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INVITED REVIEW

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Ugo Giovanni Falagario[a,b,c], Francesca Sanguedolce[d], Zach Dovey[c], Umberto Carbonara[b], Fabio Crocerossa[b], George Papastefanou[c], Riccardo Autorino[b], Marco Recchia[a], Antonella Ninivaggi[a], Gian Maria Busetto[a], Pasquale Annese[a], Giuseppe Carrieri[a] and Luigi Cormio[a]

[a]Department of Urology and Organ Transplantation, University of Foggia, Foggia, Italy; [b]Division of Urology, VCU Health System, Richmond, VA, USA; [c]Department of Urology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [d]Department of Pathology, University of Foggia, Foggia, Italy

ABSTRACT

Traditionally, diagnosis and staging of prostate cancer (PCa) have been based on prostate-specific antigen (PSA) level, digital rectal examination (DRE), and transrectal ultrasound (TRUS) guided prostate biopsy. Biomarkers have been introduced into clinical practice to reduce the overdiagnosis and overtreatment of low-grade PCa and increase the success of personalized therapies for high-grade and high-stage PCa. The purpose of this review was to describe available PCa biomarkers and examine their use in clinical practice. A nonsystematic literature review was performed using PubMed and Scopus to retrieve papers related to PCa biomarkers. In addition, we manually searched websites of major urological associations for PCa guidelines to evaluate available evidence and recommendations on the role of biomarkers and their potential contribution to PCa decision-making. In addition to PSA and its derivates, thirteen blood, urine, and tissue biomarkers are mentioned in various PCa guidelines. Retrospective studies have shown their utility in three main clinical scenarios: (1) deciding whether to perform a biopsy, (2) distinguishing patients who require active treatment from those who can benefit from active surveillance, and (3) defining a subset of high-risk PCa patients who can benefit from additional therapies after RP. Several validated PCa biomarkers have become commercially available in recent years. Guidelines now recommend offering these tests in situations in which the assay result, when considered in combination with routine clinical factors, is likely to affect management. However, the lack of direct comparisons and the unproven benefits, in terms of long-term survival and cost-effectiveness, prevent these biomarkers from being integrated into routine clinical use.

Abbreviations: ACT: Antichymotrypsin; ASCO: American Society of Clinical Oncology; AUA: American Urological Association; AUC: Area under the curve; BCR: Biochemical recurrence; BPH: Benign prostatic hypertrophy; csPCa: Clinically significant prostate cancer; DCA: Decision curve analysis; DLX1: Homeobox 1; DRE: Digital rectal examination; EAU: European Association of Urology; EPI: ExoDx Prostate IntelliScore; ERG: ETS-related gene; ERSPC: European Randomized PCa Screening Study; EV: Extracellular vesicle; f/t: Free/total; GPS: Genomic Prostate Score; GS: Gleason score; hk2: Human kallikrein-2; HOXC6: Homeobox C6; mpMRI: Multiparametric magnetic resonance imaging; mtDNA: Mitochondrial DNA; NCCN: National Comprehensive Cancer Network; NPV: Negative predictive value; p2PSA: Pro-PSA; PCa: Prostate cancer; PCA3: Prostate cancer antigen 3; PCMT: Prostate Core Mitomic Test; PHI: Prostate Health Index; PI-RADS: Prostate Imaging Reporting and Data System; PPV: Positive predictive value; PSA: Prostate-specific antigen; PSAd: Prostate-specific antigen density; PSADT: Prostate-specific antigen doubling time; PSAV: Prostate-specific antigen velocity; RP: Radical Prostatectomy; SOC: Standard of Care; TMPRSS2: Transmembrane serine protease 2; TRUS: Transrectal ultrasound

1. Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer in men, and despite a continuous reduction in its mortality over the past 20 years, it remains the third cause of death in men due to cancer [1]. The advent of multiparametric magnetic resonance imaging (mpMRI) has represented a major paradigm shift in recent years, allowing an improvement in the diagnosis and management of the disease [2–5]. At the same time, the quest for a more tailored approach to diagnosing and managing PCa has led to the introduction of several biomarkers.

Biomarkers are indicators of physiological, pathological, or biological responses to therapeutic intervention and they can be detected in a variety of samples, including blood, urine, and tissue fragments taken from biopsy or surgical specimen. In recent years we have witnessed the discovery of numerous biomarkers for a large variety of tumors, primarily breast and hematologic cancers for which typing of tumor cells for effective therapy is now essential [6,7].

With respect to PCa, biomarkers can provide diagnostic and prognostic information, guiding urologists in management of the disease. However, most of these biomarkers have not been introduced into clinical practice yet. Once it has been shown that a newly discovered biomarker is linked to a specific outcome, it is necessary to show its added value when used in conjunction with standard of care (SOC) clinical variables. Moreover, for the results to be applicable in other populations, an external validation of the predictive model based on that given

biomarker is required. From a practical point of view, its clinical utility is illustrated by a statistical method called decision curve analysis (DCA), which uses retrospective studies to identify the benefits that are obtained by using the biomarker and allows a comparison with currently available tools [8]. Some PCa biomarkers have been validated and are available all over the world, whereas others are in the validation phase with their use being expected to spread rapidly in Europe after having been introduced in the United States [9].

This review summarizes the main features of currently available PCa biomarkers that have been adopted in the European Association of Urology (EAU), National Comprehensive Cancer Network (NCCN), American Urological Association (AUA), and American Society of Clinical Oncology (ASCO) guidelines [10–12] and examines their role in the context of the common clinical scenarios in the diagnostic-therapeutic pathway of PCa patients (Figure 1).
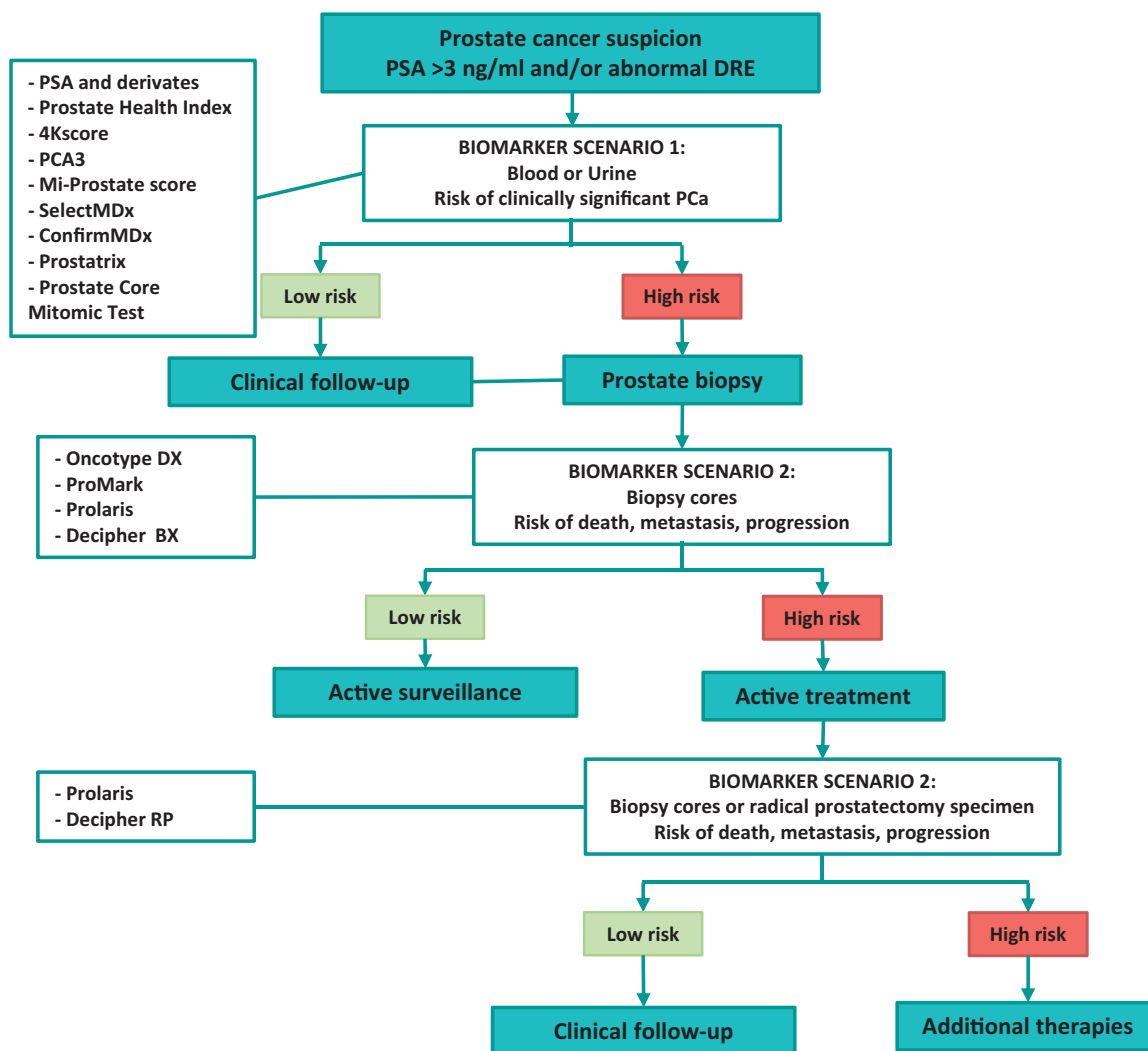
Figure 1. Flow chart summarizing biomarkers and their utility in PCA diagnosis and management. Scenario 1: When to perform a prostate biopsy? Scenario 2: When to choose active surveillance or active treatment? Scenario 3: When to start radiation therapy after radical prostatectomy?

2. Material and methods

In order to provide a readily available clinical guide of the available PCa biomarkers, we manually searched websites of major urological associations for PCa guidelines to evaluate available evidence and recommendations on the role of biomarkers and their potential contribution to PCa decision-making. A nonsystematic literature review was performed in August 2021 using PubMed and Scopus to retrieve papers related to PCa biomarkers cited and approved by guidelines.

To better examine clinical relevance, we divided our findings into three common clinical scenarios:

- Clinical scenario 1: Biomarkers for risk stratification of patients with clinical suspicion of PCa: When to perform a prostate biopsy?
- Clinical scenario 2: Biomarkers for risk stratification of patients with biopsy-proven PCa: When to choose active surveillance or active treatment?
- Clinical scenario 3: Biomarkers for disease management of high-risk PCa patients who can benefit from additional therapies after active treatment: When to start radiation therapy after radical prostatectomy (RP)?

3. Results

3.1. Clinical scenario 1: when to perform a prostate biopsy?

Prostate biopsy remains the gold standard for the diagnosis of PCa. Despite being a rapid outpatient procedure, it is not free from potential severe complications [13].

Traditionally, the decision to perform a prostate biopsy has been based on prostate-specific antigen (PSA) levels and/or digital rectal examination (DRE). In recent years, MRI has gained popularity and now guidelines recommend obtaining an mpMRI before performing prostate biopsy in case of clinical suspicion of PCa. If the mpMRI shows lesion(s) with features suggesting PCa, systematic as well as targeted biopsy should be performed [10]. However, the low specificity of MRI could potentially result in an inflation of false-positive findings and subsequent unnecessary biopsies in very low-risk patients [2,3].

Guidelines now recommend offering further risk-assessment in patients with PSA levels of 3–10 ng/mL and normal DRE [10–12]. Such risk assessment should be performed using risk-calculators and serum- or urine-based biomarkers that might aid in determining individual risk of PCa, thus reducing the number of unnecessary MRIs and biopsies. Table 1 summarizes biomarkers currently used for risk stratification of patients with clinical suspicion of PCa.

3.1.1. PSA, its derivatives, and the kallikrein family

PSA is a serine protease whose function is to keep semen fluid after ejaculation, allowing sperm to move more easily through the cervix. It is part of the kallikrein family, a group of 15 serine proteases known for their role in regulating cell growth, remodeling and degrading the extracellular matrix, and promoting cell invasion and angiogenesis [14]. Human kallikrein-3, also known as PSA, and human kallikrein-2 (hK2) are the dominant forms and allow the contents of the vas deferens to be maintained as a liquid. They exist as proproteins, pro-PSA (p2PSA) and pro-hK2, and are split to generate

Table 1. Biomarkers for risk stratification of patients with clinical suspicion of prostate cancer: when to perform a prostate biopsy?

Trade name	Sample	Biomarker	Target population	Outcome predicted
Free/total (f/t) PSA	Serum	f/t PSA ratio	First biopsy	PCa
4Kscore	Serum	Total PSA, free PSA, intact PSA, hK2, and clinical variables	First biopsy Rebiopsy	PCa csPCa
Prostate Health Index (PHI)	Serum	Total PSA, free PSA, and p2PSA	First biopsy Rebiopsy	PCa csPCa
PCA3	Urine	PCA3	Rebiopsy	PCa
Mi-Prostate score	Urine	PCA3, TMPRSS2:ERG, and clinical variables	First biopsy	PCa rebiopsy
SelectMDx	Urine	mRNA expression of HOXC6 and DLX1 against KLK3 as internal reference	First biopsy	PCa csPCa
ExoDx Prostate Intelliscore (EPI)	Urine	PCA3 and ERG normalized to SPDEF	First biopsy Rebiopsy	PCa csPCa
Prostarix	Urine	4-Metabolite assay (alanine, glycine, gluconate, and sarcosine)	First biopsy Rebiopsy	PCa csPCa
ConfirmMDx	Negative biopsy cores	Methylation of GSTP1, RASSF1, and APC	Rebiopsy	PCa rebiopsy csPCa rebiopsy
Prostate Core Mitomic Test (PCMT)	Negative biopsy cores	mtDNA mutations	Rebiopsy	PCa rebiopsy csPCa rebiopsy

active enzyme forms. Circulating levels of both kallikreins increase as the tumor becomes more poorly differentiated, perhaps due to loss of tissue architecture [15]. Furthermore, once in circulation, they are rapidly bound by antichymotrypsin (ACT) or inhibited by proteolysis. About 30% of PSA is present in the serum in a free form, whereas 70% is in a form bound to protease inhibitors, alpha-1-antichymotrypsin and alpha-2-macroglobulin.

3.1.1.1. Free/total PSA ratio.
It has been shown that the relationship between free/total (f/t) PSA is useful in the stratification of PCa risk in men with 4–10 ng/mL of total PSA and negative DRE, whereas guidelines advise against its use in patients with total PSA >10 ng/mL and in the follow-up of patients diagnosed with PCa (8). PCa was detected by biopsy in 56% of men with f/t PSA of <0.10 ng/mL, but only in 8% with f/t PSA of >0.25 ng/mL [16].

3.1.1.2. PSA density.
PSA density (PSAd) is a readily available and increasingly used parameter expressed as the PSA value (in ng/mL) divided by prostate volume (in CC). PSAd potentially identifies patients who do not have PCa but have an elevated PSA secondary to benign prostatic hypertrophy (BPH) [17]. The utility of volume and BPH parameters to predict biopsy results is well established in the literature [18–20] and several studies have shown that adding volume to the SOC clinical parameters improves the accuracy of risk calculators predicting PCa and clinically significant prostate cancer (csPCa) [21]. The optimal cutoff of PSAd to suggest a prostate biopsy is still unclear [11]. A PSAd cutoff of 0.15 ng/mL^2 was suggested in previous studies [22]. However, Nordström et al. [23] showed that a PSAd cutoff of 0.10 and 0.15 ng/mL^2 resulted in detection of only 77% and 49% of csPCa, respectively. Conversely, omitting prostate biopsy for men with PSAd $\leq$ 0.07 ng/mL^2 would save 19.7% of biopsy procedures while missing 6.9% of csPCa.

Still, PSAd has not been incorporated into early detection guidelines as a baseline measure because of the lack of precision of both PSA and prostate volume measurements using TRUS. MRI has helped to overcome this limitation and recent studies pointed out that the combination of MRI parameters and PSAd could help to predict not only prostate biopsy results [24], but also active surveillance outcomes, adverse pathologic features at RP, and biochemical recurrence (BCR) after surgical treatment [25,26].

3.1.1.3. PSA kinetics: PSA velocity and PSA doubling time.
PSA velocity (PSAV) is the absolute annual increase in serum PSA, whereas PSA doubling time (PSADT) measures the time needed for the PSA value to double. These parameters, known as PSA kinetics, provide a more dynamic picture of PCa activity compared to individual PSA values.

PSADT measurement prior to definitive treatment may provide information on the aggressiveness of PCa [27]. PSAV independently predicts high-grade disease. However, the added value of these biomarkers to SOC clinical variables is probably limited with an increase of the area under the curve (AUC) for prediction of high-grade disease from 0.626 to 0.646. Moreover, pretreatment PSAV has been shown to correlate with the development of distant metastases and PCa-specific mortality after RP, radiation therapy, and androgen-deprivation therapy [28].

3.1.1.4. 4Kscore.
Hk2 is also used as a biomarker of PCa and was introduced in the 4Kscore® Test (OPKO Health, Miami, FL, USA) [29]. The test was developed by Vickers et al. [30] using a large cohort of men from the Gothenburg arm of the European Randomized PCa Screening Study (ERSPC) and is based on a logistic model that considers four forms of kallikrein (tPSA, fPSA, intact PSA, and hK2) in order to accurately predict the presence of PCa in men with a PSA of 3.0 ng/mL or higher (AUC 0.84). The validity of the model was confirmed in a separate ERSPC cohort (Rotterdam arm) [31]. The use of the 4Kscore® test could avoid 513 per 1000 biopsies, missing only 54 of 177 low-grade tumors and 12 of 100 high-grade tumors [31]. Carlsson et al. [32] also showed that the test is able to predict the finding of aggressive disease in patients undergoing prostatectomy. Recently, the 4Kscore® test has been proposed as a risk stratification tool before prostate MRI. The negative predictive value (NPV) of a 4Kscore of <8% was 98%. Moreover, the positive predictive value (PPV) of Prostate Imaging Reporting and Data System (PI-RADS) lesions 3–5, which are considered to be positive, was 0% in patients with 4Kscore of <8%.

In this scenario, the most clinically beneficial biopsy strategy was obtaining an initial 4Kscore followed by mpMRI if the 4Kscore was >8% and a subsequent biopsy if the MRI was positive or 4Kscore was $\geq$18%. This strategy could also reduce the number of prostate MRIs performed [33]. Finally, in patients with a positive mpMRI and low 4Kscore, systematic biopsy may be omitted [34].

3.1.1.5. Prostate health index (PHI).
The Prostate Health Index (PHI), which is a diagnostic blood test approved in Europe, America, and Australia, includes

measurement of a PSA isoform that is correlated to PCa, p2PSA, in combination with the f/t PSA ratio. The PHI has been developed to predict the probability of any PCa and csPCa at prostate biopsy. Using the PHI with a cutoff of ≥25 to indicate need for biopsy could avoid 40% of biopsies and reduce 25% of Gleason score (GS) 6 diagnoses at the cost of missing 5% csPCa [35]. Three prospective multicenter studies have shown that both PHI and the 4Kscore® test have diagnostic performances higher than the f/t PSA test alone in men with a PSA between 2 and 10 ng/mL [35–37].

3.1.2. Urine-based tests

3.1.2.1. PCA3 and Mi-Prostate score.
Prostate cancer antigen 3 (PCA3) is a long-noncoding mRNA produced by prostatic cells. Although its function remains unknown, it has been shown that levels of PCA3 in malignant tissue generally far outweigh its levels in benign tissue [38].

Progensa PCA3 (Hologic, Marlborough, MA, USA) is a commercially available test based on the assay of PCA3 in a urine sample obtained following prostate massage. It has shown promising results as an indicator for a repeat biopsy, with an AUC of 0.71–0.75 in a model that also includes other clinical variables, but has yet to be validated in pre-biopsy patients. In a multivariate analysis, Salami et al. [39] found that the combination of serum PSA, PCA3 (sensitivity = 93%), and transmembrane serine protease 2:ETS-related gene (TMPRSS2:ERG) (specificity = 87%) improved PCa prediction with an AUC of 0.88, 90% specificity, and 80% sensitivity. Leyten et al. [40] published the results of a prospective multicenter study involving 497 patients and showed the addition of both PCA3 and TMPRSS2:ERG to SOC clinical variables used by the ERSPC risk calculator led to an increase in the AUC from 0.80 to 0.84. Used as a single marker, TMPRSS2:ERG has low sensitivity but high specificity. However, its combination with the other urinary marker, PCA3, has been reported to provide high specificity and sensitivity [41].

A logistic regression model combining PCA3 and TMPRSS2:ERG with SOC clinical variables to predict the risk of PCa and csPCa was proposed by the University of Michigan (MLabs, Ann Arbor, MI, USA) with the name of Mi-Prostate score [42].

3.1.2.2. SelectMDx.
SelectMDx (MDx Health, Irvine, CA, USA) is a test that calculates the probability of diagnosis of PCa and csPCa by measuring the level of gene expression of Homeobox 1 (DLX1) and Homeobox C6 (HOXC6) mRNA in a urine sample collected following DRE examination. This test is based on a study by Leyten et al. [43] who, through analysis of gene expression profiles, found 39 genes associated with PCa. Through more in-depth analysis, the combination of 3 of these 39 genes (HOXC6, TDRD1, and DLX1) showed an AUC of 0.77, greater than the Progensa PCA3 and the standard PSA (AUC of 0.68 and 0.72, respectively). Subsequently, Van Neste et al. [44] validated these results in two prospective multicenter studies, which included 519 and 386 patients, developing a predictive model with an AUC of 0.90. The model uses DLX1 and HOXC6 in association with SOC clinical variables and shows superior predictive accuracy compared to PCA3 and risk calculators. A patient with low-risk SelectMDx (NPV of 98% for csPCa) has a 10% chance of having PCa and only a 2% chance of having csPCa. From a practical point of view, DCA has shown its use would lead to a 42% reduction in all prostatic biopsies.

Recently SelectMDx was compared to mpMRI in patients undergoing MRI-guided prostate biopsies. Maggi et al. showed that SelectMDx had a higher accuracy compared to MRI in the prediction of any PCa and similar accuracy in the prediction of csPCa. In addition, the best diagnostic strategy to avoid unnecessary biopsies was to perform SelectMDx after an initial negative mpMRI. Thus, biopsy could be proposed for all cases of mpMRI PI-RADS score of 4–5 score, as well as those with PI-RADS score of 1–3 score followed by a positive SelectMDx [45,46].

3.1.2.3. ExoDx prostate IntelliScore (EPI).
The ExoDx Prostate IntelliScore (EPI) is a noninvasive urine exosome gene expression assay aiming to reduce unnecessary biopsies in men with a suspicion of PCa with or without a previous negative biopsy [47].

EPI is based on the analysis of RNA content in extracellular vehicles (EVs) isolated from urine, sampled without previous DRE, of men with suspected PCa. This test has been validated by McKiernan et al. [48] in two validation studies showing that, by applying an EPI cutoff of 15.6, the EPI resulted in an NPV for csPCa of 91% and 89% in the original validation cohort and in the second validation cohort, respectively.

In addition, the EPI is computed based solely on the expression levels of three exosomal RNAs (ERG, PCA3, and SPDEF) that play a role in PCa initiation and progression, and it provides a risk score on a scale of 0–100 that predicts the likelihood of csPCa. Its addition to a model including MRI, SOC clinical variables, or risk calculators improved the predictive accuracy for csPCa compared to a model based only on SOC clinical variables [48].

3.1.2.4. Prostarix.
Prostarix is a urine-based test that measures certain biomarkers (alanine, glycine,

gluconate, and sarcosine) providing a tool to assess the likelihood of being diagnosed for PCa after a prostatic biopsy. Recent advances in analytical technologies, such as the use of liquid chromatography-tandem mass spectrometry, have led to novel clinical applications of metabolomics [49]. These techniques allow measurement of small biochemicals specific for each cancer phenotype and related to the development and progression of PCa [50]. The elevations of sarcosine in PCa cells have been found to translate into biological fluids, especially blood and urine. This led to the development of the 4-metabolite assay known as the Prostarix clinical test, which predicts presence of PCa at biopsy. The test was validated in an external cohort showing that individuals with Prostarix scores >60 were 3.5 times more likely to have PCa detected on biopsy compared to those with Prostarix scores <40 [28]. In the case of a patient undergoing a repeat biopsy after a previous negative biopsy, the TRUS-measured prostate volume and the most recent PSA measurement can be used along with the metabolite measurements to generate a Prostarix Plus score, which can further improve risk stratification.

3.1.3. Tissue-based tests

3.1.3.1. ConfirmMDx.
ConfirmMDx (MDxHealth, Irvine, CA, USA) was designed as a risk stratification tool for men with negative prostate biopsies and aims to reduce the number of repeated biopsies [51]. This is a unique test that analyzes epigenetic changes by detecting alterations in DNA methylation patterns of key tumor suppressor genes, such as GSTP1, RASSF1, and APC, in a prostate tissue sample obtained from previous prostatic biopsy.

ConfirmMDx has been validated in a European and US cohort in which all patients had undergone two consecutive biopsies within 24–30 months, reaching an NPV of 88–90%, compared to 70% for histopathological evaluation alone [52,53].

3.1.3.2. Prostate Core Mitomic Test (PCMT).
The Prostate Core Mitomic Test (PCMT) detects mitochondrial DNA (mtDNA) mutations in prostate biopsy core specimens [54]. Specifically, the evaluated mutation is a 3.4 kb mitochondrial genome deletion associated with prostate "cancerization" that is known to be elevated in PCa cells [55]. Hence, this test can determine the presence of malignant cells in progress of cancerization by detecting underlying molecular alterations in normal-appearing tissue. Clinical validation is based on 396 patients who underwent repeat prostate biopsy, of whom 143 had a benign diagnosis and 253 were found to be affected by PCa. PCMT resulted to have a sensitivity of ∼85% and an NPV of 91% for predicting presence of cancer on repeated biopsy [56].

3.2. Clinical scenario 2: when to choose active surveillance or active treatment?

Active surveillance is considered appropriate in patients at low- and very low-risk levels. Recently, the American guidelines have proposed active surveillance in selected patients with favorable intermediate-risk as well [11,12]. However, there is some heterogeneity within each risk group, which calls for caution in patient selection, adequate counseling, and careful follow-up. Biomarkers in this context may be useful in providing prognostic information on the risk of high-grade disease, BCR, and metastatic disease in order to select patients who need active treatment [11]. Biomarkers recommended in this clinical scenario are presented in Table 2.

3.2.1. Oncotype DX
The Oncotype Dx (Genomic Health Inc., Redwood City, CA, USA) Genomic Prostate Score (GPS) is a test that

Table 2. Biomarkers for risk stratification of patients with biopsy proven PCa: when to choose active surveillance or active treatment?

Trade name	Sample	Biomarker	Target population	Outcome predicted
Oncotype DX	Biopsy cores	Real-time PCR of 12 specific PCa genes and 5 control genes	LR-FIR PCa	RP GS >3 + 4 and/or extraprostatic disease BCR
Prolaris	Biopsy cores	Real-time PCR of 31 genes associated with the cell cycle and 15 control genes	LR-FIR PCa	RP GS >3 + 4; pT3 MFS BCR CSS
ProMark	Biopsy cores	Quantitative determination of 8 PCa-related proteins	LR-FIR PCa	RP GS >3 + 4; pT3
Decipher	Biopsy cores	Microarray (whole transcriptome)	LR-FIR PCa	RP GS >3 + 4; pT3 MFS CSS

LR-FIR PCa: Low-risk and favorable intermediate-risk prostate cancer; RP GS: radical prostatectomy Gleason score; BCR: biochemical recurrence; MFS: metastasis-free survival; CSS: cancer-specific survival.

uses real-time PCR on paraffin-embedded biopsy cores. It quantifies the expression of twelve genes involved in four different pathways responsible for PCa oncogenesis, including androgen response, cellular organization, proliferation, and stromal response, as well as five genes constitutively expressed in established PCa [57]. A mathematical model produces the GPS, expressed on a scale of 0–100, which predicts the risk of having aggressive pathology (GS ≥7 or extraprostatic disease) after RP [58]. Cullen et al. [59] validated this in a cohort of 279 patients with an average follow-up of 5.2 years, confirming its predictive value against adverse pathology and adding that the GPS is able to predict the risk of BCR after prostatectomy with a hazard ratio of 2.9 ($p < 0.001$) per increase of 20 GPS units. Eggner et al. [60] prospectively validated the GPS assay as an independent predictor of adverse pathology at RP in newly diagnosed low- and intermediate-risk PCa patients. This finding mirrors prior validations studies and shows the capacity of the GPS assay to enhance prediction of adverse pathology among men with intermediate-risk disease, suggesting the assay may be particularly useful for active surveillance selection in this patient subset. GPS testing also increased physician confidence and decreased decision conflict in patients who elected RP as initial management.

3.2.2. Prolaris

The Prolaris test (Myriad Genetics Salt Lake City, UT, USA) measures the expression of 31 genes associated with the cell cycle and 15 housekeeping genes in biopsy cores of low-grade PCa. It has been recommended by European and American guidelines for use in patients with low-risk and intermediate-risk disease who have not received active treatment and have a life expectancy of 10–20 years [11,61]. This test has been validated in four different studies based on analysis of two different tissues, the prostatectomy specimen and the pre-prostatectomy biopsy specimen [62,63]. Like the Oncotype DX test, Prolaris predicts the risk of GS ≥7 and extraprostatic disease following RP, as well as the risk of metastasis and BCR after radiotherapy [64]. Furthermore, Cuzick et al. [65] showed that the test could predict specific cancer survival in a cohort of patients on active surveillance with an average follow-up of 11 years. Studies on the clinical utility of the test have shown that, in 32% of cases, it would lead to a change in the proposed treatment with the net effect of moving patients from a more aggressive approach to a more conservative one [64]. The Prolaris test may be used in Clinical scenario 2 (Prolasis on prostate biopsy cores) and 3 (Prolaris on RP specimens).

3.2.3. ProMark

ProMark (Metamark, Waltham, MA, USA) is a test that aims to predict cancer aggressiveness in patients with GS 3 + 3 or 3 + 4 at biopsy. Using immunofluorescence, it calculates the levels of eight proteins (DERL1, CUL2, SMAD4, PDSS2, PDSS2, HSPA9, FUS, pS6, and YBOX1) and algorithmically produces a score ranging from 0 to 1, which predicts the risk of having GS higher or equal to 4 + 3 or extraprostatic disease [66,67]. Blume-Jensen et al. [68] showed that the addition of the ProMark score to the NCCN risk categories increases the AUC to 0.75 in predicting adverse disease outcomes. Furthermore, using a cutoff of 0.33, a sensitivity of 90% is obtained, with an associated PPV of 83.6%, and a false negative rate of 10%.

3.3. Clinical scenario 3: when to start radiation therapy after radical prostatectomy?

Current parameters used to guide postoperative treatment include PSA levels and histopathological findings of aggressive disease. However, these results have not always proved accurate in guiding treatment decisions.

After RP, PSA should be undetectable [10]. Between 5 and 20% of men continue to have persistent PSA after RP (post-RP PSA of >0.1 ng/mL within 4 to 8 weeks of surgery), resulting from persistent local disease, preexisting metastases, or residual benign prostate tissue. Persistent PSA after RP is associated with a poor prognosis and higher overall mortality. Both salvage radiation therapy and a "wait and watch" strategy are suitable in the case of BCR, but the timing and treatment modality for PSA-only recurrences after RP remain controversial due to poor evidence. Many patients with postoperative adverse outcomes never experience an increase in PSA as many patients whose cancer recurs do not develop metastatic disease [69]. NCCN guidelines suggest the use of tissue biomarkers after RP when there is a persistently high or increasing PSA level, to aid the decision-making process regarding the use and timing of post-prostatectomy radiotherapy [11]. Biomarkers recommended in this clinical scenario are presented in Table 3.

3.3.1. Decipher

Decipher (GenomeDx Biosciences, Vancouver, BC, Canada) is a genomic test that uses a DNA microarray to measure the levels of gene expression in biopsy samples (core with the highest GS: Decipher Prostate Biopsy) and prostates (tumor nodule with the highest GS: Decipher Prostate RP). The first to be discovered and validated was the Decipher Prostate RP test [70].

Table 3. Biomarkers for disease management of high risk PCa patients who can benefit from additional therapies after active treatment: when to start radiation therapy after radical prostatectomy?

Trade name	Sample	Biomarker	Target population	Outcome predicted
Prolaris	Radical prostatectomy specimen	Real time PCR of 31 genes associated with the cell cycle and 15 control genes	Post-prostatectomy risk factors[a]	RP GS >3 + 4 and/or extraprostatic disease MFS BCR CSF
Decipher	Biopsy cores and radical prostatectomy specimen	Microarray (whole transcriptome)	Post-prostatectomy risk factors[a]	RP GS >3 + 4 and/or extraprostatic disease MFS CSS

[a]Post prostatectomy risk factors: positive margins, PSA elevation, extraprostatic disease.
RP GS: radical prostatectomy Gleason score; BCR: biochemical recurrence; MFS: metastasis-free survival; CSS: cancer-specific survival.

The score ranges from 0 to 1, dividing patients into risk classes (low, intermediate, and high) based on the expression levels of 22 genes. In a recent meta-analysis of five different studies with 855 patients in total, it was estimated that Decipher's low-, intermediate-, and high-risk categories confer a risk of 5.5%, 15.0%, and 26.7% for developing metastasis over 10 years, respectively [71]. It also predicts the risk of BCR and cancer-specific survival [72]. Several studies have shown its utility in patient counseling and the decision to start salvage radiotherapy and adjuvant therapy [73,74].

Decipher Prostate Biopsy, which is also applicable to clinical scenario 2, predicts the risk of high-grade disease (GS >3 + 4), 5-year metastasis, and specific cancer mortality at 10 years. Although this test is not mentioned in the EAU guidelines, its use in the United States is increasingly widespread. Specifically, it is recommended by the NCCN guidelines for patients who present one or more risk factors after RP, such as positive margins, PSA elevation, and extraprostatic disease, as well as for low-risk or intermediate-risk patients who are considering active surveillance [11,75].

4. Future perspectives

In the diagnostic and therapeutic pathway of PCa, there is still an unmet need for tools that can provide a more precise and personalized risk stratification in three clinical scenarios: (1) deciding whether to perform a biopsy, (2) distinguishing patients who require active treatment from those who can benefit from active surveillance, and (3) defining a subset of high-risk PCa patients who can benefit from additional therapies after RP. Several biomarkers in these settings offer promising results. Although no randomized prospective study is available that shows their usefulness, the cohorts of patients on which they have been developed and validated have reached 10 years of follow-up. Various guidelines thus recommend their use in selected cases as second-level diagnostic tests in support of SOC clinical variables of proven effectiveness. However, the clinical benefit of

these biomarkers in the context of MRI and target prostate biopsy still needs to be proven [76–78].

Some biomarkers such as Pentraxina-3 [79,80] and Stockholm-3 [81,82] are currently being validated, and there will undoubtedly be more biomarkers discovered and validated as our knowledge of the biology of PCa improves. The evolution of bioanalytical methods to quantify very small quantities of molecules in a broad range of human fluids has opened new perspectives in biomarker discovery [83].

An example of this is represented by liquid biopsy through analysis of tumor-derived cells and molecules in body fluids. In this scenario, circulating tumor cells, biomolecules (circulating tumor DNA, RNA, proteins, and mtDNA), and EVs could be used as quantitative prognostic and response biomarkers for BCR and risk stratification in localized PCa and as predictive biomarkers for targeted therapies. Indeed, the presence of circulating tumor cells in a patient with no visible extraprostatic or distant disease on imaging has been shown to identify men with micrometastatic disease at the time of diagnosis [84].

Finally, the use of artificial intelligence and predictive models capable of processing the innumerable amount of data from the omics fields will speed up this process and will form the basis for an increasingly personalized model of therapy in PCa [85].

5. Conclusions

Several validated biomarkers have been developed in recent years but only a few have undergone extensive validation and are commercially available. Still, the evidence is limited to retrospective analyses and prospective validation of these biomarkers is warranted.

Guidelines now recommend offering these tests in situations in which the assay result, when considered in combination with routine clinical factors, is likely to affect management. However, the lack of direct comparisons and the unproven benefits, in terms of long-

term survival and cost-effectiveness, prevent these biomarkers from being integrated into routine clinical use.

Authors' contribution

Research conception and design: Ugo Giovanni Falagario, Riccardo Autorino, and Luigi Cormio

Data acquisition: Ugo Giovanni Falagario, Umberto Carbonara, Fabio Crocerossa, and Antonella Ninivaggi

Data analysis and interpretation: Riccardo Autorino, Ugo Giovanni Falagario, and Ivan Jambor

Drafting of the manuscript: Ugo Giovanni Falagario, Francesca Sanguedolce, Zach Dovey, George Papastefanou, and Ivan Jambor.

Critical revision of the manuscript: Riccardo Autorino, Giuseppe Carrieri, Luigi Cormio, Ivan Jambor, Anna Lantz, Umberto Carbonara, and Fabio Crocerossa

Supervision: Riccardo Autorino, Giuseppe Carrieri, and Luigi Cormio

Approval of the final manuscript: All authors.

Disclosure statement

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