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Seminar article

Cell-free and circulating tumor cell–based biomarkers in men with metastatic prostate cancer: Tools for real-time precision medicine?

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Abstract

The recent expansion of therapeutic options for the treatment of metastatic prostate cancer highlights the need for precision medicine approaches to enable the rational selection of appropriate therapies for individual patients. In this context, circulating biomarkers in the peripheral blood are attractive as readily accessible tools for predicting and monitoring therapeutic response. In the case of circulating tumor cells and circulating tumor DNA, they may also serve as a noninvasive means of assessing molecular aberrations in tumors at multiple time points before and during therapy. These so-called "liquid biopsies" can provide a snapshot view of tumor molecular architecture and may enable clinicians to monitor the molecular status of tumors as they evolve during treatment, thus allowing for individualized precision therapeutic decisions for patients over time. In this review, we outline recent progress in the field of circulating biomarkers in metastatic prostate cancer and evaluate their potential for enabling this vision of real-time precision medicine.

Keywords: Prostate cancer; CTCs; ctDNA; Circulating biomarkers; AR

Introduction

Prostate cancer remains the second leading cause of cancer-related death in men in the United States, with an estimated 26,120 deaths in 2016 [1]. The past 6 years have seen the expansion of therapies that improve overall survival (OS) for men with metastatic castration-resistant prostate cancer (mCRPC), with other promising drugs in development [2]. However, all of these drugs ultimately have limited efficacy, and primary or acquired resistance to therapy is a significant problem. Monitoring the effectiveness of individual therapies in patients with mCRPC is a uniquely difficult problem because of the high prevalence of bone metastases, which are difficult to quantitate. There exists a need for accurate biomarkers to monitor and predict clinical response in prostate cancer, and thus enable a precision medicine approach to personalizing treatment for the individual patient. A biomarker that can reliably substitute for OS as a surrogate end point would also be useful in the design of clinical trials investigating novel therapies, especially in a disease with a growing number of available life-prolonging treatments.

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [3]. A biomarker can thus provide a clinical measurement for a specific clinical context that may correlate with patient outcomes (*prognostic* biomarker) or likelihood of response to a specific therapy (*predictive* biomarker). In many cancers, tissue biomarkers based on the molecular analysis of primary or metastatic tumors have prognostic or predictive value. However, 90% of men with mCRPC have bone metastases, and tissues from metastatic bone lesions are difficult to reliably obtain and often do not reflect the evolving biology of tumors before and after treatment [4]. Therefore, in

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the setting of metastatic prostate cancer, circulating biomarkers in the peripheral blood are particularly appealing, as they may be assessed noninvasively and repeatedly throughout therapy.

The most widely used circulating biomarker in the care of men with prostate cancer is prostate-specific antigen (PSA, also known as kallikrein-3), a serine protease produced by normal and cancerous prostate epithelial cells. Although characterized as a tumor marker, PSA is produced by normal prostate cells and by other organs in men and women and is therefore not specific for cancer, gland, or sex [5]. Most but not all prostate cancers are associated with elevated serum PSA level. PSA is regulated by circulating androgens, and its gene expression depends on activation of the androgen receptor (AR). Androgen deprivation therapy is typically associated with a decrease in serum PSA level, as well as improvement in disease-related symptoms and measurable metastatic disease. In the setting of mCRPC, PSA levels have prognostic value as an independent risk factor for mortality, and posttreatment changes in PSA level may reflect changes in tumor burden for some mCPRC therapies (reviewed in detail in Ref. [6]). However, posttreatment PSA level change has failed to satisfy the definition of a surrogate for OS for multiple therapies with varied mechanisms of action for mCRPC [7–9]. Accordingly, no therapy for prostate cancer has been approved solely based on an observed posttreatment decline in serum PSA level. This review will focus on alternative circulating biomarkers that have been proposed and studied in recent years.

Perhaps the most promising of these alternative circulating biomarkers are circulating tumor cells (CTCs) and cell-free DNA (cfDNA), so-called "liquid biopsies" that involve the noninvasive sampling and analysis of tumor-derived cells or nucleic acids in the peripheral blood [10,11]. Indeed, these approaches may not only enable the monitoring of treatment responses but may also provide detailed molecular information about their tumors that can predict response or resistance to specific treatments, and thus guide patients toward the appropriate next lines of therapy. This concept has become increasingly relevant in prostate cancer given our increased level of molecular understanding of prostate cancer through next-generation sequencing studies [12]. In this review, we provide an overview of published data regarding circulating biomarkers for men with mCRPC, with a focus on liquid biopsy approaches, their prognostic and predictive value (Table 1), and their potential to guide patient care.

Circulating tumor cells

CTCs are cancer cells that have been shed from primary or metastatic tumor deposits into the peripheral blood [13–15] and are genetically representative of the primary and metastatic tumors [16–19]. A total of 2 key limitations of CTC analyses include the rarity of CTCs, estimated at one cell per billion normal blood cells and the challenging prospect of reliable detection and isolation of these cells. In general, CTC detection strategies include (1) enrichment from blood cells by positively selecting CTCs using antibodies directed against an epithelial cell surface protein, (2) enrichment from blood cells by size-based separation, (3) depletion of blood cells using red blood cell lysis or depletion of common leukocyte antigen (CD45)–expressing leukocytes or both, and (4) CTC identification using immunofluorescence for specific proteins among a spread of the nucleated cells remaining in peripheral blood after red blood cell lysis. Details of the varied approaches to CTC isolation have been described recently in other reviews [13–15,20]. As the only Food and Drug Administration (FDA)–cleared CTC detection technology, the CellSearch assay (Veridex, USA) relies on magnetic beads coated with anti-EpCAM antibodies to capture CTCs, followed by confirmation as epithelial cells by positive expression of cytokeratin (CK)-8, CK-18, and CK-19 proteins and lack of CD45 expression by immunofluorescence staining [21]. This platform has several limitations, including its inability to capture mesenchymal CTCs that do not express EpCAM [22]. Other technologies have been developed to enable the capture of a more comprehensive range of CTC phenotypes, including the Epic Sciences platform and the negative selection–based CTC-iChip [23,24]. However, CellSearch has been the primary CTC detection platform used for large-scale patient studies that have assessed CTCs as a biomarker in mCRPC. These studies, described in more detail later, show that enumeration of CTCs correlates with clinical end points including survival and may thus serve as a prognostic biomarker (Table 1).

CTC enumeration

The prospective study that led to FDA clearance of prognostic use of the CellSearch assay in prostate cancer, IMMC38, demonstrated that CTCs are an independent predictor of OS [25]. This prospective study enrolled 276 patients with progressive mCRPC who were starting a new chemotherapy regimen. CTCs were evaluated in blood samples taken before treatment and monthly after initiation of therapy. Patients were categorized as having "unfavorable" ($\geq$5 CTCs in 7.5 ml of blood) or "favorable" ($<$5 CTCs in 7.5 ml of blood) CTC counts. IMMC38 met its primary end point, demonstrating that unfavorable posttreatment CTC counts were associated with shorter median OS when compared with favorable CTC counts (9.5 months vs. 20.7 months, hazard ratio [HR] = 4.5, $P < 0.0001$). Unfavorable pretreatment counts were also associated with decreased median OS (11.5 vs. 21.7 months). Additionally, patients who converted from unfavorable baseline CTC counts to favorable posttreatment CTC counts had improved median OS (from 6.8–21.3 months); conversely, those who converted from favorable to unfavorable CTC counts had reduced median OS (from $>$26 to 9.3 months). CTC abundance was a better predictor of OS than posttreatment changes in serum PSA levels at all time points.