Merging new-age biomarkers and nanodiagnostics for precision prostate cancer management

Kevin M. Koo[1], *Paul N. Mainwaring*[1]*, *Scott A. Tomlins*[2]* *and Matt Trau*[1,3]*

Abstract | The accurate identification and stratified treatment of clinically significant early-stage prostate cancer have been ongoing concerns since the outcomes of large international prostate cancer screening trials were reported. The controversy surrounding clinical and cost benefits of prostate cancer screening has highlighted the lack of strategies for discriminating high-risk disease (that requires early treatment) from low-risk disease (that could be managed using watchful waiting or active surveillance). Advances in molecular subtyping and multiomics nanotechnology-based prostate cancer risk delineation can enable refinement of prostate cancer molecular taxonomy into clinically meaningful and treatable subtypes. Furthermore, the presence of intertumoural and intratumoural heterogeneity in prostate cancer warrants the development of novel nanodiagnostic technologies to identify clinically significant prostate cancer in a rapid, cost-effective and accurate manner. Circulating and urinary next-generation prostate cancer biomarkers for disease molecular subtyping and the newest complementary nanodiagnostic platforms for enhanced biomarker detection are promising tools for precision prostate cancer management. However, challenges in merging both aspects and clinical translation still need to be overcome.

[1]*Centre for Personalized Nanomedicine, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia.*

[2]*Department of Pathology, Department of Urology, Michigan Centre for Translational Pathology, Comprehensive Cancer Centre, University of Michigan, Ann Arbor, MI, USA.*

[3]*School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia.*

**e-mail: drpaulmainwaring@gmail.com; tomlinss@med.umich.edu; m.trau@uq.edu.au*

https://doi.org/10.1038/s41585-019-0178-2

Prostate cancer is the most commonly diagnosed solid malignancy in men, and aggressive subtypes account for a considerable proportion of cancer-related deaths in men[1]. Prostate cancer typically affects men >65 years old, with a higher incidence in white men and men of African descent than other populations[2]. Most prostate cancers are predominantly hormone-driven, and men with aggressive disease will generally progress towards hormone-refractory or metastatic castration-resistant prostate cancer (CRPC), which is still largely influenced by androgen receptor (AR) activity[3,4]. In the past 5 years, comprehensive genomic, epigenetic, transcriptomic and proteomic analysis of prostate cancer has revealed the extent of heterogeneity within the disease. These efforts have identified common oncogenic drivers, such as point mutations in *TP53*, *SPOP* and *FOXA1*; amplifications and copy number variations in *AR* (in CRPC); aberrations in DNA repair genes and cell signalling genes such as *BRCA*, *PI3K*, *PTEN* and *MYC*; gene fusions involving the ETS gene family; and germline variants in susceptibility loci associated with predisposition to prostate cancer development and metastatic progression including *ATM*[3–17]. These endeavours have also refined our understanding of prostate cancer aetiology and progression, leading to potential advances in precision treatment strategies[18].

As most prostate cancer is localized, low-grade, indolent and unlikely to result in patient death[19], prostate cancer screening should be able to differentiate between aggressive and indolent prostate cancer subtypes[20] in order to avoid overdiagnosis and unnecessary and potentially morbid therapy. However, accurate identification and treatment of high-grade aggressive prostate cancer remain challenging and controversial conundrums in oncology. The present state of clinical prostate cancer screening and management is heavily reliant on a blood-based biomarker-driven approach that measures serum PSA levels. PSA (encoded by kallikrein 3 (*KLK3*)) is a glycoprotein enzyme produced almost exclusively by the prostate gland, but increased levels are not prostate cancer-specific and have been observed in a variety of nonprostate cancer states such as increased age, BPH or in an inflamed or infected prostate (prostatitis)[21]. Since FDA approval in 1986, an elevated serum PSA level has been used for opportunistic prostate cancer screening[22], and this use has, arguably, led to a revolution in prostate cancer management by reducing prostate cancer mortality through enabling early disease detection and treatment intervention[23]. However, the use of PSA as a prostate cancer screening biomarker is becoming increasingly controversial owing to its tendency to

Key points

- The accurate identification and personalized treatment of high-grade, clinically significant prostate cancer have been ongoing concerns since the outcomes of large international prostate cancer screening trials were published.
- The combination of next-generation prostate cancer biomarker discoveries and the emergence of companion nanodiagnostic technologies could lead to a new era of precision prostate cancer management.
- In-depth profiling of prostate cancer has resulted in the discovery of next-generation biomarkers such as *TMPRSS2–ETS* fusion genes, *PCA3* and *SCHLAP1*, which could improve molecular subtyping and risk stratification.
- Evolving nanotechnologies such as novel nanomaterials and nanoparticles might benefit clinical translation of next-generation prostate cancer biomarkers by improving detection speed and sensitivity for development of point-of-care diagnostics.
- Challenges for translating both novel biomarkers and nanotechnology platforms into the clinic still need to be overcome by bridging the gap between clinical and diagnostic disciplines.

provide false-positive and false-negative diagnoses, as well as conflicting clinical screening trial results[24].

Two notable large-scale randomized trials have assessed the potential benefit of PSA screening — the European Randomized Study of Screening for Prostate Cancer (ERSPC) trial[25] and the Prostate, Lung, Colorectal and Ovarian Cancer Screening (PLCO) trial[26]. The ERSPC trial investigated PSA screening in a largely unselected population among the screened cohort drawn from 8 different countries and reported a 27% decrease in prostate cancer mortality in men aged 55–69 years after 13 years of follow-up[27]. The PLCO trial was conducted in the USA and initially found no reduction in prostate cancer mortality and no benefit of PSA screening[26,28,29]. However, subsequent reanalysis of the PLCO data showed that PSA screening reduced risk of mortality by up to 32%[30] and suggested that initial conclusions were influenced by PSA testing contamination in the PLCO trial control group (~85% of men underwent a PSA test at least once[31,32]). Importantly, this contamination might have been a major cause for the reduction in statistical significance for the initial PLCO report. Nonetheless, the general consensus of both trials is that widespread PSA-based prostate cancer screening leads to prostate cancer overdiagnosis and overtreatment with serious implications on men's health and wellbeing[24].

In 2012, on the basis of findings of the aforementioned PSA screening trials, the United States Preventive Services Task Force (USPSTF) recommended against population-wide PSA screening for prostate cancer, regardless of age[33]. This recommendation was associated with a substantial decline in prostate cancer screening and incidence[34,35] and might reduce the harmful widespread screening effects[36] but simultaneously eliminate the known screening benefits for patients with the likelihood of developing advanced-stage prostate cancer[37]. As a result of vigorous international debate, the USPSTF issued its latest prostate cancer screening guidelines in 2018, recommending that clinicians inform men aged 55–69 years about the benefits and harms of PSA screening and offer PSA testing if they chose it[38] to enable personalized shared decision-making. Thus, an alternative approach to prostate cancer screening might not be to abandon PSA testing altogether but to design screening methodologies with improved risk stratification capabilities[39,40].

Optimal prostate cancer screening risk stratification requires molecular subtyping to yield information on disease biology, prognosis and treatment benefits. However, the molecular classification of cancer into disease subtypes for effective targeted therapies (such as HER2 (also known as ERBB2)-positive breast cancer subtype classification and trastuzumab[41]) is an ongoing effort in prostate cancer[42–49]. One notable example is the Stockholm 3 (STHLM3) study[50] of prostate cancer screening, which is the first proof-of-concept, population-based study to examine individualized risk prediction of high-grade prostate cancer (Gleason score ≥7). The study enrolled participants aged 50–69 years without prostate cancer, and each participant underwent STHLM3 and PSA screening. Men with a PSA concentration of ≥3 ng/ml or who scored as high risk using the STHLM3 model (a combination of plasma protein biomarkers (PSA, free PSA (fPSA), intact PSA, hK2, MSMB and M1C1), genetic polymorphisms (232 single-nucleotide polymorphisms) and clinical information (age, family history, previous prostate biopsy and prostate examination) were referred for prostate cancer examination and biopsy sampling. The STHLM3 model showed superior performance in predicting aggressive prostate cancer to PSA levels alone, with an area under the curve (AUC) of 0.74 compared with 0.56, and resulted in a 32% reduction in prostate biopsies. Using the existing data, the STHLM3 model has been updated using logistic regression (removal of intact PSA, inclusion of *HOXB13* and model fitting to data[50] from STHLM3 training and validation cohorts) and can reduce biopsies by 34% when used as a reflex test for men with PSA ≥3 ng/ml (REF.[51]). Other large-scale studies of primary prostate cancer molecular subtyping[43–46] and multi-institutional research collaborations into personalized metastatic prostate cancer treatments (such as Stand Up To Cancer–Prostate Cancer Foundation Dream Teams) should result in development of new biomarker-driven precision prostate cancer management approaches[52].

In light of the potential translational value of next-generation prostate cancer biomarkers, complementary cost-effective and easy-to-implement advanced detection technologies are needed. At present, prostate cancer biomarkers are characterized using laboratory-based techniques that are appropriate in a research setting or individual specialized clinical laboratories but are unsuitable for rapid, highly cost-effective and point-of-care clinical diagnostics. Nanotechnology has the potential to alleviate the current limitations of molecular testing in a single reference laboratory or reduce the need for expensive proprietary equipment, enabling point-of-care development for broad cost-effective cancer diagnostics. Furthermore, the use of nanodiagnostics for nucleic acid or protein target detection is sensitive at the single-molecule level and could be used in liquid biopsies for which high analytical sensitivity is required for low target copy number genes and protein expression levels.

In this Review, we discuss the progress of biomarker-driven prostate cancer molecular subtyping and the development of companion nanodiagnostic strategies to refine clinical biomarker detection. We also discuss the existing challenges of merging both aspects for precision prostate cancer management and provide insights into possible solutions.

Next-generation biomarkers

Advances in molecular profiling, microarray profiling and next-generation sequencing have enabled the discovery of novel prostate cancer-specific biomarkers in blood and urine with better disease-informing abilities than PSA (next-generation prostate cancer biomarkers)[53–61] (TABLES 1,2). These circulating next-generation prostate cancer biomarkers could be rapidly translated into the clinic to improve diagnostic, prognostic and predictive algorithms.

TMPRSS2–ETS *fusion*. Chromosomal rearrangements are common aberrations in prostate cancer and, through the carcinogenic processes of kataegis and chromothripsis, lead to fusions of distant genes[62–65]. In 2005, a novel bioinformatics analysis enabled the discovery that recurrent fusions between the 5′ promoter sequence of androgen-regulated *TMPRSS2* and *ETS* transcription factors (particularly the coding sequence of *ERG*) are frequently present in prostate cancer[66]. *TMPRSS2* encodes a membrane-bound serine protease[67] that is expressed on prostate cells and is involved in a signal transduction pathway associated with prostate cancer metastasis and invasion[68]. *ERG* encodes an oncogenic protein that is overexpressed in prostate cancer and drives transition of prostatic intraepithelial neoplasia (PIN) to carcinoma[69]. *TMPRSS2–ERG* fusion is a result of chromosomal translocations or interstitial deletions between the two genes, which are approximately 3 Mb apart on chromosome 21 (REFS[70–74]). As *TMPRSS2* contains androgen-sensitive elements, it was originally hypothesized that the fusion event put *ERG* expression under androgen control and caused the overexpression of oncogenic *ERG* protein in tumorigenesis[75]. However, subsequent studies revealed additional layers of complexity to this process[76–82].

TMPRSS2–ERG is present in ~50% of PSA-screened cohorts from Asia, Europe and the USA[64], and occurrences of fusions involving other *ETS* family members, such as *ETV1* or *ETV5*, are rarer[66]. Several *TMPRSS2–ERG* isoforms exist through alternate splicing and differing fusion junctions between the two genes. The most common (>90%) isoform is the T1E4 fusion between exon 1 of *TMPRSS2* and exon 4 of *ERG*[83,84]. *TMPRSS2–ERG* has superior prostate cancer specificity to PSA, with general absence in non-cancerous prostate tissues[64], and can be detected in prostate cancer precursor high-grade PIN (HGPIN) in close proximity to malignant carcinoma[85]. Hence, as one of the most prostate cancer-specific biomarkers currently available, *TMPRSS2–ERG* is highly attractive as a target for next-generation prostate cancer diagnostic development. Other studies have also suggested that *TMPRSS2–ERG*-positive prostate cancer is a distinct subtype that could be targeted by *TMPRSS2–ERG*-based or ERG-based therapeutics[86–92].

Since the initial description of *TMPRSS2–ERG*, numerous studies have investigated the clinical utility of urinary *TMPRSS2–ERG* detection[93]. The prognostic potential of urinary *TMPRSS2–ERG* is a source of debate, with some studies associating *TMPRSS2–ERG* with aggressive prostate cancer[94–96] and others showing conflicting outcomes with no correlation with worse prognosis[97]. However, as *TMPRSS2–ERG* is specifically expressed by tumour cells, increased urinary levels could be linked to increased tumour volume, which is indicative of high-grade prostate cancer[98]. To improve the performance of *TMPRSS2–ERG* for noninvasive prostate cancer stratification, a strategy using a combination of next-generation urinary prostate cancer biomarkers such as *TMPRSS2–ERG* and *PCA3* in the Mi Prostate Score (MiPS) test[99], or *ERG* with *PCA3* in the ExoDx Prostate(IntelliScore) assay[100], could be explored.

PCA3. *PCA3* is a highly prostate cancer-specific long non-coding RNA (lncRNA) biomarker that is exclusively overexpressed in cancerous prostate tissues and HGPIN[101]. *PCA3* has been postulated to be involved in prostate cancer cell survival, in part through modulating AR signalling[102]. *PCA3* was first described in 1999 (REF.[103]) and is one of the first next-generation prostate cancer screening biomarkers to be incorporated into strategies to improve the diagnostic accuracy of PSA-based prostate cancer screening[104]. *PCA3* is detectable in urine and prostatic fluid, and many studies have investigated the clinical utility of assessing urinary *PCA3* levels[105–107]. The clinical sensitivity and specificity of urinary *PCA3* for prostate cancer detection are 58–82% and ~72–79%, respectively[104,108,109]. The first major clinical contribution of *PCA3* was in combination with serum PSA; combined analysis resulted in an improvement in prostate cancer detection, with an AUC of 0.75 compared with an AUC of 0.58 for *PCA3* alone[104]. The FDA approved a quantitative *PCA3*-based prostate cancer assay (Progensa) in 2012 for men with a previous negative prostate biopsy[53]. Subsequently, this assay was refined by using combined detection of urinary *PCA3* and *TMPRSS2–ERG* RNA levels with serum PSA. The combined detection achieved an AUC, clinical sensitivity and specificity of 0.88, 80% and 90%, respectively, and performed better than each individual marker alone in

Table 1 | **Molecular biomarkers for precision prostate cancer management**

Tools	Sample source	
	Blood and/or urine	**Prostate tissue**
Existing and emerging assays	• PHI • 4Kscore • Mi Prostate Score • SelectMDx • ExoDx Prostate(IntelliScore)	• ConfirmMDx • Oncotype Dx • Prolaris • Decipher
Next-generation biomarkers	• *PCA3* • *SCHLAP1* • *TMPRSS2–ETS* fusion • *PTEN* • AR-V7	• *PCA3* • *SCHLAP1* • *TMPRSS2–ETS* fusion • *PTEN*

4Kscore, four-kallikrein; AR-V7, androgen receptor splice variant 7; PHI, prostate health index.

Table 2 | **Next-generation prostate cancer biomarkers**

Biomarker	Description	Biological function	Sampling source	Potential clinical utility and performance
TMPRSS2–ETS fusion	Chromosomal rearrangements of the *TMPRSS2* gene and ETS transcription factors	Drives the overexpression of oncogenic ETS family transcription factors such as ERG protein under androgen control in tumorigenesis	Tissue, blood and urine	• Diagnosis and potential prognosis based on transcript levels • AUC = 0.77 for prostate cancer prediction upon subsequent biopsy[110]
PCA3	Long non-coding RNA exclusively expressed in prostate tissue and overexpressed in prostate cancer	Postulated to be involved in prostate cancer cell survival, in part through modulating AR signalling	Tissue, blood and urine	• Diagnosis • AUC = 0.746 for specific prostate cancer diagnosis[104]
SCHLAP1	Long non-coding RNA that is highly overexpressed in a subset of patients with aggressive prostate cancer	Associated with cancer cell invasiveness and metastasis by antagonizing the functions of the SWI/SNF chromatin-modifying complex	Tissue and urine	• Prognosis • AUC = 0.68 for metastatic prostate cancer prediction[113]
PTEN	Frequently mutated and deleted tumour suppressor gene in prostate cancer	Activates the PI3K pathway to promote tumour initiation and growth	Tissue and blood	• Prognosis • AUC = 0.749 for lethal prostate cancer prediction[121]
AR-V7	Splice variant of AR lacking the ligand-binding domain	Maintains AR-regulated transcription in treatment-resistant prostate cancer models	Blood (CTCs)	Predicts treatment efficacy

AR, androgen receptor; AR-V7, androgen receptor splice variant 7; AUC, area under the curve; CTCs, circulating tumour cells.

predicting prostate cancer[110]. This observation further reinforces the notion of combining next-generation prostate cancer biomarkers to improve the accuracy of prostate cancer screening[111].

SCHLAP1. *SCHLAP1* is a lncRNA biomarker that was discovered using bioinformatics analysis of a subset of cancers to identify selectively upregulated lncRNAs associated with prostate cancer recurrence and progression[112]. *SCHLAP1* is not expressed in other cancers or any normal tissue[113] and is highly overexpressed in a subset of patients with aggressive prostate cancer relative to localized prostate cancer[114]. *SCHLAP1* promotes cancer cell invasiveness and metastasis presumably by antagonizing the tumour-suppressive functions of the SWI/SNF chromatin-modifying complex[112]. A large, unbiased multi-institution analysis of genes related to metastasis and mortality after radical prostatectomy for primary prostate cancer showed and subsequently validated *SCHLAP1* to be the top-ranked prognostic biomarker for metastasis development[113]. In this study, *SCHLAP1* was detectable using noninvasive methods in urine sediments from patients with prostate cancer and found to be a viable candidate urinary biomarker for discriminating high-risk aggressive prostate cancer from low-risk disease. The potential utility of *SCHLAP1* as a biomarker has been further shown in subsequent studies: *SCHLAP1* dysregulation is associated with aggressive intraductal and cribriform subpathologies of prostate cancer[115], and noninvasive detection of *SCHLAP1* in circulating tumour cells (CTCs) from blood is a potential biomarker for identifying patients with metastatic prostate cancer[116].

PTEN. *PTEN* is a frequently mutated and deleted tumour suppressor gene in 30–60% of high-grade prostate cancers[117–120]. *PTEN* loss is commonly used as a tissue biomarker in immunohistochemical detection of prostate cancer and is associated with poor prognosis and aggressive metastatic prostate cancer as well as rapid development of resistance to hormonal treatment[120,121]. Inactivation of *PTEN* results in downstream signalling of the PI3K pathway to cause increased phosphorylated AKT levels, promoting tumour cell growth, proliferation, survival and migration via multiple downstream pathways[122]. *PTEN* deletion has also been shown to occur with *TMPRSS2–ERG* fusion and drives prostate cancer progression[123–128], and combined *PTEN* loss plus *TMPRSS2–ERG* fusion is considered to reflect a particularly aggressive prostate cancer phenotype[124]. *PTEN* loss in CTCs and matched tumour tissue samples has shown strong positive correlation[129], illustrating the potential for detecting *PTEN* loss in liquid biopsies.

Androgen receptor splice variant 7. AR splice variants lack the ligand-binding domain and are capable of maintaining AR-regulated transcription in treatment-resistant prostate cancer models[130–134]. Androgen receptor splice variant 7 (AR-V7), which has an exon 7 deletion[135–137], has been implicated in the development of CRPC[138]. A 2014 study suggested that the presence of AR-V7 transcripts in CTCs from patients with CRPC treated with enzalutamide or abiraterone is associated with reduced PSA response rates and progression-free and overall survival[139]. In 2018, a correlative study reported

that patients with positive nuclear AR-V7 expression in CTCs have better overall survival when treated with taxane therapy than when treated with enzalutamide or abiraterone[140]. Thus, AR-V7 and other splice variants are promising biomarkers for predicting the sensitivity of prostate cancer to treatment. Moreover, *AR* aberrations such as chromosomal copy number variations and missense exon 8 mutations in circulating cell-free DNA have also been linked to enzalutamide and abiraterone resistance[141–143], offering another minimally invasive approach for predicting therapeutic resistance in metastatic prostate cancer.

Data from the ongoing SPARTAN[144], PROSPER[145], STAMPEDE[146] and LATITUDE[147] trials investigating different therapeutic options for metastasis-free survival are promising; thus, studies into biomarkers that are associated with treatment-prediction (such as AR-V7) will be invaluable and potentially practice changing for high-risk prostate cancer management.

Biomarker-directed screening assays

Apart from the aforementioned prominent examples, several other biomarkers are promising, including other gene fusion variants involving different *ETS* genes (such as *ETV1*, *ETV4* and *ETV5*)[64]; *GSTP1*-associated[148] and *EZH2*-associated[149] epigenetic changes; *SPINK1* (REF.[150]); *AMACR*[151]; and different variations of PSA proteins[152]. A multiomics approach combining the use of these different next-generation prostate cancer biomarkers might provide the information needed to guide therapy after initial diagnosis and/or help identify patient-specific characteristics that are important when determining therapy. Several biomarker-based diagnostic assays for supporting PSA screening outcomes are available to clinicians[153,154] (TABLE 3).

PHI and 4Kscore. Prostate cancer screening blood tests such as the prostate health index (PHI) assay (Beckman Coulter) and four-kallikrein (4Kscore) test (OPKO Laboratory) have been developed owing to the poor sensitivity of PSA alone. Both tests use combinations of different serum PSA isoforms and/or related proteins to increase prostate cancer-specific sensitivity.

PHI is an FDA-approved blood serum assay that combines the levels of total PSA, fPSA and p2PSA (a prostate cancer-specific fPSA isoform) using the formula[155]:

$$(p2PSA/fPSA) \times PSA^{0.5}$$

The clinical specificity of PHI (16%) has been shown to significantly outperform the use of total PSA and fPSA alone (clinical specificity 8.4%; $P = 0.015$)[155]. In separate multicentre studies PHI has demonstrated AUCs of 0.698 (REF.[156]) and 0.815 (REF.[157]) for detection of aggressive (Gleason score ≥7) prostate cancer.

Similar to PHI, the 4Kscore test is a Clinical Laboratory Improvement Amendments (CLIA)-certified blood test that combines the levels of total PSA, fPSA, intact PSA, human kallikrein 2 (KLK2) and clinical information[158]. The test can be administered before biopsy for improved individualized prostate cancer risk stratification, and several studies have indicated that test outcomes could identify aggressive disease (Gleason score ≥7) and predict long-term risk of cancer metastasis[158–161].

ConfirmMDx. The ConfirmMDx test (MDxHealth) is a CLIA-certified, tissue-based assay that assesses the methylation levels of a multigene panel (*GSTP1*, *APC* and *RASSF1*) from biopsy samples[148,162,163]. The underlying biological concept behind the ConfirmMDx test is the occurrence of an epigenetic field effect in which cells adjacent to cancer foci display DNA methylation changes that can be detected by the assay but are undetectable using histopathology techniques[164–167]. Tumours can be missed by initial transrectal ultrasonography-guided biopsies, meaning that patients are often subjected to multiple further biopsies[168]. Using DNA methylation analysis, the ConfirmMDx test can provide information that can be used to decide whether repeat biopsy is necessary and identify patients who have a true-negative biopsy from those who might have occult cancer[169–172]. In two retrospective studies in men with initial histopathologically negative biopsies, ConfirmMDx reached an AUC of 0.742 for detection of high-grade (Gleason score ≥7) prostate cancer on repeat biopsy[170].

Oncotype Dx. The Oncotype Dx assay (Genomic Health) is a CLIA-certified multigene assay for assessing individualized prostate cancer aggressiveness in newly diagnosed men on the basis of a comparison between tumour and healthy tissue at biopsy[173]. The assay detects the expression of 12 cancer-associated genes (*BGN, COL1A1*, *SFRP4*, *FLNC*, *GSN*, *GSTM2*, *TPM2*, *AZGP1*, *FAM13C1*, *KLK2*, *SRD5A2* and *TPX2*) representative of 4 different biological pathways (stromal response, cellular organization, androgen signalling and proliferation) and 5 reference genes (*ARF1*, *ATP5E* (also known as *ATP5F1E*), *CLTC*, *GPS1* and *PGK1*) and uses an algorithm to calculate a Genomic Prostate Score (GPS)[173–175]. The GPS can risk stratify men diagnosed with early-stage prostate cancer via prediction of probability of pathological characteristics and assist the selection of appropriate therapy, such as active surveillance or definitive treatment[176]. In an independent set of biopsy samples from 431 men, the use of GPS resulted in an AUC of 0.72 for prediction of adverse pathology[174].

Prolaris Molecular Score. The Prolaris Molecular Score assay (Myraid) is CLIA certified and directly measures cancer cell growth markers in biopsy tissues for disease prognosis and risk stratification[177]. The Prolaris Molecular Score assay estimates the proliferation of cells and predicts the risk of disease progression by normalizing measured expression levels of 31 cell cycle progression (CCP) genes with 15 housekeeping genes. The normalized expression levels were used to calculate a mathematical CCP score[177], which reflects the general expression of cell cycle regulators (low CCP score correlates with low disease progression risk)[177–179]. In a cohort of 236 men with low-risk (Gleason score ≤6) prostate cancer, the risk stratification utility of the CCP score aided in predicting 5-year biochemical-free

recurrence (PSA ≥0.2 ng/ml) with an AUC value of 0.664 (REF.[180]).

Decipher. The Decipher test (GenomeDx) is a CLIA-certified tissue-based assay that predicts the risk of metastatic disease (independent of Gleason score or PSA test results) within 5 years after radical prostatectomy surgery[181]. This prediction is achieved by analysing 22 RNA markers associated with aggressive prostate cancer that were discovered using genome-wide search algorithms of >1 million markers and has been extensively validated[182–187]. These 22 markers are associated with cell proliferation, migration, tumour motility, androgen signalling and immune system evasion. In the first meta-analysis of five separate Decipher studies consisting of a total of 975 men after prostatectomy who had individual, patient-level genomic and clinicopathological data, Decipher generated an AUC of 0.81 for prognosticating 10-year prostate cancer metastasis risk[182].

Progensa and Mi-Prostate Score. The Progensa test (Hologic), which was approved by the FDA in 2012, is the first approved urine-based assay intended to assist in making repeat biopsy decisions for men who have had a previous negative biopsy. Progensa quantifies the *PCA3*:*PSA* urinary mRNA copy number ratio (multiplied by 1,000) as a *PCA3* score[188–191]. In a study cohort of 466 men, men with a *PCA3* score <25 were 4.56 times more likely to have a negative repeat biopsy than men with a *PCA3* score ≥25 (REF.[192]). Although the Progensa test has shown promise for detecting prostate cancer (including high-grade disease) before initial biopsy, the optimal *PCA3* score cut-off values are conflicting across different studies[191,193,194], which has hindered the clinical use of Progensa beyond its current FDA-approved repeat biopsy application.

PCA3 has also been combined with *TMPRSS2–ERG* to detect early-stage prostate cancer in a CLIA-certified urinary test called MiPS provided by the University of

Table 3 | **Biomarker-directed prostate cancer assays to support PSA testing**

Prostate cancer test	Clinical utility	Biomarkers	Sampling source	Provider	Certification	Performance
PHI	Improves detection of aggressive disease	Levels of total PSA, fPSA and p2PSA	Blood	Beckman Coulter	FDA	AUC = 0.815 for aggressive (GS ≥7) prostate cancer detection[157]
4Kscore	Identifies aggressive disease and predicts long-term risk of cancer metastasis before biopsy	Levels of total PSA, fPSA, intact PSA and human *KLK2*	Blood	OPKO Laboratory	CLIA	AUC = 0.82 for high-grade (GS ≥7) prostate cancer detection[159]
ConfirmMDx	Helps guide repeat biopsy decisions	Methylation levels of *GSTP1*, *APC* and *RASSF1*	Tissue	MDxHealth	CLIA	AUC = 0.762 for high-grade (GS ≥7) prostate cancer detection[170]
Oncotype Dx	Helps decide appropriate management such as active surveillance or invasive treatment during early diagnosis	Expression of 12 cancer-associated genes (and 5 reference genes) representative of 4 different biological pathways	Tissue	Genomic Health	CLIA	AUC = 0.72 for adverse pathology prediction[174]
Prolaris	Provides disease progression risk in biopsy tissues	Expression levels of 31 (and 15 housekeeping genes) cell cycle progression genes	Tissue	Myraid Genetics	CLIA	AUC = 0.664 for predicting biochemical-free recurrence[180]
Decipher	Predicts the risk of metastatic disease after radical prostatectomy	Analysis of 22 aggressive prostate cancer-associated RNA markers	Tissue	GenomeDx	CLIA	AUC = 0.81 for predicting 10-year metastasis risk[182]
Mi Prostate Score	Provides additional information relevant to repeat biopsy decision and predicts risk of high-grade disease	Levels of *PCA3*, *TMPRSS2–ERG* and *KLK3*	Urine	MLabs	CLIA	AUC = 0.77 for predicting aggressive (GS ≥7) prostate cancer[197]
SelectMDx	Predicts presence of high-grade disease and aids in biopsy selection decisions	Levels of *HOXC6*, *DLX1* and *KLK3*	Urine	MDxHealth	CLIA	AUC = 0.90 for predicting aggressive (GS ≥7) prostate cancer[200]
ExoDx Prostate(IntelliScore)	Improves discrimination of high-grade versus low-grade prostate cancer and benign prostatic diseases on an initial biopsy	Levels of *PCA3*, *ERG* and *SPDEF*	Urinary exosomes	Exosome Diagnostics	CLIA	AUC = 0.73 for discriminating GS ≥7 from GS = 6 and low-risk prostate cancer[100]

4Kscore, four-kallikrein; AUC, area under the curve; CLIA, Clinical Laboratory Improvement Amendments; fPSA, free PSA; GS, Gleason score; p2PSA, a prostate cancer-specific fPSA isoform; PHI, prostate health index.

Michigan (MLabs) that also incorporates serum PSA. By algorithmically combining serum PSA level with urinary quantification of *KLK3*-normalized *TMPRSS2–ERG* and *PCA3* mRNA levels, MiPS provides an individualized risk estimate of prostate cancer detection upon biopsy as well as prediction of the likelihood of the development of high-grade prostate cancer[99,110,195–198]. Among 516 and 561 eligible participants in developmental and validation cohorts, respectively, the AUC value for combining serum PSA, urinary *TMPRSS2–ERG* and *PCA3* in predicting prostate cancer with Gleason score ≥7 was 0.77 (REF.[197]).

SelectMDx. SelectMDx (MDx Health) is a CLIA-certified urinary assay for detecting early-stage prostate cancer to aid selecting men for biopsy[199]. SelectMDx consists of analysis of a three-gene panel by reverse transcription PCR (RT-PCR) (*HOXC6* and *DLX1* overexpression, with *KLK3* as an internal control) in combination with risk factors including serum PSA, PSA density, digital rectal exam, age and family history of prostate cancer. The *HOXC6* and *DLX1* biomarkers were selected from a biomarker discovery study on the basis of gene expression profiling of tissue and urinary sediment samples and have been reported to be good predictors for detection of high-grade prostate cancer[199]. In two prospective multicentre studies involving an initial cohort of 492 men and a validation cohort of 371 men, SelectMDx has a reported an AUC of 0.90 for aggressive Gleason score ≥7 prostate cancer[200].

ExoDx Prostate(IntelliScore). Exosomes are nanometre-sized vesicles that are released from cells and can be found in different biofluids such as blood or urine[201]. A study in 2009 first reported that prostate cancer cell-derived exosomes in urine contain both *PCA3* and *TMPRSS2–ERG* mRNA[202]. Using advances in exosomal RNA purification, this original discovery has subsequently been developed into the CLIA-certified ExoDx Prostate(IntelliScore) assay (Exosome Diagnostics). In a study involving a training cohort of 255 men and a validation cohort of 519 men, ExoDx Prostate(IntelliScore) assessed exosomal RNA levels of *PCA3*, *ERG* and *SPDEF* by RT-PCR to derive an overall score for improved discrimination of Gleason score ≥7 from Gleason score = 6 prostate cancer and benign prostatic diseases with an AUC of 0.73 when combined with standard-of-care variables (PSA level, age, race and family prostate cancer history)[100]. Given the increased research into the biomarkers contained in urinary exosomes derived from prostate cancer, exosomes might be a viable resource for prostate cancer diagnosis and clinical management[201,203].

Multiparametric MRI

Advanced tissue imaging is emerging as a tool to supplement and enhance molecular biomarker testing for identifying aggressive tumour foci[204]. Specifically, multiparametric MRI (mpMRI) is a promising technology for prostate cancer screening, localization, staging and risk stratification. An mpMRI combines three separate parameters (imaging techniques) — T2-weighted imaging, diffusion-weighted imaging and dynamic contrast enhancement imaging — to provide detailed anatomical and functional prostate imaging[204].

In the past 3 years, studies have indicated that mpMRI improves discrimination between high-risk and low-risk prostate cancer[204–206] and enables accurate targeting of tumours for guiding biopsy sampling[207–209]. In the multicentre, randomized PRECISION trial consisting of 500 men[210], the use of mpMRI for prebiopsy risk assessment and targeted biopsy was found to be superior to standard transrectal ultrasonography (TRUS)-guided biopsy. Overall, 95 men (38%) who underwent mpMRI-targeted biopsy were diagnosed with clinically significant prostate cancer compared with 64 men (26%) who had TRUS-guided biopsy. Concurrently, overdetection of clinically insignificant prostate cancer was reduced (9% for mpMRI versus 22% for TRUS-guided biopsy), and fewer biopsy cores were required in the mpMRI group than in the TRUS-guided biopsy group. Together with molecular biomarker-driven approaches, mpMRI could aid in minimizing overdiagnosis and overtreatment of clinically insignificant prostate cancer, reducing the number of unnecessary prostate biopsies.

Nanotechnology for biomarker detection

Nanotechnology research might yield next-generation prostate cancer biomarker detection strategies that have the potential to revolutionize precision prostate cancer management. Nanotechnology applications in the field of cancer require cross-disciplinary research linking biology, chemistry, physics, engineering and medicine[211,212]. The basic rationale for using nanometre-sized materials or structures is to exploit the unique physical properties (such as optical, magnetic, electronic and structural properties) that are evident within the nanoscale range[213]. An example of the application of nanotechnology in oncology is the use of synthetic nanovectors, such as liposomes for therapeutic drug delivery to cancerous tissues (for example, liposomal doxorubicin)[214]. In addition to cancer drug delivery applications, nanotechnological cancer diagnostic approaches have an advantage at the nanoscale because the nanosensing elements require only the interaction of exceedingly few biotarget molecules of similar dimensions to rapidly generate a detection signal.

The emergence of nanotechnology-based approaches for prostate cancer screening (TABLE 4) is extremely promising owing to their highly sensitive analytical detection features, clinical utility and affordability. Essentially serving as general nucleic acid, protein or metabolite biomarker sensors, nanotechnology strategies impart remarkable detection capabilities without any specialized sample processing techniques. Many miniaturized platforms (such as integrated diagnostics, wearables and implantables enabled by nanocomponents) have been developed for disease biomarker panel analysis after comprehensive genetic screening using next-generation sequencers (FIG. 1).

Nanostructured materials. Generally, nanostructured materials refer to constructions that are nanoscale in dimension and their properties[211]. Particularly, the physical properties of nanoscale approaches are able to impart considerable improvements in detection speed and

Table 4 | **Nanomaterials and nanoparticles used in the detection of prostate cancer biomarkers**

Nanotechnology	Type	Prostate cancer biomarker	Detection limit	Detection medium
Silicon nanowires	Nanomaterial	PSA protein	0.9 pg/ml (REF.[217])	Serum
Carbon nanotubes	Nanomaterial	PSA protein	4 pg/ml (REF.[218])	Serum and tissue
Graphene	Nanomaterial	PSA protein	8 pg/ml (REF.[219])	Serum
Iron oxide paramagnetic nanoparticles	Nanoparticle	Whole prostate cancer tumour cells; various isoforms of PSA protein; and *TMPRSS2–ERG*, *PCA3* and *SCHLAP1* mRNA	• 0.1 ng/ml (protein)[227] • 1,000 copies (RNA)[232]	Urine
Quantum dots	Nanoparticle	PSA protein	0.33 ng/ml (REF.[237])	Serum
Surface-enhanced Raman scattering nanoparticles	Nanoparticle	Various isoforms of PSA protein; and *TMPRSS2–ERG* variants, *PCA3* and AR-V7 mRNA	• 12 pg/ml (protein)[242] • 100 copies (RNA)[244]	Serum and urine

AR-V7, androgen receptor splice variant 7.

sensitivity[213]. With regards to prostate cancer nanodiagnostics, which have been developed from nanostructured materials over the past decade, developments largely focus on PSA as a target for detection in clinical specimens[215]. The pioneering work that enabled the use of nanostructured material in prostate cancer detection used microcantilevers for PSA protein detection[216]. The binding of PSA proteins to antibodies on a microcantilever surface resulted in a nanomechanical deformation of the microcantilever structure. This deformation could be measured optically to achieve a clinically relevant PSA detection limit of 0.2 ng/ml in a background of human serum albumin and human plasminogen at 1 mg/ml. This development was followed by silicon-nanowire field-effect sensors that incorporated nanowires and surface PSA receptors into arrays for highly sensitive PSA protein detection[217]. Silicon nanowires are 1D semiconducting nanostructures that can be arranged into arrays, surface modified with capture antibodies and incorporated into field-effect transistors for biosensing[211]. The binding of PSA proteins to antibodies on the nanowire surface will result in a real-time electrical signal to PSA concentrations of 0.9 pg/ml in undiluted serum samples[217].

In the past 10 years, carbon nanotubes[218] and graphene[219] have emerged as new forms of superconductive nanomaterials and have been used for electrochemical PSA protein detection in human tissue and serum samples. Terminally carboxylated single-wall carbon nanotubes (SWNTs) have been shown to self-assemble in upright bundles of 20–100 nm in diameter on conductive surfaces. The SWNT nanostructures were linked to PSA antibodies and electrochemically active labels for greatly amplified electrochemical sensing of concentrations of 4 pg/ml of PSA in human serum samples[218]. Like carbon nanotubes, graphene is a nanostructured material with excellent electron transfer ability and has been used to modify a glass carbon electrode surface for an electrochemiluminescent detection limit of 8 pg/ml of PSA[219].

Nanostructured materials have demonstrated greatly improved PSA detection limits: a potentially useful property for early post-prostatectomy biochemical recurrence monitoring, which necessitates ultralow PSA detection[220]. However, clinical translation of cutting-edge nanostructured materials has not been achieved, probably owing to the lack of academic research expertise in adapting the novel nanomaterials towards actual clinical usage and slow progress towards clinical studies caused by funding issues and/or lack of diagnostic commercialization knowledge.

Nanoparticles. The most commonly used nanoparticles for molecular diagnostic applications are nanometre-sized metallic substrates of various shapes[211]. The production and use of conventional nanoparticles are now established and commercially available. Nanoparticles are an ideal substrate for maximal loading of intended biological molecules onto the nanoparticle surface owing to their high surface:volume ratio. Furthermore, as electron behaviours are constrained differently within nanostructures compared with macrostructure counterparts, nanoscale substrates have unique size-dependent magnetic, electronic and optical properties that are useful for diagnostic applications[212]. In terms of in vitro diagnostics, nanoparticles have been widely used in prostate cancer nanodiagnostic applications[221–224]. Particularly, iron oxide core paramagnetic nanoparticles have been used for isolation and purification of specific molecular targets such as nucleic acids or proteins before detection[225]. This application has been demonstrated for the detection of different prostate cancer protein biomarkers in circulation and on the cell surface[226,227]. In a demonstrative study, magnetic nanoparticles were surface modified with fluorescent dyes and monoclonal anti-prostate-specific membrane antigen antibodies to generate fluorescent magnetic biotargeting multifunctional nanoprobes (FMBMNs)[227]. The FMBMNs isolated prostate cancer cells at a capture efficiency of 97% from as low as 0.01% of an artificial cancer and red blood cell mix within 25 min.

Aside from PSA detection, progress has been made with the use of magnetic particles for detection of next-generation prostate cancer RNA biomarkers such as

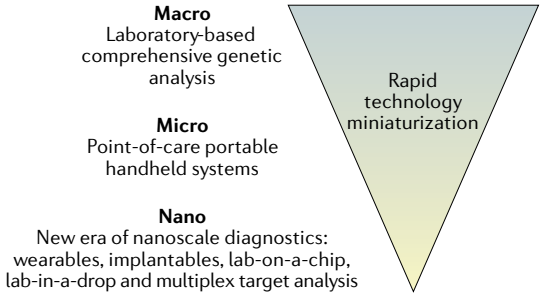

Fig. 1 | **A potential future of diagnostic miniaturization.** Continuous innovations in the nanotechnology field have resulted in the development of clinical diagnostics from next-generation sequencers with massive genetic screening capabilities to miniaturized technologies for targeted biomarker analysis. New nanoscale diagnostics could fundamentally change clinical practice though enhanced disease detection, treatment and monitoring.

TMPRSS2–ERG, *PCA3* and *SCHLAP1* (REFS[228–234]). In particular, biomolecular purification using magnetic nanoparticles has enabled magnetic isolation of *TMPRSS2–ERG* and *SCHLAP1* RNA targets from urinary RNA for amplification-free electrochemical target detection. A detection limit of 1,000 target copies was obtained within 10 min of capture probe–target hybridization[232]. The enhancement in hybridization speed was achieved using a fluidic nanomixing effect (compared with 100 min of static incubation) for enhanced concentration and hybridization of capture probes and RNA targets from bulk solution. In addition, magnetic particles have been used for the isolation and visual detection of *TMPRSS2–ERG* RNA targets from urine-isolated RNA[233]. The visual detection was achieved owing to the ability of *TMPRSS2–ERG* amplicons (from as little as 10^5 initial target copies) to initiate crosslinking of magnetic beads and flocculate out of an aqueous coloured dispersion to produce a colourless solution.

Quantum dots are nanometre-sized semiconducting particles with better enhanced fluorescence emissions than conventional organic fluorophores owing to a quantum-confinement effect of electron energy bands[235,236]. Quantum dots have tuneable, size-dependent emission wavelengths and improved photostability compared with organic fluorophores. A quantum dot-based immunochromatography test strip was developed for rapid and low-concentration PSA protein detection[237]. The platform achieved a detection limit of 0.33 ng/ml PSA within a 15 min reaction time, and clinical utility was demonstrated with clinical serum specimens. For multiplexed prostate cancer target detection, quantum dots have been used for the quadruplexed detection of *ERG* and *PTEN* status in prepared prostate tissue specimens[238]. This in situ hybridization assay featured four genomic probes individually labelled with unique haptens, which were recognized by distinct anti-hapten antibodies conjugated to different quantum dots. This labelling enabled efficient simultaneous detection of four genomic targets on patient tissue samples, and the brightness of the quantum dots enabled each target signal to be well-differentiated when viewed under a routine fluorescence microscope.

Surface-enhanced Raman scattering (SERS) nanoparticles are a class of nanoparticles that are well suited for multiplexed detection of several biomarkers within a single reaction. SERS nanoparticles generally consist of nanostructured metallic surfaces that enhance the Raman scattering signals of surface-adsorbed biomolecules[239]. Importantly, Raman spectral widths are much narrower than those of fluorescence, making SERS ideal for multiplexed biomarker detection with minimal spectral overlapping[240,241]. SERS has been used for multiplexed detection of prostate cancer biomarkers such as various forms of PSA with excellent detection sensitivity. Specifically, a duplexed SERS immunoassay was used for the determination of the free:total PSA ratio in clinical serum samples with a fPSA detection limit of 0.012 ng/ml within an hour[242]. Additionally, SERS nanoparticles have been used in the detection of next-generation prostate cancer biomarkers such as *TMPRSS2–ERG*, *PCA3* and AR-V7 for potential molecular subtyping purposes[243–245]. For RNA biomarker detection in tumour and urine samples from patients with prostate cancer, a pentaplexed SERS assay was shown to be highly sensitive (100 copies) and specific in target detection within a short time frame of 80 min[244]. As is evident from the lack of commercially available multiplexed SERS nanoparticles in the current market, a drawback of SERS is the lack of a synthesis strategy to generate a commercially viable output of SERS nanoparticles[246]. Thus, ongoing research efforts into large-scale synthesis methods of SERS nanoparticles with high reproducibility and stability are required[247].

Miniaturized integrated systems. As well as the synthesis of novel nanomaterials and nanoparticles, nanotechnology has enabled the design of miniaturized device-based lab-on-a-chip or solution-based lab-in-a-drop systems (FIG. 2). These systems downscale and integrate different aspects of biomarker analysis workflow (such as patient sample preparation, target copy amplification and target detection readout) onto a singular platform, minimizing patient sample requirement and sample-to-outcome turnaround time[240].

Lab-on-a-chip systems typically consist of microfluidic channels and various miniaturized components such as fluid chambers, valves or electrodes that perform a multitude of biomarker analysis tasks such as sample processing, fluid mixing, biomarker isolation and detection[248]. For entire sample-to-targeted-gene analysis from prostate cancer urine and serum liquid biopsies, an integrated biochip has been developed for simultaneous detection of *TMPRSS2–ERG*, *PCA3*, *SCHLAP1* and *KLK2* RNA within 30 min[234]. Specifically, the biochip uses separate microchambers to enable rapid electrical release of cellular contents for parallel target capture, isothermal amplification and electrochemical readout. The microchambers also contain customized microelectrode patterning to induce physical nanofluidic enhancement of solid-phase target amplification. This biochip achieved a detection limit of 50 target copies[234]. Presently, as is evident from the limited number of

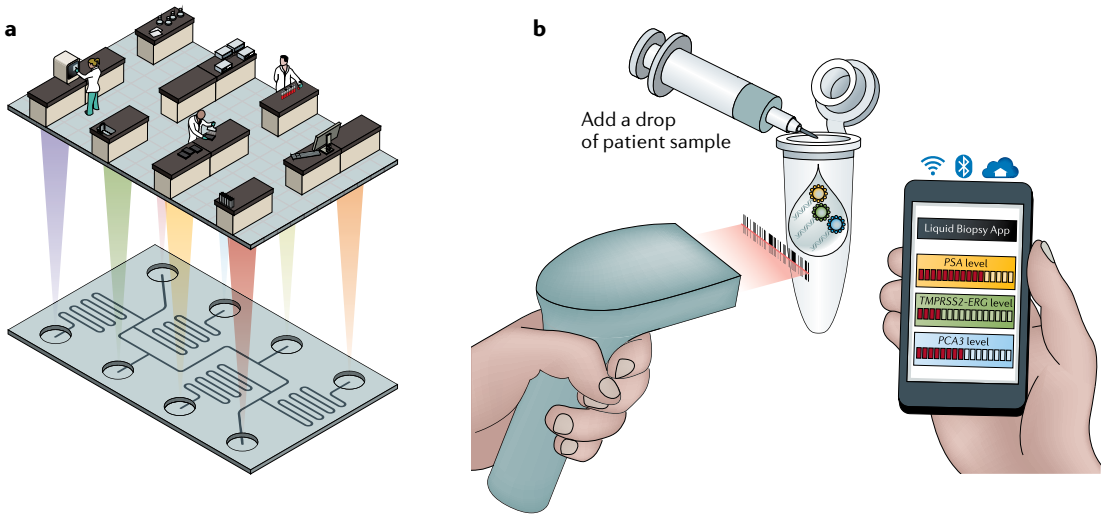

Fig. 2 | **Miniaturized integrated systems. a** | Lab-on-a-chip systems are nanodiagnostic platforms that miniaturize an entire laboratory-based biomarker analysis workflow (crude sample preparation, target copy amplification and biomarker detection readout)[240]. **b** | In lab-in-a-drop systems, different barcoded nanoparticles are used for multiplex quantification of various prostate cancer biomarkers within a single fluid droplet.

successfully commercialized lab-on-a-chip products, the translation of such systems into widespread clinical use is hampered by costly sophisticated chip engineering[249].

Lab-in-a-drop systems are alternative nanodiagnostic tools that miniaturize an entire laboratory-based biomarker analysis workflow within a single fluid droplet[240]. The main advantage of lab-in-a-drop systems is the removal of the need for costly and specialized precise engineering associated with lab-on-a-chip systems. A key example in prostate cancer is a microtube-based assay called FusBLU, which couples isothermal target amplification with an enzyme-catalysed readout to detect *TMPRSS2–ERG* RNA[231]. By performing the target amplification and detection readout in a miniaturized fluidic format, FusBLU achieves single-cell-level detection sensitivity in 75 min with flexible colorimetric, optical and electrochemical readouts[231]. By circumventing the limitations of specialized chip fabrication and sophisticated on-chip microfluidic flow associated with lab-on-a-chip platforms, lab-in-a-drop systems[240] could be smoothly translatable into practical commercial products for personalized disease applications.

Challenges in clinical translation

The described nanosized platforms for biomarker measurement are nanotechnological progress towards the optimization of prostate cancer screening. However, prostate cancer nanodiagnostics are still at an early stage of development, and more work is necessary for successful translation into viable precision clinical screening tools.

Validation of biomarker panels. The availability of high-throughput next-generation sequencing technologies for whole-exome and transcriptomic sequencing of cancer specimens means that the discovery of new and improved biomarkers for prostate cancer and other cancer types has progressed at an exponential rate but may be nearing saturation[250]. Given the heterogeneity of prostate cancer, no single biomarker is likely to be sufficient for disease risk stratification[56]. A panel of several biomarkers is probably required, and the main challenge is the assembly of such a molecular target panel for precision prostate cancer management[251]. For prostate cancer, the ideal biomarker-driven screening test needs to show superior clinical sensitivity and specificity and demonstrate considerable improvement to the receiver operating characteristic (ROC) curve in comparison with the PSA test and routinely available clinicopathological variables. To ensure maximal clinical sensitivity and specificity for clinical use, proper and in-depth biomarker validation needs to be performed using well-designed and well-conducted clinical trials with validation across different institutions. Moreover, from a clinical perspective, a prostate cancer diagnostic biomarker panel might need to be modified for patients with different germline heritage, especially in underrepresented populations (for example, drivers of prostate cancer are different in men of African descent from those in white men)[252]. For treatment biomarkers, trial designs such as adaptive enrichment approaches[253,254] might help identify prostate cancer biomarkers that can predict benefit from specific treatments.

Clinical verification of nanodiagnostics. To date, nanodiagnostic platforms have achieved only limited success in translation from research concept to clinical use. The foremost issue is that nanodiagnostic tools are currently being evaluated mostly on the basis of their analytical performance in research laboratories, with lack of continued evaluation in a clinical setting. The establishment of analytical detection sensitivity, selectivity and repeatability is no doubt crucial, but the subsequent clinical evaluation of these nanodiagnostics to test robustness, reproducibility, standardization and applicability for actual patient use is generally being overlooked.

The general expertise of academic researchers lies in the development of innovative nanotechnologies geared towards solving medical challenges. However, technological transfer into clinical use requires robust technical and/or clinical validation using a specific disease model and relevant patient cohorts, both of which are outside the traditional nanotechnological research domains. Thus, in the absence of adequate clinical collaborations and commercialization knowledge and funding, which have to be externally sourced, promising nanodiagnostic innovations of today will be confined to research environments and not translated for patient benefit.

Clinical evaluation involves the testing of novel nanotechnological tools on a cohort of patient samples (of appropriate size and context) to establish clinical sensitivity, specificity, positive predictive value, negative predictive value and AUC to demonstrate clinical utility. Lately, progress has been made in clinical evaluation of prostate cancer nanodiagnostics by using a clinically effective MiPS biomarker model (which has been independently shown to provide individualized prostate cancer risk stratification[197]) to comprehensively verify the clinical performance of a SERS nanodiagnostic technology[255]. This study enabled a urinary risk scoring system to be established by relating the SERS nanodiagnostic detection signals to gold-standard patient biopsy findings. The risk scoring system exhibited clinical sensitivity, specificity and AUC values of 0.91, 0.87 and 0.94, respectively, as well as 0.87, 0.90, 0.84, respectively, in independent training (n = 80) and validation (n = 40) human sample cohorts for differentiating high-risk (Gleason score ≥7) and low-risk (Gleason score <7) prostate cancer. The evaluation of these clinical parameters will enable the generation of clinically relevant biomarker detection limits, which are notably different from their analytical counterparts. For instance, a detection limit of a single molecule is an outstanding limit of detection analytically; however, this detection level might not be of great clinical utility if no implication for disease management exists (that is, no clinical action is required) when an oncogenic biomarker is present at a single copy. The determination of clinically relevant detection limits might be especially pertinent in prostate cancer, in which indolent disease is extremely common and overtreatment needs to be avoided[24].

Acceleration of validation processes. The time frame for comprehensive clinical validation and translation of novel prostate cancer biomarkers and new biomarker-based nanotechnologies needs to be effectively accelerated to provide faster patient benefit — it currently typically takes 5–10 years for each biomarker and nanotechnology to be translated. A feasible way to streamline this process might be the simultaneous combined evaluation of new nanodiagnostic technologies and novel biomarkers to investigate potential clinical benefits, leading to a reduced overall time frame for validation. This change could be realized using carefully designed clinical studies with well-established prospectively defined research objectives regarding the choice of nanotechnology platform and biomarker panel and relevant patient cohorts and samples.

Standardization of protocols. Protocols related to prostate cancer biomarker analysis and nanodiagnostic development are now varied across different research institutions owing to diverse practices within the sample-to-outcome pathway, such as human sample collection and handling approaches, biomarker extraction techniques and biomarker analysis parameters. This lack of protocol standardization is concerning, as it will hinder direct comparison and validation of study outcomes, resulting in inaccurate conclusions. In collaborative biomarker studies within large consortia, care must be taken to minimize systematic and random variation with proper control conditions to enable head-to-head comparisons and cross-validation of related studies. For the development of nanodiagnostic technology, standardization of nanocomponent synthesis processes and storage conditions is needed to minimize batch-to-batch variability and ensure optimal functions during applications. Particularly, the standardization of patient sample processing and molecular testing processes in biomarker analysis is pivotal. In 2017, a combination of a standardized whole-urine sample collection protocol, an automated urinary RNA extraction and a PCR platform contributed to robust analytical validation of an RNA-based urinary assay (for *DLX1*, *HOXC6* and *KLK3*) across two independent laboratories[256]. The analysis of 99 whole-urine samples at both laboratories indicated a highly positive correlation (r = 0.997; P < 0.001) in assay analytical performance, and the detection outcome in terms of absolute likelihood difference for high-grade prostate cancer upon biopsy was <2%.

Multidisciplinary collaboration. The successful realization of novel clinical prostate cancer diagnostic tests involving next-generation prostate cancer biomarker detection requires complementary cutting-edge detection approaches. In this regard, the role of nanotechnology in enabling rapid, highly sensitive, accurate and affordable biomolecular target sensing is greatly encouraging. Many innovative nanotechnology-based biosensing techniques have focused on only improving PSA detection as a prostate cancer diagnosis model owing to relatively poor awareness of next-generation prostate cancer biomarkers in the nanotechnology research field. Thus, improved interaction between the fields of clinical and nanodiagnostic research in the form of multidisciplinary conferences and fora are required so that nanotechnology researchers are kept up to date with the most relevant clinical information on biomarkers and clinicians are updated with nanodiagnostic advances. This interaction will enable state-of-the-art diagnostic nanotechnologies to be designed for the most promising and exciting cancer biomarkers available, directing efforts to address the most relevant and pressing need in clinics.

Conclusions

A single biomarker is unlikely to be sufficient to achieve the required diagnostic sensitivity and specificity to enable accurate prostate cancer risk stratification. Emerging evidence using various next-generation prostate cancer biomarkers shows that effective prostate cancer classification into different molecular subtypes is potentially

amenable for precision treatment strategies. As well as detection of early-stage disease, the use of prostate cancer molecular subtyping could extend to the guidance of treatment decisions. Research efforts in the next decade will probably focus on the clinical evaluation and translation of different combinations of next-generation prostate cancer biomarkers in order to elucidate a comprehensive multiomics panel that can identify clinically significant prostate cancer subtypes with high accuracy.

The importance of developing improved detection technologies to target these biomarkers in human specimens is as equally essential as translation of biomarkers into clinical use in order to justify changing clinical and regulatory screening paradigms. Thus, the unique benefits of nanotechnological approaches could be exploited for increased detection sensitivity, accuracy and speed at a reduced cost. The urinary detection of prostate cancer biomarkers could offer a truly noninvasive form of prostate cancer liquid biopsy that could overcome the challenges of tumour heterogeneity associated with surgical tissue biopsies. Preliminary results of several urine-based prostate cancer tests in the clinic have been promising, but the possibility of more powerful urine-based diagnostics that could provide an accurate overall disease molecular snapshot requires further clinical evaluation before clinical translation. With the many innovative developments in nanodiagnostic tools, the potential of cancer nanotechnology in improving prostate cancer care is highly optimistic. To translate these nanotechnologies into clinical use in the near future, researchers must properly evaluate their methodologies in relevant patient cohorts, establish clinically relevant detection limits and comprehensively evaluate clinical performance parameters.

Lastly, optimal precision outcomes for patients with prostate cancer will probably be derived from a synergy of ideas and knowledge brought together under an umbrella of cross-disciplinary collaborations. This Review bridges the knowledge gap between the prostate cancer clinical and nanodiagnostic research fields by informing clinicians about future possibilities and benefits of prostate cancer nanotechnology-based screening and alerting nanotechnology researchers to the availability of next-generation prostate cancer biomarkers for integration into their nanodiagnostic research.

Published online 8 April 2019

1. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2018. *CA Cancer J. Clin.* **68**, 7–30 (2018).
2. DeSantis, C. E. et al. Cancer statistics for African Americans, 2016: progress and opportunities in reducing racial disparities. *CA Cancer J. Clin.* **66**, 290–308 (2016).
3. Quigley, D. A. et al. Genomic hallmarks and structural variation in metastatic prostate cancer. *Cell* **174**, 758–769 (2018).
4. Robinson, D. et al. Integrative clinical genomics of advanced prostate cancer. *Cell* **161**, 1215–1228 (2015).
This cohort study provides multi-institutional clinical sequencing information that could effect treatment decisions for patients with metastatic CRPC.
5. Robinson, D. R. et al. Integrative clinical genomics of metastatic cancer. *Nature* **548**, 297–303 (2017).
6. Gundem, G. et al. The evolutionary history of lethal metastatic prostate cancer. *Nature* **520**, 353–357 (2015).
This study details the complex patterns of metastatic spread arising from prostate tumours in ten patients.
7. Beltran, H. et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat. Med.* **22**, 298–305 (2016).
8. Fraser, M. et al. Genomic hallmarks of localized, non-indolent prostate cancer. *Nature* **541**, 359–364 (2017).
9. Hong, M. K. H. et al. Tracking the origins and drivers of subclonal metastatic expansion in prostate cancer. *Nat. Commun.* **6**, 6605 (2015).
10. Kumar, A. et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. *Nat. Med.* **22**, 369–378 (2016).
11. Smith, B. A. et al. A basal stem cell signature identifies aggressive prostate cancer phenotypes. *Proc. Natl Acad. Sci. USA* **112**, E6544–E6552 (2015).
12. Carreira, S. et al. Tumor clone dynamics in lethal prostate cancer. *Sci. Transl Med.* **6**, 254ra125 (2014).
13. Barbieri, C. E. et al. The mutational landscape of prostate cancer. *Eur. Urol.* **64**, 567–576 (2013).
14. Taylor, R. A. et al. Germline BRCA2 mutations drive prostate cancers with distinct evolutionary trajectories. *Nat. Commun.* **8**, 13671 (2017).
15. Spratt, D. E., Zumsteg, Z. S., Feng, F. Y. & Tomlins, S. A. Translational and clinical implications of the genetic landscape of prostate cancer. *Nat. Rev. Clin. Oncol.* **13**, 597–610 (2016).
This is a review of molecular prostate cancer drivers and remaining clinical translation challenges.
16. Schumacher, F. R. et al. Association analyses of more than 140,000 men identify 63 new prostate cancer susceptibility loci. *Nat. Genet.* **50**, 928–936 (2018).
17. Mijuskovic, M. et al. Rare germline variants in DNA repair genes and the angiogenesis pathway predispose prostate cancer patients to develop metastatic disease. *Br. J. Cancer* **119**, 96–104 (2018).
18. Wedge, D. C. et al. Sequencing of prostate cancers identifies new cancer genes, routes of progression and drug targets. *Nat. Genet.* **50**, 682–692 (2018).
19. Attard, G. & Beltran, H. Prioritizing precision medicine for prostate cancer. *Ann. Oncol.* **26**, 1041–1042 (2015).
20. Irshad, S. et al. A molecular signature predictive of indolent prostate cancer. *Sci. Transl Med.* **5**, 202ra122 (2013).
21. Polascik, T. J., Oesterling, J. E. & Partin, A. W. Prostate specific antigen: a decade of discovery -what we have learned and where we are going. *J. Urol.* **162**, 293–306 (1999).
22. Catalona, W. J. et al. Measurement of prostate-specific antigen in serum as a screening-test for prostate cancer. *N. Engl. J. Med.* **324**, 1156–1161 (1991).
23. Bibbins-Domingo, K., Grossman, D. C. & Curry, S. J. The US preventive services task force 2017 draft recommendation statement on screening for prostate cancer: an invitation to review and comment. *JAMA* **317**, 1949–1950 (2017).
24. Pinsky, P. F., Prorok, P. C. & Kramer, B. S. Prostate cancer screening -a perspective on the current state of the evidence. *N. Engl. J. Med.* **376**, 1285–1289 (2017).
25. Schroeder, F. H. et al. Screening and prostate-cancer mortality in a randomized European study. *N. Engl. J. Med.* **360**, 1320–1328 (2009).
26. Andriole, G. L. et al. Mortality results from a randomized prostate-cancer screening trial. *N. Engl. J. Med.* **360**, 1310–1319 (2009).
27. Schroder, F. H. et al. Screening and prostate cancer mortality: results of the European randomised study of screening for prostate cancer (ERSPC) at 13 years of follow-up. *Lancet* **384**, 2027–2035 (2014).
28. Andriole, G. L. et al. Prostate cancer screening in the randomized prostate, lung, colorectal, and ovarian cancer screening trial: mortality results after 13 years of follow-up. *J. Natl Cancer Inst.* **104**, 125–132 (2012).
29. Pinsky, P. F. et al. Extended mortality results for prostate cancer screening in the PLCO trial with median follow-up of 15 years. *Cancer* **123**, 592–599 (2017).
30. Tsodikov, A. et al. Reconciling the effects of screening on prostate cancer mortality in the ERSPC and PLCO trials. *Ann. Intern. Med.* **167**, 449–455 (2017).
31. Shoag, J. E., Mittal, S. & Hu, J. C. Reevaluating PSA testing rates in the PLCO trial. *N. Engl. J. Med.* **374**, 1795–1796 (2016).
32. Pinsky, P. F. et al. Assessing contamination and compliance in the prostate component of the prostate, lung, colorectal, and ovarian (PLCO) cancer screening trial. *Clin. Trials* **7**, 303–311 (2010).
33. Moyer, V. A. U.S. Preventive Services Task Force. Screening for prostate cancer: U. S. Preventive Services Task Force recommendation statement. *Ann. Intern. Med.* **157**, 120–134 (2012).
34. Jemal, A. et al. Prostate cancer incidence and PSA testing patterns in relation to USPSTF screening recommendations. *JAMA* **314**, 2054–2061 (2015).
35. Sammon, J. D. et al. Prostate-specific antigen screening after 2012 US preventive services task force recommendations. *JAMA* **314**, 2075–2077 (2015).
36. Kearns, J. T. et al. PSA screening, prostate biopsy, and treatment of prostate cancer in the years surrounding the USPSTF recommendation against prostate cancer screening. *Cancer* **124**, 2733–2739 (2018).
37. Weiner, A. B., Matulewicz, R. S., Eggener, S. E. & Schaeffer, E. M. Increasing incidence of metastatic prostate cancer in the United States (2004–2013). *Prostate Cancer Prostatic Dis.* **19**, 395–397 (2016).
38. U.S. Preventive Services Task Force et al. Screening for prostate cancer: US Preventive Services Task Force recommendation statement. *JAMA* **319**, 1901–1913 (2018).
39. Carter, H. B. et al. Early detection of prostate cancer: AUA guideline. *J. Urol.* **190**, 419–426 (2013).
40. Gandaglia, G. et al. The problem is not what to do with indolent and harmless prostate cancer-the problem is how to avoid finding these cancers. *Eur. Urol.* **70**, 547–548 (2016).
41. Hudis, C. A. Trastuzumab — mechanism of action and use in clinical practice. *N. Engl. J. Med.* **357**, 39–51 (2007).
42. Carlsson, S. V. & Kattan, M. W. Prostate cancer: personalized risk — stratified screening or abandoning it altogether? *Nat. Rev. Clin. Oncol.* **13**, 140–142 (2016).
43. Grasso, C. S. et al. Integrative molecular profiling of routine clinical prostate cancer specimens. *Ann. Oncol.* **26**, 1110–1118 (2015).
44. Abeshouse, A. et al. The molecular taxonomy of primary prostate cancer. *Cell* **163**, 1011–1025 (2015).
This is a comprehensive molecular analysis of molecular heterogeneity among primary prostate cancers.

45. Tomlins, S. A. et al. Characterization of 1577 primary prostate cancers reveals novel biological and clinicopathologic insights into molecular subtypes. *Eur. Urol.* **68**, 555–567 (2015).
46. Kaffenberger, S. D. & Barbieri, C. E. Molecular subtyping of prostate cancer. *Curr. Opin. Urol.* **26**, 213–218 (2016).
47. Paulo, P. et al. Molecular subtyping of primary prostate cancer reveals specific and shared target genes of different ETS rearrangements. *Neoplasia* **14**, 600–611 (2012).
48. Smith, S. C. & Tomlins, S. A. Prostate cancer subtyping biomarkers and outcome: is clarity emerging? *Clin. Cancer Res.* **20**, 4733–4736 (2014).
49. Na, R. et al. Germline mutations in ATM and BRCA1/2 distinguish risk for lethal and indolent prostate cancer and are associated with early age at death. *Eur. Urol.* **71**, 740–747 (2017).
50. Gronberg, H. et al. Prostate cancer screening in men aged 50–69 years (STHLM3): a prospective population-based diagnostic study. *Lancet Oncol.* **16**, 1667–1676 (2015).
This is a biomarker-based screening model to examine individualized risk prediction of high-grade prostate cancer in the Swedish population.
51. Ström, P. et al. The Stockholm-3 model for prostate cancer detection: algorithm update, biomarker contribution, and reflex test potential. *Eur. Urol.* **74**, 204–210 (2018).
52. Barbieri, C. E., Chinnaiyan, A. M., Lerner, S. P., Swanton, C. & Rubin, M. A. The emergence of precision urologic oncology: a collaborative review on biomarker-driven therapeutics. *Eur. Urol.* **71**, 237–246 (2017).
53. Prensner, J. R., Rubin, M. A., Wei, J. T. & Chinnaiyan, A. M. Beyond PSA: the next generation of prostate cancer biomarkers. *Sci. Transl Med.* **4**, 127rv3 (2012).
This paper provides an introduction to the use of next-generation prostate cancer biomarkers.
54. Dijkstra, S., Mulders, P. F. A. & Schalken, J. A. Clinical use of novel urine and blood based prostate cancer biomarkers: a review. *Clin. Biochem.* **47**, 889–896 (2014).
55. Wu, D. et al. Urinary biomarkers in prostate cancer detection and monitoring progression. *Crit. Rev. Oncol. Hematol.* **118**, 15–26 (2017).
56. Velonas, V. M., Woo, H. H., dos Remedios, C. G. & Assinder, S. J. Current status of biomarkers for prostate cancer. *Int. J. Mol. Sci.* **14**, 11034–11060 (2013).
57. Jedinak, A. et al. Novel non-invasive biomarkers that distinguish between benign prostate hyperplasia and prostate cancer. *BMC Cancer* **15**, 259 (2015).
58. Cuzick, J. et al. Prevention and early detection of prostate cancer. *Lancet Oncol.* **15**, E484–E492 (2014).
59. Hessels, D. & Schalken, J. A. Urinary biomarkers for prostate cancer: a review. *Asian J. Androl.* **15**, 333–339 (2013).
60. Narayan, V. M., Konety, B. R. & Warlick, C. Novel biomarkers for prostate cancer: an evidence-based review for use in clinical practice. *Int. J. Urol.* **24**, 352–360 (2017).
61. Ploussard, G. & de la Taille, A. Urine biomarkers in prostate cancer. *Nat. Rev. Urol.* **7**, 101–109 (2010).
62. Prensner, J. R. & Chinnaiyan, A. M. Oncogenic gene fusions in epithelial carcinomas. *Curr. Opin. Genet. Dev.* **19**, 82–91 (2009).
63. Kumar-Sinha, C., Kalyana-Sundaram, S. & Chinnaiyan, A. M. Landscape of gene fusions in epithelial cancers: seq and ye shall find. *Genome Med.* **7**, 129 (2015).
64. Kumar-Sinha, C., Tomlins, S. A. & Chinnaiyan, A. M. Recurrent gene fusions in prostate cancer. *Nat. Rev. Cancer* **8**, 497–511 (2008).
65. Edwards, P. A. W. Fusion genes and chromosome translocations in the common epithelial cancers. *J. Pathol.* **220**, 244–254 (2010).
66. Tomlins, S. A. et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* **310**, 644–648 (2005).
67. Paoloni-Giacobino, A., Chen, H. M., Peitsch, M. C., Rossier, C. & Antonarakis, S. E. Cloning of the TMPRSS2 gene, which encodes a novel serine protease with transmembrane, LDLRA, and SRCR domains and maps to 21q22.3. *Genomics* **44**, 309–320 (1997).
68. Wilson, S. et al. The membrane-anchored serine protease, TMPRSS2, activates PAR-2 in prostate cancer cells. *Biochem. J.* **388**, 967–972 (2005).
69. Carver, B. S. et al. ETS rearrangements and prostate cancer initiation. *Nature* **457**, E1 (2009).
70. Linn, D. E., Penney, K. L., Bronson, R. T., Mucci, L. A. & Li, Z. Deletion of interstitial genes between TMPRSS2 and ERG promotes prostate cancer progression. *Cancer Res.* **76**, 1869–1881 (2016).
71. Clark, J. et al. Diversity of TMPRSS2-ERG fusion transcripts in the human prostate. *Oncogene* **26**, 2667–2673 (2007).
72. Clark, J. P. & Cooper, C. S. ETS gene fusions in prostate cancer. *Nat. Rev. Urol.* **6**, 429–439 (2009).
73. Barbieri, C. E. & Rubin, M. A. Genomic rearrangements in prostate cancer. *Curr. Opin. Urol.* **25**, 71–76 (2015).
74. Mani, R. S. et al. Inflammation-induced oxidative stress mediates gene fusion formation in prostate cancer. *Cell Rep.* **17**, 2620–2631 (2016).
75. Adamo, P. & Ladomery, M. R. The oncogene ERG: a key factor in prostate cancer. *Oncogene* **35**, 403–414 (2016).
76. Kron, K. J. et al. TMPRSS2-ERG fusion co-opts master transcription factors and activates NOTCH signaling in primary prostate cancer. *Nat. Genet.* **49**, 1336–1345 (2017).
77. Bose, R. et al. ERF mutations reveal a balance of ETS factors controlling prostate oncogenesis. *Nature* **546**, 671–675 (2017).
78. Kim, J., Wu, L., Zhao, J. C., Jin, H. J. & Yu, J. TMPRSS2-ERG gene fusions induce prostate tumorigenesis by modulating microRNA miR-200c. *Oncogene* **33**, 5183–5192 (2014).
79. Wu, L. T. et al. ERG is a critical regulator of Wnt/LEF1 signaling in prostate cancer. *Cancer Res.* **73**, 6068–6079 (2013).
80. Kedage, V. et al. An interaction with Ewing's sarcoma breakpoint protein EWS defines a specific oncogenic mechanism of ETS factors rearranged in prostate cancer. *Cell Rep.* **17**, 1289–1301 (2016).
81. Lucas, J. M. et al. The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. *Cancer Discov.* **4**, 1310–1325 (2014).
82. Tian, T. V. et al. Identification of novel TMPRSS2: ERG mechanisms in prostate cancer metastasis: involvement of MMP9 and PLXNA2. *Oncogene* **33**, 2204–2214 (2014).
83. St. John, J., Powell, K., Conley-LaComb, M. K. & Chinni, S. R. TMPRSS2-ERG fusion gene expression in prostate tumor cells and its clinical and biological significance in prostate cancer progression. *J. Cancer Sci. Ther.* **4**, 94–101 (2012).
84. Hu, Y. et al. Delineation of TMPRSS2-ERG splice variants in prostate cancer. *Clin. Cancer Res.* **14**, 4719–4725 (2008).
85. Mosquera, J. M. et al. Characterization of TMPRSS2-ERG fusion high-grade prostatic intraepithelial neoplasia and potential clinical implications. *Clin. Cancer Res.* **14**, 3380–3385 (2008).
86. Attard, G. et al. Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer. *Cancer Res.* **69**, 2912–2918 (2009).
87. Wang, X. J. et al. Development of peptidomimetic inhibitors of the ERG gene fusion product in prostate cancer. *Cancer Cell* **31**, 532–548 (2017).
88. Chatterjee, P. et al. The TMPRSS2-ERG gene fusion blocks XRCC4-mediated nonhomologous end-joining repair and radiosensitizes prostate cancer cells to parp inhibition. *Mol. Cancer Ther.* **14**, 1896–1906 (2015).
89. Furusato, B. et al. ERG oncoprotein expression in prostate cancer: clonal progression of ERG-positive tumor cells and potential for ERG-based stratification. *Prostate Cancer Prostatic Dis.* **13**, 228–237 (2010).
90. White, N. M., Feng, F. Y. & Maher, C. A. Recurrent rearrangements in prostate cancer: causes and therapeutic potential. *Curr. Drug Targets* **14**, 450–459 (2013).
91. Hessels, D. & Schalken, J. A. Recurrent gene fusions in prostate cancer: their clinical implications and uses. *Curr. Urol. Rep.* **14**, 214–222 (2013).
92. Kissick, H. T., Sanda, M. G., Dunn, L. K. & Arredouani, M. S. Development of a peptide-based vaccine targeting TMPRSS2:ERG fusion-positive prostate cancer. *Cancer Immunol. Immunother.* **62**, 1831–1840 (2013).
93. Sanguedolce, F. et al. Urine TMPRSS2: ERG fusion transcript as a biomarker for prostate cancer: literature review. *Clin. Genitourin. Cancer* **14**, 117–121 (2016).
94. Wang, J. H., Cai, Y., Ren, C. X. & Ittmann, M. Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. *Cancer Res.* **66**, 8347–8351 (2006).
95. Wang, J. H. et al. Pleiotropic biological activities of alternatively spliced TMPRSS2/ERG fusion gene transcripts. *Cancer Res.* **68**, 8516–8524 (2008).
96. Park, K. et al. TMPRSS2:ERG gene fusion predicts subsequent detection of prostate cancer in patients with high-grade prostatic intraepithelial neoplasia. *J. Clin. Oncol.* **32**, 206–211 (2014).
97. Culig, Z. TMPRSS:ERG fusion in prostate cancer: from experimental approaches to prognostic studies. *Eur. Urol.* **66**, 861–862 (2014).
98. Young, A. et al. Correlation of urine TMPRSS2:ERG and PCA3 to ERG plus and total prostate cancer burden. *Am. J. Clin. Pathol.* **138**, 685–696 (2012).
99. Tomlins, S. A. et al. Urine TMPRSS2:ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA. *Sci. Transl. Med.* **3**, 94ra72 (2011).
100. McKiernan, J. et al. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. *JAMA Oncol.* **2**, 882–889 (2016).
101. Day, J. R., Jost, M., Reynolds, M. A., Groskopf, J. & Rittenhouse, H. PCA3: From basic molecular science to the clinical lab. *Cancer Lett.* **301**, 1–6 (2011).
102. Ferreira, L. B. et al. PCA3 noncoding RNA is involved in the control of prostate-cancer cell survival and modulates androgen receptor signaling. *BMC Cancer* **12**, 507 (2012).
103. Bussemakers, M. J. G. et al. DD3: A new prostate-specific gene, highly overexpressed in prostate cancer. *Cancer Res.* **59**, 5975–5979 (1999).
104. Groskopf, J. et al. APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. *Clin. Chem.* **52**, 1089–1095 (2006).
105. Cui, Y. et al. Evaluation of prostate cancer antigen 3 for detecting prostate cancer: a systematic review and meta-analysis. *Sci. Rep.* **6**, 25776 (2016).
106. Lee, G. L., Dobi, A. & Srivastava, S. Diagnostic performance of the PCA3 urine test. *Nat. Rev. Urol.* **8**, 123–124 (2011).
107. Deras, I. L. et al. PCA3: a molecular urine assay for predicting prostate biopsy outcome. *J. Urol.* **179**, 1587–1592 (2008).
108. Marks, L. S. et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology* **69**, 532–535 (2007).
109. Tinzl, M., Marberger, M., Horvath, S. & Chypre, C. DD3PCA3 RNA analysis in urine—a new perspective for detecting prostate cancer. *Eur. Urol.* **46**, 182–186 (2004).
110. Salami, S. S. et al. Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. *Urol. Oncol.* **31**, 566–571 (2013).
111. Laxman, B. et al. A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. *Cancer Res.* **68**, 645–649 (2008).
112. Prensner, J. R. et al. The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. *Nat. Genet.* **45**, 1392–1398 (2013).
113. Prensner, J. R. et al. RNA biomarkers associated with metastatic progression in prostate cancer: a multi-institutional high-throughput analysis of SChLAP1. *Lancet Oncol.* **15**, 1469–1480 (2014).
114. Mehra, R. et al. Overexpression of the long non-coding RNA SChLAP1 independently predicts lethal prostate cancer. *Eur. Urol.* **70**, 549–552 (2016).
115. Chua, M. L. K. et al. A prostate cancer "nimbosus": genomic instability and schlap1 dysregulation underpin aggression of intraductal and cribriform subpathologies. *Eur. Urol.* **72**, 665–674 (2017).
116. Yin, C. Q. et al. Molecular profiling of pooled circulating tumor cells from prostate cancer patients using a dual-antibody-functionalized microfluidic device. *Anal. Chem.* **90**, 3744–3751 (2018).
117. Di Cristofano, A. & Pandolfi, P. P. The multiple roles of PTEN in tumor suppression. *Cell* **100**, 387–390 (2000).
118. Yoshimoto, M. et al. Interphase FISH analysis of PTEN in histologic sections shows genomic deletions in 68% of primary prostate cancer and 23% of high-grade prostatic intra-epithelial neoplasias. *Cancer Genet. Cytogenet.* **169**, 128–137 (2006).
119. Murphy, S. J. et al. Integrated analysis of the genomic instability of PTEN in clinically insignificant and significant prostate cancer. *Mod. Pathol.* **29**, 143–156 (2016).

120. Phin, S., Moore, M. W. & Cotter, P. D. Genomic rearrangements of PTEN in prostate cancer. *Front. Oncol.* **3**, 00240 (2013).
121. Lotan, T. L. et al. PTEN loss detection in prostate cancer: comparison of PTEN immunohistochemistry and PTEN FISH in a large retrospective prostatectomy cohort. *Oncotarget* **8**, 65566–65576 (2017).
122. Carnero, A., Blanco-Aparicio, C., Renner, O., Link, W. & Leal, J. F. M. The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. *Curr. Cancer Drug Targets* **8**, 187–198 (2008).
123. Krohn, A. et al. Genomic deletion of PTEN is associated with tumor progression and early PSA recurrence in ERG fusion-positive and fusion-negative prostate cancer. *Am. J. Pathol.* **181**, 401–412 (2012).
124. Yoshimoto, M. et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. *Mod. Pathol.* **21**, 1451–1460 (2008).
125. Han, B. et al. Fluorescence in situ hybridization study shows association of PTEN deletion with ERG rearrangement during prostate cancer progression. *Mod. Pathol.* **22**, 1083–1093 (2009).
126. Ahearn, T. U. et al. A prospective investigation of PTEN Loss and ERG expression in lethal prostate cancer. *J. Natl. Cancer Inst.* **108**, djv346 (2016).
127. King, J. C. et al. Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. *Nat. Genet.* **41**, 524–526 (2009).
128. Carver, B. S. et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. *Nat. Genet.* **41**, 619–624 (2009).
129. Punnoose, E. A. et al. PTEN loss in circulating tumour cells correlates with PTEN loss in fresh tumour tissue from castration-resistant prostate cancer patients. *Br. J. Cancer* **113**, 1225–1233 (2015).
130. Haile, S. & Sadar, M. D. Androgen receptor and its splice variants in prostate cancer. *Cell. Mol. Life Sci.* **68**, 3971–3981 (2011).
131. Hu, R. et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res.* **69**, 16–22 (2009).
132. Hornberg, E. et al. Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival. *PLOS ONE* **6**, e19059 (2011).
133. Ware, K. E., Garcia-Blanco, M. A., Armstrong, A. J. & Dehm, S. M. Biologic and clinical significance of androgen receptor variants in castration resistant prostate cancer. *Endocr. Relat. Cancer* **21**, T87–T103 (2014).
134. Dehm, S. M. & Tindall, D. J. Alternatively spliced androgen receptor variants. *Endocr. Relat. Cancer* **18**, R183–R196 (2011).
135. Sun, S. H. et al. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. *J. Clin. Invest.* **120**, 2715–2730 (2010).
136. Watson, P. A. et al. Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. *Proc. Natl Acad. Sci. USA* **107**, 16759–16765 (2010).
137. Liu, L. L. et al. Mechanisms of the androgen receptor splicing in prostate cancer cells. *Oncogene* **33**, 3140–3150 (2014).
138. Guo, Z. Y. et al. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. *Cancer Res.* **69**, 2305–2313 (2009).
139. Antonarakis, E. S. et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N. Engl. J. Med.* **371**, 1028–1038 (2014).
140. Scher, H. I. et al. Assessment of the validity of nuclear-localized androgen receptor splice variant 7 in circulating tumor cells as a predictive biomarker for castration-resistant prostate cancer. *JAMA Oncol.* **4**, 1179–1186 (2018).
141. Azad, A. A. et al. Androgen receptor gene aberrations in circulating cell-free dna: biomarkers of therapeutic resistance in castration-resistant prostate cancer. *Clin. Cancer Res.* **21**, 2315–2324 (2015).
142. Salvi, S. et al. Circulating cell-free AR and CYP17A1 copy number variations may associate with outcome of metastatic castration-resistant prostate cancer patients treated with abiraterone. *Br. J. Cancer* **112**, 1717–1724 (2015).
143. Wyatt, A. W. et al. Genomic alterations in cell-free DNA and enzalutamide resistance in castration-resistant prostate cancer. *JAMA Oncol.* **2**, 1598–1606 (2016).
144. Smith, M. R. et al. Apalutamide treatment and metastasis-free survival in prostate cancer. *N. Engl. J. Med.* **378**, 1408–1418 (2018).
145. Hussain, M. et al. Enzalutamide in men with nonmetastatic, castration-resistant prostate cancer. *N. Engl. J. Med.* **378**, 2465–2474 (2018).
146. James, N. D. et al. Abiraterone for prostate cancer not previously treated with hormone therapy. *N. Engl. J. Med.* **377**, 338–351 (2017).
147. Fizazi, K. et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. *N. Engl. J. Med.* **377**, 352–360 (2017).
148. Van Neste, L. et al. The epigenetic promise for prostate cancer diagnosis. *Prostate* **72**, 1248–1261 (2012).
149. Xu, K. et al. EZH2 oncogenic activity in castration-resistant prostate cancer cells is polycomb-independent. *Science* **338**, 1465–1469 (2012).
150. Tomlins, S. A. et al. The role of SPINK1 in ETS rearrangement-negative prostate cancers. *Cancer Cell* **13**, 519–528 (2008).
151. Rubin, M. A. et al. α-Methylacyl coenzyme a racemase as a tissue biomarker for prostate cancer. *JAMA* **287**, 1662–1670 (2002).
152. Meany, D. L., Zhang, Z., Sokoll, L. J., Zhang, H. & Chan, D. W. Glycoproteomics for prostate cancer detection: changes in serum PSA glycosylation patterns. *J. Proteome Res.* **8**, 613–619 (2009).
153. Sartori, D. A. & Chan, D. W. Biomarkers in prostate cancer: what's new? *Curr. Opin. Oncol.* **26**, 259–264 (2014).
154. Feng, F., Schaich, M. & Hughes, L. Current and future applications of genetic prostate cancer screening in the urologic clinic. *Urol. Nurs.* **34**, 281–289 (2014).
155. Catalona, W. J. et al. A multicenter study of -2 pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. *J. Urol.* **185**, 1650–1655 (2011).
156. Loeb, S. et al. The prostate health index selectively identifies clinically significant prostate cancer. *J. Urol.* **193**, 1163–1169 (2015).
157. de la Calle, C. et al. Multicenter evaluation of the prostate health index to detect aggressive prostate cancer in biopsy naive men. *J. Urol.* **194**, 65–72 (2015).
158. Vickers, A. J. et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European randomized study of prostate cancer screening in Göteborg, Sweden. *BMC Med.* **6**, 19 (2008).
159. Parekh, D. J. et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. *Eur. Urol.* **68**, 464–470 (2015).
160. Braun, K., Sjoberg, D. D., Vickers, A. J., Lilja, H. & Bjartell, A. S. A four-kallikrein panel predicts high-grade cancer on biopsy: independent validation in a community cohort. *Eur. Urol.* **69**, 505–511 (2016).
161. Vickers, A. et al. Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication. *J. Clin. Oncol.* **28**, 2493–2498 (2010).
162. Henrique, R. et al. Epigenetic heterogeneity of high-grade prostatic intraepithelial neoplasia: clues for clonal progression in prostate carcinogenesis. *Mol. Cancer Res.* **4**, 1–8 (2006).
163. Zhou, M., Tokumaru, Y., Sidransky, D. & Epstein, J. I. Quantitative GSTP1 methylation levels correlate with Gleason grade and tumor volume in prostate needle biopsies. *J. Urol.* **171**, 2195–2198 (2004).
164. Trujillo, K. A., Jones, A. C., Griffith, J. K. & Bisoffi, M. Markers of field cancerization: proposed clinical applications in prostate biopsies. *Prostate Cancer* **2012**, 12 (2012).
165. Slaughter, D. P., Southwick, H. W. & Smejkal, W. "Field cancerization" in oral stratified squamous epithelium. Clinical implications of multicentric origin. *Cancer* **6**, 963–968 (1953).
166. Braakhuis, B. J. M., Tabor, M. P., Kummer, J. A., Leemans, C. R. & Brakenhoff, R. H. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res.* **63**, 1727–1730 (2003).
167. Mehrotra, J. et al. Quantitative, spatial resolution of the epigenetic field effect in prostate cancer. *Prostate* **68**, 152–160 (2008).
168. Djavan, B., Remzi, M., Schulman, C. C., Marberger, M. & Zlotta, A. R. Repeat prostate biopsy: who, how and when?: a review. *Eur. Urol.* 42, (93–103 (2002).
169. Partin, A. W. et al. Clinical validation of an epigenetic assay to predict negative histopathological results in repeat prostate biopsies. *J. Urol.* **192**, 1081–1087 (2014).
170. Van Neste, L. et al. Risk score predicts high-grade prostate cancer in DNA-methylation positive, histopathologically negative biopsies. *Prostate* **76**, 1078–1087 (2016).
171. Stewart, G. D. et al. Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies: results of the MATLOC study. *J. Urol.* **189**, 1110–1116 (2013).
172. Wojno, K. J. et al. Reduced rate of repeated prostate biopsies observed in ConfirmMDx clinical utility field study. *Am. Health Drug Benefits* **7**, 129–134 (2014).
173. Dall'Era, M. A. et al. Utility of the Oncotype DX® prostate cancer assay in clinical practice for treatment selection in men newly diagnosed with prostate cancer: a retrospective chart review analysis. *Urol. Pract.* **2**, 343–348 (2015).
174. Cullen, J. 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. *Eur. Urol.* **68**, 123–131 (2015).
175. Klein, E. A. et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. *Eur. Urol.* **66**, 550–560 (2014).
176. Canfield, S. E. et al. A guide for clinicians in the evaluation of emerging molecular diagnostics for newly diagnosed prostate cancer. *Rev. Urol.* **16**, 172–180 (2014).
177. Cuzick, J. et al. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. *Lancet Oncol.* **12**, 245–255 (2011).
178. Cuzick, J. et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. *Br. J. Cancer* **106**, 1095–1099 (2012).
179. Whitfield, M. L. et al. Identification of genes periodically expressed in the human cell cycle and their expression in tumors. *Mol. Biol. Cell* **13**, 1977–2000 (2002).
180. Tosoian, J. J. et al. Prognostic utility of biopsy-derived cell cycle progression score in patients with national comprehensive cancer network low-risk prostate cancer undergoing radical prostatectomy: implications for treatment guidance. *BJU Int.* **120**, 808–814 (2017).
181. Marrone, M., Potosky, A. L., Penson, D. & Freedman, A. N. A 22 gene-expression assay, Decipher(R) (GenomeDx Biosciences) to predict five-year risk of metastatic prostate cancer in men treated with radical prostatectomy. *PLOS Curr.* https://doi.org/10.1371/currents.eogt.761b81608129ed61b0b48d42c04f92a4 (2015).
182. Spratt, D. E. et al. Individual patient-level meta-analysis of the performance of the decipher genomic classifier in high-risk men after prostatectomy to predict development of metastatic disease. *J. Clin. Oncol.* **35**, 1991–1998 (2017).
183. Karnes, R. J. et al. Validation of a genomic risk classifier to predict prostate cancer-specific mortality in men with adverse pathologic features. *Eur. Urol.* **73**, 168–175 (2018).
184. Klein, E. A. et al. Decipher genomic classifier measured on prostate biopsy predicts metastasis risk. *Urology* **90**, 148–152 (2016).
185. Klein, E. A. et al. A genomic classifier improves prediction of metastatic disease within 5 years after surgery in node-negative high-risk prostate cancer patients managed by radical prostatectomy without adjuvant therapy. *Eur. Urol.* **67**, 778–786 (2015).
186. Badani, K. K. et al. Effect of a genomic classifier test on clinical practice decisions for patients with high-risk prostate cancer after surgery. *BJU Int.* **115**, 419–429 (2015).
187. Badani, K. 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* **4**, 600–609 (2013).

188. Haese, A. et al. Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. *Eur. Urol.* **54**, 1081–1088 (2008).
189. Chun, F. K. et al. Prostate cancer gene 3 (PCA3): development and internal validation of a novel biopsy nomogram. *Eur. Urol.* **56**, 659–667 (2009).
190. Ploussard, G. et al. Prostate cancer antigen 3 score accurately predicts tumour volume and might help in selecting prostate cancer patients for active surveillance. *Eur. Urol.* **59**, 422–429 (2011).
191. Roobol, M. J. et al. Performance of the prostate cancer antigen 3 (PCA3) gene and prostate-specific antigen in prescreened men: exploring the value of PCA3 for a first-line diagnostic test. *Eur. Urol.* **58**, 475–481 (2010).
192. Gittelman, M. C. et al. PCA3 molecular urine test as a predictor of repeat prostate biopsy outcome in men with previous negative biopsies: a prospective multicenter clinical study. *J. Urol.* **190**, 64–69 (2013).
193. Wei, J. T. et al. Can urinary PCA3 supplement PSA in the early detection of prostate cancer? *J. Clin. Oncol.* **32**, 4066–4072 (2014).
194. Hessels, D. & Schalken, J. A. The use of PCA3 in the diagnosis of prostate cancer. *Nat. Rev. Urol.* **6**, 255–261 (2009).
195. Tomlins, S. A. et al. Urine TMPRSS2:ERG plus PCA3 for individualized prostate cancer risk assessment. *Eur. Urol.* **70**, 45–53 (2016).
196. Merdan, S. et al. Assessment of long-term outcomes associated with urinary prostate cancer antigen 3 and TMPRSS2:ERG gene fusion at repeat biopsy. *Cancer* **121**, 4071–4079 (2015).
197. Sanda, M. G. et al. Association between combined TMPRSS2:ERG and PCA3 RNA urinary testing and detection of aggressive prostate cancer. *JAMA Oncol.* **3**, 1083–1093 (2017).
198. Leyten, G. et al. Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. *Eur. Urol.* **65**, 534–542 (2014).
199. Leyten, G. et al. Identification of a candidate gene panel for the early diagnosis of prostate cancer. *Clin. Cancer Res.* **21**, 3061–3070 (2015).
200. Van Neste, L. et al. Detection of high-grade prostate cancer using a urinary molecular biomarker-based risk score. *Eur. Urol.* **70**, 740–748 (2016).
201. Duijvesz, D., Luider, T., Bangma, C. H. & Jenster, G. Exosomes as biomarker treasure chests for prostate cancer. *Eur. Urol.* **59**, 823–831 (2011).
202. Nilsson, J. et al. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. *Br. J. Cancer* **100**, 1603–1607 (2009).
203. Overbye, A. et al. Identification of prostate cancer biomarkers in urinary exosomes. *Oncotarget* **6**, 30357–30376 (2015).
204. Futterer, J. J. et al. Can clinically significant prostate cancer be detected with multiparametric magnetic resonance imaging? a systematic review of the literature. *Eur. Urol.* **68**, 1045–1053 (2015).
205. Mehralivand, S. et al. A magnetic resonance imaging-based prediction model for prostate biopsy risk stratification. *JAMA Oncol.* **4**, 678–685 (2018).
206. Ahmed, H. U. et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. *Lancet* **389**, 815–822 (2017).
207. Pokorny, M. R. et al. Prospective study of diagnostic accuracy comparing prostate cancer detection by transrectal ultrasound-guided biopsy versus magnetic resonance (MR) imaging with subsequent MR-guided biopsy in men without previous prostate biopsies. *Eur. Urol.* **66**, 22–29 (2014).
208. Moore, C. M. et al. Image-guided prostate biopsy using magnetic resonance imaging-derived targets: a systematic review. *Eur. Urol.* **63**, 125–140 (2013).
209. Siddiqui, M. M. et al. Comparison of MR/ultrasound fusion-guided biopsy with ultrasound-guided biopsy for the diagnosis of prostate Cancer. *JAMA* **313**, 390–397 (2015).
210. Kasivisvanathan, V. et al. MRI-targeted or standard biopsy for prostate-cancer diagnosis. *N. Engl. J. Med.* **378**, 1767–1777 (2018).
211. Ferrari, M. Cancer nanotechnology: opportunities and challenges. *Nat. Rev. Cancer* **5**, 161–171 (2005).
212. Nie, S. M., Xing, Y., Kim, G. J. & Simons, J. W. Nanotechnology applications in cancer. *Annu. Rev. Biomed. Eng.* **9**, 257–288 (2007).
This paper gives a broad introductory review of various nanotechnologies for cancer applications.
213. Smith, S. J., Nemr, C. R. & Kelley, S. O. Chemistry-driven approaches for ultrasensitive nucleic acid detection. *J. Am. Chem. Soc.* **139**, 1020–1028 (2017).
214. Barenholz, Y. Doxil® — the first FDA-approved nano-drug: lessons learned. *J. Control. Release* **160**, 117–134 (2012).
215. Kang, B. J. et al. Diagnosis of prostate cancer via nanotechnological approach. *Int. J. Nanomed.* **10**, 6555–6569 (2015).
216. Wu, G. H. et al. Bioassay of prostate-specific antigen (PSA) using microcantilevers. *Nat. Biotechnol.* **19**, 856–860 (2001).
This paper details the pioneering use of nanotechnology to enhance PSA biomarker detection performance.
217. Zheng, G. F., Patolsky, F., Cui, Y., Wang, W. U. & Lieber, C. M. Multiplexed electrical detection of cancer markers with nanowire sensor arrays. *Nat. Biotechnol.* **23**, 1294–1301 (2005).
218. Yu, X. et al. Carbon nanotube amplification strategies for highly sensitive immunodetection of cancer biomarkers. *J. Am. Chem. Soc.* **128**, 11199–11205 (2006).
219. Xu, S. J., Liu, Y., Wang, T. H. & Li, J. H. Positive potential operation of a cathodic electrogenerated chemiluminescence immunosensor based on luminol and graphene for cancer biomarker detection. *Anal. Chem.* **83**, 3817–3823 (2011).
220. Tilki, D., Kim, S. I., Hu, B., Dall'Era, M. A. & Evans, C. P. Ultrasensitive prostate specific antigen and its role after radical prostatectomy: a systematic review. *J. Urol.* **193**, 1525–1531 (2015).
221. Grubisha, D. S., Lipert, R. J., Park, H. Y., Driskell, J. & Porter, M. D. Femtomolar detection of prostate-specific antigen: an immunoassay based on surface-enhanced Raman scattering and immunogold labels. *Anal. Chem.* **75**, 5936–5943 (2003).
222. Mani, V., Chikkaveeraiah, B. V., Patel, V., Gutkind, J. S. & Rusling, J. F. Ultrasensitive immunosensor for cancer biomarker proteins using gold nanoparticle film electrodes and multienzyme-particle amplification. *ACS Nano* **3**, 585–594 (2009).
223. Liu, X. et al. A one-step homogeneous immunoassay for cancer biomarker detection using gold nanoparticle probes coupled with dynamic light scattering. *J. Am. Chem. Soc.* **130**, 2780–2782 (2008).
224. Wang, J., Koo, K. M., Wang, Y. & Trau, M. "Mix-to-go" silver colloidal strategy for prostate cancer molecular profiling and risk prediction. *Anal. Chem.* **90**, 12698–12705 (2018).
225. Gupta, A. K., Naregalkar, R. R., Vaidya, V. D. & Gupta, M. Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications. *Nanomedicine (Lond.)* **2**, 23–39 (2007).
226. Sarkar, P., Ghosh, D., Bhattacharyay, D., Setford, S. J. & Turner, A. P. F. Electrochemical immunoassay for free prostate specific antigen (f-PSA) using magnetic beads. *Electroanalysis* **20**, 1414–1420 (2008).
227. Song, E. Q. et al. Fluorescent-magnetic-biotargeting multifunctional nanobioprobes for detecting and isolating multiple types of tumor cells. *ACS Nano* **5**, 761–770 (2011).
228. Koo, K. M., Carrascosa, L. G., Shiddiky, M. J. A. & Trau, M. Poly(A) extensions of miRNAs for amplification-free electrochemical detection on screen-printed gold electrodes. *Anal. Chem.* **88**, 2000–2005 (2016).
229. Koo, K. M., Carrascosa, L. G., Shiddiky, M. J. A. & Trau, M. Amplification-free detection of gene fusions in prostate cancer urinary samples using mRNA-gold affinity interactions. *Anal. Chem.* **88**, 6781–6788 (2016).
230. Koo, K. M., Carrascosa, L. G. & Trau, M. DNA-directed assembly of copper nanoblocks with inbuilt fluorescent and electrochemical properties: application in simultaneous amplification-free analysis of multiple RNA species. *Nano Res.* **11**, 940–952 (2018).
231. Koo, K. M., Wee, E. J. H. & Trau, M. Colorimetric TMPRSS2-ERG gene fusion detection in prostate cancer urinary samples via recombinase polymerase amplification. *Theranostics* **6**, 1415–1424 (2016).
232. Koo, K. M., Dey, S. & Trau, M. Amplification-free multi-RNA-type profiling for cancer risk stratification via alternating current electrohydrodynamic nanomixing. *Small* **14**, 1704025 (2018).
This paper describes a nanodevice for amplification-free detection of multiple types of prostate cancer RNA biomarkers.
233. Koo, K. M., Wee, E. J. H., Mainwaring, P. N. & Trau, M. A simple, rapid, low-cost technique for naked-eye detection of urine-isolated TMPRSS2: ERG gene fusion RNA. *Sci. Rep.* **6**, 30722 (2016).
This paper describes a miniaturized solution-based technique for visual detection of urinary *TMPRSS2–ERG* status.
234. Koo, K. M., Dey, S. & Trau, M. A sample-to-targeted gene analysis biochip for nanofluidic manipulation of solid-phase circulating tumor nucleic acid amplification in liquid biopsies. *ACS Sens.* **3**, 2597–2603 (2018).
This paper details an integrated biochip platform for complex liquid biopsy sample-to-targeted genetic analysis.
235. Brus, L. Quantum crystallites and nonlinear optics. *Appl. Phys. A* **53**, 465–474 (1991).
236. Chinen, A. B. et al. Nanoparticle probes for the detection of cancer biomarkers, cells, and tissues by fluorescence. *Chem. Rev.* **115**, 10530–10574 (2015).
237. Li, X. et al. Rapid and quantitative detection of prostate specific antigen with a quantum dot nanobeads-based immunochromatography test strip. *ACS Appl. Mater. Interfaces* **6**, 6406–6414 (2014).
238. Zhang, W., Hubbard, A., Brunhoeber, P., Wang, Y. & Tang, L. Automated multiplexing quantum dots in situ hybridization assay for simultaneous detection of ERG and PTEN gene status in prostate cancer. *J. Mol. Diagn.* **15**, 754–764 (2013).
239. Fleischmann, M., Hendra, P. J. & McQuillan, A. J. Raman spectra of pyridine adsorbed at a silver electrode. *Chem. Phys. Lett.* **26**, 163–166 (1974).
240. Koo, K. M., Wee, E. J. H., Wang, Y. & Trau, M. Enabling miniaturised personalised diagnostics: from lab-on-a-chip to lab-in-a-drop. *Lab. Chip* **17**, 3200–3220 (2017).
This paper provides an introduction to the concept of miniaturized 'lab-in-a-drop' systems.
241. Zong, C. et al. Surface-enhanced Raman spectroscopy for bioanalysis: reliability and challenges. *Chem. Rev.* **118**, 4946–4980 (2018).
242. Cheng, Z. et al. Simultaneous detection of dual prostate specific antigens using surface-enhanced raman scattering-based immunoassay for accurate diagnosis of prostate cancer. *ACS Nano* **11**, 4926–4933 (2017).
This paper details the duplexed evaluation of free to total PSA ratio in clinical serum samples.
243. Koo, K. M., McNamara, B., Wee, E. J. H., Wang, Y. & Trau, M. Rapid and sensitive fusion gene detection in prostate cancer urinary specimens by label-free surface-enhanced Raman scattering. *J. Biomed. Nanotechnol.* **12**, 1798–1805 (2016).
244. Koo, K. M., Wee, E. J. H., Mainwaring, P. N., Wang, Y. & Trau, M. Toward precision medicine: a cancer molecular subtyping nano-strategy for RNA biomarkers in tumor and urine. *Small* **12**, 6233–6242 (2016).
This paper details the pentaplexed detection of next-generation prostate cancer RNA biomarkers in clinical patient samples.
245. Wang, J., Koo, K. M., Wee, E. J. H., Wang, Y. L. & Trau, M. A nanoplasmonic label-free surface-enhanced Raman scattering strategy for non-invasive cancer genetic subtyping in patient samples. *Nanoscale* **9**, 3496–3503 (2017).
246. Mir-Simon, B., Reche-Perez, I., Guerrini, L., Pazos-Perez, N. & Alvarez-Puebla, R. A. Universal one-pot and scalable synthesis of SERS encoded nanoparticles. *Chem. Mater.* **27**, 950–958 (2015).
247. Kim, H. M. et al. Large scale synthesis of surface-enhanced Raman scattering nanoprobes with high reproducibility and long-term stability. *J. Ind. Eng. Chem.* **33**, 22–27 (2016).
248. Whitesides, G. M. The origins and the future of microfluidics. *Nature* **442**, 368 (2006).
249. Chin, C. D., Linder, V. & Sia, S. K. Commercialization of microfluidic point-of-care diagnostic devices. *Lab. Chip* **12**, 2118–2134 (2012).
250. Armenia, J. et al. The long tail of oncogenic drivers in prostate cancer. *Nat. Genet.* **50**, 645–651 (2018).
251. Brooks, J. D. Translational genomics: the challenge of developing cancer biomarkers. *Genome Res.* **22**, 183–187 (2012).
252. Yamoah, K. et al. Novel biomarker signature that may predict aggressive disease in African American men with prostate cancer. *J. Clin. Oncol.* **33**, 2789–2796 (2015).
253. Simon, N. & Simon, R. Adaptive enrichment designs for clinical trials. *Biostatistics* **14**, 613–625 (2013).
254. Simon, R. & Simon, N. Inference for multimarker adaptive enrichment trials. *Stat. Med.* **36**, 4083–4093 (2017).

255. Koo, K. M. et al. Design and clinical verification of surface enhanced Raman spectroscopy diagnostic technology for individual cancer risk prediction. *ACS Nano* **12**, 8362–8371 (2018).
This is the first comprehensive clinical evaluation of a prostate cancer nanodiagnostic technology.
256. Hessels, D. et al. Analytical validation of an mRNA-based urine test to predict the presence of high-grade prostate cancer. *Transl Med. Commun.* **2**, 5 (2017).

Acknowledgements
K.M.K. acknowledges support from the Australian Government Research Training Program Scholarship and the University of Queensland Graduate School International Travel Award. Although not directly funding this work, K.M.K., P.N.M. and M.T. acknowledge funding from the National Breast Cancer Foundation of Australia (CG 1207), Australian Research Council (DP 140104006 and DP 160102836) and Royal Brisbane Women's Hospital Foundation (2018 Research Project Grant). These grants have considerably contributed to the environment that stimulates this work. Prostate cancer research in the laboratory of S.A.T. is supported by the US National Institutes of Health, the US Department of Defense and the Prostate Cancer Foundation. S.A.T. is supported by the A. Alfred Taubman Medical Research Institute. The authors thank all patients for their consent, which progresses the research developments mentioned herein.

Author contributions
K.M.K. researched data for and wrote the manuscript. All authors made substantial contributions to discussion of content and reviewed and edited the manuscript before submission.

Competing interests
The University of Michigan has been issued a patent on *ETS* gene fusions in prostate cancer on which S.A.T. is a coinventor. The diagnostic field of use has been licensed to Hologic (which acquired Gen-Probe), which has sublicensed rights to Roche (which owns Ventana Medical Systems). S.A.T. has received travel support from, and had a sponsored research agreement with, Compendia Bioscience (which was acquired by Life Technologies, which was acquired by ThermoFisher Scientific). S.A.T. has sponsored research agreements with Astellas and GenomeDx. S.A.T. has served as a consultant for and received honoraria from Roche, Ventana Medical Systems, Almac Diagnostics, Janssen, AbbVie, Sanofi and Astellas (which acquired Medivation). S.A.T. is a cofounder of, consultant for and the Laboratory Director of Strata Oncology. P.N.M. and M.T. are co-founders of XING Technologies, which has licensed intellectual property from the University of Queensland, Australia. P.N.M. is a shareholder of XING Technologies. P.N.M. has served as a consultant, advisory board member and lecturer for and received honoraria, travel support and grant support from Astellas, Bristol-Myers Squibb, Ipsen, Janssen, Medivation, Merck, Novartis, Pfizer and Genentech (a subsidiary of Roche). K.M.K. declares no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Reviewer information
Nature Reviews Urology thanks T. van der Kwast and other anonymous reviewer(s) for their contribution to the peer review of this work.