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Next generation biomarkers in prostate cancer

Jennifer A. Locke, Peter C. Black

Vancouver Prostate Centre and Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

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1. ABSTRACT

A wide spectrum of non-protein based biomarkers are under development that promises to revolutionize the care of prostate cancer (CaP) patients. In the context of CaP detection we highlight the potential value of the urine tests PCA3 and Prostarix^{TM}, especially for their ability to stratify patient risk with previous negative biopsy for occult cancer. The search for such markers is made more complex by the development of MRI and image-fusion technology that can help focus biopsy on specific prostatic lesions. Tissue-gene signatures are finding utility in predicting recurrence and progression after radical prostatectomy or identifying patients with apparent low-risk disease who may harbor occult higher-risk disease that would warrant definitive intervention over active surveillance. Furthermore, serum-based microRNA, cell-free DNA and circulating tumor cells are under investigation in clinical trials, especially in the setting of metastatic castration-resistant CaP, for their ability to predict response to novel therapies and patient survival. The meticulous testing of these biomarkers by incorporation into current clinical trials will aid in their widespread use and ability to guide CaP management.

2. INTRODUCTION

Despite evidence of benefit in large prospective randomized clinical trials such as the European Randomized Study of Screening for Prostate Cancer, screening for prostate cancer (CaP) with prostate specific antigen (PSA) remains controversial (1-3). PSA does, however, have an established role in monitoring CaP progression after diagnosis as well as in monitoring response to treatment (4). In general, from diagnosis of CaP to management of castration-resistant prostate cancer (CRPC), there are few other biomarkers used routinely by physicians.

Prostate cancer risk stratification after primary diagnosis is currently based on serum PSA, clinical staging, Gleason score and the extend of disease on prostate biopsy. In combination these properties determine initial management of the disease, whether it be active surveillance, single modality surgery or radiation, or multimodal therapy (5). Unfortunately, many patients are currently over-treated or under-treated based on these properties. This is because multiple factors limit the prognostic and predictive capacity of these parameters, including the innate heterogeneity of the disease, the multifocality of CaP and the incomplete sampling of the cancer with current biopsy techniques.

In the context of advanced CaP we have seen a veritable explosion in the number of new drugs approved for clinical use over the past several years. Biomarkers are essential to tailor therapy to the individual patient and to enable prediction of response to therapy. Advanced CRPC poses particular challenges because tissue is rarely available for molecular interrogation, and the biology of the disease is vastly different than it was at the time of diagnostic biopsy prior to therapy. Non-invasive

Table 1. Areas of need: biomarkers in the management of prostate cancer

How do we distinguish aggressive disease from indolent disease in patients on active surveillance ?
How do we identify patients who will benefit from adjuvant radiation therapy?
How do we effectively select drugs for patients with castration-resistant prostate cancer in the context of several recently approved new agents?

markers to guide therapy of men with CRPC are a particularly important focus of ongoing research.

Here we highlight some important aspects of next generation biomarkers under development that promise to enhance the future landscape of personalized care of CaP. This is not intended as a comprehensive review of all available biomarkers, but instead highlights new technologies that have demonstrated clinical utility in multiple cohorts.

3. CLINICAL SCENARIOS

The clinical utility of next generation biomarkers will be dependent on how well these markers address clinical questions in specific clinical scenarios. In Table 1 we highlight some of the most pertinent unmet clinical needs requiring novel biomarkers.

3.1. How do we distinguish aggressive disease from indolent disease in patients on active surveillance?

Currently in 25-40% of patients who are deemed eligible for active surveillance the biopsy pathology underestimates tumor grade or extent, resulting in treatment delay and potentially decreased chance of cure (6,7). The use of multiple repeat biopsies in active surveillance protocols presents significant morbidity for patients. In fact, 6.7.% of low risk patients develop sepsis after biopsy and one-third of patients require self-medicating analgesia (8,9). New biomarkers, particularly from urine and blood, may benefit active surveillance patients by identifying aggressive disease from indolent disease at the time of initial diagnosis, thereby reducing biopsy-associated morbidity and allowing those with truly low risk disease to undergo surveillance with fewer biopsies while those with more aggressive disease seek early curative treatment.

3.2. How do we identify patients who will benefit from adjuvant radiation therapy?

In patients with high-risk, lymph node negative disease, especially in those with adverse pathologic findings at radical prostatectomy (e.g. seminal vesicle invasion, positive surgical margins, extraprostatic extension), adjuvant radiation therapy reduces the risk of biochemical recurrence (10-12). The SWOG trial on adjuvant radiation was the only trial to demonstrate an improvement in metastasis-free and overall survival, and it has been criticized for underutilizing early salvage radiation (13,14). Thompson *et al.* demonstrated the number needed to treat with radiation to prevent one case of metastatic disease at a median follow-up of 12.6. years was 12.2, which is considered relatively unfavorable, especially when the potential adverse effects of radiation are considered (13). Acute and late toxicities include genitourinary effects (e.g. urinary incontinence and sexual dysfunction), gastrointestinal effects, and secondary cancers (15,16). With the controversy surrounding the benefit of adjuvant radiation, and the relatively high number needed to treat, it is crucial that novel biomarkers be identified and validated to select those who will benefit from adjuvant radiation therapy from those who will not. Also, novel biomarkers may offer prediction of those patients who may develop specific side effects of adjuvant radiation therapy (17,18).

3.3. How do we effectively select drugs for patients with castration-resistant prostate cancer in the context of several recently approved new agents?

The management of CRPC is evolving rapidly, and patients experiencing progression on androgen deprivation therapy have several new therapeutic options including abiraterone, enzalutamide, cabazitaxel, Radium-223 and sipuleucel-T (19-23). Several other agents are being investigated in this population of patients, and optimal drug sequencing and combination is becoming a major challenge. In this climate of evolving therapy, new biomarkers will be essential to identify those patients who will have a survival benefit from specific drugs or drug combinations.

4. TISSUE-BASED GENE SIGNATURES

Several gene signatures reflective of the underlying biology of CaP progression are being developed in biopsy material and radical prostatectomy specimens (Table 2). Each offers unique value to risk stratification that improves upon established clinical and pathologic risk parameters. An important technical breakthrough that has enabled the development of clinical grade gene signature tests is the ability to analyze RNA expression on routine, archived, formalin-fixed, paraffin-embedded tissue samples (24). Further advances have included the ability to conduct such analysis on the small amounts of tissue available from prostate biopsy specimens.

The Prolaris Score™ from Myriad and the OncotypeDx™ Genomic Prostate Score have been developed to use prostate biopsy tissue to predict

Table 2. Tissue-based gene signatures

Test	Definition	Description	Clinical Evidence
Myriad Genetics Prolaris Score™	46-gene RNA expression signature in formalin-fixed paraffin-embedded (FFPE) biopsy specimens (RT-PCR)	Cell cycle progression genes	1) Predictor of BCR after RP in 3 cohorts of CaP patients (n=582) (25,82,83) 2) Predictor of BCR after EBRT in 141 CaP patients (84) 3) Predictor of OS especially when combined CAPRA-S* score in 413 CaP patients (83)
Genome Health OncotypeDx™ Genomic Prostate Score (GPS)	22-gene RNA expression signature in FFPE biopsy specimens (RT-PCR)	Genes selected representing biological pathways with a known role in prostate tumorigenesis (androgen pathway, cellular organization, proliferation, and stromal response (85)	1) Discovery in 441 RP and 167 biopsy specimens, validation in 395 biopsy specimens from men with low and intermediate risk patients suitable for AS but undergoing RP (discovery study) (28). In the same population the addition of GPS was shown to reclassify many men stratified to high risk based on CAPRA-S≥6 alone. Patients with both high GPS and high CAPRA-S risk scores were at markedly elevated post-RP risk for lethal CaP (27)
GenomeDx Biosciences Decipher™	22-gene RNA expression signature in FFPE RP specimens (gene expression microarray)	Unbiased whole transcriptome expression analysis; includes 19 non-coding genes (30)	1) Predictor of early metastasis in 545 high-risk RP patients (discovery study) (30) 2) Predictor of early metastasis in 219 high-risk RP patients (case-cohort validation study), especially when combined with CAPRA-S score (33) 3) Predictor of early metastasis in 85 men with BCR after RP (32) 4) Predictor of BCR and metastasis in 139 high-risk RP patients post-adjuvant radiation (86) 5) Decipher™ results affect decision making with respect to post-RP adjuvant radiation clinical utility testing (87)
NF-kB–activated recurrence predictor 21 (NARP21)	21-gene signature in in RP specimens (RT-PCR)	Identified from a nonmalignant NF-kB activated androgen depleted transgenic mouse prostate model (29)	1) Predictor of metastasis-free and disease specific survival in previously publically archived dataset of 596 RP samples (29)
*CAPRA-S (Cancer of the Prostate Risk Assessment post-Surgical; RP (radical prostatectomy)			

adverse pathologic features that would make a patient inappropriate for active surveillance (25-27). A critical consideration for tissue markers in this disease context is undersampling of the prostate with the risk of missing a more threatening tumor. Both of the indicated genomic tests report evidence for a field effect in CaP that allows the tests to predict the presence of high-stage and grade disease even if the index lesion is not sampled (28).

Decipher™ from GenomeDx, the Prolaris Score™ and the NF-kB signature NARP21 have been designed to predict clinical outcome after radical prostatectomy. The NF-kB signature predicts for metastasis-free survival and disease-specific survival (29). The Prolaris Score predicts for metastasis and Decipher™ predicts for cancer-specific mortality (30,31). These tests have been developed by various investigators on large annotated tissue repositories from the Mayo Clinic. Decipher™ has been widely validated in multiple cohorts and has demonstrated some potential to influence the clinical decision to recommend post-radical prostatectomy adjuvant radiation (28,32-34). In particular this test may aid in identifying patients who are at very low risk of clinical progression and therefore would likely have marginal or no benefit from adjuvant therapy. However, it will require analysis in prospective clinical trials to determine if patients found to be at high risk of clinical progression would benefit from current adjuvant therapies (34).

All of these markers have been developed in retrospective studies. Optimal clinical validation to prove clinical utility will require prospective clinical trials. One step short of this “gold” standard would be retrospective testing in prospectively conducted clinical trials. Clinical implementation of these tests will likely only be successful if high-level evidence supports the use of the tests to address a specific clinical question. A major limitation of the tests will be cost as previously observed in breast cancer management (35).

5. BLOOD-BASED BIOMARKERS

Blood-based markers have the potential to overcome the inherent heterogeneity of CaP and capture the tumor in its entirety (Table 3). In this respect, blood-based markers could fulfill the goal of a “liquid

Table 3. Blood-based biomarkers

Test	Description	Clinical Evidence
microRNA	Small noncoding RNAs found in tissue and serum samples that are involved in post-transcriptional regulation of a large number of biological processes (38)	1) A signature of 2-3 differentially expressed mRNAs had a high positive predictive value for biochemical failure in 105 CaP patients at time of **RP** (43) 2) Serum miR-141 is associated with **higher GS** in 170 patients undergoing prostate **biopsy** (41) 3) Serum miR-16 levels is useful in **discriminating CaP from BPH** in 47 patients undergoing **biopsy** (39) 4) In **serum** higher miR levels are correlated with **CaP** in a series of patients (78 with CaP and 28 without CaP) (40) 5) **Serum** miR-375, miR-141 are significantly overexpressed in 30 **high risk localized CaP** patients and 26 **metastatic CRPC** patients (43) 6) miR-182 expression is associated with **biochemical and clinical progression** free survival in various samples (60 **RP**, 273 **biopsies**, and 92 **urine**) from CaP patients (88) 7) Serum mRNA signature is associated with docetaxel chemotherapy outcome in 97 CRPC patients (44)
RP (radical prostatectomy)		

Table 4. Urine-based biomarkers

Name	Definition	Clinical Evidence
PCA3	CaP-specific gene located on chromosome 9q21-22	1) PCA3 demonstrates a sensitivity of 69% and specificity of 79% for predicting CaP diagnosis in 143 men undergoing biopsy (91) 2) PCA3 levels are predictive of those patients who need a repeat biopsy in men who have elevated PSA and a prior negative biopsy (63) 3) PCA3 score is associated with higher volume CaP and high-grade disease in 387 men on an active surveillance protocol (65)
TMPRSS2-ERG	Gene fusion involving the 5' untranslated region of the androgen-regulated gene TMPRSS2 with ERG or ETV1	1) TMPRSS2-ERG detection has yielded a specificity of 93% and a positive predictive value of 94% in predicting a diagnosis of CaP in 78 men with PSA>3ng/mL and/or abnormal DRE (92) 2) TMPRSS2-ERG detection independently predicts Gleason score and clinical tumor stage in 497 men undergoing biopsy (67) 3) TMPRSS2-ERG score is associated with higher volume CaP and high-grade disease in 387 men on an active surveillance protocol (65)
Metabolin Prostarix^{TM}	4 metabolite signature in urine determined by liquid chromatography mass spectrometry (68)	1) Urine Prostarix^{TM} score is associated with metastases in patients with CaP bone metastases n=20, normal bone n=14, malignant prostate tissue n=13, benign prostate tissue n=17 and plasma samples n=15 (93) 2) RP Prostarix^{TM} score is associated with disease free survival rates in 148 CaP patients (94) 3) Biopsy Prostarix^{TM} score is associated with increased risk of CaP in 1122 CaP patients (69)

biopsy", enabling non-invasive, real-time monitoring of disease status and response to therapy, especially in the context of metastatic CRPC. While the measurement of various proteins in blood, including PSA, other PSA derivatives and other kallikreins remain important, cell free DNA, microRNA and circulating tumor cells (CTCs) represent the next generation of blood-based biomarkers (Table 4) (36,37). The use of cell free DNA, microRNA and CTCs has become main stage in CaP due to key technologic advances. Key technologic advances include the ability to perform detailed molecular analysis on small amounts of nucleic acids and small numbers of CTCs.

MicroRNAs are small noncoding RNAs found in tissue and serum samples that are involved in post-transcriptional regulation of a large number of biological processes (38). MicroRNAs, which are measured by Q-RT-PCR in serum and tissue samples, are quite stable in blood and thus may offer a useful biomarker in CaP disease (39). To date, various microRNAs have found utility in deciphering BPH from CaP, categorizing patients with CaP, predicting biochemical failure and treatment outcome (38,40-44).

Small amounts of cell free DNA found in plasma might constitute a source of genetic material for the identification of tumour-associated molecular

Table 5. CTCs Platforms (54)

Platform	Method
ApoCell ApoStream	Separation based on dielectric footprint
Biocept Cell Enrichment and Extraction OncoCell CEE-BR	Biotin-tagged antibodies that target CTCs
BioFluidica Microtechnologies CTC Detection System	Polymer based microfluidic chamber with affinity-coated surface, integrated with conductivity sensor for label-free counting)
Cynevenio Integrated System for Molecular Analysis of CTCs	Microfluidic system employing biomagnetic separation from whole blood
CytoTrack FM3 Scanner	Images fluorescently labeled cells captured by antibody on glass discs
Fluxion BioSciences IsoFlux	Magnetic beads coupled to antibodies separate cells from leukocytes in small volume, cells are recoverable and viable
On-Q-Ity Circulating Cancer Capture and Characterization Chip	Microfluid dual capture platform isolates cells based on EpCAM affinity and size
Rare Cells Diagnostics ISET	Isolation by filtration based on size, not antigen selection
ScreenCell MB	Filtration device equipped with different buffers depending on which downstream analysis to be done),
Silicon Biosystems DEPArray	Microarray containing dielectrophoretic cages
Veridex CellSearch CTC	Captures based on EpCAM affinity

alterations (45). To date, cell free DNA has been found to be useful in differentiating patients with CaP from BPH (46). In those patients already diagnosed with CaP, cell free DNA has been correlated with tumor stage and category and is useful in predicting shorter biochemical recurrence free survival (47-50). Furthermore, cell free DNA may have clinical utility in CRPC patients. The presence of androgen receptor (AR) gene aberrations in cell free DNA has been correlated to radiographic/clinical disease progression on enzalutamide (AR directed therapy) (50). The detection of AR copy number gain and AR L702H mutation in cell free DNA has been associated with resistance to abiraterone (CYP17A1 inhibitor) and a mutation in F876L detected in cell free DNA has been related to resistance to the novel drug ARN-509 (AR competitive antagonist) (51,52). Identification of molecular and genomic aberrations in cell free DNA has the potential to provide guidance in determining optimal treatment for patients with CRPC.

Circulating tumor cells (CTC) are found in the peripheral blood and may have an important role in tumour dissemination and progression (45). CTCs are currently detected using commercially available systems including the FDA-approved CellSearch™ method (tumor cells are isolated based on surface expression of EpCAM and identified by positive cytokeratin expression and negative CD45 expression) and many novel methods summarized in Table 5 (53,54). The CellSearch™ and several other technologies are limited by their dependence on epithelial surface antigens that may be lost in epithelial to mesenchymal transition. Some of the newer devices focus on mechanical features of CTC such as cell size and deformability in order to enable an antigen-agnostic selection of CTC. These platforms can be limited by their inability to extract viable separated cells. A novel microfluidic ratchet mechanism is one possible technique that is designed to overcome this shortcoming (55). Novel methods for identifying CTCs promise to enhance the dynamic range of CTC enumeration as a clinical predictor, but also to enhance the molecular analysis of CTCs to fulfill the goal of a “liquid biopsy” reflective of evolving tumor biology in response to treatment.

CTCs have found several roles in CaP management. They are rarely detected in localized CaP, but they have been shown to predict bone metastasis and overall survival in CRPC (56-59). Furthermore, investigators are also evaluating CTCs to predict response to novel agents in CRPC disease (e.g. NCT00974311, NCT01961843, NCT01084655), and specific markers in CTCs have further helped categorize patients (60,61). In particular, the splice variant of the androgen receptor AR-V7 in CTCs was associated with lower PSA response rate, shorter PSA progression free survival and shorter overall survival in 62 CRPC patients treated with enzalutamide and abiraterone (62). Clinical implementation of these findings will require validation in an independent cohort with a higher number of patients.

Validation of microRNA, cell free DNA and CTC assays are being pursued in most large scale CRPC clinical trials while investigational work is focusing on deciphering the genetic alterations, predictive protein markers and signaling profiles associated with CTCs as improved markers in CaP management. The potential to monitor molecular changes in response to therapy may allow continuous monitoring of drug targets and guide corresponding alterations in therapy, which represents the essence of precision oncology. Robust and reliable

methodology will be critical in ensuring that biomarker discovery and validation keep up with advances in therapeutics.

6. URINE-BASED BIOMARKERS

Urine is perhaps the most easily obtained specimen for biomarker development (Table 4). It is available in large quantities and can be collected non-invasively. Urine markers are particularly attractive when the prostate is intact, especially in the setting of screening and early stage disease.

PCA3 is a CaP-specific gene located on chromosome 9q21-22, whose mRNA can be easily isolated and quantified using available molecular assays (63,64). There is reasonable retrospective evidence for the clinical utility of PCA3 and it has therefore already been adopted to some degree in routine clinical practice. Specifically, PCA3 has become a useful marker to determine the need for repeat biopsy in those patients with rising PSA and previous negative biopsy (63). It has also been tested as a predictor of progression in patients on active surveillance and found to be associated with high-grade and higher volume disease (65).

TMPRSS2-ERG gene fusions involve the 5' untranslated region of the androgen-regulated gene TMPRSS2 with ERG or ETV1 (66). TMPRSS2-ERG levels can be measured in urine, where its levels been shown to correlate with biopsy Gleason score and clinical tumor stage (67). Like PCA3, it has also been tested as a predictor of progression in patients on active surveillance and found to be associated with high-grade and higher volume disease (65).

Prostarix^{TM} is a 4-metabolite signature in urine determined by liquid chromatography mass spectrometry (68). It has found utility in determining the need for initial or repeat biopsy in men with an elevated PSA but negative DRE (69).

These urine biomarkers are proving useful in the diagnosis of CaP and have potential applications in risk stratification of men considering active surveillance. The development of these markers is occurring in parallel with advances in CaP imaging, especially with multiparametric MRI. It remains to be seen if enhanced visualization of index lesions in the prostate with concomitant targeted biopsy will reduce the clinical utility of urine markers in the setting of active surveillance and the management of localized disease, or whether they will have complementary utility (70).

7. IMAGE-BASED BIOMARKERS

Multiparametric MRI consists of anatomic imaging with T1 and T2-weighted imaging combined with one or more functional analyses, including diffusion weighted imaging (DWI), dynamic contrast enhancement (DCE) and/or spectroscopy. The clinical use of MRI in CaP diagnosis and staging is rapidly evolving due to improvements in imaging technologies as well as the recognition that the failure to identify small, low-risk cancers is a potential advantage and not a shortcoming of this modality.

Multiparametric MRI is being studied in the context of primary imaging before biopsy and has already demonstrated utility in guiding repeat biopsy in patients with previously negative biopsy and elevated PSA (70-73). In the context of active surveillance, MRI appears to enable more careful staging with reduction of the risk of missing occult higher risk disease (74). Staging of disease to assess for extraprostatic extension prior to radical prostatectomy is also being trialed, although the negative predictive value of MRI in this context limits its utility (75-77).

Utilization of MRI and ultrasound for quantitative image analysis is a novel concept that is in the early stages of development. Rather than using the signals just for creation of images, the raw data generated from each pixel of an imaging modality can be used for quantitative analysis. Specialized imaging protocols may be required, but in principle the data is already being generated and only requires capture and analysis with specialized software. In essence an imaging "signature" can be derived for each pixel and correlated to subsequent pathologic findings in the same area of the prostate. Machine learning can then be applied for pattern recognition, and algorithms can be developed to predict disease presence, stage and grade based strictly on imaging criteria. One recent study, for example, employed quantitative analysis of DCE MRI to distinguish triple negative breast cancer from non-triple negative breast cancer (78). Quantitative image analysis can also be used to measure tumor response to therapy, which in turn can guide clinical decision-making (79).

8. THE FUTURE OF NEXT GENERATION BIOMARKERS

A wide spectrum of novel, non-protein based biomarkers is under development that promises to revolutionize future patient care, especially as these next generation biomarkers arise in parallel to significant improvements in CaP therapeutics. We have highlighted some of these biomarkers in the context of the clinical unmet needs that they address (Table 6), and their potential to advance the field moving forward.

In the setting of prostate cancer diagnosis, urine PCA3 and Prostarix^{TM} help guide the use of repeat biopsy in men with an elevated PSA and prior negative biopsy. MRI and fusion technologies may prove to be effective in determining specific target lesions for biopsy

Table 6. Summary of biomarkers in different clinical scenarios

Clinical Scenario	Recommendation
Active surveillance	1) PCA3- to predict patients who need a repeat biopsy in those men who have elevated PSA and a prior negative biopsy (*prognostic*) (63) 2) Prostarix™- to stratify the risk of a patient with previous negative biopsy to have occult cancer and thus would warrant further biopsy (*prognostic*) (69) 3) Tissue gene signatures (e.g. Prolaris Score™, OncotypeDx™)- to identify patients with apparent low risk disease who may harbor occult higher risk disease that would warrant definitive intervention over active surveillance (*prognostic*) (25,28,82,83) 4) Serum based microRNA and cell free DNA- to discriminate CaP from BPH (*prognostic*) (39,46) 5) MRI- to help focus biopsy on specific prostatic lesions (*prognostic*).
Localized prostate cancer	1) Tissue gene signatures (e.g. Decipher™)- to predict recurrence and progression after radical prostatectomy (*prognostic*) (30, 32, 33, 86, 87) 2) Serum based microRNA- to predict biochemical and clinical progression (*prognostic*) (43) 3) Cell free DNA- to predict biochemical recurrence (*prognostic*) (49)
Castration-resistant prostate cancer	1) Serum based microRNA- to predict outcome on docetaxel chemotherapy (*prognostic and predictive*) (44) 2) Cell free DNA- to predict resistance to enzalutamide, abiraterone and ARN-509 (*predictive*) (50,51,95) 3) Circulating tumor cells- to predict survival and response to enzalutamide and abiraterone (*prognostic and predictive*) (62)

when repeat biopsy is indicated by PCA3 or Prostarix™ results. Future validation of these tests may reduce the need for additional biopsies and thereby prevent the associated morbidity.

Gene signatures in biopsy specimens have potential utility in distinguishing indolent from aggressive disease, and similar signatures in RP can predict important disease outcomes. In particular, Prolaris Score™, OncotypeDx™ Genomic Prostate Score, Decipher™ and NF-kB NARP21 gene signatures have been evaluated in these settings. To date, Decipher™ has undergone the most rigorous validation of these gene signatures, but it still requires validation in the context of specific clinical questions such as the need for adjuvant therapy after RP.

Serum-based microRNA, cell free DNA and CTCs are currently being evaluated in large clinical trials as biomarkers for metastasis, treatment response and overall survival, especially in men with CRPC. In the future these “liquid biomarkers” offer the most promise for enabling specific modifications of systemic therapies according to evolving molecular changes in an individual’s CaP. Developing validated predictive markers to guide selection and sequencing of systemic therapy in CRPC is an ongoing research priority.

The evolution of next generation biomarkers further augments the already exciting advances ongoing in the management of patients with CaP. Two significant barriers that need to be overcome in order to implement these biomarkers into routine clinical practice are the need for careful clinical validation and the associated cost of the tests. More precise delivery of care, however, may ultimately reduce cost (80,81). Incorporation of these biomarkers into ongoing and future clinical trials will be essential in their development and clinical implementation.

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Send correspondence to: Peter Black, Department of Urologic Sciences, Faculty of Medicine, University of British Columbia Gordon & Leslie Diamond Health Care Centre Level 6, 2775 Laurel St., Vancouver, BC, V5Z 1M9, Fax: 604-875-4637, E-mail: pblack@mail.ubc.ca