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Does the molecular classification of breast cancer point the way for biomarker identification in prostate cancer?

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Abstract

There is significant variation in clinical outcome between patients diagnosed with prostate cancer (CaP). Although useful, statistical nomograms and risk stratification tools alone do not always accurately predict an individual's need for and response to treatment. The factors that determine this variation are not fully elucidated. In particular, cellular response to androgen ablation and subsequent paracrine/autocrine adaptation is poorly understood and despite best therapies, median survival in castrate resistant patients is only approximately 35 mo. We propose that one way of understanding this is to look for correlates in other comparable malignancies, such as breast cancer, where markers of at least 4 distinct gene clusters coding for 4 different phenotypic subtypes have been identified. These subtypes have been shown to demonstrate prognostic significance and successfully guide appropriate treatment regimens. In this paper we assess and review the evidence demonstrating parallels in the biology and treatment approach between breast and CaP, and consider the feasibility of patients with CaP being stratified into different molecular classes that could be used to complement prostate specific antigen and histological grading for clinical decision making. We show that there are significant correlations between the molecular classification of breast and CaP and explain how techniques used successfully to predict response to treatment in breast cancer can be applied to the prostate. Molecular phenotyping is possible in CaP and identification of distinct subtypes may allow personalised risk stratification way beyond that currently available.

Key words: Prostate cancer; Molecular classification; Biomarker; Breast cancer; Prognostic

Core tip: This paper demonstrates that prostate cancer (CaP) has defined molecular subtypes in a similar manner to breast cancer. The molecular classification

and subsequent personalised treatment of breast cancer has revolutionised its management. It is becoming increasingly apparent that the same principles may be applied to CaP, allowing more individualised treatment and informing clinical decision making.

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INTRODUCTION

Prostate cancer (CaP) is the most common male malignancy in the United Kingdom with an incidence of 134 per 100000 in 2010[1]. It is the second most common male cancer worldwide[2] and confers significant morbidity and mortality. With rising incidence it is a tremendous health economic burden, with annual expenditure in the United Kingdom of £94.2 million and in the United States of $11.5 billion in 2010 alone[3].

Diagnosis of CaP is based on clinical examination of the prostate, serum prostate specific antigen (PSA) levels and histological Gleason assessment International Society of Urological Pathology consensus[4]. Correlation between these factors and subsequent cancer outcomes has led to the development of well validated risk stratification tools[5] that broadly classify newly diagnosed patients into low (47%), intermediate (38%) and high (15%) risk groups[6,7]. These tools, and meticulously populated nomograms[8,9], continue to inform clinical decision making during the investigation, management and follow up of CaP[10].

However it is increasingly apparent that these tools alone are not sufficient to determine an individual's likelihood of being affected by clinically significant disease, particularly in the large "intermediate" risk group. Some patients require radical treatment but in others their disease is likely to remain indolent, having no demonstrable effect on their quality of life, or indeed life expectancy.

The factors that determine this variation in cancer aggression between patients are not fully elucidated. In particular, cellular response to androgen ablation and subsequent paracrine/autocrine adaptation is poorly understood and despite best therapies, median survival in castrate resistant patients is only approximately 35 mo[11].

CaP is an extremely heterogeneous disease process and further work is required to characterise its complex molecular biological mechanisms and genetic aberrations. This heterogeneity presents obstacles and opportunities for identifying and developing more accurate diagnostic and prognostic tests and new therapeutic avenues. There is now a realisation that CaP has an intricate relationship with its stromal microenvironment[12,13] and it may develop from different progenitor cells resulting in cancers with basal and luminal lineages, leading to divergent disease pathways[14].

One way of approaching this is to look for correlates in other comparable malignancies, such as breast cancer, where at least 4 distinct gene clusters coding for 4 different phenotypic subtypes were identified. These comprise luminal oestrogen receptor (ER) positive, basal [cytokeratin 5/14 positive, ER and human epidermal growth factor receptor 2 (HER2) negative], HER2/neu2 positive, and a "normal-like" phenotype[15]. These phenotypes reflect the tumour cell of origin and cell signalling pathways involved in disease progression and are generally characterised by differing patient outcomes[16-18]. But the biggest impact on survivorship has arguably resulted from advances in targeted adjuvant therapy and use of the humanised antibody, trastuzumab, to inhibit growth and metastasis in patients with cancers expressing HER2[19].

There are similarities in the treatment approach used for CaP, but the repertoire of therapeutic options is more restricted. The mainstay curative treatments for localised CaP are surgical prostatectomy alone or radiotherapy combined with a period of chemical castration involving hormonal-based androgen deprivation therapy (ADT) whose primary purpose is to prevent testosterone-responsive growth in any residual viable tumour cells[20]. However 20%-30% of patients treated for localised CaP will fail therapy and require long term ADT[21]. Unfortunately castrate-refractory disease is essentially inevitable at some point along the disease pathway, associated with poor prognosis due to metastasis formation. Therefore there remains an unmet need to inhibit metastasis formation, possibly resulting from circulating tumour cells[22] or the activation of dormant disseminated tumour cells (DTCs) present at the time of diagnosis[23]. Crucially, it has been proposed that the biology of DTCs is fundamentally different to the primary tumour supporting the need for characterisation of DTCs so that appropriate therapeutic approaches can be designed to successfully neutralise the threat posed by DTCs[24]. Novel combinative strategies that targets the primary and DTCs may be required to achieve significant improvement in treatment success.

The ability to identify those patients at significant risk of disease progression is required to tailor personalised therapy. This review examines the current evidence for biomarkers and their use for assessing disease detection, risk of progression, and prognosis. Moreover, we assess parallels in the biology and treatment approach between breast and CaP, and consider the feasibility of patients with CaP being stratified into different molecular classes that could be used to complement PSA and histological grading for clinical decision making.

PROSTATE EPITHELIAL CELL LINEAGE

Anatomically the prostate has a lobular structure, with

lateral and anterior lobes. However, seminal work by McNeil described a zonal architecture, with each zone demonstrating different characteristics and propensity to develop cancers. Secretory glandular tissue is arranged in ill-defined lobules forming the peripheral and inner periurethral zones. Glands have a papillary architecture and contain three main cellular populations comprising tall luminal columnar epithelial cells that line the approximately 30 prostatic ducts, the smaller basal epithelial cells on which the luminal cells rest and small numbers of neuroendocrine cells within the basal layer[25,26]. In addition, anteriorly is a mixture of smooth muscle and fibrous tissue, the fibromuscular stroma. Maintenance of prostatic epithelium is hormone (testosterone) dependent and in its absence the columnar cells change to a more squat cuboidal form accompanied by a reduction in secretory function.

There is accumulating evidence that malignant potential, disease aggression and prognosis may be determined by the subset of cells the cancer is derived from. The majority of malignancies are thought to develop in epithelial cells located in the peripheral zone, whereas the majority of benign prostatic hyperplasia develops in the transitional zone.

There has been considerable debate as to the cell lineage pathways of prostate epithelium. It is becoming apparent that the basal compartment contains a pool of multipotent stem cells[27-30] that are capable of differentiation into basal and secretory luminal epithelium. These different cellular subtypes can be identified through discrete expression patterns of certain cell surface proteins. For example luminal cells commonly express cytokines (CKs) 8 and 18, whereas basal cells express CK 5 and 14[26]. However, further work has demonstrated an "intermediate" cell type that co-express these markers along with others such as CD24[31]. This intermediate population is believed to represent a transition or amplification stage in the progression from multipotent stem cells to more differentiated basal and luminal epithelium[31].

Further work has shown that human basal cells *in vivo* can be triggered to develop CaP when exposed to common gene mutations[32]. This evidence fits with a hypothesis that stem cells are highly likely to be the origin of CaP as they have an inherent ability to self-renew, and their subsequent longevity provides sufficient time for repeated genetic mutations to finally trigger carcinogenesis.

However, the question remains at which point in the cellular differentiation pathway CaP is initiated. Evidence is growing that there are also populations of luminal cells that retain some stem-cell like qualities, perhaps because they are still "early" in the differentiation phase[33], or because they derive from an entirely separate stem cell population[34,35]. The lineage of prostate epithelial cell development has certainly not yet been fully mapped, and as a result the exact cell, or cells, of origin of CaP remain uncertain. Another unanswered question is whether the cell of origin determines tumour aggression, metastatic potential and likelihood of developing castrate resistance as it has recently been proposed that selective clonal stem cell expansion is associated with CaP aggressiveness[36].

Three clinical stages of CaP progression have been proposed[37]: (1) Low stage/endocrine-driven phase involving androgen receptor (AR) activation by testosterone derived dihydrotestosterone (DHT); (2) Progression stage/paracrine driven phase where AR signalling pathways are still important but different mechanisms are involved to endocrine induced activation. Increased expression of oncogenes (PI3K/Akt) and loss of tumour suppressor (*PTEN*) genes are detected and this stage represents a pathway terminating in metastasis and resistance to ADT (castrate resistance); and (3) Tumour cell autonomy. Here the cancer has adapted to its new environment, e.*g.,* bone tissue, and tumour cell proliferation is independent of AR mediated cell signalling. At this stage, tumours have neuroendocrine features with an acinar to small cell histology phenotype, and show *TMPRSS-ERG g*ene fusion[38].

Late stage disease in breast and CaP is characterised by altered ER and AR activity, involving loss of dependence on their natural ligands estrogen and testosterone respectively.

In vitro single cancer cell line models cannot replicate the multi-stage dynamic process involved in cancer cell progression through to metastasis. To overcome this deficiency, models have been refined by the use of co-cultured cell techniques, cancer cell/matrix techniques, or the use of *in vivo* animal models. With the first two approaches, surrogate functional markers of used to assess metastasis potential, comprising cell proliferation and migration.

MOLECULAR CLASSIFICATION OF BREAST CANCER AND THE PARALLELS TO CAP

CaP and breast cancer share a number of characteristics including common genetic, biochemical and growth factors[39]. They are both hormonally manipulated, the stromal microenvironment plays an integral role in each and they are more common in the presence of certain gene mutations such as BRCA1 and BRCA2[40,41]. Clearly, it is important to review the evidence for the existence of different molecular phenotypes in CaP to assess if classification could lead to similar risk profiling and specific targeted therapies utilised in breast cancer.

It is recognised that breast cancer has multiple genetic phenotypes that were initially identified by gene expression profiling (GEP)[15] and hierarchical clustering models to define four molecular classes: Normal breast, luminal (ER positive), basal-like and HER2 (epithelial growth factor receptor 2; ERBB2 gene/neu).

Subsequent work demonstrated further subtypes such as luminal A and B and claudin-low[42].

An important aspect of this work was the association between molecular subtype and cancer specific survival, allowing the development of risk assessment and personalised targeted therapy based on gene/protein expression profiling.

Luminal-like CaP

Similar to breast cancer, over the last decade many studies concluded that CaP derives mainly from terminally differentiated luminal cells, based on the observation that the majority of cancer specimens stained negative for basal cell markers and the cell surface protein p63[43,44]. However, other studies suggest that CaP is by no means a homogenous entity. While the absence of P63 is used as an adjunct in the histological classification of CaP[45] it is however occasionally expressed in CaP tissue, with elevated expression in tissue with higher Gleason scores[44].

AR signalling is critical in the development of normal prostate tissue. Like the estrogen receptor (ER) in breast cancer the AR plays a key role in mediating the various stages of CaP and subsequent castrate resistance. AR "promiscuity" is likely to contribute to this process by triggering transcriptional activation in response to antiandrogens or other endogenous hormones[46].

Development of castrate resistance in advanced CaP is associated with poor clinical outcome. Identifying which patients will succumb is currently a key research objective to aid clinical management and identify novel targets for therapy. The AR receptor and related genes are implicated in the durability of ADT treatment. Fujimura et *al*[47] proposed two panels of gene expression markers for determining clinical failure (defined by PSA recurrence) and cancer specific survival in treatment naïve CaP patients with bone metastasis. They found expression of *Sox2, Her2* and *CRP* in cancer cells to be predictive of clinical failure; panels comprising *Oct1, TRIM36, Sox2 and c-Myc AR*, *Klf4* and *ERα* were found to be prognostic of survival in cancer and stromal cells respectively.

Basal-like CaP

In breast cancer, the basal phenotype has been shown to be associated with more aggressive disease, poor patient outcomes and as yet has no specific targeted treatment[48,49]. The basal phenotype is commonly defined by a lack of expression of ER, progesterone receptor and HER2 and for this reason is referred to as the "triple negative" phenotype. Although the two terms are frequently used interchangeably the basal and triple negative types may actually be 2 distinct groups, albeit with similar poor clinical outcomes[50,51]. In CaP the steroid nuclear AR is expressed in luminal, basal and stromal cells but importantly its regulatory function varies with each cell population. It enhances cell survival in luminal cells, stimulates proliferation and metastases in stromal cells and suppresses proliferation and metastasis in basal cells respectively[52].

There is strong evidence demonstrating the basal phenotype as a cell of origin for some CaPs. Recent work has suggested that genetic signatures commonly associated with embryonic stem cells (ESCs) are up regulated in the tumours of patients with more poorly differentiated CaPs[53,54]. Markert et *al*[53] proposed three stem cell genotypes: (1) ESC; (2) Induced pluripotent stem cells (IPSC); and (3) Polycomb repressive complex-2 (PRC2). Interestingly the same characteristic ESC signature, identified in 13% CaP patients, has been found in high grade breast cancers, particularly the basal subtype[55]. ESC+ CaPs have been associated with loss of p53 and PTEN function, TMPRSS-ERG gene fusion, higher Gleason scores (8-10) and poorer prognosis. An IPSC signature (30% patients) is represented across all Gleason scores, whereas a PRC2 signature (44% patients) was frequently found in patients with a low Gleason cancer. Moreover, the population of phenotypically positive prostate stem cells appears increased in metastatic bone cancer compared to the primary CaP[56]. Colombel et *al*[57] suggest using the putative stem cell markers integrin alpha-2 or -6 in combination with c-met and a 5% cutoff threshold to predict reduced survival associated with bone metastasis. Interestingly, these markers appear to be confined to stem cells localised in the basal cell layer of normal and benign prostate hyperplasia tissue[57].

But contradicting the existence of a pure basal class of CaP is the observation that basal CaP cells tend to lose their basal-defining cell marker characteristics and transform into a more luminal phenotype. However, although appearing histologically homogenous, CaPs still maintain lineage-specific genetic signatures. In contrast to basal-like breast cancer, CaPs retaining a basal phenotype appear to be a rarer event and may actually have a better prognosis than their luminal cell derived counterparts[14]. But, identifying the legacy of basal-transformed cells presents difficulties and limits its clinical usefulness.

HE

HER2 CaPs

Given the similarities between hormonally mediated prostate and breast cancer it is unsurprising that the HER2 oncogene has demonstrated an association with outcome in CaP. HER2 overexpression has been found in approximately 20% of localised, untreated CaPs, and this rises to over 60% in metastatic disease and those cancers treated with ADT, although there is significant variation between studies, based on definition of "overexpression" and also the assay used[58]. Increased expression of HER2 in CaP has been associated with higher Gleason grade, cancer stage and rate of proliferation (as demonstrated by the Ki67 index)[59] and also poorer outcome[60]. However, anti HER2 antibodies such as trastuzumab (Herceptin) that have proven extremely effective in HER2-positive breast cancer has

Table 1 A summary of some of the key similarities between breast and prostate cancer

CaP	Breast cancer
Incidence - 134 per 100000/yr (United Kingdom)	Incidence - 164 per 100000/yr (United Kingdom)
Risks - Increasing age, ethnicity (Black African and Black Caribbean men have highest risk), family history, obesity	Risks - Increasing age, family history, smoking, obesity
AR - Prostate epithelial cells are primarily androgen dependent and AR mutation, promiscuity and hypersensitivity are key stages in cancer progression	AR - Certain subgroups of triple negative breast cancers express the AR. These patients have been shown to have worse prognosis than AR - ve groups
BRCA1 and *BRCA2* - Epidemiological studies have demonstrated a link between breast and CaP within families. However only a small proportion of CaPs can be linked to these gene mutations	*BRACA1* and *BRACA2* - Extremely important genes involved in cell cycle regulation. Responsible for many breast cancers - particularly in younger patients
ESCs - Genetic signatures associated with ESCs have been linked to poorly differentiated CaP. Loss of action of PTEN and P53 and TMPRSS-ERG fusions are frequently observed in these cells	ESC - The ESC signature has recently been demonstrated to be present in some high grade breast cancers
HER2 - The *HER2* oncogene is expressed in 20% of localised CaP. This rises to 60% of metastatic CaP. It has been associated with increased cell proliferation and poorer outcomes in some studies, however the use of anti HER2 antibodies has not demonstrated improved patient outcomes	*HER2* - *HER2* expression has been clearly demonstrated to define a molecular sub group of breast cancer with characteristic survival. An example of early success in the development of "targeted therapy" anti-HER2 antibodies such as trastuzumab (Herceptin) have revolutionised HER2 positive breast cancer treatment

CaP: Prostate cancer; AR: Androgen receptor; BRACA: Breast cancer; ESC: Embryonic stem cell; HER2: Human epidermal growth factor receptor 2.

not shown any clinical efficacy in CaP. Interestingly trastuzumab is most effective in breast cancers in which HER2 overexpression is mediated by gene amplification. In CaP, while HER2 expression is upregulated, gene amplification is uncommon, and thus the target may not be as important in this disease[61].

In summary, breast and CaPs share many common features (see Table 1 for a brief summary of key similarities). Stratification of CaP based on similar principles to that used for the molecular classification of breast cancer may be conceptually possible for the luminal and basal classes but they do not represent the full heterogeneity seen in progressive CaP disease. Based on current academic knowledge and the development of breast cancer therapy, future clinical management of CaP is going to require an individualised approach built on assessment of cell signalling biomarkers that inform about cell functional activity. These will be briefly reviewed.

BIOMARKERS WITH PREDICTIVE AND PROGNOSTIC CAPABILITY

The development and progression of CaP is an extremely complex process involving a varying combination of genetic abnormalities, oxidative stress, cellular inflammation, altered epithelial - stromal interaction and androgen receptor signalling. Previous studies have failed to determine the critical time point when metastasis occurs during carcinogenesis. This could be addressed by prospectively collecting blood samples in patient cohorts pre- and post-diagnosis for CaP to determine the window of opportunity for tumour containment needed for metastasis prevention. Isolation of circulating CaP cells has been shown to be prognostic of survival[22,62], and predictive of disease dissemination[63]. Also, the activation of dormant DTCs and consequential metastasis involves a balance between three opposing processes: Cellular dormancy (mitotic arrest); angiogenic dormancy (vascular-delivered nutrient restriction); and immune-mediated dormancy resulting from immune system cytotoxicity[64]. Procedures exist for the isolation of DTCs[65] and biomarkers have been proposed for assessing their functional state.

In breast cancer, early stratification studies of patients using GEP revealed an association between tumour biology genotype, tumour behaviour and response to targeted therapy[15]. This approach has been refined and it is the case that whilst a single biomarker can inform about likely response to targeted therapy (theranostics, e.*g.,* ER status and candidature for tamoxifen treatment), panels of biomarkers are needed to inform about individualised risk of disease progression and survival. Risk assessment can be used to assist chemotherapy decision-making. For example, a 21 multi-gene PCR-based assay (Oncotype DX Breast Cancer Test) was developed for predicting tumour recurrence in tamoxifen treated, node negative, ER expressing breast cancer[66].

The Oncotype DX CaP Test is a multigene PCR-based assay that assesses risk of disease progression in patients with apparent low risk disease. This 17 gene profile assesses 4 distinct biological process targets: The androgen pathway, cellular organisation, proliferation and stromal response[67]. This assay gives a "genomic prostate score" that predicts the likelihood of high grade or high stage disease at diagnosis[68]. This array, like its competitors "Prolaris" and "Decipher" are not yet widely used and a recent systematic review concluded that they have yet to clearly demonstrate any significant advantage over more established predictive nomograms as a general clinical application[62].

Single biomarkers are currently used for diagnostic and predictive assessment of CaP. The most widely used and evidence-based is PSA, a 34 kD serine protease encoded by a gene on chromosome 19 and uniquely

produced by prostate epithelial cells. A raised PSA level can indicate an increased risk of CaP, although presence of other factors such as urinary tract infection and significant lower urinary tract symptoms can cause similar rises. There is no absolute value above which CaP is present, but studies have shown that a PSA of 4 ng/mL confers approximately a 25% risk of cancer[69]. PSA is particularly useful in monitoring response to treatment in CaP because a rise in PSA in a patient who has undergone radical treatment is an early indicator of disease recurrence. Another marker, less widely used and mainly as a diagnostic adjunct, is CaP antigen-3 (PCA3). It is a non-coding segment of mRNA produced by prostate epithelial cells approximately 60 to 100 times more in CaP than benign tissue. The most common assay is marketed as Progensa[70]. Samples for analysis are collected in the urine after prostatic massage and a ratio of PCA3 to PSA mRNA is calculated and a CaP risk is determined.

Assessment of cell proliferation in CaP has received much attention because proliferation is a key requirement for tumour growth and disease progression but is not readily assessed in prostate patients. Unregulated cell turnover occur as a result of genetic abnormalities at all stages of tumour development and can broadly be grouped by where in this pathway they occur. For example, alterations to genes such as[71] ER-1B[72] and NKX3-1[73,74] have been linked to disregulated cell proliferation. Loss or mutation of genes such as HPC1 (codes for the tumour suppressor protein RNaseL) are thought to lead to altered apoptotic processes in response to cellular stress[75,76].

The immunohistochemical Ki67 marker applied to histological tumour sections can be used to detect CaP cells undergoing proliferation (G1, S, G2, and mitosis phase) in conservatively treated patients. Using a cutoff of > 5% cancer nuclei positively stained, Ki67 is prognostic of cancer specific death in tissues derived by trans-urethral resection of prostate[71]. Similar findings were obtained using a 10% nuclei cutoff in diagnostic biopsies[77]. A recent investigation found high (> 6.2%) levels of histologically detected Ki67 were prognostic of disease specific death, metastasis and biochemical failure (rising PSA) in low to intermediate (PSA < 20 ng/mL) patients treated with a combination of short term ADT and radiotherapy[78]. Ki67 is also a component of the cell cycle progression signature proposed by the transatlantic prostate group for independently predicting CaP specific death[79]. We can confirm that immunohistochemical analysis of Ki67 has the potential for providing a cost-effective and robust laboratory technique applicable to routinely processed pathology samples (manuscript submitted: Green et *al,* 2015).

Further important genes implicated in CaP include downregulation of the tumour suppressor gene p53 and deletions/mutations of PTEN (phosphatase and tensin homologue), which acts as a cell cycle regulator. PTEN deletions have been demonstrated in 5% of localised CaP but over 30% of metastatic CaP[80], suggesting it may be an important target in the molecular transition between organ confined and widespread disease.

Transforming growth factor beta (TGF-β) is a protein involved in proliferation and cellular differentiation in many cells and its dysregulation plays an integral part in the development and propagation of CaP. The distal-less homeobox (*DLX)* gene family has been implicated in triggering this dysregulation. The DLX family is a group of six genes that are involved in embryonic development, tissue homeostasis, lymphocyte development, cell cycle and apoptosis. A recent study showed that DLX2 is involved in shifting TGF-β from a tumour suppressor to a tumour promoting function by repressing TGF-βRII and the cell cycle inhibitor p21CIP1, and simultaneously increasing the mitogenic transcription factor c-Myc and epidermal growth factor[81]. The impact of this has been shown to increase tumour growth and metastasis formation in melanoma and lung cancer and work is ongoing to further elucidate the role of DLX2 in CaP[82]. Another key genetic abnormality in CaP is TMPRSS2-ERG fusion, which is found to occur in at least 50% of CaP patients[83]. It has been shown to promote cancer invasion and metastasis and some groups have linked TMPRSS2-ERG fusion to poorer overall prognosis, particularly in those patients in a "watchful waiting" cohort[84]. However, in patients undergoing surgery for their CaP no clear difference in cancer specific survival caused by this gene fusion has yet been demonstrated[85].

Further work employing hierarchical clustering techniques has identified expression of the gene product of Hey2 as being an independent predictor of biochemical failure, local recurrence and distant metastasis in CaP[86]. The same group has demonstrated another gene (CYP4Z1) is an independent predictor of indolent disease.

CONCLUSION

There is a need for more accurate markers of disease outcome in CaP. Currently many patients undergo highly invasive and expensive treatments that carry significant side effects and may have been unnecessary, as their disease would never have become clinically apparent or life threatening. Others will initially be stratified as low or intermediate risk but will subsequently develop highly aggressive disease.

Understanding the cell lineage of CaP and applying the highly successful techniques used in breast cancer research has led to the development of gene signature arrays that reveal different molecular classifications in CaP. Emerging evidence suggests that molecular phenotyping is possible in CaP and identification of distinct subtypes may allow personalised risk stratification way beyond that currently available. This approach needs supporting with the identification and development of treatment regimens directed at theranostic targets, especially in patients assessed as high risk for castrate resistant disease.

While initial results are promising, further work is required to define a robust panel of predictive markers

in CaP; this may involve selection of predictive/prognostic biomarkers that inform about the potential biological behaviour of circulating and DTCs in addition to those detected in the primary organ (prostate). The use of gene expression arrays coupled with bioinformatic techniques has led to the identification of clinically useful multigene PCR assays and protein-based biomarkers. The former are generally more complex and require specialised tissue processing. Protein based assays are mostly applied to routinely processed histological based samples or liquid samples. Currently, all could be used in-conjunction with nomograms and risk algorithms employed by clinicians managing patients with CaP.

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