Abstract

Despite recent advances, prostate cancer continues to be a leading cause of cancer-related death among men. While the standard management options of surgery, radiotherapy, and androgen deprivation therapy are well established, there are still significant unmet needs. For example, which patients would best be served by active surveillance at the time of diagnosis versus proceeding with definitive therapy is still not well understood. Additionally, more accurate means of monitoring patients' responses to therapy and remission statuses following therapy are needed. Since all patients with metastatic disease ultimately progress to castration-resistant prostate cancer, new treatment options for this population are also required. As in other areas of oncology, greater personalisation of care holds the potential for more effective treatment while also reducing the risk of adverse effects and morbidity. This review addresses three topics currently under investigation related to the personalised management of prostate cancer: the use of circulating tumour cells in both diagnosis and monitoring of treatment response, the role of poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors, and the emerging role of genomic assays for risk stratification at the time of diagnosis.

INTRODUCTION

Prostate cancer is the second most commonly diagnosed cancer in men worldwide and the fifth leading cause of cancer-related deaths. The majority of patients are diagnosed with localised disease, which is managed with radical prostatectomy (RP) or radiotherapy (RT). Despite high progression-free survival (PFS) rates, up to approximately 30% of patients treated with surgery and 30–50% of those treated with RT eventually experience disease recurrence.

The heterogeneous biology of prostate cancer has led to increased interest in personalised approaches to management of the disease. This narrative review focuses on three areas of active investigation: the use of circulating tumour cells (CTC) in diagnosis and monitoring of treatment response, the role of poly(adenosine diphosphateribose) polymerase (PARP) inhibitors, and the potential applicability of multigene assays to aid in risk stratification at diagnosis. The information detailed in this review was obtained via PubMed searches for the terms “prostate cancer”, “circulating tumour cells”, “PARP inhibitor”, and “multigene assay”.

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CIRCULATING TUMOUR CELLS IN THE DIAGNOSIS AND MANAGEMENT OF PROSTATE CANCER

CTC are cancer cells shed from the primary tumour or from a metastatic focus into the circulation, and enumeration and analyses of CTC are being used in the diagnosis and management of various malignancies. Although the presence of these cells was recognised in the 19th century, several technical challenges limited the feasibility of CTC use until the last decade. These include the relative rarity of CTC in the bloodstream, in part due to their short lifespan in this environment, as well as the presence of multiple subpopulations of CTC in the parent tumour.

Identification of Circulating Tumour Cells

The number of CTC present in patients with solid tumour malignancies is known to be low. Frequencies as low as 1 CTC per 7.5 mL of blood from patients with metastatic prostate cancer have been reported. Thus, much of the work directed at improving the clinical applicability of CTC has aimed at improving the sensitivity of technologies for their detection. Several techniques have been developed to isolate CTC by exploiting their unique physical and immunologic properties.

Immunoaffinity is one of the methods used to isolate CTC, based on cell-surface markers such as the epithelial cell adhesion molecule (EpCAM). Various devices have been developed utilising specialised beads, microposts (also known as CTC-chip), and in vivo wires or needles. CELLSEARCH® (Menarini Silicon Biosystems, Inc., Bologna, Italy) is a platform that was developed to identify breast, colon, and prostate cancer CTC using anti-EpCAM antibodies attached to ferrofluid nanoparticles. After the antibodies bind to the target cells, they are removed from solution using a magnet. The isolated cells are stained with 4',6-diamidino-2-phenylindole (DAPI) nuclear stain, as well as antibodies directed against cytokeratin (CK) and CD45. CTC are identified by their staining pattern as EpCAM-positive, DAPI-positive, CK-positive, and CD45-negative.

The microfluidics system CTC-chip uses a chamber with thousands of microposts embedded with antibodies to Ep-CAM. Cells passed through the CTC-chip can then be detected using immunofluorescence techniques. Stott et al. isolated prostate cancer CTC using the CTC-chip, then flowed a rabbit antibody specific to prostate-specific antigen (PSA) through the chip, followed by a goat antibody that was fluorescently tagged to rabbit immunoglobulin G.

The above techniques are based on cell-surface markers and so their effectiveness is compromised by the heterogeneity of expression in a particular CTC population. Since EpCAM is a generic epithelial marker, systems based on this antigen have reduced sensitivity in the presence of other epithelial cells. In addition, cells have to be fixed for identification with the CELLSEARCH platform, so live CTC cannot be isolated by this method.

Several other techniques for CTC isolation are based on differences in size and deformability. Generally, CTC are larger and more rigid than benign cells; this has led to approaches using filters such as membranes or adjustable cell traps. These platforms allow recovery of live CTC but still have limitations of sensitivity and specificity, and require staining to confirm identification.

Monitoring of Disease Status

Prostate cancer CTC have been used in a variety of settings, from localised to metastatic disease. Some of the studies are summarised here.

Localised Disease

Puche-Sanz et al. demonstrated that CTC could be detected in prostate cancer patients at the time of diagnosis. CTC were isolated from 86 patients with clinical suspicion of prostate cancer who met the criteria for prostate biopsy (based on PSA >10 ng/mL or PSA 4-10 ng/mL with a free/total PSA ratio <0.2). The Carcinoma Cell Enrichment and Detection Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) was used, which involves magnetic beads labelled with a multi-CK-specific antibody. In this population with a low burden of disease, the rate of CTC detection was 18.6%.
Kuske et al.\textsuperscript{10} combined the CELLSEARCH platform with two other techniques to enhance the sensitivity and specificity. The CellCollector\textsuperscript{®} (GILUPI, Potsdam, Germany) uses a sterile stainless steel medical wire coated with antibodies to EpCAM, enabling collection of CTC \textit{in vivo}.\textsuperscript{11} A third EpCAM-independent assay, EPISPOT, is an adapted enzyme-linked immunospot assay.\textsuperscript{12} By combining these approaches, the detection rate improved from 37.0\% to 59.0\% for the individual assays, and to 81.3\% for the combined approach.

### Metastatic Hormone-Sensitive Prostate Cancer

Currently, patients who undergo curative treatment with either surgery or radiation are monitored for recurrence via serum PSA level, typically in the setting of androgen deprivation therapy. CTC have been investigated as additional biomarkers of response. Roviello et al.\textsuperscript{13} investigated the correlation of CTC detectability with clinical recurrence in patients with hormone-sensitive disease who had received curative treatment. Using the CELLSEARCH assay, CTC were detected in 14 of the 42 (33.3\%) patients enrolled. This group was found to have a significantly higher mean PSA level and was significantly more likely to have bone metastases compared to the CTC-undetectable group. In addition, Josefsson et al.\textsuperscript{14} collected CTC from 46 of the 53 (87\%) patients with metastatic hormone-sensitive prostate cancer who were assayed with the CELLSEARCH platform. The presence of CTC was found to be associated with shorter PFS. In particular, expression of epidermal growth factor receptor on CTC demonstrated significant negative prognostic value, with a PFS of 5 months versus 11 months for those in whom epidermal growth factor receptor was not detected.

### Metastatic Castration-Resistant Prostate Cancer

Much interest has focussed on the utility of CTC as leading indicators of the emergence of resistance to androgen blockade, or as prognostic markers in the management of patients already known to have castration-resistant prostate cancer (CRPC). An important study was conducted by de Bono et al.\textsuperscript{15} in which peripheral blood from 231 patients with CRPC was assayed for CTC, both before and after initiating each new line of therapy. Patients were divided into groups of favourable or unfavourable CTC counts, based on whether <5 or ≥5 CTC were collected in a 7.5 mL sample. Using this method, median overall survival was found to be significantly longer in the favourable group (20.7 months) versus the unfavourable group (9.5 months). CTC count was demonstrated to be more accurate in predicting overall survival than the alternative measure of PSA decrease.

One mechanism for the development of castration resistance is the amplification of the androgen receptor gene (\textit{AR}). Podolak et al.\textsuperscript{16} collected both peripheral blood CTC and biopsy tissue from a group of 25 patients with metastatic CRPC and measured \textit{AR} amplification in the samples. Twenty-four (96\%) of the patients demonstrated concordance of \textit{AR} status, an encouraging result that suggests a CTC assay could ultimately replace biopsy in some cases.

Another key mechanism of castration resistance is the development of the abnormally spliced androgen receptor AR-V7. Antonarakis et al.\textsuperscript{17} studied a population of 202 patients with metastatic CRPC being treated with abiraterone or enzalutamide, looking at three separate subgroups: CTC-negative, CTC-positive/AR-V7-negative, and CTC-positive/AR-V7-positive. Clinical and radiographic PFS, the primary endpoint of the study, was significantly different, measuring 13.9 months for the CTC-negative group versus 7.7 months for the CTC-positive/AR-V7-negative group and 3.1 months for the CTC-positive/AR-V7-positive group. These results suggest that the presence of CTC is itself a poor prognostic factor, with the presence of AR-V7-positive CTC having a particularly negative implication. The aforementioned investigations demonstrate the increasing role and applicability of CTC in the management of prostate cancer, potentially from the time of diagnosis and continuing through advanced disease.
Significant effort has been directed towards personalised management of the population of patients with metastatic CRPC, an entity that exhibits considerable genetic heterogeneity. Approximately 90% of these cancers contain actionable mutations. Robinson et al.\textsuperscript{18} reported an analysis of 150 cases that demonstrated that 22.7% of patients had alterations in DNA repair genes. The observation that some cases of CRPC contained mutations in\textit{BRCA1},\textit{BRCA2}, and\textit{ATM} suggested that these cancers might be sensitive to treatment with inhibitors of backup DNA repair pathways, an approach referred to as synthetic lethality. The development of PARP inhibitors facilitated this new approach to management of CRPC.

**Castration-Resistant Prostate Cancer with Germline DNA Repair Mutations**

Initial studies of\textit{BRCA1} and\textit{BRCA2}-mutated prostate cancer focussed on germline mutations, which were found to be associated with a higher Gleason score (range: 2–10; higher score represents more aggressive disease), T stage, likelihood of nodal involvement, and likelihood of metastatic disease at diagnosis, as compared with wild-type cancers.\textsuperscript{19} The development of PARP inhibitors heralded a novel targeted approach, with a proof-of-concept basket trial of\textit{BRCA1} and\textit{BRCA2}-mutated cancers. Fong et al.\textsuperscript{20} treated a cohort containing some patients with germline\textit{BRCA1} and\textit{BRCA2}-mutated ovary, breast, and prostate cancer with the PARP inhibitor olaparib. Only mutation carriers responded and less toxicity was observed than with standard chemotherapy options. Further studies targeting germline\textit{BRCA2}-mutated prostate cancer alone also demonstrated a response to olaparib.\textsuperscript{21}

**Inducing ‘BRCAness’**

The degree to which a given cancer is deficient in homologous recombination, such that it is sensitive to treatment with PARP inhibitors, has been referred to as ‘BRCAness’. Only a minority of CRPC contain DNA repair mutations and ongoing studies are investigating whether the quality of BRCAness can be induced therapeutically. Significantly, AR signalling pathways have been demonstrated to regulate the expression of DNA repair genes;\textsuperscript{23} additionally, PARP-1 has been found to promote the activity of AR.\textsuperscript{24}

Based on preclinical work using the antiandrogen enzalutamide, Li et al.\textsuperscript{25} hypothesised that blocking this pathway in CRPC cells would enhance their BRCAness and render them more susceptible to PARP inhibition. A lead-in strategy was used in which prostate cancer cells from the cell lines VCaP and LNCaP (American Type Culture Collection, Manassas, Virginia, USA) and CWR22Rv1 (Memorial Sloan Kettering Cancer Center, New York City, New York, USA) were treated with enzalutamide for 24 hours, then with a combination of enzalutamide and olaparib for 48 hours. These were compared with cells

**Castration-Resistant Prostate Cancer with Sporadic DNA Repair Mutations**

With the activity of PARP inhibitors in germline-mutated CRPC established, interest developed regarding their applicability to CRPC with sporadic mutations of DNA repair genes. A pivotal study by Mateo et al.\textsuperscript{22} (the TOPARP-A trial) examined the use of olaparib in a cohort of patients with CRPC of unknown DNA repair mutation status. Subjects underwent a prospective series of biomarker studies, including whole-genome sequencing and transcriptome analysis; CTC analysis was performed using the CELLSEARCH platform. Of the 49 patients evaluated, 16 had an objective response (33% response rate), while 14 patients (29% response rate) showed a reduction in CTC count. Next-generation sequencing revealed that 16 patients had mutations in DNA repair genes (referred to as biomarker-positive), including 7 patients with alterations of\textit{BRCA2}. Notably, patterns of response were different between the groups, with significantly higher response rates in biomarker-positive patients. Eighty-eight percent of patients with DNA repair mutations responded to olaparib, whereas only 6% of the biomarker-negative patients responded. Additionally, olaparib was generally well tolerated, with the most common Grade 3–4 adverse effect being anaemia, which 20% of patients presented with.
treated with enzalutamide and olaparib concomitantly, and the lead-in strategy was found to be more effective at impairing cell growth.

**GENOMIC TESTING FOR RISK STRATIFICATION**

Contemporary treatment options for newly diagnosed localised prostate cancer include surgery (RP with or without pelvic lymph node dissection), radiation (external beam RT or brachytherapy) with androgen deprivation therapy, or active surveillance. The choice among these options is guided by the degree of risk associated with the individual patient’s disease, an assessment that is largely based on the aggressiveness of cells in the prostate biopsy (reported as Gleason score). However, the accuracy of biopsy is limited due to tumour heterogeneity and sampling errors. Hence, a growing area of interest in personalised prostate cancer care relates to predictive models to improve risk stratification. Ongoing efforts are focussed on the development and use of multigene assays to better characterise prostate cancer, starting at the time of diagnosis, an approach somewhat analogous to that used currently in the management of breast cancer.

**Genomic Prostate Score Assay**

Klein et al. developed a 17-gene assay called the genomic prostate score (GPS) to risk-stratify newly diagnosed localised prostate cancer using a multistep methodology. First, 441 prostatectomy samples were analysed to help generate candidate genes for the multigene assay. Second, a validation study was conducted comprising 167 patients who initially had a biopsy revealing prostate cancer and then underwent prostatectomy. In total, 732 genes were analysed, of which 288 were found to be predictive of recurrence and 198 were predictive of aggressive disease. Seventeen genes (12 genes associated with prostate cancer aggressiveness and 5 reference genes) were included in the finalised assay, after which the third component (prospective validation study) was conducted, which included 395 patients, all of whom were candidates for surveillance but elected to undergo prostatectomy within 6 months of the biopsy. A higher GPS value (scale: 0–100) was found to be associated with poorer clinical outcome when adjusted for Cancer of the Prostate Risk Assessment (CAPRA) score. Specifically, each 20-point increase in GPS was predictive of a 2.3-fold increased risk of high-grade disease at prostatectomy and was also associated with a 1.9-fold increased risk of non-organ-confined disease. A cut-off GPS value was not suggested.

A follow-up study by Cullen et al. validated the GPS retrospectively in a group of 431 patients diagnosed with very low-to-intermediate-risk prostate cancer. Of this cohort of patients, 20% were African American. GPS was found to be predictive of outcome, including time to biochemical recurrence and time to metastasis, and median GPS was the same (30.3) for both African American and Caucasian patients.

**Stratification of Patients with Intermediate-Risk Disease**

Sinnott et al. noted that the grading of prostate biopsy involves some degree of interobserver variability, and many patients are diagnosed with Gleason score 7 (intermediate-risk) disease. This group of patients is heterogeneous, with a wide range of prognoses, and traditionally they have been risk-stratified according to Gleason score 3+4 versus 4+3 disease (where the first score indicates the dominant histologic pattern present and the second score indicates the non-dominant histologic pattern). To better characterise this particular group, Sinnott et al. developed a 30-gene signature specifically for use in patients with Gleason score 7 disease. Whole-transcriptome gene expression profiling was performed on 113 prostate cancer specimens (either from RP or transurethral resection of the prostate) from patients who died of the disease or developed distant metastases. Subsequently, another 291 samples were analysed from patients with indolent disease who did not develop metastases and died of non-prostate cancer-related causes. All patients in both groups were classified as having intermediate-risk Gleason score 7 tumours. A signature containing 157 genes was developed using these gene expression profiles, which was narrowed to 30 genes. The score generated ranged 0–1, with lower scores more similar to Gleason score ≤6 disease, while higher scores were more similar to Gleason score ≥8.
When compared to Gleason score alone, the 30-gene score was found to be more predictive of aggressive disease. The score was a stronger predictor of lethality than Gleason score 3+4 or 4+3 status, although this difference was not statistically significant.

**Methylation-Based Assay**

Epigenetic factors, such as methylation, have been recognised as playing a role in carcinogenesis. Vasiljević et al. analysed 13 genes in 367 men with localised prostate cancer, specifically looking at the methylation status. Twelve of the 13 genes analysed were associated with prostate cancer-related death and the hazard ratios increased with the degree of methylation. Subsequently, the same group developed a methylation score for risk stratification of low-to-intermediate-risk prostate cancer. Six genes from the previous set of 13 were selected for inclusion in the methylation score. Transurethral resection of the prostate samples from 385 patients with low-to-intermediate-risk CAPRA scores were assayed to determine the methylation statuses. The methylation score was found to be a stronger predictor of prostate cancer-related death than CAPRA score and the difference was statistically significant, with hazard ratios of 2.72 and 1.62, respectively.

**CONCLUSION**

Prostate cancer is a common malignancy and a major contributor to the global burden of cancer-related morbidity and death. One of the striking features of the disease is its heterogeneity in presentation and clinical course, with some patients having indolent disease and minimal symptom burden while others experience highly aggressive disease. This review has summarised some of the efforts and data collected regarding greater personalisation of care for patients with prostate cancer, with the goal of avoiding overtreatment of lower-risk patients while targeting higher-risk patients for more appropriate management.

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