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Commercialized Biomarkers: New Horizons in Prostate Cancer Diagnostics

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Abstract

Limitations with current clinical tools available for diagnosis and prognosis of prostate cancer have resulted in over-diagnosis and costly overtreatment which is impacting on the outcomes and quality of life of men. The biotech industry is investing significant resources into developing more specific biomarkers for prostate cancer detection and patient stratification which would greatly advance the decision-making processes behind prostate cancer management and treatment. In this review we focus on those biomarkers which have been translated into commercial tests available to clinicians. Since these tests aim to fill specific gaps during the decision-making process of prostate cancer management, we have grouped them based on the clinical question they claim to address i.e. improved prostate cancer screening, false negative biopsy dilemma, prognostic tests following a positive biopsy and tests predicting relapse/metastases after surgery. We evaluate each test with respect to its development, platform, clinical validation, biomatrix, regulatory approval status and cost.

Key words

Patented biomarkers, commercialized prostate cancer tests, prostate cancer screening, false-negative biopsy dilemma, clinical utility

1. Introduction

Prostate cancer (PCa) is the second most common cancer worldwide with an estimated 1,100,000 new cases diagnosed in 2012 [1]. In the United States alone, more than \$8 billion is spent annually to treat PCa. The global market for PCa prevention and treatment was valued at \$26.1 billion in 2011 and it is expected to reach \$50.3 billion in 2017 [2].

2. Novel diagnostics for prostate cancer

The current management of PCa is summarised in Table 1. Due to the limitations of current clinical tools used for diagnosis and prognosis, many research groups have focused on the discovery and validation of novel biomarkers. In 2012, we reviewed PCa biomarkers which had been successfully patented [3]. This article focusses on biomarkers which have been translated into commercially available tests. Since these tests aim to fill specific gaps during the decision-making process of PCa management, they are grouped based on the clinical question they claim to address as follows: improved PCa screening, the false negative biopsy dilemma, prognostic tests following a positive biopsy and tests predicting relapse/metastases after surgery (Figure 1). We have also evaluated each test with respect to its development, platform, clinical validation, biomatrix, regulatory approval status and cost (Table 2).

2.1 Improved PCa screening

A number of new tests are aimed at improving PCa screening by guiding initial biopsy decision and reducing the number of unnecessary biopsies following PSA rise and positive digital rectal examination (DRE).

2.1.1 4Kscore Prostate Cancer Test (OPKO Health)

Immunoassay blood test measuring a panel of kallikrein markers: Total PSA, Free PSA, Intact PSA and human kallikrein 2 combined using an algorithm. The 4KScore test has been launched in Europe in partnership with International Health Technology (Cambridge, UK), as part of IHT's ProstateCheck early detection programme. The test is available through clinics in the UK, Ireland, Sweden and Denmark.

This panel is based on markers used in a series of studies conducted in Europe and the U.S, assessing risk for clinically significant PCa [4-10]. The OPKO panel has been demonstrated in over 8,500 patients to predict the probability of cancer-positive biopsies in men suspected of having PCa. Retrospective studies have shown that the use of the panel could eliminate over 50% of unnecessary biopsies, with a probability of delaying diagnosis of a high grade cancer of only 0.6% (and this small population of men would be followed with active surveillance) [6,9].

2.1.2 Prostarix (Metabolon)

This quantitative liquid chromatography-mass spectroscopy based test measures the concentrations of four amino acids in the cell pellet of urine spun down post-DRE. A Prostarix score is then generated that can improve the sensitivity and specificity of PCa risk

stratification in men with negative DREs and moderately elevated PSA levels (4-10 ng/ml). While this test is mainly aimed at improving PCa screening, it can also be performed on fluid in which prostate biopsy cores are soaked prior to processing by the pathologist, giving additional information about the aggressiveness of a patient's PCa. In October 2013, Metabolon announced an exclusive Marketing Agreement with Bostwick Laboratories to market the Prostarix test [11].

This test identifies metabolic correlates to post-surgical endpoints that are associated with 15-year PCa specific mortality (Gleason score, extracapsular extension, and extraprostatic invasion). Studies profiled metabolomic features of prostatectomy tissues from two independent cohorts of PCa patients (331 prostate tumour-containing samples and 178 tumour-free prostate samples) [12]. They found that prostate tumours had significantly altered metabolite profiles compared to cancer-free prostate tissues, including biochemicals associated with cell growth, energetics, stress, and loss of prostate-specific biochemistry. Many metabolites were further associated with clinical findings of aggressive disease. Moreover, when added to multiparametric nomograms, metabolites improved prediction of organ confinement (area under the curve (AUC) from 0.53 to 0.62) and 5-year recurrence (AUC from 0.53 to 0.64).

2.1.3 Prostate Health Index, phi (Beckman Coulter)

This immunoassay based test measures and combines several forms of PSA in blood; PSA, free PSA and a precursor form of PSA - p2PSA. An algorithm calculates the patient's personalized PCa risk assessment, or Prostate Health Index (phi). phi does not replace PSA testing, but provides better specificity for PCa detection compared to PSA and % free PSA alone. It is performed by laboratories equipped with Beckman Coulter UniCel and Access immunoassay analyzers.

Two retrospective studies (total n=879) [13,14] and one prospective study (n=2,034)¹⁵ have demonstrated that when p2PSA measurements are combined with PSA and free PSA measurements, the resulting index demonstrates a significant improvement in clinical specificity for PCa detection, relative to PSA and % fPSA alone, in the PSA range 2-10 ng/mL in men 50 years of age or older and with non-suspicious DREs. The phi test is now FDA approved for men 50 years and older with a total PSA value between 4-10 ng/mL and negative DRE.

2.2 The false-negative biopsy dilemma

PSA screening has led to a high number of false-positives which results in a high number of unnecessary prostate biopsies [15]. The majority of men will receive a negative biopsy result. However, due to sampling errors, cancer can be missed in up to 25% of cases [16] leading to uncertainty of diagnosis and a high rate of repeat biopsies with the associated discomfort and risk. A number of new tests on the market claim to address this dilemma.

2.2.1 ProgenSA PCA3 (Gene-Probe)

This RT-PCR based test assesses the expression of a single non-coding RNA in urine. The output is a PCA3 score which is the ratio between PCA3 and PSA mRNA found in urine samples following DRE. A PCA3 score <25 indicates a decreased probability of positive biopsy; PCA3 score >25 indicates an increased probability of positive biopsy. The test is CE Marked and FDA approved and is currently performed in 72 laboratories across the world [17].

PCA3, (or DD3), was discovered in 1999 by comparing gene expression of tumour tissue from radical prostatectomies (RP) (n=56) with the adjacent non-neoplastic tissue [18]. DD3 mRNA was over-expressed in tumour tissue compared with benign and was prostate-specific. Further studies have proven PCA3's clinical utility [19-23]. The most recent, leading to FDA approval of the test, was a prospective multi-center study on 466 patients from 14 centers [24,25]. This study showed that the risk of positive biopsy correlated with the PCA3 score and supplements PSA and other clinical information to provide more accurate prediction of repeat biopsy outcome.

2.2.2 ConfirmMDx (MDxHealth)

This epigenetic test utilises quantitative methylation-specific PCR to measure levels of GSTP1, APC and RASSF1 in "normal" tissue surrounding the cancer. The test requires FFPE tissue obtained within 24 months of biopsy. In July 2013, MDxHealth and Bostwick Laboratories entered into a marketing agreement to commercialize ConfirmMDx. In October 2013, MDxHealth signed a deal with two health insurance providers FedMed & America's Choice Provider Network, to expand coverage of their test [26].

A 2006 study tested the methylation of the promoters of three genes (APC, GSTP1, and RAR β 2) involved in prostate carcinogenesis by quantitative methylation-specific PCR in tissue-derived DNA from 30 prostate carcinomas, 128 high-grade PIN, and 30 normal prostate tissue samples dissected from 30 RP specimens [27]. They showed that aberrant APC and RAR β 2 promoter methylation are early events during prostate carcinogenesis. Higher levels of GSTP1 promoter methylation were also observed in carcinoma and some high-grade PIN lesions compared with normal prostate samples. The performance of GSTP1 and APC DNA methylation in the setting of repeat biopsy in men with an initially negative biopsy but a high index of suspicion for missed PCa was evaluated in a subsequent study [28]. This was the first prospective study to evaluate performance of DNA methylation markers in a clinical cohort of men undergoing repeat biopsy (n=86) and showed that APC had a better negative predictive value than GSTP1 (0.96 vs. 0.80). In October 2013, MDxHealth announced that its ConfirmMDx for Prostate Cancer test met all of the primary endpoints in the recently completed multicentre, blinded DOCUMENT (Detection of Cancer Using Methylated Events in Negative Tissue) clinical validation trial. This study, conducted at five major U.S. urologic centres, involved analyzing tissue from initial negative biopsies and comparing assay results to cancer detection in subsequent biopsies within 24 months. ConfirmMDx was used to analyze the epigenetic profile of prostate biopsy cores from 350 men screened by PSA. [29].

2.2.3 Prostate Core Mitomic Test (Mitomics)

This RT-PCR test detects large-scale mitochondrial DNA (mtDNA) deletions in FFPE biopsy samples, enabling a more accurate discrimination between benign and malignant tissue. It uses the tumour field effect to identify molecular signatures of increased levels of mtDNA genomic mutations in histologically benign cells adjacent to tumour tissue [30]. Launched in 2001, it is available through Mitomics and QDX Pathology Services as the “QPredict PCMT”.

The discovery and validation process involved 396 patients and nearly 1,700 prostate cores including 143 patients with benign and 253 patients with malignant histology. External validation was performed by the National Institute of Standards and Technology under the Early Detection Research Network of the National Cancer Institute. An additional study was conducted within the framework of a clinical trial (a retrospective blinded study). In this study 94 patients with benign histology results contributed 553 cores; within 14 months, each patient underwent a second round of biopsies. The study showed that QPredict PCMT predicted the outcome of the repeat biopsy, with a sensitivity of 84% and a negative predictive value of 91% [30]. It can anticipate the onset and/or presence of hidden or occult cancer by detecting a distinct molecular signature not detectable by conventional biopsy [31].

2.3 Distinguishing aggressive cancers versus indolent ones

To avoid overtreatment, watchful waiting with active surveillance (AS) has been increasingly used in men with low-risk PCa. However, AS is not without risks including under-treatment of occult aggressive cancers. A critical need exists for new tests that can predict and monitor PCa grade, stage, and aggressiveness with better accuracy, less morbidity and less cost. A number of new tests on the market are addressing this.

2.3.1 Prolaris (Myriad Genetic Laboratories)

The Prolaris genomic test measures the cell cycle progression (CCP) signature of 46 genes (31 cell cycle genes and 15 house-keepers) used to predict 10-year PCa-specific disease progression and mortality. This RT-PCR based test is performed on FFPE material (biopsies or resected tumours). The test is prognostic at the point of diagnosis and in the post-surgery setting. It can also help men decide between AS and treatment. When used on post-prostatectomy patients that have higher risk features, it better estimates risk of biochemical recurrence (BCR), so that monitoring or additional therapy can be adjusted. Prolaris is FDA approved for use in low-risk men with Gleason score 6, and for patients post prostatectomy who are at high risk for PCa recurrence [32].

CCP genes were originally identified as having RNA expression levels that oscillated as cells progressed through various stages of the cell cycle [33] and have proven prognostic value in breast cancer [34-39]. Their utility to predict BCR in a retrospective cohort of patients (n=366) following RP was tested⁴⁰. Next, the ability of this same CCP signature to predict death from PCa in a conservatively treated cohort diagnosed from a transurethral resection of the prostate (TURP) specimen was evaluated (n=337) [40]. The CCP signature was a highly significant predictor of outcome in both cohorts. The signature was tested at disease diagnosis in two conservatively managed cohorts from the UK (n=337 and 349), after RP in two cohorts from the U.S. (n=366 and n=413), and after external beam radiation therapy

(n=141). The CCP signature was a highly significant predictor of outcome in all these studies [40-43].

2.3.2 OncotypeDx Prostate Cancer Assay (Genomic Health)

The Oncotype Dx Prostate Cancer Assay is a genomic test measuring 17 genes across 4 biological pathways (12 cancer-related genes and 5 house-keepers). This RT-PCR based test on FFPE biopsy material can stratify indolent versus aggressive disease. The assay reports a quantitative Genomic Prostate Score (GPS) result ranging from 0-100 which is representative of the biologic potential of the tumour. The GPS results are combined with standard clinical parameters which results in a more accurate prediction of favourable pathology, helping patients choosing between AS or immediate treatment [44].

RT-PCR analysis of 732 candidate genes in a development study (n=441 patients) identified 288 genes predictive of clinical recurrence regardless of Gleason patterns in separately-sampled FFPE PCa tissue specimens. For each patient, two tumour specimens were separately analyzed to represent the primary and highest Gleason patterns sampled from the patient's RP tissue [45]. Eighty-one genes taken forward into needle biopsy study of low/intermediate-risk patients (n=167 patients) confirmed strong association of the genes with adverse pathology. Multivariate analysis of both studies yielded 17 genes across multiple biological pathways and a GPS algorithm [46]. In an independent validation study (n=395), GPS, assessed in biopsies with as little as 1mm tumour length from patients suitable for AS, strongly predicted ($p<0.005$) high grade and/or pT3 disease after adjusting for Cancer of the Prostate Risk Assessment (CAPRA) score or other standard pretreatment factors [47]. Importantly, approximately 10% of patients originally classified as very low or low risk by clinical factors were identified by GPS as having more aggressive disease, which would be considered for immediate treatment.

2.3.3 OurView Prognostic Panel (OPP) (Oppenheimer Urologic Reference CLIA Laboratory OURLab)

This predictive test measures clinical features, Gleason Grade and three biomarkers (PTEN, proliferation Ki67 and DNA ploidy) which have been extensively associated with prostate carcinogenesis [48, 49]. Although these mechanisms are well documented in the literature, no validation studies have been performed by the company to show combined value. The results of this test are incorporated into a pathology second opinion report on a biopsy that improves risk assessment and treatment decision-making. The clinical information required includes PSA, age, clinical stage and a biopsy report. An OPP score is derived from mathematical models and is based on weighted proportions of significant quantitative and qualitative factors. Biopsies of less aggressive tumours will have OPP scores less than 50, and be identified in the lower risk section of the OURView diagram. The accuracy of the test depends on the accuracy of the biopsy [50].

2.4 Tests predicting relapse/metastases after surgery

Following RP, it is of crucial importance to monitor the risk of BCR in order to be able to timely intervene should a relapse occur. Currently, two tests claim to address this clinical need. Moreover, one test is available to monitor response to treatment in castrate-resistant PCa.

2.4.1 Decipher (GenomeDX)

This genomic test measures the expression levels of 22 markers (both coding and non-coding RNAs) in FFPE samples. The aim is to classify an individual patient's risk of clinical metastasis after RP [51].

The Decipher gene-signature was discovered on a cohort of 545 patients that received RP for primary prostatic adenocarcinoma. A genome classifier (GC) of 22 genes was generated from a high-density transcriptome-wide microarray study, using a random forest machine-learning algorithm on a training set (n=359). The GC was then validated on an independent cohort (n=186), outperforming clinical variables and other gene signatures [52]. The GC was clinically validated in two studies on 1095 patients who were clinically high-risk and developed BCR after RP [53, 54]. Decipher could independently forecast which patients developed metastasis. Decipher clinical utility was demonstrated in a prospective, pre-post design study assessing urologist treatment recommendations following RP in both the adjuvant and salvage settings [55]. This study showed that treatment recommendations changed from pre-GC to post-GC in 43% of adjuvant, and in 53% of salvage setting case evaluations.

2.4.2 NADiA ProsVue (IRIS International)

This test, which utilises a combination of RT-PCR and an immunoassay, determines the rate of change of total serum PSA (tPSA) over a period of time to help identify patients at low risk of recurrence following RP. No results are to be reported until the slope has been calculated from three serum samples collected between six weeks and 20 months post-RP. The assay is not intended for patients with tPSA values greater than 0.1 ng/mL in the first serum sample. All three samples are tested in the same NADiA ProsVue assay run which received 510(k) FDA clearance and CE Mark approval in late 2011 [56].

This test was assessed as an independent prognostic marker for identifying men at reduced risk of clinical recurrence of PCa after RP (N=304) [57]. A NADiA ProsVue PSA slope of ≤ 2.0 pg/mL/mo after prostatectomy was prognostic for a reduced risk of PCa recurrence and added predictive power to the established risk factors. IRIS Personalized Medicine is currently conducting a Field Experience Trial designed to show ProsVue clinical utility.

2.4.3 CellSearch CTC kit (Janssen Diagnostic, previously Veridex)

This test aims to predict prognosis and monitor the anti-tumour effects of treatment in castration resistant PCa. It may also serve as an intermediate end point for drug approval. The test measures circulating tumour cells (CTCs) of epithelial origin (CD45-, EpCAM+, & cytokeratins 8, 18+, and/or 19+) via the CellSearch (FDA approved in 2004) Circulating Tumor Cell Kit. The test is based on a combination of immunomagnetic separation and immunofluorescence detection [58].

Several studies showed that CTCs could be used as indicators of prognosis in metastatic PCa [59-63] leading to its FDA approval. One prospective study [63] assessed whether CTC enumeration pre- and post- a new cytotoxic treatment could predict overall survival (OS). Patients with histologically confirmed metastatic PCa (n=231) were stratified into predetermined favorable or unfavorable groups based on CTC count (<5 and >5 CTC/7.5mL). Patients with unfavorable pretreatment CTC counts (57%) had shorter OS (median OS, 11.5 versus 21.7 months; Cox hazard ratio, 3.3; P < 0.0001). Unfavorable post-treatment CTC counts also predicted shorter OS (median OS, 6.7-9.5 versus 19.6-20.7 months; Cox hazard ratio, 3.6-6.5; P < 0.0001). The prognosis for patients with (a) unfavorable baseline CTC counts who converted to favorable CTC counts improved (6.8 to 21.3 months); while those with (b) favorable baseline CTC counts who converted to unfavorable counts worsened (>26 to 9.3 months).

3. Discussion

There has been a steady rise in PCa diagnoses, especially in countries with access to PSA testing. Rates of over-diagnosis are estimated to be as high as 50% [64] and overtreatment costs are an estimated \$3 billion each year in the US alone [65]. The biotech industry is investing significant resources into developing more specific biomarkers which would advance the decision-making processes behind PCa management and treatment.

3.1 Biomatrices

The tests reviewed use a range of biomatrices including blood, urine and prostate tissues (Figure 2). The tests aiming to improve screening by avoiding unnecessary biopsies are based on less invasive fluids such as blood and post-DRE urine. Some tests dealing with the negative biopsy dilemma use tissues from the first negative biopsy, while others look at urine markers. While urine markers tend not to be prostate-specific, they completely circumvent biopsy sampling error. All the tests addressing false negative biopsies use biopsy tissue but propose ways around the sampling errors by looking at “halo” or “field effects” either in the stroma close to cancer cells (ConfirmMDx and PPCA) or in benign cells adjacent to tumour tissues (Mitomic). Those tests which aim to distinguish between indolent versus aggressive PCa are all based on biopsy tissue. However, due to the disease heterogeneity and sampling errors, serum biomarkers would be more informative. Finally, in the post-operative and metastatic clinical setting, two assays are based on blood samples while one examines gene expression in tissues. Specifically in the metastatic setting, a liquid biopsy is the only biomatrix which can be used since metastases biopsies are not routinely carried out.

3.2 Technical platforms

The majority of tests use RT-PCR to quantify their biomarkers of interest. The main benefit of RT-PCR is the use of single chemistry as well as the highly reproducible nature of the technology, along with high detection specificity and good sensitivity. Moreover, the combination of RT-PCR with specific algorithms to analyse gene expression represents a powerful tool with clear cut-off values easily commercialized. However, a thorough morphological evaluation of the sample combined with macrodissection is recommended to

avoid sample contamination with stroma or benign tissue. The requirements for acceptable biopsy samples are very specific. Prolaris requires that FFPE blocks should include at least 2 mm of linear tumour on diagnostic H&E slides so that a pathologist can identify the tumour area and isolate RNA only from that area. In contrast, those tests using immune-assays on full slides which conserve their original morphology do not require tumour macrodissection. This platform is prone to pre-analytical tissue processing issues such as formalin fixation, antigen retrieval and antibody specificity. Some of these limitations are discussed in a recent study in which a set of published prognostic markers for prostate cancer were systematically verified using immuno-histochemistry (IHC) [66]. This study showed that only 14% of markers were verified as independently prognostic for PSA relapse following radical prostatectomy which would advocate for more robust validation of IHC biomarkers and codes of best practice such as the REMARK guidelines [67]. One key issue with tests based on IHC is antibody specificity. This issue can be overcome by establishing strict criteria for the pragmatic validation of antibodies for use in immunohistochemical assays as recently reviewed [68]. Antibody specificity is also common to ELISA assays which would otherwise have the advantage of being cheap, simple and reproducible. Finally, platforms which require changes in clinical practice or require more expensive machinery and dedicated personnel will not fit smoothly into current clinical practice and, therefore, will have a lower market penetrance.

3.3 Validation of tests and potential economic impact

3.3.1 Reducing unnecessary biopsies

PSA screening is associated with a high risk of over-diagnosis leading to an estimated number of 750,000 unnecessary biopsies each year in the US [9]. The 4Kscore™ blood test has now been validated in more than 8,500 patients, demonstrating a statistically significant improvement of 8-10% in predictive accuracy. The test developers estimate that about \$1 billion in healthcare costs could be saved in the US annually by its implementation [69]. Numerous retrospective and prospective multicentre studies suggest that Beckman Coulter's phi test substantially improves the predictive value of PSA in the detection of early stage PCa. Another study on a hypothetical health plan with 100 000 male members aged 50-75, showed that although the addition of phi increased the total cost of blood tests, these costs were offset by a reduction in the number of required office visits, laboratory tests and prostatic biopsies, resulting in a 1 year net saving of \$356,647 (for PSA threshold of ≥ 2 ng/mL) and \$94,219 (for PSA threshold of ≥ 4 ng/mL) [70]. Metabolon test has yet to be validated in independent multicentre studies.

3.3.2 The false-negative biopsy dilemma

Several studies have reported that PCa can be found in 10%-35% of repeat biopsies [71-76]. One of the most serious risks of a repeat biopsy is the diagnosis of indolent, low-volume/low-grade prostate cancer, potentially leading to patient anxiety and overtreatment [77]. The ConfirmMDx and Prostate Core Mitomic tests are tissue-based, but it remains to be seen if they have overcome biopsy sampling error. The Gen-Probe test has overcome biopsy sampling error completely by developing a non-invasive urine test. Their test has also undergone the most clinical validation and is FDA approved. Currently, the decision to perform a repeat biopsy relies on the physician's best clinical judgment to estimate the

patient's risk of PCa and patient preference. A recent study (n=1,024) showed that the combination of best clinical judgment with PCA3 would have avoided 64% of repeat biopsies compared with 26% for best clinical judgment alone and 55% for PCA3 alone [77]. The diagnostic accuracy for Gleason sum ≥ 7 PCa of the best clinical judgement with PCA3 scenario was superior to that of the other scenarios, with a negative predictive value of 99%.

3.3.3 Distinguishing aggressive versus indolent cancers

A critical need exists for new tests that can predict and monitor PCa grade, stage, and aggressiveness with better accuracy, less morbidity and less cost. The Prolaris and Oncotype Dx tests may be useful in identifying which men may confidently opt for AS and those which should consider immediate treatment. The OncotypeDx biopsy-based test claims to more accurately stratify indolent versus aggressive disease enabling low risk patients to avoid invasive treatments. It also claims to have overcome issues with tumour heterogeneity and biopsy under-sampling. Only one validation study has been published but the authors showed that their test shifted about half of the men into either a lower or a higher risk category. However, the test is only aimed at newly diagnosed patients with early stage disease and is carried out on biopsy tissue acquired within six months of diagnosis.

The Prolaris test has undergone more clinical validation including two clinical utility studies. They show the test leads to significant reductions or increases in interventional treatments based on patients' unique risk profiles [78, 79]. In one study, 150 clinicians completed surveys in 305 cases assessing the influence of the Prolaris score on clinical decision making. In 65% of cases, physicians changed their intended therapy based on the Prolaris test score [79]. The Prolaris test has been validated to predict PCa-specific mortality, risk of metastasis and BCR. Studies have identified both low-risk patients who can be managed with AS and high-risk patients who may benefit from more aggressive treatment. However, the accuracy of this test depends on accuracy of the biopsy.

3.3.4 Tests predicting relapse/metastases after surgery

After surgery, up to 50% of men exhibit one or more clinical risk factors increasing their chance of metastasis and death from PCa [80]. These men are candidates for secondary treatment which carries significant costs and risks. However, only a minority with adverse pathology and BCR post-RP experience metastasis and die [81]. The current clinical tools cannot accurately determine the true risk of metastasis. The Decipher test has been validated in two published case-cohort design studies and outperforms current clinicopathologic variables and existing nomograms. A prospective study showed that urologists across a range of practice settings changed treatment decisions when presented with the results of the Decipher test following RP. Implementation of this genomic risk stratification test into routine clinical practice may better direct treatment decision-making post-RP. While the NADiA ProVue test has received FDA 510(k) clearance, it has only been validated in one published multicentre retrospective study and the results of a field experience trial designed to show the clinical utility of the test are not published. More validation studies in additional populations are necessary for both tests.

Monitoring patients with metastatic cancer throughout treatment is critical to making informed clinical decisions. The CELLSEARCH test is the only FDA-cleared blood test for enumerating CTCs in patients with metastatic breast, colorectal, and PCa. Its advantages include semi-automation and proven reproducibility, reliability, sensitivity, linearity and accuracy [82, 83]. Clinical studies have demonstrated that the number of CTCs correlates to disease prognosis and can provide an important marker for assessing patients' status throughout the continuum of treatment.

4. Conclusion

Successfully translating a biomarker into routine clinical use requires a collaborative effort between the research laboratory and diagnostic industry (who develop and translate a concept onto a practical reliable tool), the clinicians (who identify the unanswered clinical questions) and the clinical laboratory (which evaluates the tool in real-life practice) [84]. Rigorous validation steps should be followed to confirm clinical utility of a new test in prospective studies on large multicentre cohorts. In addition, prognostic tests should be robustly validated in terms of their reliability and reproducibility with extensive optimization to achieve desirable analytical sensitivity, specificity, recovery, accuracy, precision and stability [3]. Although the decision to introduce a new biomarker is influenced by different regulatory and reimbursement policies in different healthcare systems, introduction into routine clinical practice requires rigorous assessment from three different perspectives – those of the clinician, the clinical laboratory and the healthcare funding organization. From all perspectives, the implementation of a new test should be evidence-based. From the clinician, the clinical laboratory and the healthcare funding organization. From all perspectives, the implementation of a new test should be evidence-based. From the healthcare provider standpoint, a new test must be cost-effective such as facilitating a reduction in patient admissions. From a clinical perspective, a new test must provide information that adds to or replaces information available from existing gold standards and improves patient outcome. Finally, from a hospital laboratory viewpoint, it must be possible to incorporate the new test easily into the routine workflow (e.g. it must have reasonably robust pre-analytical specimen handling requirements). Although the tests discussed in this review may ultimately help to improve the diagnosis and treatment of PCa, they are still new and their use should be considered cautiously. The true clinical role of these novel markers needs to be carefully evaluated in the context of numerous large, multicentre, prospective trials with full health economic analyses before any advice can be made about their use in routine clinical practice.

5. Expert commentary

Limitations with the gold standard clinical tools available for the diagnosis and prognosis of prostate cancer result in both over diagnosis and costly overtreatment and impacts on the outcomes and quality of life of men. Numerous research groups have focused their work on the discovery and validation of novel biomarkers for prostate cancer diagnostics which are urgently needed to help both clinicians and their patients to make appropriate treatment decisions for the optimal management of the disease. In the last few years a number of new diagnostic tests have been brought to the marketplace. These tests will ultimately help to improve the diagnosis and treatment of PCa, however they are still new and their use should be considered cautiously. The true clinical role of these novel markers needs to be carefully

evaluated in the context of numerous large, multicentre, prospective trials with full health economic analyses before any advice can be made about their use in routine clinical practice.

6. 5-Year View

The advent of PSA heralded a new era in the management of PCa. However, decades later, controversy still persists regarding its appropriate use. PCa is highly heterogeneous, so in order to diagnose and treat patients in an appropriate way, future tests will need to (a) incorporate a panel of biomarkers and (b) address a specific clinical question. However, a striking discrepancy still exists between biomarker discovery and the number of biomarkers that actually make it into routine clinical practice. The driving forces for the successful commercialisation of any new test include the analytical validity of the assay and its cost-effectiveness. However, in order for any new test to be successfully incorporated into routine clinical practice, a significant amount of convincing clinical validation studies are also required. These studies will need to definitively show that by measuring the biomarkers, a doctor would change his/her decision, and that this changed decision would benefit the patient. As evidenced in our review, the most significant challenge facing these tests is the level (and convincing nature) of the clinical validation published to date. Our view is that in the next 5 years, those tests which will achieve the most convincing validation of their clinical utility will be incorporated into routine clinical practice providing new diagnostic tools to clinicians, ultimately making prostate cancer diagnosis and prognosis more accurate and reliable.

7. Key issues

- Limitations with the gold standard clinical tools available for the diagnosis and prognosis of prostate cancer result in both over diagnosis and costly overtreatment.
- In the past few years new diagnostic/prognostic biomarkers have been translated into commercial diagnostic tests for prostate cancer.
- Each new test should be tailored to address a specific clinical question for which limited diagnostic options are currently available.
- Tests aimed at improving PCa screening include 4Kscore Prostate Cancer Test, Prostarix and Prostate health index, phi.
- Cancer can be missed in up to 25% of men who receive a negative biopsy result. Tests addressing this dilemma include Progensa PCA3, ConfirmMDx and the Prostate Core Mitomic™ Test.
- Tests that can predict and monitor PCa grade, stage, and aggressiveness with better accuracy, less morbidity include Prolaris, OncotypeDx Prostate Cancer Assay and OurView Prognostic Panel.
- Current clinical tools cannot accurately determine a patient's true risk of metastasis or monitor patients with metastatic cancer throughout their treatment. Tests addressing these dilemmas include: Decipher, NADiA ProVue and the CellSearch CTC kit.

- Based on the clinical question, a test should be both specific and sensitive in order to avoid under-treatment and over-diagnosis.
- Tests achieving the most convincing clinical validation will be incorporated into routine clinical practice providing new diagnostic tools to clinicians, ultimately making prostate cancer diagnosis and prognosis more accurate and reliable.

8. Declaration of interest

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MP has been on secondment from University College Dublin to OncoMark from 1.09.2013 to 31.08.2014, where her official status is that of employee; she does not hold any stock in this company. WMG is Chief Scientific Officer at OncoMark, holds stock and is a Director of the company

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Reference annotations

- Reference #3 (**): This review gives an overview of the newest patented biomarkers in the prostate cancer space, leading the way to novel commercial tests for prostate cancer diagnostics.
- Reference #64 (**): Although far from being an ideal biomarker, PSA is still the gold standard test for prostate cancer diagnosis and response to treatment. This paper presents a quantitative estimate of prostate cancer overdiagnosis due to PSA testing.
- Reference #81 (**): This review gives an overview of what it takes for a biomarker to make it into routine clinical practice both in terms of analytical performance of the biomarker and its cost-effectiveness.
- Reference #66 (*): Meta-analysis of 7 separate trials shows the Kallikrein Panel, is superior in ruling out any cancer and high-grade cancers in a variety of at risk patient populations. It also improves the performance characteristics of a clinical laboratory diagnostic model regardless of the patient's testing history.
- Reference #74 (*): This example of a clinical utility study shows that the combination of best clinical judgement with PCA3 testing can reduce the number of repeat biopsies while maintaining the sensitivity to detect Gleason sum ≥ 7 PCa.

- Reference #75 (*): This clinical utility study shows that Prolaris adds meaningful new information to the risk assessment of localized prostate cancer patients and can lead to changes in interventional treatment.

Figures and Tables

Figure 1 Commercial biomarkers in the context of prostate cancer management. T Tissue from FFPE material, U Urine sample, B Blood sample. Tests in dash-line box are under development. Abbreviations: PSA, prostate specific antigen, DRE, digital rectal examination, AS, active surveillance, OC, organ confined, NOC, non-organ confined, RP, radical prostatectomy, ADT, androgen deprivation therapy.

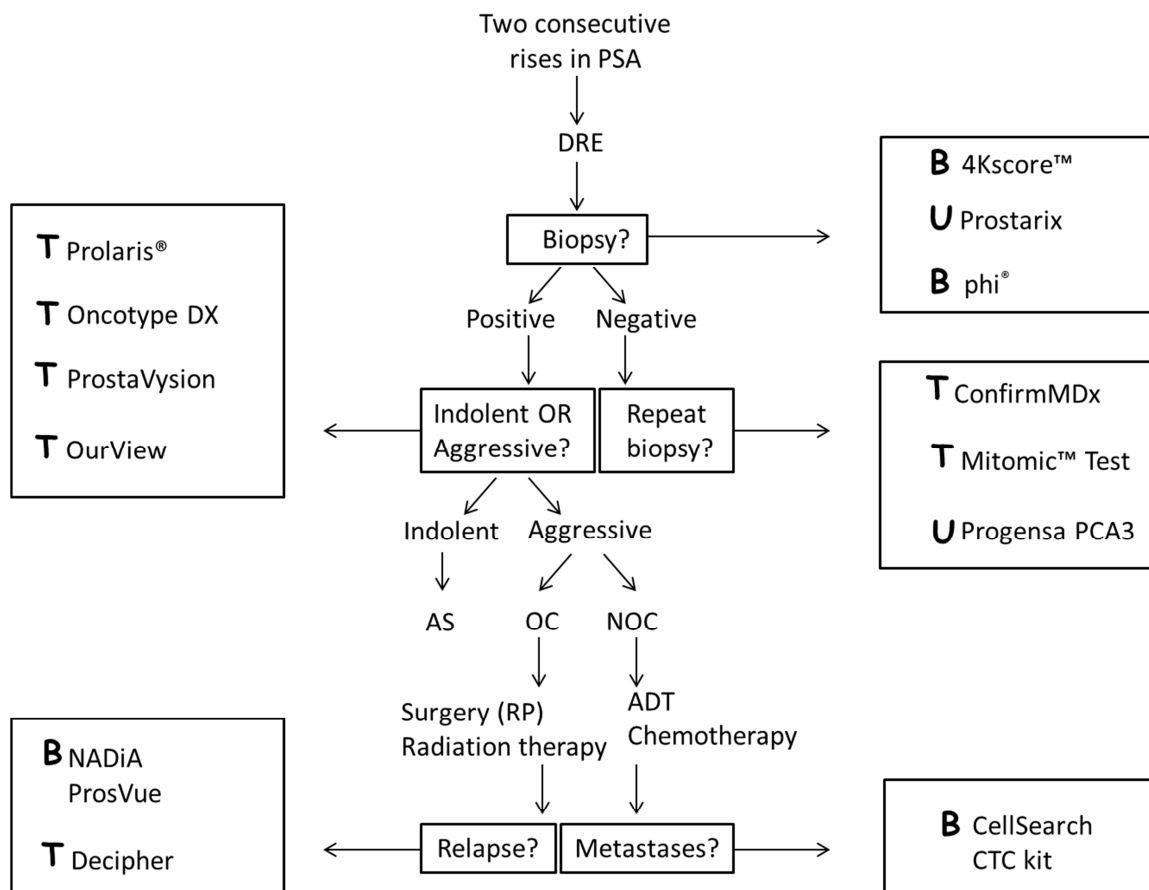


Table 1 Current management of prostate cancer

	Current Methods	Limitations
Diagnosis	Concerning PSA velocity, PSA above a threshold or concerning DRE leading to a TRUS biopsy	PSA has low specificity (false positives) and low sensitivity (false negatives). TRUS biopsies are invasive procedures usually requiring patient sedation. The rate of false negatives is high as only 0.1-0.2% of the entire prostate is sampled.
Prognosis	Various treatments are available based on disease grade and stage: active surveillance, radical prostatectomy, radiation treatment or androgen deprivation therapy.	Biopsy sampling error is an issue. Gleason score is subject to great inter-observer variability, especially when limited material is available for analysis.
PCa recurrence	Following treatment, the patient is monitored via PSA and radiology/MRI for recurrence in order to intervene with ADT and docetaxel.	Imaging tools are not sensitive enough to detect small metastases. This represents a problem not only at this stage of the disease, but also in the pre-operative setting where missing small metastases can lead to performing radical prostatectomies on patients with metastatic disease who should be offered ADT instead.
Prediction of response to treatment	PSA is the only biomarker currently in use to predict response to treatment	Please see above for PSA limitations.

Table 2. Overview of novel commercial biomarkers

Test (Manufacturer)	Clinical Utility	Sample	Technology	No. of analytes	Validation	Regulatory Approval	Cost
4Kscore™ Prostate Cancer Test (OPKO Health, Inc)	Improved PCa screening	Blood	Immunoassay	4 kallikrein markers	7 studies involving more than 10,000 patients from the US and Europe.	LDT CLIA	250£
Prostarix (Metabolon Inc.)	Improved PCa screening	Urine	Quantitative liquid chromatography-mass spectrometry	4 amino-acids	The discovery study (n=509) showed that when added to multiparametric nomograms, Prostarix improved prediction of organ confinement (AUROC from 0.53 to 0.62) and 5-year recurrence (AUROC from 0.53 to 0.64). No validation studies have been carried out yet.	LTD CLIA	
Prostate health index, phi® (Beckman Coulter)	Improved PCa screening	Blood	Immunoassay	Combination of total PSA, free PSA and p2PSA	3 Retrospective studies (n=879) and one prospective study (n=2034)	FDA	
Progensis PCA3 (Gene-probe Inc)	Helps to determine need for repeat prostate biopsies	Urine	RT-PCR	1 non-coding RNA	6 Studies for a total of 1365 patients from several centers in the US and Europe. According to the last prospective, multicentre study, a PCA3 score cutoff of 25 yielded 77.5% sensitivity, 57.1% specificity, and negative and positive predictive values of 90% and 33.6%, respectively.	FDA CE Mark	300-400£
ConfirmMDx (MDxHealth)	Helps to determine need for repeat prostate biopsies	FFPE	Quantitative methylation-specific PCR	GSTP1, APC and RASSF1	2 Prospective studies for a total of 436 patients. The results from the second multicentre study from 5 hospitals in the US will be published as soon as analysis is completed.	LTD CLIA	From 2,000\$
Prostate Core Mitomic™ Test (Mitomics™)	The aim of the test is to eliminate false-negative biopsies	FFPE	RT-PCR	Levels of mitochondrial DNA deletions (a 3.4kb deletion)	1 validation study with 94 patients. PCMT predicted the outcome of the repeat biopsy with a sensitivity of 84%, a specificity of 54% and a negative	LTD CLIA	

					predictive value of 91%.		
Prolaris® (Myriad Genetics Inc.)	On biopsies: Can stratify indolent vs. aggressive disease On prostatectomies: Predicts risk of biochemical recurrence post-prostatectomies	FFPE	RT-PCR	46 (31 cell cycle genes+15 house keepers)	5 Retrospective studies for a total of 1606 patients from the US and UK. The CCP signature was a highly significant predictor of outcome in all five studies	FDA	3,400\$
Oncotype DX (Genomic Health)	Can stratify indolent vs. aggressive disease	FFPE	RT-PCR	17 (12 cell cycle genes+5 house keepers)	One validation study with 395 patients. According to the company website, in multivariate analysis, GPS was found to strongly predict adverse pathology at RP, (p-value 0.002).	Awaiting FDA approval	3,820\$
OurView Prognostic Panel (OPP) (OURLab)	Improves risk assessment and treatment decision-making	FFPE	Various	Measures clinical features, Gleason Grade, PTEN, Ki67 and DNA Ploidy	Although these mechanisms are well documented in the literature, no validation studies have been performed by the company in relation to the specific assay that they are offering.	LTD CLIA	\$350
ProstaVysion (Bostwick laboratories®)	Provides a molecular analysis of prostate cancer aggressiveness and long-term patient prognosis	FFPE	Various	DNA methylation of HOXD3, gene fusion/translocation of ERG and loss of PTEN	Although these mechanisms are well documented in the literature, no validation studies have been performed by the company in relation to the specific assay that they are offering.	LTD CLIA	~ 1,350\$
Decipher (GenomeDX)	Predicts patient's risk of clinical metastasis after radical prostatectomy	FFPE	RT-PCR	22 genes	In addition to the discovery study, 2 validation studies for a total of 1095 patients from the US were performed. Both studies showed that GC outperformed any clinical variable or prediction model for metastasis (AUC=0.79).	LTD CLIA	

NADiA ProsVue (IRIS International, Inc.)	Helps identifying patients at low risk of recurrence following RP	Blood	Immuno-PCR (combination of immunoassay and RT-PCR)	Rate of change of serum total PSA over a period of time	1 validation study with 304 patients from 4 clinical sites in the US showed a NPV of 92.7% and a PPV of 78%.	FDA CE Mark	~ 3,500\$
CellSearch CTC kit (Janssen Diagnostic, Llc)	Predict prognosis and monitors the antitumor effects of treatment in castration resistant prostate cancer	Blood	Immunomagnetic & immunofluorescence	CTC count	1 prospective study (n=231) showed that patients with unfavorable pretreatment CTC (>5 CTC/7.5mL) had shorter OS (median OS, 11.5 vs. 21.7 months; Cox hazard ratio, 3.3; P < 0.0001). Unfavorable post-treatment CTC counts also predicted shorter OS (median OS, 6.7-9.5 vs. 19.6-20.7 months; Cox hazard ratio, 3.6-6.5; P < 0.0001).	FDA	