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.\(^5\) 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.\(^6\) 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 these particular fields of 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. 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.

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, radical prostatectomy (RP), neurovascular bundle preservation, and pelvic lymph node dissection omission during RP or radiotherapy. Posttreatment nomograms also exist, providing estimates of biochemical progression-free survival (PFS) after RP or the potential success of salvage radiation therapy after RP. However, the use of nomograms has been criticized, particularly nomograms developed in academic centers that may generalize results for patient population. Nomograms may also incorporate subjective or intermediate endpoints and could be affected by changing diagnostic procedures.

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. 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). Furthermore, higher PSA levels are associated with the risk of cancer, high-grade disease, tumor stage, and the presence of metastatic disease. 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. 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. 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.

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. 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. Similarly, in the PC Prevention Trial, 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). 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/cm³ (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. Finally, the clinical utility of age-specific PSA reference ranges remains uncertain.

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. 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. Nonetheless, PSA levels still remain the first-line biomarker option for the detection of PC. As the humorous title of Vickers and Lilja’s 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. This facilitated the development of selective immunodetection assays for PSA that was not bound to plasma proteins. Thus, the measurement of fPSA and the calculation of fPSA percentage over total
PSA (tPSA = fPSA + PSA bound to α1-antichymotrypsin) became possible. 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. 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. 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. By contrast, benign PSA is a multichain form featuring internal peptide bond cleavages. 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. 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. Multiple studies have suggested a role for this isoform in early detection of PC, as well as for identifying aggressive forms of the disease. Moreover, the velocity of certain isoforms, such as fPSA and proPSA, appears promising in increasing the detection of early PC.

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. 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. 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. 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.

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.” 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 and has been added into the smartphone application of the multi-variable Rotterdam risk calculator used for clinical decisions.

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. 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. 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. The 2015 NCCN guidelines 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 or as part of nomograms. PSA also reflects the burden of disease in CRPC. Prior to the start of docetaxel chemotherapy in the setting of CRPC, several studies showed that PSA doubling time is prognostic for OS. 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. Baseline PSA and dynamic PSA changes, such as doubling time and velocity, are associated with PC recurrence and the emergence of metastases. PSA can also improve the correlation of clinical stage and biopsy Gleason sum to the pathological stage at RP. 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. However, PSA is not a reliable marker of response for this specific immunotherapy.

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. In hormone-sensitive patients treated with androgen deprivation or CRPC patients treated with chemotherapy, PSA progression is associated with OS. 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. 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. Others emerge from fishing approaches where unselected genes are filtered to correlate genes and phenotypes. 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. 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.
| Biomarker | Description | Use | Sample Type | Further Studies Needed |
|-----------|-------------|-----|-------------|-----------------------|
| 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 | 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 ≥ 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 | FSN |
| ConfirmMDx Multiplex DNA methylation assay | To help with repeat biopsy decisions | Tissue | Yes | FSN | 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 | 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 | FSN |
| Circulating tumor cells (CTCs) Cancer cells found in peripheral circulation | Detection media for various biomarkers | Serum | Yes | Yes | FSN | 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 <2.5 is associated with a decreased likelihood of true PC. 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%. Another recent meta-analysis pooled 11 heterogeneous studies. 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, Boudoumis et al 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. Prostarix measures the concentration of four urinary metabolites, sarsosine, 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.

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 and appears to be very specific to PC, with a positive predictive value of 94%. Some studies have suggested a prognostic utility for this fusion gene, but it appears to vary according to specific disease contexts. Boström et al 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. Alternatively, the negative prognostic impact of TMPRSS2:ERG has been reported in watchful waiting cohorts, early-onset disease, and high-grade disease.

The TMPRSS2:ERG rearrangement has also been studied as a predictor of response to therapy. Danila et al 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. Moreover, ERG expression is also associated with better response to androgen suppression, although some studies have not supported this correlation.

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.

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. 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. This test uses a fully automated, quantitative, multiplex immunofluorescence assay. The recent clinical validation study met its two coprimary endpoints, separating favorable from unfavorable 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.

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. 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). 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. Furthermore, Wojno et al 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. 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. 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%. 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. In the validation study, GPS was shown to be associated with high-grade and high-stage disease at surgical pathology, as well as high-grade and/or high-stage disease after controlling for established clinical factors. Recently, the GPS was 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. The GPS has also shown clinical utility in a study by Dall’Era et al 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 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. Two other studies reported Prolaris to be an independent prognostic factor for biochemical relapse and metastatic progression after RP. Finally, Freedland et al determined that Prolaris correlated with biochemical relapse and disease-specific survival definitive external beam radiation therapy. When combined with a multivariable score representing postprostatectomy clinical and pathological risk (CAPRA-S score), Prolaris added incremental prognostic information when compared to traditional clinical models. In a recent study, physicians completed surveys regarding treatment recommendations before and after they received CTC 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. However, this expensive test has been criticized for the lack of cost-effectiveness data. According to the 2015 NCCN PC guidelines, the clinical utility of Oncotype DX and Prolaris awaits evaluation by prospective, randomized clinical trials, which remain unlikely to be conducted.

**Decipher PC test.** GenomicDX Biosciences created the Decipher PC test. Conducted on tissue sample, this test measures 22 RNA biomarkers in multiple biological pathways in order to classify post-surgery 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. Clinical utility trials with Decipher were also favorable. In a report by the DECODE 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. In the PRO-ART study, 146 PC patients with adverse pathological features following RP were evaluated. 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. 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. In other recent studies, Decipher was able to predict the presence of lymph node metastasis in pre-and post-RP patients as well as metastasis in patients undergoing postoperative, salvage radiation therapy. Decipher can also be recalibrated for time-to-event data.

**Circulating tumor cells.** CTCs have been detected in a majority of epithelial cancers and have emerged as interesting prognostic biomarkers. 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. 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. 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. 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. In this study, CTC conversion was associated with an improvement in OS as early as four weeks posttreatment. 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. 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. Furthermore, CTC counts after treatment with radium 223 may be helpful for monitoring treatment response.

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. One group was even able to sequence the whole genome of CTCs from four patients. 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. Second, the dependency on a human operator for CTC counts may introduce a bias. Finally, the required equipment is costly and not broadly available. 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. 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 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. 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 galantrene (a new steroidal antiandrogen) in men with mCRPC whose tumors express the AR-V7 splice variant.

**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. 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. Inversely, normalization of the same bone markers within three months of treatment initiation were associated with reduced risks of skeletal complications. Higher levels of bone-specific alkaline phosphatase in serum were also associated with a decrease in OS in men with androgen-independent PC. 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.

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 compared to placebo. 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.

**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. 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. 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. 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-infiltrating 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. Concordantly, many biomarkers associated with prostatic inflammation diseases are also present in PC. 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. Further evidence for association between PC progression and two key cytokines, IL-8 and stromal cell-derived factor-1 (CXCL12), has been reviewed.

IL-6 can stimulate the growth of androgen-independent cancer cells while suppressing androgen-dependent cells and plays a role in promoting skeletal prostatic tumor growth by interacting with RANKL. IL-6 has also been described in metastatic and CRPC patients, but the association with OS remains uncertain. 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. In particular, TGF-β may play a significant role in the progression of PTEN-mutant PC. 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. 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, 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 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.

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. In metastatic patients, high serum CRP level (≥10 mg/L) was associated with significantly worse OS. In CRPC patients treated with docetaxel and several phase II chemotherapeutic regimens, CRP was independently associated with OS. 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. 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. Reports on the prognostic impact of TLRs in the postdiagnostic setting have been mixed since both upregulation and downregulation have been associated with high PC recurrence. Nonetheless, TLRs now represent a promising therapeutic target.

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

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, 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. Other researchers, such as Elkord et al, 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. | Serum | No | Yes | Yes | FSN | FSN |
| | | Prediction of responses with chemotherapy, vaccines and sipuleucel-T. | | | | | | |
| 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 lymphocyte 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.
Biomarkers in Cancer 2016:8(s2)

Unfortunately, the prognostic and predictive impact of these response did not directly correlate with local tissue response. Interestingly, the magnitude of the circulating immune system might yield other potential candidates, although the first report failed to reach its OS primary endpoint. 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 incident 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 CRPC patients 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 al202 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, NDUF51, 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 al215 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. Therefore, these responses might hold pharmacodynamic as well as prognostic impacts in this particular population. Antonarakis et al. also looked at humoral responses to Sipuleucel-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. This is not surprising since PC generally shows low levels of PD-L1 expression. However, CD8+ T-lymphocytes infiltrating the prostate have been shown to express PD-1. Interestingly, works by Bishop et al. 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. 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-1+ Tim-3- CD4 lymphocytes, higher PD-1+ Tim-3+ CD8 lymphocytes, and higher CTLA-4+ T-lymphocytes were associated with a longer OS. In patients treated with ipilimumab as a single agent, increases in CD4+ effector cells, Tregs, PD-1+ CD4+ effector cells, and PD-1+ CD8+ T-cells were observed but were not associated with OS. However, low pretreatment levels of PD-1+ CD4+ effector cells were related to a longer OS. Moreover, when infiltrating T-cells were analyzed following preoperative Sipuleucel-T, the cells were identified as PD-1+ and Ki-67+, consistent with activated T-cells.

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. B7-H3 may also have a predictive potential; it has been noted to increase in response to hormone therapy in PC patients after RP. CD73. CD73 is an ectonucleotidase involved in the hydrolysis of extracellular adenosine monophosphate to adenosine, an immunosuppressive molecule. 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. Furthermore, the therapeutic potential of CD73 blockade in PC is suggested by preclinical models where anti-CD73 mAbs significantly enhanced the activity of both anti-CTLA-4 and anti-PD-1 mAbs against different subcutaneous tumors, including PC.
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.

Acknowledgments

We would like to acknowledge the support of the Canadian Institutes for Health Research (CIHR) and the Medical Imaging Trials Network of Canada (MITNEC) funded by the CIHR. Dr. Fred Saad is a principal investigator who is a part of MITNEC.

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.

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]; 2013 [cited June 7, 2015]. Available at: http://globocan.iarc.fr
2. Society CC. Prostate Cancer Statistics, 2015 [cited June 7, 2015]. Available at: http://www.cancer.ca/en/cancer-information/cancer-type/prostate/statistics/regionqc
3. Society AC. What are the Key Statistics About Prostate Cancer? 2015 [cited June 7, 2015] Available at: http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-key-statistics
4. Surveillance E, End Results Program (SEER). Surveillance, Epidemiology, and Ends Results Program. Fast Stats; 2015 [cited June 7, 2015]. Available at: http://seer.cancer.gov/faststats/selections.php
5. Montie JD, Roehl KA, Loeb S, et al. Which is the most important risk factor for prostate cancer: race, family history, or baseline PSA level? J Urol 2008; 179(4):148.
6. Gittes RF. Carcinoma of the prostate. N Engl J Med. 1991;324(4):236–245.
7. Borstweg DG. Gleason grading of prostatic needle biopsies. Correlation with grade in 316 matched prostatectomies. Am J Surg Pathol. 1994;18(8):796–803.
8. D’Amico AV, Whittington R, Malkowicz SB, et al. Biochemical outcome after radical prostatectomy or external beam radiation therapy for patients with clinically localized prostate carcinoma in the prostate specific antigen era. Cancer. 2003;95(2):281–286.
9. Kattan MW, Eastham JA, Wheeler TM, et al. Counseling men with prostate cancer: a nomogram for predicting the presence of small, moderately differentiated, confined tumors. J Urol. 2003;170(5):1792–1797.
10. Center MS-KC. Prostate Cancer Nomograms; 2015 [cited July 11, 2015]. Available at: http://www.riskcalc.org/nomograms.html
11. Graefen M, Haese A, Pichlmieier U, et al. A validated strategy for side specific prediction of organ confined prostate cancer: a tool to select for nerve sparing radical prostatectomy. J Urol. 2001;165(3):857–863.
12. Briganti A, Chun FK, Salonia A, et al. A nomogram for staging of exclusive non-nervous lymph node metastases in patients with localized prostate cancer. Eur Urol. 2007;51(1):112–119; discussion 9–20.
13. Jeldres C, Suardi N, Wale J, et al. Validation of the contemporary Epstein criteria for insignificant prostate cancer in European men. Eur Urol. 2008;54(6):1306–1313.
14. Greenle KL, Meng MV, Elkin EP, et al. Validation of the Kattan preoperative nomogram for prostate cancer recurrence using a community based cohort: results from the cancer of the prostate strategic urological research endeavor (cap-sure). J Urol. 2004;171(6 pt 1):2255–2259.
15. Partin AW, Mangold LA, Lamm DM, Walsh PC, Epstein JJ, Pearson JD. Contemporaneous update of prostate cancer staging nomograms (Partin Tables) for the new millennium. Urolgy. 2001;58(6):843–848.
16. Surveillance E, End Results Program (SEER). SEER Stat Fact Sheets: Prostate Cancer, 2015 [cited June 7, 2015]. Available at: http://seer.cancer.gov/statfacts/html/prost.html
17. Catalona WJ, Smith DS, Ratliff TL, Basler JW. Detection of organ-confined prostate cancer is increased through prostate-specific antigen-based screening. JAMA. 1993;270(8):948–954.
18. Antenoe JA, Han M, Roehl KA, Nadler RB, Catalona WJ. Relationship between initial prostate specific antigen level and subsequent prostate cancer detection in a longitudinal screening study. J Urol. 2004;172(1):90–93.
19. Thompson IM, Pauker DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate–specific antigen level < or =4.0 ng per milliliter. N Engl J Med. 2004;350(22):2239–2246.
20. Parekh DJ, Ankerst DP, Toyer D, Srivastava S, Thompson IM. Biomarkers for prostate cancer detection. J Urol. 2007;178(6):2252–2259.
21. Thompson IM, Ankerst DP, Chi C, et al. Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. JAMA. 2005;294(1):66–70.
22. Benson MG, Whang IS, Olsson CA, McMahon DJ, Cooner WH. The use of prostate specific antigen density to enhance the predictive value of intermediate levels of serum prostate specific antigen. J Urol. 1992;147(3 pt 2):817–821.
23. Carter HB, Pearson JD, Metter EJ, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. JAMA. 1992;267(16):2215–2220.
24. Oesterling JE, Jacobsen SJ, Cooner WH. The use of age–specific reference ranges for serum prostate specific antigen in men 60 years old or older. J Urol. 1995; 153(4):1160–1163.
25. Schmid HP. Tumour markers in patients on deferred treatment: prostate specific antigen doubling times. Cancer Surv. 1995;23:157–167.
97. Blume-Jensen P, Berman DM, Rimm DL, et al. Development and clinical validation of a genomic classifier for prostate cancer risk stratification in men with a negative biopsy. Eur Urol. 2016;69(1):136–145.

98. Stewart GD, Van Neste L, Delvenne P, et al. Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies. J Clin Oncol. 2013;31(11):1428–1434.

99. Grande E, Gonzalez-Billalabeitia E, Duran I, et al. Phase II multicenter study to validate the prognostic role of the surrogate enrichment factor in patients with metastatic castration resistant prostate cancer patients and response to docetaxel treatment. Prostate. 2014;74(12):1222–1230.

100. Saylor PJ, Karoly ED, Smith MR. Prospective study of changes in the metabolic profiles of men during their first three months of androgen deprivation therapy. J Urol. 2015;193(6):1848–1853.

101. Cooperberg MR, Simko JP, Cowan J, et al. Validation of a cell cycle progress gene panel to improve risk stratification in a contemporary prostatectomy cohort. J Clin Oncol. 2013;31(11):1428–1434.

102. Frezza C. The role of mitochondria in the oncogenic signal transduction. Int J Biochem Cell Biol. 2014;48:11–17.

103. Robinson K, Creed J, Reguly B, et al. Accurate prediction of repeat prostate biopsy outcomes by a mitochondria DNA deletion assay. Prostate Cancer Prostatic Dis. 2010;13(2):126–131.

104. Klein EA, Cooperberg MR, Magi-Galluzzi C, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multicentricity, and biopsy under sampling. Eur Urol. 2014;66(5):550–560.

105. Cullen J, Rosner BL, Brandt TC, et al. A biopsy-based 17-gene genomic prostate score predicts recurrence after radical prostatectomy and adverse surgical pathology in a racially diverse population of men with clinically low- and intermediate-risk prostate cancer. BJU Int. 2012;110(10):1549–1556.

106. Dall Eara MA, Dones B, Lawrence HJ, et al. Clinical utility of a 17-gene genomic prostate score (GPS) for treatment selection in men with newly diagnosed prostate cancer (PCa). ASCO Proc. 2015;33(15_suppl):e16124.

107. Cuzick J, Berney DM, Fisher G, et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. Br J Cancer. 2012;106(6):1095–1099.

108. Bishoff JT, Freidland SJ, Gerber L, et al. Prognostic utility of the cell cycle progression score generated from biopsy in men treated with prostatectomy. J Urol. 2014;192(2):409–414.

109. Cooperberg MR, Simko JP, Cowan J, et al. Validation of a cell cycle progress gene panel to improve risk stratification in a contemporary prostatectomy cohort. J Clin Oncol. 2013;31(11):1428–1434.

110. Freedland SJ, Gerber L, Reid J, et al. Prognostic utility of cell cycle progression score in men with prostate cancer after primary external beam radiation therapy. J Urol Radiol. 2012;336(6084):1040–1044.

111. Cooperberg MR, Hilsen JF, Carroll PR. The CAPRA-S score: a straightforward tool for improved prediction of outcomes after radical prostatectomy. Cancer. 2011;117(22):5039–5046.

112. Crawford ED, Scholz MC, Kar AJ, et al. Cell cycle progression score and treatment decisions in prostate cancer: results from an ongoing registry. Med Care. 2014;52(10):1025–1031.

113. Bianconi M, Faloppi L, Zizzi A, et al. Multiplexing in incidentally and clinically detected prostate cancer. ASCO Proc. 2015;33(15_suppl):e16170.

114. Oncology NCCNCPG. Prostate Cancer Version 1.2015, 2015 [cited June 30, 2015]. Available at: www.nccn.org/professionals/physician_gls/pdf/prostate.pdf.

115. Cooperberg MR, Davicioni E, Crisan A, Jenkins RB, Ghadessi M, Karnes RJ. Combined value of validated clinical and genomic risk stratification tools for predicting prostate cancer mortality in a high-risk prostatectomy cohort. Eur Urol. 2015;67(2):326–333.

116. Den RB, Feng FY, Showalter TN, et al. Genomic prostate cancer classifier predicts biochemical failure and metastases in patients after postoperative radiation therapy. Int J Radiat Oncol Biol Phys. 2014;89(5):1038–1046.

117. Den RB, Yousef K, Tribulsi EJ, et al. Genomic classifier identifies men with adverse pathology after radical prostatectomy who benefit from adjuvant radiation therapy. J Clin Oncol. 2015;33(8):944–951.

118. Karnes RJ, Bergstralh EJ, Davicioni E, et al. Validation of a genomic classifier that predicts metastasis following radical prostatectomy in an at risk patient population. J Urol. 2013;190(2):1947–1953.

119. Klit HR EA, Vousif K, Haldal Z, et al. A genomic classifier improves prediction of metastatic disease within 5 years in patients with grade-negative high-risk prostate cancer patients managed by radical prostatectomy without adjuvant therapy. Eur Urol. 2015;67(4):778–786.

120. Moshou D, Johnson MF, Choeurng V, et al. Novel biomarker signature may predict aggressive disease in African American men with prostate cancer. J Clin Oncol. 2015;33(25):2789–2796.

121. Badani K, Thompson DJ, Buerki C, et al. Impact of a genomic classifier of metastatic risk on postoperative treatment recommendations for prostate cancer patients: a report from the DECIDE study group. Oncotarget. 2013;4(48):600–609.

122. Michalopoulos SN, Kella N, Payne R, et al. Influence of a genomic classifier on post-operative treatment decisions in high-risk prostate cancer patients: results from the PRO-ACT study. Curr Med Res Opin. 2014;30(9):1547–1556.

123. Badani KK, Thompson DJ, Brown G, et al. Effect of a genomic classifier test on clinical practice decisions for patients with high-risk prostate cancer after surgery. BJU Int. 2015;115(3):419–429.

124. Lee HJ, Godbeu E, Raheem O, et al. Evaluation of a genomic classifier in primary tumor and lymph node metastases in pre- and post-radical prostatectomy tissue specimens from men with high grade node positive prostate cancer. ASCO Proc. 2015;33(15_suppl):e16087.

125. Davicioni E, Choeurng V, Luo B, et al. Recalibration of genomic risk prediction models in prostate cancer to improve individual-level predictions. ASCO Proc. 2015;33(15_suppl):e16122.

126. Pasteau K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. Nat Rev Cancer. 2008;8(5):329–340.

127. Yu M, Stort T, Toner M, Maheswaran S, Haber DA. Circulating tumor cells: approaches to isolation and characterization. J Cell Biol. 2011;192(3):373–382.
...
200. McNeel DG, Nguyen LD, Storer BE, Vessella R, Lange PH, Disis ML. Anti-neutrophil membrane  protein-lipid ratio in men with metastatic castration-resistant prostate cancer. *Clin Genitourin Cancer*. 2014;12(3):317–324.

201. Liu W, Deng B, Lu Y, Wu W, Qian W, Zhang JY. Autoantibodies to tumor-associated antigens as biomarkers in cancer immunodiagnostics. *Autoimmun Rev*. 2011;10(6):331–335.

202. Shi FD, Zhang JY, Liu D, et al. Preferential humoral immune response in prostate cancer to cellular proteins p90 and p62 in a panel of tumor-associated anti-gens. *Prostate*. 2005;64(1):252–257.

203. Minelli A, Ronquist G, Carlsson L, Mearini E, Nilsson O, Larsson A. Antiproteasome antibody titres in benign and malignant prostate disease. *Anticancer Res*. 2005;25(6):4399–4402.

204. Parmigiani RB, Bettrun P, Grossi DM, et al. Antibodies against the cancer-tester antigen CTSP-1 are frequently found in prostate cancer patients and are an independent prognostic factor for biochemical-recurrence. *Int J Cancer*. 2008;122(10):2385–2390.

205. Roscilli G, Cappellini M, De Vitis C, et al. Circulating MMP11 and specific antibody responses in prostate and breast cancer patients. *J Transl Med*. 2014;12:54.

206. Boveman K, Qul, Zhou H, et al. Circulating tumour cell-derived proteins uncover a distinct pattern of prostate cancer serum immunoreactivity. *Proteomics*. 2003; (3):2200–2207.

207. Wang X, Yu, J, Sreekumar A, et al. Autoantibody signatures in prostate cancer. *N Engl J Med*. 2005;353(12):1224–1235.

208. Massionner P, Lueking A, Goehler H, et al. Serum-autoantibodies for discovery of prostate cancer specific biomarkers. *Prostate*. 2012;72(4):427–436.

209. Wandall HH, Blatz O, Tarp MA, et al. Cancer biomarkers defined by auto-antibodies to signatures of aberrant O-glycoprotein epitopes. *Cancer Res*. 2010;70(4):1306–1313.

210. Burford B, Gentry-Mahara J, Graham R, et al. Autoantibodies to MUC1 glycopeptides cannot be used as a screening assay for early detection of breast, ovarian, lung or pancreatic cancer. *Br J Cancer*. 2013;108(3):2045–2055.

211. Maricque BB, Eichhoff JC, McNeel D. Antibody responses against prostate-specific antigens in patients with prostatectomy and prostate cancer. *Prostate*. 2011;71(12):134–146.

212. O’Rourke DJ, DiJohnon DA, Ciaiazzo RJ Jr, et al. Autoantibody signatures as biomarkers to distinguish prostate cancer from benign prostatic hyperplasia in patients with advanced serum prostate specific antigen. *Clin Chim Acta*. 2012;415(6–7):561–567.

213. Nesslering NJ, Ng A, Tsang KY, et al. A viral vaccine encoding prostate-specific antigen induces antigen spreading to a common set of self-proteins in prostate cancer patients. *Proc Natl Acad Sci U S A*. 2011;108(25):10082–10087.

214. Kronik N, Kogan Y, Elisheveren M, Halevi-Tobias K, Vuk-Pavlovic S, Agur Z. Predicting outcomes of prostate cancer immunotherapy by personalized mathematical models. *PLoS One*. 2010;5(12):e15482.

215. Smith HA, Maricque BB, Eberhardt J, et al. IgG responses to tissue-associated antigens as biomarkers of immunological treatment efficacy. *J Biomed Biotechnol*. 2011;2011:45861.

216. GuhaThakurta D, Shikhel NA, Fan LQ, et al. Humoral immune response against nontargeted tumor antigens after treatment with sipuleucel-T and its association with improved clinical outcome. *Clin Cancer Res*. 2015;21(18):3619–3630.

217. Amangozari FS, Khet al AR, Gesu OW, et al. Immune responses and clinical outcomes in STAND, a randomized phase 2 study evaluating optimal sequential of sipuleucel-T (sip-T) and androgen deprivation therapy (ADT) in biochemically-recurrent prostate cancer (BRPC) after local therapy failure. *ASCO Meet Abstr*. 2015;33(15_suppl):TS5080.

218. Topoladin SL, Hodi FS, Brahmer J, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366(26):2443–2454.

219. Carosella ED, Plougaard G, LeMaout J, Desgrandchamps F. A systematic review of immunotherapy in urologic cancer: evolving roles for targeting of CTLA-4, PD-1/PD-L1, and HLA-A: *Eur Urol*. 2015;68(2):267–279.

220. Snors KS, Bruno TC, Meeker AK, De Marzo AM, Isaacs WB, Drake CG. Human prostate-infiltrating CD8+ T lymphocytes are oligoclonal and PD-1+ Prostate. 2009;69(15):1649–1703.

221. Bishop JL, Sio A, Angeles A, et al. PD-L1 is highly expressed in Enzalutamide resistant prostate cancer. *Oncotarget*. 2015;6(3):234–242.

222. Bishop J, Thaper DL, Vahidi S, Johansson MH, Zoubeidi A. Reduction in PD-L1 expression by STAT3 inhibition with GPA500 in enzalutamide-resistant prostate cancer. *ASCO Meet Abstr*. 2015;33(15_suppl):e16075.

223. Jochems C, Tucker JA, Tsang KY, et al. A combination trial of vaccine plus ipilimumab in metastatic castration-resistant prostate cancer patients: immune correlates. *Cancer Immunol Immunother*. 2014;63(4):407–418.

224. Kwek SS, Lewis J, Zhang L, et al. Preexisting levels of CD4 T cells expressing PD-1+ are associated with improved clinical outcome in metastatic castration-resistant prostate cancer. *JUrol*. 2011;108(1):2045–2055.

225. Yuan H, Wei X, Zhang G, Li C, Zhang X, Hou J. B7-H3 over expression in prostate cancer tissue promotes tumor cell progression. *J Urol*. 2011;186(3):1093–1099.

226. Yang X, Thompson RH, Al-Ahmade HA, et al. B7-H3 and B7x are highly expressed in human prostate cancer and associated with disease spread and poor outcome. *Proc Natl Acad Sci U S A*. 2007;104(49):19456–19461.
227. Chavin G, Sheinin Y, Crispen PL, et al. Expression of immunosuppressive B7-H3 ligand by hormone-treated prostate cancer tumors and metastases. *Clin Cancer Res*. 2009;15(6):2174–2180.

228. Stagg J, Beavis PA, Divisekera U, et al. CD73-deficient mice are resistant to carcinogenesis. *Cancer Res*. 2012;72(9):2190–2196.

229. Leclerc BC, Charlebois R, Chouinard G, et al. CD73 expression is an independent prognostic factor in prostate cancer. *Clin Cancer Res*. 2015;22(1):158–166.

230. Allard B, Pommey S, Smyth MJ, Stagg J. Targeting CD73 enhances the anti-tumor activity of anti-PD-1 and anti-CTLA-4 mAbs. *Clin Cancer Res*. 2013;19(20):5626–5635.

231. Troy A, Davidson P, Atkinson C, Hart D. Phenotypic characterisation of the dendritic cell infiltrate in prostate cancer. *J Urol*. 1998;160(1):214–219.

232. Shimura S, Yang G, Ebara S, Wheeler TM, Frolov A, Thompson TC. Reduced infiltration of tumor-associated macrophages in human prostate cancer: association with cancer progression. *Cancer Res*. 2000;60(20):5857–5861.

233. Ebelt K, Babaryka G, Figel AM, et al. Dominance of CD4+ lymphocytic infiltrates with disturbed effector cell characteristics in the tumor microenvironment of prostate carcinoma. *Prostate*. 2008;68(1):1–10.

234. Hussein MR, Al-Assiri M, Musalam AO. Phenotypic characterization of the infiltrating immune cells in normal prostate, benign nodular prostatic hyperplasia and prostatic adenocarcinoma. *Exp Mol Pathol*. 2009;86(2):108–113.

235. Ebelt K, Babaryka G, Frankenberger B, et al. Prostate cancer lesions are surrounded by FOXP3+, PD-1+ and B7-H1+ lymphocyte clusters. *Eur J Cancer*. 2009;45(9):1664–1672.

236. Ness N, Andersen S, Valkov A, et al. Infiltration of CD8+ lymphocytes is an independent prognostic factor of biochemical failure-free survival in prostate cancer. *Prostate*. 2014;74(14):1452–1461.

237. Taverna G, Giusti G, Seveso M, et al. Mast cells as a potential prognostic marker in prostate cancer. *Dis Markers*. 2013;35(6):711–720.

238. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014;371(23):2189–2199.

239. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell*. 2015;160(1–2):48–61.

240. Anastasopoulou EA, Voutsas IF, Keramitsoglou T, et al. A pilot study in prostate cancer patients treated with the AE37 Ir-key-HER-2/neu polypeptide vaccine suggests that HLA-A*24 and HLA-DRB1*11 alleles may be prognostic and predictive biomarkers for clinical benefit. *Cancer Immunol Immunother*. 2015;64(9):1123–1136.