presence of *BRCA* mutations and GIS in ovarian cancer.⁴³

FoundationOneCDx assay provides information on genomic LOH (gLOH) score. This score is calculated as percentage of LOH genome, with gLOH of 16 or higher considered as “LOH high.” FoundationOneCDx assay has been used for tumor tissue testing in the PROFound phase 3 trial, which compared the efficacy of treatment with olaparib versus physician’s choice in patients with mCRPC with alterations in HRD-related genes who progressed to a previous treatment with ARSI, showing prolonged PFS and OS for patients with *BRCA1*, *BRCA2*, and *ATM* alterations.^{15,44} In a cohort of 3476 PCas molecularly characterized using the FoundationOneCDx assay,⁵ gLOH scores were high in tumors harboring *BRCA1*, *BRCA2*, *ATR*, and *FANCA* alterations, whereas only a minority of *CDK12*-altered tumors presented a high score.⁵

Classifier of Homologous Recombination Deficiency (CHORD) is a genome-wide random forest-based approach, developed to detect tumor chromosomal instability.⁴⁵ In a cohort of 3504 solid tumors, analyzed by WGS, the CHORD was able to distinguish between “*BRCA1*-like” (*BRCA1* alterations) and “*BRCA2*-like” (*BRCA2*, *PALB2*, and *RAD51 C* alterations) phenotypes.⁴⁵ Another supervised learning algorithm, HRDetect,

is a lasso logistic regression model developed to identify *BRCA1* and *BRCA2* mutational signatures in breast cancer tumors.^{46,47} This algorithm, applied to a cohort of 311 PCa samples analyzed by WGS, correctly discriminated samples with biallelic *BRCA1/2* mutations, as well as identified further *BRCA1/2* nonmutant cases with a high HRDetect scores (>0.7). HRDetect showed lower specificity when applied to WES data from the same cohort.⁴⁷

MICROSATELLITE INSTABILITY TESTING

Alterations in MMR genes have been reported in 4% to 5% of mCRPC.^{2,3,5} MSI status has been classically assessed by IHC for MLH1, MSH2, MSH6, and PMS2 proteins.^{48–50} For IHC scoring, a product of intensity of the staining (0–3) and percentage of positive cells (0–3, 0 [0%], 1 [1%–33%], 2 [34%–66%], and 3 [67%–100%]) is calculated, with a product score of 3 or less classified as “loss of protein expression.”⁵¹ An alternative strategy is to assess MSI status by sequencing of specific microsatellite (or tandem repeats) loci using polymerase chain reaction (PCR). With this approach, panels of 5 (Bethesda,⁵² OncoMate MSI Dx Analysis System⁵³) to 8 (LMR MSI Analysis System⁵⁴) microsatellite loci have been developed. In colorectal cancer (CRC), MSI-H detection by IHC has shown a 91.9% agreement with the detection by PCR, with high negative predictive and low positive predictive values, when compared with PCR.⁵⁵ Over the past years, NGS approach has enabled parallel assessment of multiple microsatellite loci.⁵⁶ Most of these NGS panels have been optimized for CRC (MSIPlus,⁵⁷ ColoSeq⁵⁸). In a cohort of 91 PCas, with MSI status additionally tested by deep targeted sequencing of the MMR genes, 5-marker PCR panel had a sensitivity of 72.4% and a specificity of 100%, and larger NGS panels (>60 markers) showed a sensitivity of 93.1% and a specificity of 98.4%.⁵⁶ MSIsensor is an algorithm developed to detect somatic microsatellite alterations from paired normal-tumor-targeted NGS data.^{59,60} A cohort of 1033 localized PCas and mPCas with available normal tumor NGS data (MSK-Impact)⁶¹ has been analyzed with this algorithm, uncovering MSI-H or dMMR tumors in 3.1% of the cases, with 29.1% of these samples harboring pathogenic germline alterations in Lynch syndrome-related genes.²⁹ Half of these patients with MSI-H/dMMR showed more than 50% PSA declines under anti-PD1/PD-L1 ICIs.²⁹ MSI status in PCa can be also evaluated by liquid biopsy and cfDNA analysis (Guardant360 CDx,⁶² FoundationOne Liquid CDx⁶³) (Table 1).

Table 1
Summary of available homologous recombination deficiency and microsatellite instability tissue-based assays

Method	Assay/Analysis Method	Score and Threshold	Interpretation
HRD tumor testing			
Targeted NGS	Myriad Genetics MyChoice CDx	<ul style="list-style-type: none"> • LOH + LST + TAI (threshold ≥ 42) • Variants and large rearrangements in 15 genes (<i>ATM</i>, <i>BARD1</i>, <i>BRCA1</i>, <i>BRCA2</i>, <i>BRIP1</i>, <i>CDK12</i>, <i>CHEK1</i>, <i>CHEK2</i>, <i>FANCL</i>, <i>PALB2</i>, <i>PPP2R2A</i>, <i>RAD51 B</i>, <i>RAD51 C</i>, <i>RAD51D</i>, and <i>RAD54 L</i>). 	<ul style="list-style-type: none"> • GIS • Pathogenicity of variants
	Myriad Genetics MyChoice CDx Plus	<ul style="list-style-type: none"> • LOH + LST + TAI (threshold ≥ 42) • Variants and large rearrangements in <i>BRCA1</i> and <i>BRCA2</i> 	<ul style="list-style-type: none"> • GIS • Pathogenicity of variants
	TruSight Oncology 500 HRD	<ul style="list-style-type: none"> • SNV, indels, CNV in 523 genes, rearrangements in 55 genes • MSI and TMB 	<ul style="list-style-type: none"> • Genomic alterations • MSI and TMB • GIS
	FoundationOneCDx	<ul style="list-style-type: none"> • LOH + LST + TAI (threshold ≥ 42) • SNV, indels, CNV in 324 genes, rearrangements in selected genes • MSI and TMB • gLOH ≥ 16 	<ul style="list-style-type: none"> • Genomic alterations • MSI and TMB • gLOH low/high
Genome-wide NGS (WGS, WES)	CHORD	<ul style="list-style-type: none"> • Biallelic loss (deep deletion), presence of LOH, pathogenicity of variants • Threshold ≥ 0.5 	<ul style="list-style-type: none"> • Probability of <i>BRCA1/2</i> deficiency • HRD
	HRDetect	<ul style="list-style-type: none"> • Mutational signatures analysis, HRD index score, analysis of variants in <i>BRCA1/2</i> and other HRR-related genes • Threshold > 0.7 	<ul style="list-style-type: none"> • Probability of <i>BRCA1/2</i> deficiency • HRD
MSI testing			
IHC of MMR proteins	MLH1, MSH2, MSH6, and PMS2	<ul style="list-style-type: none"> • Intensity of staining: 0–3 • Percentage of positivity: 0–3 • Product score (threshold ≤ 3) 	<ul style="list-style-type: none"> • Loss of MMR protein expression (dMMR)

PCR of microsatellites	Bethesda panel	<ul style="list-style-type: none"> • 5 microsatellite markers: 2 mononucleotide (Bat25, Bat26) and 3 dinucleotide (D2S123, D5S346, and D17S250) • Threshold: ≥ 2 markers positive for shifts in the allelic bands 	<ul style="list-style-type: none"> • MSS • MSI-L (1 marker) • MSI-H (≥ 2 markers)
	MSI Analysis System Version 1.2/OncoMate MSI Dx Analysis System	<ul style="list-style-type: none"> • 5 SMR markers (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) and 2 pentanucleotide repeat markers (Penta C and Penta D) • Threshold: ≥ 2 markers positive for shifts in the allelic bands 	<ul style="list-style-type: none"> • MSS • MSI-L (1 marker) • MSI-H (≥ 2 markers)
	LMR MSI Analysis System	<ul style="list-style-type: none"> • 4 SMR markers (BAT-25, BAT-26, NR-21, and MONO-27), 4 LMR markers (BAT-52, BAT-56, BAT-59, and BAT-60), and 2 pentanucleotide repeat markers (Penta C and Penta D) • Threshold: ≥ 3 markers positive for shifts in the allelic bands 	<ul style="list-style-type: none"> • MSS • MSI-L (1–2 markers) • MSI-H (≥ 3 markers)
NGS	MSIPlus	<ul style="list-style-type: none"> • Optimized for CRC • 16 microsatellite markers and hotspots in KRAS, NRAS, and BRAF 	<ul style="list-style-type: none"> • MSI-H (following mSINGS score)
	ColoSeq	<ul style="list-style-type: none"> • Optimized for CRC • SNV, deletions or rearrangements in MMR-related genes (MLH1, MSH2, MSH6, PMS2, EPCAM, APC, MUTYH) and 24 additional genes 	<ul style="list-style-type: none"> • Variant interpretation
	NGS-targeted panels including MMR genes (eg, MSK-Impact)	<ul style="list-style-type: none"> • MSISensor score ≥ 10 	<ul style="list-style-type: none"> • MSI-H
Targeted sequencing of MMR genes	Any NGS-targeted panel covering MMR genes	<ul style="list-style-type: none"> • SNV, indels, CNV in MMR genes 	<ul style="list-style-type: none"> • Variant interpretation

Abbreviations: CNV, copy number variations; LMR, long mononucleotide repeats; LST, large-scale transitions; MSI-L, MSI-low; MSS, microsatellite stable; SMR, single mononucleotide repeats; SNV, single nucleotide variants; TAI, telomeric allelic imbalance; TMB, tumor mutational burden.

GERMLINE HOMOLOGOUS RECOMBINATION DEFICIENCY TESTING

In the PROfound phase 3 study,¹⁵ BRCAanalysis CDx identified germline *BRCA1/2* alterations in blood samples of the 16% of the included patients. This HRD germline population constituted 53.5% of all patients with tumor *BRCA1/2* alterations in the study. When considering the 62 evaluable patients with a positive BRCAanalysis CDx test for germline *BRCA1/2* alterations, their PFS was 10.12 versus 1.87 months for olaparib versus physician's choice (hazard ratio, 0.08, $P < 0.0001$).^{64,65} For tumor tissue testing, FoundationOneCDx assay was used in the study.¹⁵ Myriad's BRCAanalysis CDx is currently FDA approved for patients with ovarian, breast, and pancreatic cancers and PCa who meet criteria for germline testing to identify pathogenic *BRCA1* and *BRCA2* mutations. For PCa, the NCCN guidelines recommend germline testing for patients with personal history of PCa, diagnosed at any age and starting from high-risk localized stage, as well as for patients with familial history of PCa (Table 2). It is recommended that germline panels include the Lynch syndrome-related genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*, and the HRD genes *BRCA1*, *BRCA2*, *ATM*,

PALB2, and *CHEK2*.¹⁴ Other genes, such as *HOXB13*, should also be considered.^{14,66}

DISCUSSION

Over the past years, tumor molecular characterization has been progressively integrated into the clinical management of patients with localized PCa and mPCa.¹⁴ For localized PCa, multiple pre-treatment risk stratification algorithms are available based on clinical and pathologic features (eg, GS, PSA level, clinical T stage).⁶⁷ For patients with biopsied PCas and NCCN low to favorable intermediate risk, genomic classifiers, independently or in combination with multiparametric MRI,⁶⁸ can help identify better candidates for active surveillance, although further validation is needed for some of these classifiers.^{14,34} For patients who have biochemical recurrence after RP, the NCCN guidelines recommend that physicians consider adding androgen deprivation therapy (ADT) to salvage radiotherapy for patients; the use of genomic classifiers such as Decipher may help identify patients most likely to benefit from the addition of ADT to salvage radiotherapy in this setting. Across the spectrum of localized PCa, several gene expression classifiers are

Table 2

National Comprehensive Cancer Network guidelines recommendation for germline testing in patients with diagnosis of prostate cancer

Family history			Personal history	
Family members*	Tumor type	Age at diagnosis	Tumor type	Age at diagnosis
Germline testing is recommended			Germline testing is recommended	
At least 1 (1st degree)	PCa**	≤ 60	PCa (from high risk localized to metastatic)	Any
At least 1 (1st, 2nd or 3rd degree)	Breast, CRC or endometrial	≤ 50	Breast	Any
	Male breast, ovarian, exocrin pancreatic	Any	Germline testing may be considered	
	PCa (from high risk localized to metastatic)	Any	PCa (intermediate risk and intraductal/criform histology)	Any
At least 2 (1st, 2nd or 3rd degree)	PCa** or Breast	Any	PCa and previous other cancer ***	Any
At least 3 (1st or 2nd degree)	Lynch-related cancers	Especially if < 50		
Mutation (pathogenic/likely pathogenic) in <i>BRCA1</i> , <i>BRCA2</i> , <i>ATM</i> , <i>PALB2</i> , <i>CHEK2</i> , <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i> , <i>PMS2</i> , <i>EPCAM</i>				
Ashkenazi Jewish ancestry				

available as prognostic tools to aid in risk stratification for clinical decision making.

For advanced PCa, recent advances in targeted therapeutic approaches have increased the clinical need to screen mPCa tumors for targetable molecular alterations. Most relevant therapeutic advances have been made for DDR^{15–17} and MSI-H tumors,^{20,21} as well as CDK12-altered PCAs.²² However, despite these relevant advances, targeted treatment with PARP inhibitors and immunotherapy is successful only in a subset of DDR,¹⁷ MSI-H,²⁹ and CDK12-altered PCAs,^{22,69} and further development and validation of solid predictive biomarkers is needed. For instance, distinct HRD genotypes harbor different degrees of sensitivities to PARP inhibitors.^{15,17} Beyond germline alterations in *BRCA1* and *BRCA2*, biomarker analysis from the TOPAR-B phase 2 trial showed that PCAs with homozygous *BRCA2* and *PALB2* deficiency, as well as tumors with loss of ATM protein expression, had most benefit from treatment with olaparib.¹⁷ In ovarian cancer, first-line maintenance treatment with olaparib in combination with bevacizumab is indicated for patients with HRD tumors assessed by Myriad Genetics MyChoice CDx, based on the results of the PAOLA-1 phase 3 trial.⁷⁰ In PCa, still limited studies are available correlating GIS score with efficacy of PARP inhibitors.^{15,42}

For MSI-H/dMMR tumors, the KEYNOTE-158 phase 2 study assessed the efficacy of pembrolizumab in distinct MSI-H/dMMR tumor entities, including 6 patients with mPCa, showing an overall response rate of 34.3% and a median OS of 23.5 months (95% confidence interval, 13.5–not reached) for the entire study cohort.⁷¹ MSI status was assessed either by IHC or 5 microsatellite loci PCR panel.⁷¹ Based on this and other studies, pembrolizumab is currently approved for MSI-H/dMMR mPCAs, which progressed after at least 1 prior systemic treatment line.⁷² Moreover, for patients with uncovered pathogenic or likely pathogenic mutations in Lynch syndrome-associated genes, germline counseling and/or testing is recommended, as well as for patients with personal or familial history of PCa (see **Table 2**). Clinical and molecular features of CDK12-altered mPCAs have been analyzed in a retrospective study, which included 60 patients, 51.7% of them harboring a biallelic alteration in *CDK12*.²² The study showed that CDK12-altered tumors had poor responses to ARSI and taxane-based chemotherapy, lack of response to PARP inhibitors, and variable responses to PD-1 inhibitors (pembrolizumab and nivolumab).²² Mechanistically, the lack of response to PARP inhibitors of this molecular subtype of PCa has been correlated with a genomic instability phenotype distinct from HRD, characterized by tandem duplications and gene

fusions.²² Finally, recent studies have been assessing the role of liquid biopsy for molecular subtyping and identification of predictive biomarkers in advanced PCa.^{62,63,73}

Surgical pathologists play a critical role in triaging tissue for molecular biomarker testing in PCa. It is important for pathologists to understand when biomarker testing may be appropriate, and how these tests are performed. Pathologists should be aware that preanalytic and histopathologic factors may affect these tests. Because these assays are validated only on FFPE specimens containing untreated PCa, specimens that were previously frozen or fixed in nonformalin fixatives should not be used for these tests. In addition, tumors that have been treated with radiation or ADT are not eligible for these assays. When choosing tissue for these tests, pathologists should pick the most representative tissue blocks with the highest Gleason grade and largest tumor volume. Pathologists should also make sure there is sufficient tumor in the tissue submitted for testing.

SUMMARY

Molecular tumor profiling has gained relevance in personalized clinical management and precision oncology treatment of localized PCa and mPCa. Use of gene expression assays for genomic risk stratification can support decision algorithm regarding active surveillance or indication of intensification of therapy in localized PCa. For mPCa, tumor molecular characterization by NGS, as well as by assays assessing HRD and MSI status, are essential to predict benefit from molecularly targeted therapies, such as PARP inhibitors and immunotherapy. Moreover, because PCa tumor responses to targeted treatment are still highly heterogeneous, further development and validation of robust predictive biomarkers is required.

CLINICAL CARE POINTS

- Molecular tumor testing is essential in metastatic PCa, in order to identify patients with targetable alterations, such as HRD and dMMR/MSI-H tumors.
- Genomic classifiers may help identify patients most likely to benefit from the addition of ADT to radiotherapy in the biochemical recurrence setting.

- Germline testing should be offered to patients with metastatic or nodal positive PCa, as well as to a subset of patients with high risk localized PCa.

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DISCLOSURE

D. Akhoundova and C.C. Pritchard declare no conflicts of interests. F.Y. Feng serves on the Scientific Advisory Board of Artera, BlueStar Genomics, SerImmune, and the Immuno-Oncology Program for Bristol Myers Squibb, and has consulted for Foundation Medicine, Tempus, Janssen, Astellas, Bayer, Myovant, Roivant, and Novartis. M.A. Rubin is a coinventor on patents in the area for diagnosis and therapy in prostate cancer for ETS fusions (University of Michigan and the Brigham and Women's Hospital), SPOP mutations (Cornell University), and EZH2 (University of Michigan).

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