The Present and Future of Biomarkers in Prostate Cancer: Proteomics, Genomics, and Immunology Advancements

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Supplementary Issue: Biomarkers and their Essential Role in the Development of Personalised Therapies (A)

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ABSTRACT: Prostate cancer (PC) is the second most common form of cancer in men worldwide. Biomarkers have emerged as essential tools for treatment and assessment since the variability of disease behavior, the cost and diversity of treatments, and the related impairment of quality of life have given rise to a need for a personalized approach. High-throughput technology platforms in proteomics and genomics have accelerated the development of biomarkers. Furthermore, recent successes of several new agents in PC, including immunotherapy, have stimulated the search for predictors of response and resistance and have improved the understanding of the biological mechanisms at work. This review provides an overview of currently established biomarkers in PC, as well as a selection of the most promising biomarkers within these particular fields of development.

KEYWORDS: prostate cancer, biomarker, genomics, proteomics, immunology

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Introduction

According to the International Agency for Cancer Research, prostate cancer (PC) is the second most common cancer in men.[1] It is estimated that 1.1 million men worldwide were diagnosed with PC in 2012, accounting for 15% of the cancers diagnosed in men. In North America, PC remains the most common noncutaneous solid tumor. It ranks third in Canada and second in USA as a leading cause of death by cancer in males.[2,3] Overall, the five-year survival rate is excellent (98.9%) but drops considerably in the metastatic context (28.5%).[4] Fortunately, 80.4% are diagnosed with localized disease.[4] This is due in large part to improvements in screening methods, highlighting the role of biomarkers such as prostate-specific antigen (PSA). A baseline PSA value is a stronger predictive factor of PC than family history or ethnicity.[5] The utility and importance of such biomarkers is underlined by the importance of a personalized approach to PC, given the variability of disease behavior, the diversity of treatments, and the related impairment of quality of life. Aside from their diagnostic and prognostic utilities, biomarkers that are predictive of treatment response are emerging as essential guiding tools, particularly after the expansion of therapeutic options for the castrate-resistant PC (CRPC) population. Furthermore, high-throughput technology platforms in proteomics, genomics, and immunology fields have accelerated the development of biomarkers. This article provides an overview of the currently established biomarkers in PC, as well as a selection of the most promising exploratory biomarkers in development.

Standard Biomarkers for Risk Stratification in PC

Initial patient evaluation and treatment decisions are currently based on a risk stratification scheme that incorporates the three most important prognostic biomarkers at diagnosis: clinical stage, biopsy Gleason grade/score, and serum PSA. Clinical stage is based on the TNM system and is associated with patient survival.[6] The Gleason grade describes the histological features of the cancer cells, from grade 1 (well differentiated) to grade 5 (poorly differentiated), and correlates with clinical behavior also. Since PC is often heterogeneous, a Gleason score (ranging from 2 to 10) is calculated from the sum of the two Gleason grades representing the primary and secondary

histological patterns of each biopsy sample. Higher scores are associated with a greater likelihood of having nonorgan-confined disease and a worse outcome after treatment of localized disease.[7] The 2010 version AJCC/UICC staging system uses clinical stage, biopsy Gleason score, and pretreatment serum PSA to define prognostic groups for prostate adenocarcinoma. The risk groups can predict the probability of biochemical failure after definitive local therapy and, therefore, are used as guides to select the most appropriate therapeutic approach. Such risk groups have been published and validated in multiple publications and provide superior prognostic information than clinical stage alone.[8]

Prognostic nomograms. A nomogram is an instrument that associates a set of input data to a particular outcome. The predictive power of a nomogram can be superior to the risk groups alone because they combine a greater number of prognostic variables specific to an individual patient. They usually incorporate information, such as clinical stage, PSA, and pathological information, such as Gleason score and number of positive biopsy scores. Numerous nomograms have been developed for different clinical situations, such as treatment decision making for patients eligible for active surveillance,[9] radical prostatectomy (RP),[10] neurovascular bundle preservation,[11] and pelvic lymph node dissection omission during RP[12] or radiotherapy.[10] Posttreatment nomograms also exist, providing estimates of biochemical progression-free survival (PFS) after RP[13] or the potential success of salvage radiation therapy after RP.[10] However, the use of nomograms has been criticized, particularly nomograms developed in academic centers that may generalize results for patient population.[14] Nomograms may also incorporate subjective or intermediate endpoints and could be affected by changing diagnostic procedures.[15]

Prostate-specific Antigen

PSA as a screening tool. PSA is the most widely used biomarker for the early detection of PC. Since the introduction of PSA testing, PC diagnoses have increased, but at the same time, the number of patients dying from the disease has decreased.[16] PC detected by elevated PSA levels has a better chance of being confined to the prostate than PC detected with a digital rectal examination (DRE).[17] Furthermore, higher PSA levels are associated with the risk of cancer, high-grade disease, tumor stage, and the presence of metastatic disease.[18] However, PSA does not represent an ideal biomarker. First, commercial assays measuring PSA are not standardized for direct comparison, and repeat testing is usually necessary. Second, PSA levels are not specific to PC and can be modulated by many factors, such as age, infection, trauma, ejaculation, instrumentation, and medication use (eg, 5-alpha-reductase inhibitors and corticosteroids). Third, there is no absolute value below which there is a negligible risk, and PSA levels cannot distinguish between indolent and aggressive diseases. In the PC Prevention Trial, approximately 15% of men with a PSA below 4 ng/mL were at risk for PC, and 15% of these men had high-grade disease.[19] However, when the PSA level was less than 1 ng/mL, the risk of high-grade disease was very low. Moreover, PSA levels above the traditional cutoff of 4 ng/mL reveal the presence of cancer on biopsies in only 25%–30% of patients.[20] Hence, there is no PSA cutoff point with high sensitivity and specificity for PC monitoring in healthy men but rather a continuum of PC risk at all values of PSA.[21]

PSA derivatives. Refinements to PSA measurements have been proposed, including PSA velocity (rate of change in PSA over time), PSA density (PSA to prostate volume ratio), age-specific PSA levels, and PSA doubling time.[22–25] However, these have not replaced PSA levels, because they have not been shown to add any incremental value. In the European Randomized Study of Screening for PC (ERSPC), PSA velocity did not independently predict cancer after adjusting for PSA level.[26] Similarly, in the PC Prevention Trial,[27] it was determined that using PSA velocity would increase the number of unnecessary biopsies while missing more high-grade cancers that would be identified just by lowering the PSA cutoff. As for PSA density, measurements are not very convenient because they require transrectal ultrasound or magnetic resonance imaging, and the results are not superior to those obtained with the percentage of free PSA (%fPSA).[28] Moreover, the sensitivity of this test is limited. This was shown by data from a large multicenter screening trial determining that a cutoff of 0.15 ng/mL/cm^3 (a commonly recommended cutoff value) of PSA density would miss nearly 50% of PCs detected in patients with a normal DRE and PSA levels between 4.0 and 10.0 ng/mL.[29] Finally, the clinical utility of age-specific PSA reference ranges remains uncertain.[30]

PSA screening controversy. PC screening with PSA levels has been a subject of debate and controversy due to its potential toward overdetection and overtreatment, which can induce patient anxiety. Indeed, the ability of PSA levels to reduce mortality has produced mixed results in recent randomized screening trials.[31,32] Uncertainty also exists in the practical considerations of testing, such as the age at which to initiate and discontinue the testing, along with its frequency. Various guidelines addressing PC screening have highlighted these issues of uncertainty, prompting the US Preventive Services Task Force to recommend *against* the use of PSA levels for screening in 2012.[33] Nonetheless, PSA levels still remain the fist-line biomarker option for the detection of PC. As the humoristic title of Vickers and Lilja's[34] article points out: "We need a better marker for PC. How about renaming PSA."

PSA isoforms. In the 1990s, it was discovered that the predominant form of PSA in the serum was in complex with α-1-antichymotrypsin.[35] This facilitated the development of selective immunodetection assays for PSA that was not bound to plasma proteins.[36] Thus, the measurement of fPSA and the calculation of fPSA percentage over total

PSA (tPSA = fPSA + PSA bound to α-1-antichymotrypsin) became possible.[37] The %fPSA has been associated with enhanced specificity for early PC detection for men with tPSA between 4 and 10 ng/mL and was initially associated with negative prostate biopsy in several single and multicentric studies.[38] A multicentric trial on the clinical utility of %fPSA reported a sensitivity and specificity of 95% and 20%, respectively, when a single cutoff of 25% was applied for men with tPSA values between 4.0 and 10.0 ng/mL.[39] Consequently, the FDA approved the use of the %fPSA for the early detection of PC in men with PSA levels between 4.0 and 10.0 ng/mL.

fPSA itself contains the following three distinct isoforms: (1) proPSA, (2) intact fPSA, and (3) benign PSA. ProPSA and intact fPSA are incompletely processed, single-chain forms that retain some parts of the propeptide sequence.[40] By contrast, benign PSA is a multichain form featuring internal peptide bond cleavages.[41] Most of the current research has focused on proPSA. Initially, most of the studies evaluating the [–5] and [–7]proPSA isoforms discovered that these molecules were no better than fPSA or other PSA-based measurements, in improving PC detection rates, especially in men with a tPSA below 10 ng/mL.[42] However, proPSA interest was rekindled by the identification of [–2]proPSA, a truncated form of proPSA, which has been described as the most prevalent form in tumor extracts and is preferentially expressed in cancerous prostatic epithelium.[43] Multiple studies have suggested a role for this isoform in early detection of PC, as well as for identifying aggressive forms of the disease.[44,45] Moreover, the velocity of certain isoforms, such as fPSA and proPSA, appears promising in increasing the detection of early PC.[46]

Prostate health index. The [–2]proPSA isoform has recently been incorporated in a test known as the prostate health index (PHI), which is developed by Beckman Coulter Inc., in partnership with the NCI's Early Detection Research Network. This test is a mathematical formula of three biomarkers: ([–2]proPSA/fPSA) × $PSA^{1/2}$. The purpose of this test is to distinguish benign and malignant prostatic conditions in patients aged 50 years and older, with a serum PSA between 4 and 10 ng/mL and a normal DRE. Recently, Lazzeri et al[44] have demonstrated that %[–2]proPSA and PHI accurately predict PC in men with a family history of PC and correlate with aggressiveness of the disease. This group also showed that [–2]proPSA, %[–2]proPSA, and PHI values can discriminate PC from chronic histologic prostatic inflammation and benign prostatic hyperplasia, in patients with a PSA between 4 and 10 ng/mL and a normal DRE.[47] Furthermore, meta-analyses published to date demonstrate that the PHI appears to outperform both PSA and %fPSA for the detection of overall and high-grade PC on biopsy.[48,49]

Testing with proPSA and PHI has been approved by the FDA since 2012. PHI, in particular, has been gaining acceptance worldwide, with regulatory approval in more than 50 countries and integration into some PC guidelines. Although PHI is discussed in the 2015 National Comprehensive Cancer Network (NCCN) guidelines for early PC detection, the panel does not recommend its use as first-line screening of all patients because of "limited prospective analyses in US populations."[50] Nonetheless, the panel states that a PHI score of >35 provides an estimate of the probability of PC and is "potentially informative in patients who have never undergone a biopsy or after a negative biopsy." The PHI is also discussed in the Melbourne Consensus Statement[51] and has been added into the smartphone application of the multivariable Rotterdam risk calculator used for clinical decisions.[52]

4K Score. OPKO Lab Inc. developed the 4K Score, which represents a combination of the following four kallikrein proteins: tPSA, fPSA, intact PSA, and kallikrein-related peptide 2 (hK2), the last of which distinguishes this test from the PHI score. The 4K Score also incorporates clinical information (such as age and history of prior negative biopsy) and provides an estimate of the probability of PC on a given prostatic biopsy. In a retrospective study using the Swedish Cancer Registry, Vickers et al[53] reported that the 4K Score enhanced the predictive accuracy for clinically diagnosed PC when compared to total PSA and age. Another retrospective study conducted on the Rotterdam arm of the European Randomized Study of Screening for PC showed further evidence that the 4K Score provided good accuracy in predicting aggressive disease.[54] A recent meta-analysis from aggregated studies evaluating the four-kallikrein panel showed a statistically significant improvement of 8%–10% in predictive accuracy of PC on biopsy. According to the authors, 48%–56% of biopsies could be avoided using this prediction tool, resulting in substantial financial savings.[55] The 2015 NCCN guidelines[50] do not recommend the 4K Score for first-line screening of all patients, but as stated for the PHI score, the 4K Score is also "potentially informative in patients who have never undergone a biopsy or after a negative biopsy." However, the 4K Score is not yet FDA approved.

Prognostic and predictive values of PSA. Despite being criticized as a PC screening biomarker, PSA has many other roles that are broadly accepted. As part of the initial clinical evaluation at diagnosis, PSA is a strong prognostic marker. It is associated with overall survival (OS) alone[56] or as part of nomograms.[57] PSA also reflects the burden of disease in CRPC.[58] Prior to the start of docetaxel chemotherapy in the setting of CRPC, several studies showed that PSA doubling time is prognostic for OS.[59,60] Associations of PSA with survival in CRPC patients with bone metastases have also been described, and it correlates with bone disease progression and skeletal-related events, regardless of treatment with bone-targeted therapy.[61] Baseline PSA and dynamic PSA changes, such as doubling time and velocity, are associated with PC recurrence and the emergence of metastases.[56,62–64] PSA can also improve the correlation of clinical stage and biopsy Gleason sum to the pathological stage at RP.[65] In the

IMPACT phase III trial evaluating Sipuleucel-T immunotherapy in CRPC patients, the greatest benefit was observed among patients with better baseline prognostic factors, particularly those with lower baseline PSA values.[66] However, PSA is not a reliable marker of response for this specific immunotherapy.[67]

PSA can also be used as a dynamic response biomarker. For instance, PSA is commonly accepted as a response indicator within initial androgen deprivation therapy.[68] In hormone-sensitive patients treated with androgen deprivation or CRPC patients treated with chemotherapy, PSA progression is associated with OS.[69] Similarly, in CRPC treated with abiraterone, PSA kinetics, such as PSA nadir, response rate, time to progression, and doubling time, were highly associated with OS, thus suggesting that PSA might even be used as a surrogate endpoint in this particular population.[70] However, it should be noted that these observations may be treatment specific.

Biomarkers Under Development and Evaluation

Proteomic/genomic biomarkers. In the last decade, proteomic and genomic advancements have accelerated the understanding of PC biology with such technologies as microarray analyses and next-generation genome-wide sequencing. As these platforms have become more available and affordable, an explosion of data has emerged. New approaches now include mostly diagnostic and prognostic panels that integrate somatic mutation signatures. Chosen gene alterations for these panels may be based upon well-known carcinogenic pathways in PC.[71] Others emerge from *fishing* approaches where unselected genes are filtered to correlate genes and phenotypes.[72] Considerable biostatistic support is required for such strategies to exclude the risk of chance associations. Adherence to standard criteria (such as Reporting of Recommendations for Tumor Marker Prognostic Studies) is also required to reduce potential biases.[73] Other approaches search for key genetic and epigenetic alterations in the peripheral blood, such as circulating tumor cells (CTCs). Most of the proteomic and genomic biomarkers in current development are reported on the NCI's Early Detection Research Network website (http://edrn.nci.nih.gov). The following selection, summarized in Table 1, represents the most promising tests, some of which are already commercially available. However, comparative studies between these tests do not currently exist.

PC antigen 3 and Progensa. PC antigen 3 (PCA3 or DD3) is a noncoding RNA that is specific to prostate tissue and highly expressed in the presence of malignant disease. PCA3 is of interest because it may be more accurate in outcome prediction than other methods for the early detection of PC at both initial and repeat biopsies. Accordingly, Hologic Inc. has produced the Progensa assay, which has been approved by the FDA since 2012 to help determine whether a repeat biopsy is necessary after a previous negative result. Progensa is a nucleic acid amplification test measuring the concentration of PCA3

Table 1. Summary of selected contemporary proteomic/genomic prostate cancer biomarkers.

BIOMARKER	DESCRIPTION	APPLICATIONS	SAMPLING SPECIMEN	COMMERCIAL AVAILABILITY	PROGNOSTIC	PREDICTIVE	PHARMACODYNAMIC	SURROGATE
Prostate specific antigen (PSA)	Prostate specific antigen	Diagnostic of PC PC follow-up	Serum	Yes	Yes	Yes	Yes	FSN
Free PSA (fPSA)	PSA not bound to plasma proteins	Diagnostic of PC	Serum	Yes	Yes	FSN	FSN	FSN
[–2]proPSA	Incompletely processed single-chain form of PSA	Diagnostic of PC	Serum	Yes	Yes	FSN	FSN	FSN
Prostate health index (PHI)	Mathematical formula of three biomarkers: ([–2]proPSA/fPSA) × $PSA^{1/2}$	To distinguish benign and malignant prostatic conditions in patients ≥50 years old with a serum PSA between 4–10 ng/mL and a normal digital rectum examination	Serum	Yes	Yes	FSN	FSN	FSN
4K Score	Immunoassay of four kallikrein: tPSA, fPSA, intact PSA and kallikrein-related peptide 2 (hK2)	To provide an estimate of the probability of having aggressive PC (GS ≥ 7) on first or repeat biopsy	Blood plasma	Yes	Yes	FSN	FSN	FSN
Progensa Prostate cancer antigen 3 (PCA3) Assay	Nucleic acid amplification test measuring the concentration of PCA3 and PSA RNA molecules in urine	To help with repeat biopsy decisions in men ≥50 years old with ≥1 negative prostate biopsies	Urine	Yes	FSN	FSN	FSN	FSN

Prostarix	Logistic regression algorithm combining four urinary metabolites: sarcosine, alanine, glycine and glutamate	To provide help in the decision for initial or repeat biopsy in patients with a negative digital rectal examination and mildly elevated PSA levels	Urine	Yes	Yes	FSN	FSN	FSN
TMPRSS2: ERG	Fusion gene of ERG and Transmembrane Protease, Serine 2	Prognostic and predictive utility at different stages of disease	Urine, Blood, Tissue	No	Yes	Yes	FSN	FSN
Mi-Prostate Score urine test	Multiplex analysis of urine tests for PCA3, TMPRSS2-ERG and PSA levels	To determine the probability of detecting prostate and aggressive disease (GS $\geq$ 6) on needle biopsy after PSA testing	Urine	Yes	Yes	FSN	FSN	FSN
ProMark	8-biomarker proteomic assay	To differentiate indolent from aggressive disease on intact tissue biopsies	Tissue	Yes	Yes	FSN	FSN	FSN
ConfirmMDx	Multiplex DNA methylation assay	To help with repeat biopsy decisions	Tissue	Yes	FSN	FSN	FSN	FSN
Prostate Core Mitomic Test	Genomic test measuring molecular alterations based on mitochondrial DNA	To help with repeat biopsy decisions	Tissue	Yes	FSN	FSN	FSN	FSN
Oncotype DX	Measures the expression of 17 genes related to 4 different molecular pathways	To personalize PC treatment based on assessment of disease aggressiveness	Tissue	Yes	Yes	FSN	FSN	FSN
Prolaris	Cell cycle progression (CCP) score based on the expression of 46 genes	To personalize PC treatment based on assessment of disease aggressiveness	Tissue	Yes	Yes	FSN	FSN	FSN
Decipher	Genomic test measuring 22 RNA biomarkers in multiple biological pathways	To classify post-surgery, intermediate- and high-risk patients into genomic risk categories for metastasis	Tissue	Yes	Yes	FSN	FSN	FSN
Circulating tumor cells (CTCs)	Cancer cells found in peripheral circulation	Detection media for various biomarkers	Serum	Yes	Yes	Yes	FSN	Yes
Androgen receptor splice variant 7 (AR-V7)	Androgen receptor variant	Possible predictive utility in patients receiving enzalutamide, abiraterone, or galaterone	Tissue	No	FSN	Yes	FSN	FSN

Abbreviations: PC, prostate cancer; FSN, further studies needed.

and PSA RNA molecules in urine after a DRE. A ratio of PCA3 RNA to PSA RNA is then calculated to provide the PCA3 score. In patients with an initially negative prostate biopsy, a PCA3 score of <25 is associated with a decreased likelihood of true PC.[74] In a meta-analysis of 11 combined clinical studies, of which 7 studies used the Progensa test, the sensitivity ranged from 53% to 69% and the specificity ranged from 71% to 83%.[75] Another recent meta-analysis pooled 11 heterogeneous studies.[76] In the group, including high-grade prostatic intraepithelial neoplasia and atypical small acinar proliferation, the sensitivity and specificity were 72% and 53%, respectively, when using a PCA3 score cutoff of 20. If the cutoff score was increased to 35, the sensitivity and specificity decreased to 49% and 35%, respectively. Recently, Bourdoumis et al[77] conducted a prospective observational study in CRPC patients and demonstrated a strong association between hormonal treatment and the absence of PCA3 expression. The Progensa PCA3 assay has been included in the EAU guidelines for repeat biopsy decision making.

Prostarix Risk Score. Boswick Laboratories offers the Prostarix Risk Score. This test aims to help physicians decide if an initial or repeat biopsy is necessary for patients with a negative DRE and mildly elevated PSA levels.[43] Prostarix measures the concentration of four urinary metabolites, sarcosine, alanine, glycine, and glutamate, which are combined in a logistic regression algorithm. As with the PCA3 assay, the urine is collected after a vigorous DRE. The first studies conducted on such metabolomic profiles have provided evidence that they may serve as promising diagnostic and prognostic tools.[78–81]

TMPRSS2:ERG. Gene rearrangements have been described in many cancers, particularly in hematologic malignancies. The fusion of ERG, a protooncogene of the erythroblast transformation-specific (ETS) family, and transmembrane protease, serine 2 (TMPRSS2) was first reported in 2005[82] and appears to be very specific to PC, with a positive predictive value of 94%.[43]

Some studies have suggested a prognostic utility for this fusion gene, but it appears to vary according to specific disease contexts. Boström et al[73] recently reviewed the evidence of its prognostic value in patients following RP and discovered that only 4 out of 10 studies had a significant association with outcome. Furthermore, a recent meta-analysis in this particular population did not show significant association with biochemical relapse or lethal disease.[83] Alternatively, the negative prognostic impact of TMPRSS2:ERG has been reported in watchful waiting cohorts,[84,85] early-onset disease,[86] and high-grade disease.[87]

The TMPRSS2:ERG rearrangement has also been studied as a predictor of response to therapy. Danila et al[88] showed that the rearrangement did not predict the response to abiraterone in CRPC patients, as measured in CTCs. However, the RNA expression of TMPRSS2:ERG in a similar population decreased in 86% of patients undergoing docetaxel chemotherapy.[89] Moreover, ERG expression is also associated with better response to androgen suppression,[90] although some studies have not supported this correlation.[91,92] In the upcoming PREMIERE-SOGIG phase II trial in metastatic CRPC (mCRPC) patients receiving enzalutamide, the primary objective will be to assess the value of TMPRSS2:ETS in primary tumors and CTCs in predicting PFS.[93]

Mi-Prostate Score urine test. Although TMPRSS2:ERG is specific for PC, most tumors have multiple foci and are heterogeneous in TMPRSS2:ERG expression. To overcome this limitation, TMPRSS2:ERG has been combined with other biomarkers. The University of Michigan MLabs has developed the Mi-Prostate Score, a multiplex analysis of urine tests for PCA3, TMPRSS2:ERG, and PSA levels, producing a risk assessment for aggressive disease. In a recent validation study, models applying PCA3 and TMPRSS2:ERG to cohort samples improved the association of PSA with PC and high-grade disease on biopsy.[94] This test is not yet FDA approved.

ProMark. Metamark Genetics Inc. has developed the ProMark test which is based on the prostate pathology and comprises an eight-biomarker (CUL2, DERL1, FUS, HSPA9, PDSS2, pS6, SMAD4, and YBX1) proteomic assay for intact tissue biopsies.[95] The test uses a fully automated, quantitative, multiplex immunofluorescence assay.[96] The recent clinical validation study met its two coprimary endpoints, separating favorable from nonfavorable pathology and Gleason score 6 versus non-Gleason score 6 pathology and showing that ProMark provided independent prognostic information relative to current risk stratification systems.[97]

ConfirmMDx. MDxHealth offers the ConfirmMDx multiplex DNA methylation assay. This test evaluates epigenetic biomarkers, especially the methylation of glutathione *S*-transferase pi 1, adenomatous polyposis coli, and Ras association (RalGDS/AF-6). The test aims to predict true negative prostate biopsies from those with possible occult cancer.[43] Two validation studies have been conducted thus far. In the retrospective MATLOC trial, a multivariate model showed that this epigenetic assay was significantly associated with patient outcome with an odds ratio of 3.17 (95% confidence interval 1.81–5.53).[98] In the DOCUMENT study, the assay was independently associated with PC detection in a repeat biopsy collected at an average of 13 months after an initial negative result and demonstrated an 88% negative predictive value.[99] Furthermore, Wojno et al[100] provided evidence for the clinical utility of the test, showing that in 138 patients with a negative initial prostate biopsy and a negative ConfirmMDx test, only 6 patients had repeat biopsies, with no evidence of disease. The current PASCUAL study, which is a controlled prospective study to track the clinical utility of the ConfirmMDx assay in US urologic practices, is expected to be complete in 2017.

Prostate Core Mitomic Test. There is emerging evidence linking mitochondrial function with regulation by oncogenes and tumor suppressors.[101] MDNA Life Sciences Inc. has created the Prostate Core Mitomic Test. The goal of this test is to

correctly identify true negative prostate biopsies by utilizing a cancerization field effect to identify the molecular changes in the mitochondrial DNA.[102] In a clinical validation study, this test was associated with a negative predictive value of 91%, a sensitivity of 84%, and a specificity of 54%.[103] This test has not been reviewed by the FDA.

Oncotype DX. Genomic Health Inc. has developed the Oncotype DX PC assay's Genomic Prostate Score (GPS). This tissue-based assay measures the expression of 17 genes related to the following four different molecular pathways: androgen (FAM13C, KLK2, AZGP1, and SRD5A2), stromal response (BGN, COL1A1, and SFRP4), cellular organization (FLNC, GSN, TPM2, and GSTM2), and proliferation (TPX2). Gene expression was quantified by reverse transcription-polymerase chain reaction in the following three studies: a discovery prostatectomy study, a biopsy study, and a prospectively designed, independent clinical validation study testing retrospectively collected needle biopsies from contemporary patients with low to intermediate clinical risk who were candidates for active surveillance.[104] In the validation study, the GPS was associated with high grade and high stage at surgical pathology, as well as high-grade and/or high-stage disease after controlling for established clinical factors.[104] Recently, the GPS correlated with biochemical relapse (after adjusting for NCCN risk group) and time to metastases and was strongly associated with adverse pathology in patients with very low, low, or intermediate risk after RP.[105] The GPS has also shown clinical utility in a study by Dall'Era et al[106] where GPS provided a net increase in recommendations and/or adoption of active surveillance in patients with newly diagnosed PC.

Prolaris score. Myriad Genetics has developed the Prolaris score, which produces a cell cycle progression (CCP) score based on the expression of 46 genes, consisting of 31 cell cycle progression genes and 15 housekeeping genes. The test was first elaborated in 2011[71] and has since been validated in four studies. In the first validation study, the gene panel was associated with PC death in a conservatively managed cohort that had been diagnosed by biopsy and transurethral resection of the prostate.[107] Two other studies reported Prolaris to be an independent prognostic factor for biochemical relapse and metastatic progression after RP.[108,109] Finally, Freedland et al[110] determined that Prolaris correlated with biochemical relapse and disease-specific survival definitive external beam radiation therapy. When combined with a multivariable score representing postprostratectomy clinical and pathological risk (CAPRA-S score), Prolaris added incremental prognostic information when compared to traditional clinical models.[111] In a recent study,[112] physicians completed surveys regarding treatment recommendations before and after they received and discussed the CCP test results with patients. Overall, 65% of cases showed a change between intended treatment before and after CCP reporting. Recently, the Prolaris panel has also been shown to detect subtle gene expression differences between incidental and clinically detected PCs.[113] However, this expensive test has been criticized for the lack of cost-effectiveness data.[73] According to the 2015 NCCN PC guidelines,[114] the clinical utility of Oncotype DX and Prolaris awaits evaluation by prospective, randomized clinical trials, which remain unlikely to be conducted.

Decipher PC test. GenomeDX Biosciences created the Decipher PC test. Conducted on tissue sample, this test measures 22 RNA biomarkers in multiple biological pathways in order to classify postsurgery patients with intermediate- and high-risk PC into genomic risk categories for metastasis. Decipher demonstrated better associations with metastatic disease than clinical-based models alone in multiple studies.[115–120] Clinical utility trials with Decipher were also favorable. In a report by the DECIDE study group, urologists were presented pathology reports and Decipher test results for 24 patients from a previous validation cohort. Following the Decipher genomic classifier results, treatment recommendations changed in 43% of adjuvant and 53% of salvage setting cases.[121] In the PRO-ACT study, 146 PC patients with adverse pathological features following RP were evaluated.[122] After reviewing the genomic classifier test, 60% of high-risk patients were re-classified as low risk. Furthermore, 42.5% of patients who were initially recommended adjuvant therapy were then recommended for observation. In a similar study, Badani et al[123] reported that recommendations for observation after RP increased by 20% for patients who were at low risk for metastasis, whereas recommendations for treatment increased by 16% for patients at high risk for metastasis. Similar to the Prolaris test, the Decipher gene panel's prognostic accuracy was at its highest when combined with clinical models, such as the CAPRA-S score.[115] In other recent studies, Decipher was able to predict the presence of lymph node metastasis in pre- and post-RP patients[124] as well as metastasis in patients undergoing postoperative, salvage radiation therapy.[117] Decipher can also be recalibrated for time-to-event data.[125]

Circulating tumor cells. CTCs have been detected in a majority of epithelial cancers and have emerged as interesting prognostic biomarkers.[126,127] This subsequently led to the FDA approval of the Veridex Cell Search platform, based on immunomagnetic selection of EPCAM-positive and CD45-negative cells. The number of CTCs has been shown to correlate with OS in PC patients.[128–131] The IMMC38 study was conducted using the Cell Search platform among CRPC patients receiving chemotherapy and was the first to report the prognostic and predictive role of CTCs. Patients with unfavorable pre- and posttreatment CTC enumeration (<5 CTC per 7.5 mL of blood) had shorter OS, and CTC counts had a stronger association with OS than PSA decrement algorithms at all time points.[128] The results of this study also demonstrated the prognostic value of baseline CTC counts as a continuous variable, before and after the initiation of treatment.[131] Subsequently, the prognostic and predictive values of CTCs in the IMMC38 study were prospectively validated in the phase III COU-AA-301 trial, which evaluated abiraterone

versus placebo in patients who had received docetaxel.[132] In this study, CTC conversion was associated with an improvement in OS as early as four weeks posttreatment.[133] Further analyses from the same trial revealed that at the individual patient level, a panel containing CTC number and lactate dehydrogenase level served as a surrogate for survival.[134] Additional trials are ongoing to validate these post hoc determined findings. These will require multivariate analyses to determine the value of this biomarker relative to already established, prognostic clinical and laboratory features.

In addition to abiraterone chemotherapy, the prognostic and predictive potential of CTCs appears promising for many new treatments for mCRPC. In patients treated with enzalutamide in the AFFIRM phase III trial, conversion from unfavorable to favorable CTC counts correlated with the OS benefit.[135] Furthermore, CTC counts after treatment with radium 223 may be helpful for monitoring treatment response.[136]

The CTCs are particularly interesting since no flare has been described so far, and changes in CTC numbers often occur before increases in PSA levels, highlighting their potential as a promising therapy monitoring marker.[132] One group was even able to sequence the whole genome of CTCs from four patients.[137] However, technical issues remain. First, the Cell Search detection process is dependent on the epithelial phenotype of CTCs, which can miss cells that have a mesenchymal transformation, and the sensitivity of currently available assays is fairly low.[132] Second, the dependency on a human operator for CTC counts may introduce a bias.[138] Finally, the required equipment is costly and not broadly available.[68] Nonetheless, CTC enumeration could be improved with new technologies that are not dependent on EPCAM detection.

Androgen receptor splice variant 7. Perhaps one of the most promising breakthroughs in predictive biomarkers is described in the work of Antonarakis et al.[139] This group examined the clinical relevance of androgen receptor variants from 31 enzalutamide-treated and 31 abiraterone-treated CRPC patients. Specifically, the androgen receptor splice variant 7 (AR-V7) mRNA status was established by reverse transcription-polymerase chain reaction on the CTCs of individual patients. Among the men who received enzalutamide or abiraterone, none of the AR-V7-positive patients had a PSA response. Patients had significantly shorter PSA PFS, clinical or radiographic PFS, and OS. These associations were maintained after adjustment for expression of full-length androgen receptor mRNA. Another study by Steinestel et al[140] also validated the predictive value of AR-V7 and other AR modifications in CTCs. A more recent analysis has shown that AR-V7-positive patients respond to taxane-based chemotherapy in a similar fashion as AR-V7-negative patients. This is one of the first attempts to personalize treatment choice in mCRPC.[141] Large-scale validation of these results are ongoing and will require testing for statistical interaction since clinical endpoints remain the primary objectives of the trial. ARMOR-3-SV phase III trial is the first phase III study to integrate a biomarker in patient selection for specific PC treatment. The study will evaluate the efficacy of galaterone (a new steroidal antiandrogen) in men with mCRPC whose tumors express the AR-V7 splice variant.[142]

Bone turnover biomarkers. The prognostic value of bone turnover markers has been evaluated in many studies. Bone-specific alkaline phosphatase and urinary N-telopeptide (Ntx) were associated with skeletal-related events, bone disease progression, and death in patients with solid tumors (including PC) in the placebo arm of two randomized phase III studies.[143] Similar results were reproduced in patients (411 patients with PC) treated with zoledronic acid, and high levels of Ntx were associated to a four- to sixfold increase in the risk of death.[144] Inversely, normalization of the same bone markers within three months of treatment initiation were associated with reduced risks of skeletal complications.[145] Higher levels of bone-specific alkaline phosphatase in serum were also associated with a decrease in OS in men with androgen-independent PC.[146] However, this was not the case for Ntx in that same study. Recent reviews support the utility of bone marker levels to assess disease progression in the metastatic setting and to evaluate bone health during hormonal therapy and response to bisphosphonate therapy.[147,148]

Furthermore, bone turnover markers can potentially guide response to therapy. In a prespecified, exploratory analysis of a multicenter phase III trial, levels of serum type 1 C-telopeptide, tartrate-resistant alkaline phosphatase 5b, and procollagen 1 N-terminal telopeptide decreased significantly in androgen-deprived patients treated with denosumab when compared to placebo.[149]

Immunologic biomarkers. Initially, PC was not considered as immunogenic in its nature, and first attempts to stimulate an immune response in the prostate were unsuccessful.[150] However, recent evidence demonstrated that PC generates a variety of tumor-associated antigens (TAAs), including PSA, prostatic acid phosphatase, and prostatic-specific membrane antigen, which are capable of producing a clinical response through immunogenicity.[151] Ultimately, this was translated into OS benefits in three phase III clinical trials, including the IMPACT trial, of Sipuleucel-T, an autologous antigen-presenting cell-based vaccine, and led to its approval by the FDA in 2010 and the European Medicine Agency in 2014 for the treatment of asymptomatic or minimally symptomatic mCRPC.[152–154] Multiple new immunotherapies, including other vaccines and immune checkpoint inhibitors, are currently under investigation, and the demand for predictive and surrogate biomarkers will most certainly increase in the forthcoming years. Such biomarkers could identify responders in the earlier phases of treatments, in which the full effects are often not apparent before weeks to months after initiation. Because OS benefits are generally better demonstrated with immunotherapy than PFS benefits, such biomarkers could

also provide surrogate endpoints to trials that would otherwise take years to complete.

Multiple categories of immune biomarkers have already been investigated in PC and include multiple inflammatory biomarkers and mediators, such as cytokines, various cellular and humoral immune responses and signatures, immune checkpoints and regulators, and tumor-infliltrating lymphocytes and other immune cells of the tumor microenvironment. Of note, the study of cellular and humoral immune parameters has produced interesting findings in response to PC itself as well as in the context of different immunotherapies, yielding potential prognostic, predictive, and/or pharmacodynamic biomarkers. New fields are also being developed, such as the genomics of immunological responses. Selected immunological biomarkers are summarized in Table 2.

Individual cytokines and other inflammatory proteins as biomarkers. Evidence from molecular, experimental, and clinical data suggests that inflammation can contribute or promote prostate carcinogenesis.[155] Concordantly, many biomarkers associated with prostatic inflammation diseases are also present in PC.[156] Among the inflammatory mediators are cytokines, a broad category of small molecules involved in cell signaling, such as chemokines, interferons, interleukins, lymphokines, and tumor necrosis factor. Multiple cytokines have been studied as biomarkers in the context of PC; most of them have generated interest as diagnostic or prognostic tools. Elevated IL-8, TNF-α, and MCP-1 were associated with poorer OS in metastatic PC patients who had started on androgen-deprivation therapy.[157] Further evidence for association between PC progression and two key cytokines, IL-8 and stromal cell-derived factor-1 (CXCL12), has been reviewed.[158]

IL-6 can stimulate the growth of androgen-independent cancer cells while suppressing androgen-dependent cells[159] and plays a role in promoting sketetal prostatic tumor growth by interacting with RANKL.[160] IL-6 has also been described in metastatic and CRPC patients, but the association with OS remains uncertain.[161]

Transforming growth factor-β1 (TGF-β1) has multiple functions, including cell-mediated immunity. TGF-β1 has been associated with biochemical recurrence post-RP, high Gleason score, and extent of disease.[162–164] In particular, TGF-β may play a significant role in the progression of PTEN-mutant PC.[165] Moreover, it might have a role as a predictive biomarker for immunotherapy. For instance, TGF-β1 is inversely correlated with in vivo and in vitro immunologic responses to the AE37 peptide vaccination in PC.[166]

Similar to recent strategies in the proteomic/genomic field, some cytokines have been combined with other serum biomarkers in a nomogram. In a study by Shariat et al,[167] preoperative plasma levels of TGF-β, IL-6, soluble IL-6 receptor, vascular endothelial growth factor, vascular cell adhesion molecule-1, endoglin, urokinase-type plasminogen activator, and plasminogen activator inhibitor-1 improved the accuracy of standard models associated with biochemical recurrence after RP.

Aside from their prognostic significance, cytokines may also be relevant as predictive biomarkers for chemotherapy. IL-6 and MIC-1 had previously raised interest in the context of docetaxel resistance, and Mahon et al[168] recently reported that in metastatic PC treated with docetaxel, changes in the levels of seven circulating cytokines were associated with progressive disease after completion of one cycle. Moreover, immunological responses in a subset of patients enrolled in the Sipuleucel-T IMPACT phase III trial demonstrated that OS correlated with cytokines and chemokines that were associated with activated antigen-presenting cells activated under secondary to Sipuleucel-T treatment.[169]

C-reactive protein (CRP) is an acute-phase protein of the pentraxin family of innate immune regulators involved in inflammation, necrosis, and carcinogenesis. It appears to have prognostic value in different stages of disease. In localized PC patients treated with radiotherapy, elevated CRP was associated with cancer-specific survival, OS, and clinical disease-free survival.[170] In metastatic patients, high serum CRP level (≥10 mg/L) was associated with significantly worse OS.[171] In CRPC patients treated with docetaxel and several phase II chemotherapeutic regimens, CRP was independently associated with OS.[172,173] A recent meta-analysis pooled the results of six studies correlating CRP with OS, in which a statistically significant association was observed between high CRP level and mortality.[174] Based on these analyses, the best estimated CRP cutoff was 12 mg/L.

Toll-like receptors (TLRs) are a family of transmembrane proteins that can recognize highly conserved molecules in invading pathogens. TLR-9 is reportedly increased in poorly differentiated prostate tumors.[175] Reports on the prognostic impact of TLRs in the postdiagnostic setting have been mixed since both upregulation[176] and downregulation[177] have been associated with high PC recurrence. Nonetheless, TLRs now represent a promising therapeutic target.[178]

The negative prognostic impact of a high neutrophil-to-lymphocyte ratio in pre-[179] and postdocetaxel mCRPC patients[180] has also been documented. High neutrophil-to-lymphocyte ratio also holds prognostic and predictive value in mCRPC patients during enzalutamide treatment.[181]

Cellular immune responses to PC. Detectable helper T-cell immune correlates of PC were established more than a decade ago when studies, such as McNeel et al,[182] showed that PC patients developed specific responses to PSA and prostatic acid phosphatase of the Th-1 subtype. Remarkably, PSA has also been demonstrated to be immunosuppressive through T-lymphocyte-mediated mechanisms.[183] Other researchers, such as Elkord et al,[184] have shown an impaired PSA-specific, cytotoxic T-cell response in PC patients. However, the prognostic value of PSA-specific, cytotoxic T-cells in peripheral blood outside of therapeutic interventions remains unknown. In one study, it was correlated to circulating prostate-specific,

Table 2. Summary of selected contemporary immunologic prostate cancer biomarkers.

BIOMARKER	DESCRIPTION/ EXAMPLES	APPLICATIONS	SAMPLING SPECIMEN	ROUTINE CLINICAL AVAILABILITY	PROGNOSTIC	PREDICTIVE	PHARMACODYNAMIC	SURROGATE
Inflammatory biomarkers								
Individual inflammatory cytokines	IL-6, IL-8, TGF-β1	Diagnostic and prognostic utility in various stages of disease Prediction of responses with chemotherapy, vaccines and Sipuleucel-T	Serum	No	Yes	Yes	FSN	FSN
C-reactive protein (CRP)	Acute-phase protein involved in inflammation, necrosis and carcinogenesis	Prognostic utility in various stages of disease	Serum	Yes	Yes	FSN	FSN	FSN
Toll-like receptors (TLRs)	Family of transmembrane proteins that can recognize highly conserved molecules in invading pathogens	Post-diagnostic prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Neutrophil-to-lymphocyte ratio	Ratio of peripheral neutrophil to lymphocite count	Post-diagnostic prognostic utility Possible predictive value in enzalutamide-treated-patients	Serum	Yes	Yes	Yes	FSN	FSN
Cellular response to PC								
Increase in Th1 T cell response	Subtype of T-helper cell response	Possible favorable prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Increase in Th2 T cell response	Subtype of T-helper cell response	Possible negative prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Cellular response to immunotherapeutic agents								
Increase in various T cell responses	Cytotoxic and T-helper lymphocytes	Possible prognostic and pharmacodynamic utility in patients treated with vaccines	Serum	No	Yes	FSN	Yes	FSN
Decrease in Treg response	Regulatory T cells	Role to be defined in patients treated with ipilimumab, Sipuleucel-T, and other vaccines	Serum	No	FSN	FSN	FSN	FSN
Increase in eosinophil response	Peripheral eosinophil count	Possible prognostic and predictive utility in Sipuleucel-treated patients	Serum	No	Yes	FSN	FSN	FSN
Humoral response to PC								
Tumor-associated antigens (TAAs) other than PSA	p90, p62	Possible diagnostic and prognostic utility	Serum	No	Yes	FSN	FSN	FSN
Auto-antibody signatures	Combination of various serum auto-antibodies	Possible diagnostic and prognostic utility	Serum	No	Yes	FSN	FSN	FSN

Humoral response to immunotherapeutic agents								
Antigen spreading	Vaccine-associated response to ubiquitously expressed self-antigens	Possible pharmacodynamic, prognostic and predictive utilities in patients treated with vaccines including Sipuleucel-T	Serum	No	FSN	FSN	FSN	FSN
Immune checkpoints								
PD-1/PD-L1 (B7-H1)	PD-1: Immunoglobulin superfamily member PD-L1: Ligand of PD-1, member of the B7 superfamily of costimulatory molecules	Predictive role in patients treated with Anti-PD-L1 and Anti-PD-1 monoclonal antibodies Possible predictive role in enzalutamide-resistant patients Possible prognostic role in ipilimumab- and Sipuleucel-T- treated patients	Tissue	Yes	Yes	Yes	FSN	FSN
CD276 (B7-H3)	Member of the B7 superfamily of costimulatory molecules	Possible post-diagnostic, prognostic and predictive roles New immunotherapy target	Tissue	No	Yes	Yes	FSN	FSN
CD73	Ectonucleotidase catabolizing the hydrolysis of extracellular adenosine monophosphate (AMP) to adenosine	Possible post-diagnostic, prognostic and predictive roles New immunotherapy target	Tissue	No	Yes	Yes	FSN	FSN
Immunologic biomarkers of tumor microenvironment								
Tumor-associated macrophages (TAMs)		Possible adverse prognostic role	Tissue	No	Yes	FSN	FSN	FSN
Cytotoxic CD8 tumor-infiltrating lymphocytes (TILs)		Possible adverse prognostic role	Tissue	No	Yes	FSN	FSN	FSN
Treg tumor-infiltrating lymphocytes (TILs)		Possible adverse prognostic role	Tissue	No	FSN	FSN	FSN	FSN
Mast cells		Role remains to be defined	Tissue	No	FSN	FSN	FSN	FSN

Abbreviations: PC, prostate cancer; FSN, further studies needed.

PSA-expressing cells, but not to PSA serum levels.[184] Another study did, however, link a Th-1 profile to a lower risk of PC in a prediagnostic setting.[185] Conversely, a Th-2 profile was observed in post-RP patients who had a worse prognosis.[186]

Cellular immune responses to immunotherapeutic agents. Most of the cancer vaccine studies to date have used biomarkers based on T-cell responses to TAAs. Early reports, such as Heiser et al,[187] showed that human autologous dendritic cells that were transfected with RNA encoding PSA stimulated prostate-specific cytotoxic T-cells in vitro. Using a PSA-specific DNA vaccine in vivo, cellular immune responses, including a Th-1-skewed response, were reported.[188] In an HLA-A24$^+$ PC patient vaccinated with cytotoxic T-lymphocyte-directed peptides, including PSA, humoral and Th-1 subtype immune responses were elicited.[189] Another group associated superior OS with delayed-type hypersensitivity immune responses in advanced PC patients treated with prostate stem cell antigen and PSA peptide-loaded dendritic cells.[190] In a phase II study of the Onyvax vaccine in mCRPC patients, a Th-1 cytokine release profile was noted in patients responding to restimulation with vaccine lysate.[191] A recent phase II study using the PROSTVAC vaccine showed a trend toward increased OS in patients with a greater than sixfold increase in T-cell response.[192] The immune monitoring within the PROSPECT randomized phase III study with this vaccine may yield further interesting candidates.[193]

Recently, cellular responses to ipilimumab, a fully human monoclonal antibody targeting the CTLA-4 checkpoint, were examined in a few studies. In bladder cancer patients treated with anti-CTLA therapy and cystoprostatectomy, there was a higher frequency of CD4$^+$ ICOS(hi) T-cells and higher levels of IFN-γ mRNA, observed in nonmalignant prostate tissue and incidental prostate tumor.[194] In a phase II clinical study of neoadjuvant ipilimumab (NCT01194271), the primary endpoints included the ratio of effector T-cells/Treg-cells, CD4$^+$ ICOS$^+$ and CD8$^+$ ICOS$^+$ T-cell counts, the presence of NY-ESO-1 antibodies, and total lymphocyte count in peripheral blood, which might emerge as predictive or surrogate biomarkers. Phase III trials of ipilimumab in the CRPC population might yield other potential candidates, although the first reported trial failed to reach its OS primary endpoint.[195]

The success of Sipuleucel-T also stimulated the search for meaningful biomarkers. In a phase II clinical trial of preoperative Sipuleucel-T, mCRPC patients receiving treatment had increased T-cell proliferation and IFN-γ in peripheral blood, as well as an increase in infiltrating CD3$^+$, CD4$^+$, FOXP3$^-$, and CD8$^+$ T-cells in RP tissues compared to pretreatment biopsies. Interestingly, the magnitude of the circulating immune response did not directly correlate with local tissue response.[196] Unfortunately, the prognostic and predictive impact of these changes remains unknown. Another group has reported on the samples of patients who participated in three randomized clinical trials using Sipuleucel-T. Interestingly, a transient increase in serum eosinophils at week 6 following treatment correlated with an induced immune response, a longer PC-specific survival, and a trend in OS.[197] Thus, transient increases in eosinophil count might hold prognostic and predictive values. Recent analyses from the NeoACT trial report that Sipuleucel-T supports a treatment-induced T-cell migration into the prostate tissue.[198] Other analyses from the STRIDE trial suggest that concurrent or sequential enzalutamide treatment does not impair the Sipuleucel-T immune response.[199]

Humoral immune responses to PC. Antibody immunity to PC was demonstrated by McNeel et al,[200] who showed that antibody immunity to PSA and HER-2/neu was significantly higher in PC patients compared to control populations. This response was also increased in patients with androgen-independent disease. Immunoscreening for PC has been successful with multiple TAAs, such as p90 and p62,[201,202] or antiprostasome antibodies.[203] Prognostic value has also been demonstrated for many antibodies including those against cancer-testis antigen CTSP-1,[204] matrix metalloprotease 11,[205] and of course, PSA (see the prognostic and predictive values of PSA section).

Autoantibody signatures and panels for PC screening. Autoantibody signatures and panels have been developed and may have a prominent role in PC detection. Microarrays of tumor cell-derived proteins in PC patients also uncovered a distinct pattern of immunoreactivity.[206] Shi et al[202] developed a panel of six TAAs, including p90 and p62, yielding positive reactions of 92.5% in PC patients. One group developed a phage protein microarray to analyze serum samples of PC patients. The 22-phage-peptide detector had 88.2% specificity and 81.6% sensitivity in discriminating between the group with PC and the control group, performing better than PSA testing.[207] Similar studies followed, all of which confirmed the potential of different autoantibody panels to discriminate between PC and benign disease.[208–212]

Humoral immune responses to immunotherapeutic agents. Many studies have reported humoral immune responses to new immunotherapeutic agents, although the firm distinction between pharmacodynamic, prognostic, and predictive values of these findings remains to be established. In a phase II randomized clinical trial of combined radiotherapy and a poxvirus-based vaccine encoding PSA, 7 out of 33 patients demonstrated a phenomenon known as *antigen spreading*, in which a vaccine-associated autoantibody response was induced by four ubiquitously expressed self-antigens, DIRC2, NDUFS1, MRFAP1, and MATN2.[213] The efficacy of vaccine immunotherapies may be enhanced by using predictive, individualized regimens that are tailored by mathematical models encompassing the basic interactions of the vaccine, immune system, and PC cells.[214] Smith et al[215] used a machine-learned Bayesian belief network along with phage immunoblots to identify the patterns of IgG following three months of treatment with different agents. In this report, androgen deprivation showed a different antigen recognition pattern compared to DNA and poxvirus vaccine therapies.

Humoral responses in patients from the IMPACT and ProACT Sipuleucel-T studies were evaluated. After treatment, antigen spreading against multiple secondary antigens occurred in treated patients but not in controls, and the responses to PSA and LGALS3 were associated with an improved OS in the IMPACT trial.[216] Therefore, these responses might hold pharmacodynamic as well as prognostic impacts in this particular population. Antonarakis et al[217] also looked at humoral responses to Sipeuleucel-T in the context of the phase II STAND trial and found that induction of a PA2024 antibody response may correlate with longer time to PSA progression.

Immune checkpoints and regulators.

PD-1 and PD-L1. Programmed cell-death receptor 1 (PD-1) is an immunoglobulin superfamily member shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The PD-1–ligand interaction is a major pathway hijacked by tumors to suppress immune control. Immunotherapies targeting this pathway, such as pembrolizumab and nivolumab, have shown promising results in many different tumor types. In the context of CRPC, PD-L1 is emerging as a potential predictive biomarker. In a phase I trial of nivolumab in multiple tumor sites, no objective responses were observed in mCRPC patients (n = 17), but only two of those patients had a biopsy, which were PD-L1 negative.[218] This is not surprising since PC generally shows low levels of PD-L1 expression.[219] However, $CD8^+$ T-lymphocytes infiltrating the prostate have been shown to express PD-1.[220] Interestingly, works by Bishop et al[221] established that resistance to enzalutamide (androgen receptor antagonist) is associated with PD-L1- and PD-L2-positive dendritic cells in both patients and preclinical models and that this resistance mechanism could be overcome by STAT3 inhibition.[222] Further studies are necessary to validate the possible impact of these biomarkers with anti-PD-1/anti-PD-L1 immune checkpoint therapy.

PD-1 has also been reported to be of relevance in the context of other immunotherapeutic agents, such as ipilimumab, the anti-CTLA-4 immune checkpoint inhibitor. In a phase I trial combining ipilimumab and the PROSTVAC vaccine in CRPC patients, lower $PD\text{-}1^+$ $Tim\text{-}3^-$ CD4 lymphocytes, higher $PD\text{-}1^-$ $Tim\text{-}3^+$ CD8 lymphocytes, and higher CTLA-Treg lymphocytes were associated with a longer OS.[223] In patients treated with ipilimumab as a single agent, increases in $CD4^+$ effector cells, Tregs, $PD\text{-}1^+$ $CD4^+$ effector cells, and $PD\text{-}1^+$ $CD8^+$ T-cells were observed but were not associated with OS. However, low pretreatment levels of $PD\text{-}1^+$ $CD4^+$ effector cells were related to a longer OS.[224] Moreover, when infiltrating T-cells were analyzed following preoperative Sipuleucel-T, the cells were identified as $PD\text{-}1^+$ and $Ki\text{-}67^+$, consistent with activated T-cells.[196]

B7-H3 (CD276). A new immune checkpoint protein, B7-H3 (CD276), represents a promising therapeutic target and has been reported as an adverse prognostic biomarker in PC. Indeed, high levels of B7-H3 have been associated with tumor progression and poor clinical outcomes.[225,226] B7-H3 may also have a predictive potential; it has been noted to increase in response to hormone therapy in PC patients after RP.[227]

CD73. CD73 is an ectonucleotidase involved in the hydrolysis of extracellular adenosine monophosphate to adenosine, an immunosuppressive molecule.[228] CD73 is expressed in many types of tissues and has been shown to be upregulated in cancer. The CD73–adenosine axis may have adverse prognostic implications in PC patients.[229] Furthermore, the therapeutic potential of CD73 blockade in PC is suggested by preclinical models where anti-CD73 mAb significantly enhanced the activity of both anti-CTLA-4 and anti-PD-1 mAbs against different subcutaneous tumors, including PC.[230]

Tumor-infiltrating lymphocytes and the tumor microenvironment. In parallel with other solid tumors, the PC microenvironment has gained increasing attention in the last few years. Early studies already showed immune disturbances where dendritic cells were statistically less in PC compared to normal tissue.[231] In another study, tumor-associated macrophages adjacent to cancer cells were positively associated with Gleason score.[232] Dominance of $CD4^+$ T-cell infiltrates with disturbed effector cell function was also noted by Ebelt et al.[233] Compared to benign nodular prostatic hyperplasia, high-grade prostatic adenocarcinoma had a significantly decreased total immune cell count.[234] The increasing interest in immunosuppressive functions of T-cells further revealed that PC tissue was surrounded by Tregs as well as $PD\text{-}1^+$ and $PD\text{-}L1^+$ cells.[235] The expression of PD-1 by T-cells may explain why some groups have reported significantly shorter biochemical failure-free survival that is associated with a high density of $CD8^+$ lymphocytes.[229,236] Other possible mechanisms include CTLA-4 or CD73 expression and IL-35 production. Of note, mast cells have also been studied in the PC tissue, but their clinical significance is still a matter of controversy.[237] Predictive biomarkers in PC tissue are actively investigated in the context of anti-PD-1 immune checkpoint therapy.

Immune biomarkers and genomics. A landmark study by Snyder et al[238] was published in *The New England Journal of Medicine* in late 2014. This group performed whole exome sequencing on tumors and matched blood samples of 64 melanoma patients treated with the anti-CTLA-4 agents, ipilimumab or tremelimumab. Using genomewide somatic neoepitope analysis and patient-specific HLA typing, candidate tumor neoantigens were identified for each patient, and a neoantigen landscape that was specifically present in tumors with a significant response to treatment was elucidated. Another remarkable study conducted by Rooney et al[239] recently identified 35 recurrently mutated genes showing a positive association with T-cell cytolytic activity, including β-2-microglobulin, HLA-A, -B, and -C, and caspase 8. Genetic amplifications were also associated with high

cytolytic activity, including immunosuppressive factors, such as PD-L1/2 and ALOX12B/15B. These genes reveal potential genetic biomarkers for predicting the outcome as well as candidate targets for immunotherapy. Such strategies hold high potential and are beginning to emerge in the specific context of PC. For example, Anastasopoulou et al[240] showed that patients with HLA-A*24 and HLA-DRB*11 alleles had increased immune responses as well as a higher OS after treatment with the AE37 li-key-HER-2/neu polypeptide vaccine, suggesting the potential prognostic and predictive impact of these alleles.

Conclusion and Future Perspectives on PC Biomarkers

Biomarkers in PC is a rapidly expanding field, and recent developments of proteomic/genomic platforms, as well as the rise of immunotherapy (and its mostly unmet need for adequate biomarkers), provide meaningful research opportunities for the upcoming years. Other promising innovations, such as imaging biomarkers, are also being developed. Nonetheless, many challenges still lie ahead. These include the harmonization and validity of assays used in biomarker development, the need for comparative studies for biomarker assays used in similar contexts, the association of correlative immune parameters with clinical endpoints, the development of panels applicable to multiple clinical contexts and therapies, as well as the sample sizes and the cost effectiveness of these tests. Furthermore, selected biomarkers have to provide additional, independent information from already established clinical and pathological variables. Finally, some areas of biomarker research remain largely unexplored and could provide clinically useful information, such as biomarkers predictive of treatment toxicity. Overall, it is rather unlikely that a single biomarker will be able to guide future clinical decisions, and recent trends point to the development of panels combining many different markers, with an underlying statistical complexity that should be designed a priori in clinical trials or meta-analyses. Based on the available body of literature, exciting discoveries in PC biomarker research most certainly lie in the near future.

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Author Contributions

Wrote the first draft of the article: P-OG. Contributed to the writing of the article: P-OG, JS, DS, and FS. Agreed with manuscript results and conclusions: P-OG, JS, DS, and FS. Jointly developed the structure and arguments for the article: POG and FS. Made critical revisions and approved the final version: P-OG, JS, DS, and FS. All authors reviewed and approved the final article.

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