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INVITED REVIEW

Prostate Cancer

Utility of cell-free nucleic acid and circulating tumor cell analyses in prostate cancer

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Prostate cancer is characterized by bone metastases and difficulty of objectively measuring disease burden. In this context, cell-free circulating tumor DNA (ctDNA) and circulating tumor cell (CTC) quantitation and genomic profiling afford the ability to noninvasively and serially monitor the tumor. Recent data suggest that ctDNA and CTC quantitation are prognostic for survival. Indeed, CTC enumeration using the CellSearch® platform is validated as a prognostic factor and warrants consideration as a stratification factor in randomized trials. Changes in quantities of CTCs using CellSearch also are prognostic and may be employed to detect a signal of activity of new agents. Molecular profiling of both CTCs and ctDNA for androgen receptor (AR) variants has been associated with outcomes in the setting of novel androgen inhibitors. Serial profiling to detect the evolution of new alterations may inform drug development and help develop precision medicine. The costs of these assays and the small quantities in which they are detectable in blood are a limitation, and novel platforms are required to address this challenge. The presence of multiple platforms to assay CTCs and ctDNA also warrants the consideration of a mechanism to allow comparison of data across platforms. Further validation and the continued development and standardization of these promising modalities will facilitate their adoption in the clinic.
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INTRODUCTION

With over one million cases diagnosed yearly worldwide, prostate cancer represents a spectrum of clinical presentations ranging from indolent localized cancers that may never require therapy to rapidly progressive and incurable metastatic castration-resistant disease.[1] In addition to established markers such as prostate-specific antigen (PSA) and Gleason score, newer technologies have been sought to assist in clarifying prostate cancer prognosis and predicting and monitoring response to therapy.[2,3] As early as the 1860s, it was recognized that circulating tumor cells (CTCs) could be identified in the blood of patients with solid tumors.[4] In the last decade, techniques for detecting and interrogating CTCs[5] and cell-free circulating tumor DNA (ctDNA) have expanded. In this review, we outline the technologies available for identifying and characterizing CTCs and ctDNA and examine the data supporting their use in caring for patients with prostate cancer. We will also discuss strategies for developing these technologies to advance the management of men with prostate cancer.

RATIONALE FOR IDENTIFYING CIRCULATING TUMOR COMPONENTS

Advanced cancers have long been known to demonstrate genomic heterogeneity, both temporally with new mutations developing over the course of or in response to therapeutic interventions, and spatially, with unique genetic alterations detected in different biopsy samples within the same patient.[6] This genetic diversity has been well documented in patients with localized prostatic adenocarcinoma.[7,8] It has been more difficult to assess clonal heterogeneity in patients with multiple sites of metastatic disease, because many of their osseous lesions present challenges to biopsy.[9] Additionally, processing of bone is known to damage the extracted genomic material. Robinson *et al.*[10] recently conducted a prospective whole-exome and transcriptome sequencing of bone and/or soft-tissue tumor biopsies from a cohort of 150 patients with metastatic castration-resistant prostate cancer. This study provides important insights and identified potentially clinically actionable aberrations in 89% of studied individuals, including 62.7% with alterations in androgen receptor (AR), 56.7% with recurrent E26 transformation-specific (ETS) fusions, 53.3% with alterations in tumor protein p53 (TP53), 40.7% with alterations in phosphatase and tensin homolog (PTEN), 22.7% with abnormalities in the DNA repair/recombination pathway (including BRCA1, BRCA2, serine/threonine kinase [ATM]), and abnormalities in the phosphoinositide 3-kinase (PI3K) pathway, B-Raf proto-oncogene, serine/threonine kinase (BRAF), and RB transcriptional corepressor 1 (RB1).[10] Identifying these abnormalities without the invasiveness of tissue biopsy(s) is one driver behind the pursuit of circulating tumor components.

DETECTION OF CTCS

Individual cells sloughed from a primary malignancy and spread through the vasculature or lymphatic channels have been implicated as drivers of metastasis.[11] These CTCs have been detected in patients with almost every type of solid tumor and are often characterized by a transition from epithelial to mesenchymal gene-expression patterns that has been proposed as a mechanism for cancer spread.[12] There are

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a variety of means for detecting CTCs as they must be isolated from the diverse cellular components of blood. One of the most widely employed means for isolating CTCs is through immunoaffinity for cell surface markers.[13,14] Magnetic beads or chip-based systems are coated with antibodies that help identify CTCs through both positive and negative selection.[15] A common positive target for these antibodies is epithelial cell adhesion molecule (EpCAM), a membrane glycoprotein present on epithelial tissues and strongly expressed on epithelial carcinomas.[16] The most validated and currently only FDA-approved commercially available immunoaffinity platform for identifying CTCs is the CellSearch® system (Janssen Diagnostics, LLC, Raritan, NJ, USA).[17] This is a commercial method in which 7.5 ml samples of blood are collected into proprietary preservative tubes and then centrifuged to separate solid components from plasma. Ferrofluid nanoparticles with antibodies directed at EpCAM are then used to isolate CTCs. The candidate CTCs are then exposed to antibodies against cytokeratin (CK), antibodies against CD45 (a leukocyte-specific marker), and a 4',6-diamidino-2-phenylindole (DAPI) nuclear stain. Candidate cells that stain for EpCAM, CK, and DAPI but are negative for CD45 are presented to an operator for final review and confirmed as CTCs.[18]

Immunoaffinity-based systems, like the CellSearch® platform, are dependent on the antibody target for detection of CTCs. As CTCs that have completed epithelial-to-mesenchymal transition may downregulate EpCAM, they may not always be detected by such an antibody-based approach designed to identify epithelial cells.[19] Alternative means for identifying CTCs have been developed to isolate cells capitalizing on their larger size, lower elasticity, and different dielectric properties, in many cases accommodating unfixed specimens and allowing greater manipulation of the identified cells.[14] Representative examples of such platforms include ApoStream® (ApoCell) and the Parsortix system (Angle).[20,21]

Another group of emerging competitors in the race to develop technologies that will allow rapid quantification and characterization of CTCs are platforms that incorporate high-resolution scanning algorithms followed by molecular characterization of the identified CTCs. Epic Sciences™ and Cynvenio™ are examples of such platforms that have been incorporated into a variety of trials including in prostate cancer that not only identify CTCs but can also be deployed to carry out further molecular characterization.[5,22] CTC genomes can be amplified and analyzed by immunofluorescence staining for protein expression, RNA sequencing or reverse transcription (RT)-polymerase chain reaction (PCR) for transcriptome alterations and expression (RNA), fluorescence *in situ* hybridization (FISH) for gene amplification, and next-generation sequencing (NGS) to detect point mutations.[23]

CTDNA

An alternative or perhaps complementary technology to analysis of CTCs is identification and manipulation of cell-free ctDNA. DNA and RNA were both noted to be circulating in the blood of healthy individuals as early as the 1940s, and in 1977, the levels of free DNA in many patients with metastatic cancer were observed to be significantly higher than in patients with localized neoplastic disease or healthy controls.[24,25] Later work identifying particular genomic aberrations in patients' circulating DNA specific to their known metastatic cancers helped confirm tumoral origin of at least a fraction of the circulating cell-free DNA (cfDNA).[26] Tumor-derived circulating cfDNA has been shown to be more fragmented than circulating DNA from healthy patients. In addition, circulating cfDNA appears in numerous structural forms including nucleosomes, microvesicular exosomes, apoptotic bodies, and DNA linked to serum proteins.[27] Much like the race to identify and characterize CTCs, emerging platforms have been developed for optimally detecting and manipulating ctDNA.

Guardant Health's Guardant 360™ assay is one platform representative of the technologies rapidly being incorporated into ongoing clinical trials focusing on evaluation of ctDNA. Completing this assay involves collection of two 10 ml tubes of whole blood in proprietary fixative that prevents degradation of white blood cells and release of germ-line DNA, thus enriching for ctDNA. ctDNA fragments are isolated from plasma and individual fragments are converted into digital sequence libraries that are subsequently amplified. A NGS panel is employed to identify point mutations or single-nucleotide variants (SNVs) in an expanding list of cancer-related potentially actionable genes, copy number variants in 18 genes, and 6-gene fusions.[28] In addition, a platform by Foundation Medicine® is also offered commercially to analyze a panel of genes for base substitutions, insertions/deletions (indels), copy number variations, and rearrangements/fusions.[29] Notably, ctDNA-based detection of specific EGFR gene mutations (Cobas®) is approved by the FDA as complementary assays to administer erlotinib (Exon 19 deletions and L858R mutations) or osimertinib (T790M mutations) to selected patients with advanced nonsmall cell lung cancer.[30]

CTCS VERSUS CTDNA

Both CTCs and ctDNA can be quantified and further analyzed to aid in management of oncologic disease, but there are unique advantages and disadvantages to working with each platform (**Table 1**). Both

Table 1: A comparison of circulating tumor cell versus circulating tumor DNA analyses

	CTCs	*ctDNA*
Target for analysis	Intact individual tumor cells circulating in peripheral blood[11]	Fragments of DNA released from necrotic, apoptotic, and viable tumor cells[27]
Methodologies for detection	CTCs detected/enriched from blood through assays focusing on immunoaffinity for cell-surface markers, size, elasticity, dielectric properties, *etc.*,[12,13,18–20]	ctDNA fragments isolated from plasma using PCR or next-generation sequencing-based techniques[28,29]
Downstream applications	Can be used to facilitate RNA, DNA, protein, and functional analyses[21,20]	Primarily DNA-based analyses[28]
Relative advantages	Only platform currently with FDA approval (CellSearch®)[18] allows greater spectrum of downstream analysis including potential for patient CTC culture in animal models potentially allowing testing of therapeutics[32,33]	Technically easier to isolate than CTCs so potentially cheaper platforms;[32] Greater sensitivity in many settings than CTC analysis;[31] ctDNA analysis theoretically offers snapshot of the entire genomic landscape of tumor burden[26]
Relative disadvantages	Low abundance of CTCs can inhibit detection; CTCs that have completed epithelial-to-mesenchymal transition may be missed by standard antibody-based detection;[18,19] Individual isolated CTCs may represent only a small fraction of genomic heterogeneity of tumor burden	Require identification of known genomic abnormalities to prove with certainty tumor origin;[26] ctDNA-based assays do not generally allow functional analyses

ctDNA: circulating tumor DNA; CTCs: circulating tumor cells; FDA: Food and Drug Administration; PCR: polymerase chain reaction

CTCs and ctDNA may capture alterations pooled from all sites of disease, but all malignant clones may not shed CTCs or ctDNA at the same level. In general, isolation and characterization of CTCs is more technically challenging than isolating ctDNA. In a trial of metastatic breast cancer patients, Dawson *et al.*[31] serially followed CTC count, ctDNA analysis, and serum CA 15-3 protein levels over the course of therapy. The presence of ctDNA was more sensitive for metastatic disease than CTCs, and concentration of ctDNA appeared to show greater correlation with changes in tumor burden than either CTC count or CA 15-3 level. ctDNA analysis may in some cases be more efficient and cost-effective than analysis of CTCs. In contrast, CTC technologies may offer other advantages such as allowing the functional analysis of downstream mRNA and protein, which is not feasible when analyzing ctDNA.[32] Early studies have suggested that it may be feasible to culture freshly harvested CTCs and then test them for drug sensitivity *ex vivo*.[33] As clinician comfort level improves with CTC and ctDNA-based technologies, both types of assay may be employed as complementary noninvasive platforms for molecular profiling of the malignancy and to expand patient care options.

APPLICATION OF CTC/CTDNA TECHNOLOGIES IN THE CLINICAL MANAGEMENT OF PROSTATE CANCER

As the number of modalities for detecting and interrogating CTCs and ctDNA has expanded, so too have the potential indications for these techniques in the clinical management of prostate cancer. A growing number of clinical trials have incorporated CTC and ctDNA technology into correlative or even primary endpoints. Early trials in this realm looked at quantifying CTCs and then ctDNA primarily as prognostic markers.[34] Newer trials have used both quantification and more detailed genomic analysis of CTCs and ctDNA to identify potential predictive biomarkers for response to a variety of treatment interventions. We will present a snapshot of some of the available data supporting the incorporation of CTC/ctDNA analysis into management of metastatic and localized prostate cancer.

Quantification of CTCs/ctDNA in determining prognosis of metastatic prostate cancer

Because both CTCs and ctDNA are most readily identifiable in the setting of advanced neoplastic disease, it is in the setting of metastatic prostate cancer that these technologies have been most thoroughly evaluated. de Bono *et al.*[34] used the previously discussed CellSearch® platform to enumerate CTCs in 276 patients with metastatic castration-resistant prostate cancer (mCRPC) before starting a new line of docetaxel chemotherapy and monthly thereafter. Patients were stratified into "favorable" and "unfavorable" categories determined by baseline CTC counts <5 or ≥5 CTCs per 7.5 ml blood, respectively. Patients with "unfavorable" CTC counts at baseline had median overall survival of 11.5 months versus 21.7 months in patients with "favorable" counts ($P < 0.0001$). In addition, subsequent CTC counts between 2 and 20 weeks after initiating therapy correlated with overall survival better than existing PSA decrement algorithms at all time points.[34] Expanding on this early work, subsequent CTC analysis built into randomized trials such as SWOG SD0421, a phase III trial of docetaxel with or without atrasentan for mCRPC, confirms the value of baseline CTC count and increases within 3 weeks as prognostic for survival.[35] Similarly, CTC count changes within 12 weeks were also reported to be prognostic in the setting of abiraterone acetate administered postdocetaxel in the COU-AA-301 trial.[36] More limited studies have also suggested a prognostic value to baseline CTC counts in the setting of metastatic hormone-sensitive prostate cancer.[37] Similar to CTCs, baseline ctDNA levels have also been identified as potentially prognostic of overall survival in mCRPC.[38]

Predictive abilities in the setting of advanced disease

It is the potential for CTC or ctDNA analysis to predict responses to therapy that has the most appeal in altering clinician prostate cancer management. In addition to prognosis, simple quantification of CTCs and ctDNA has some role in predicting early response to therapy. Thalgott *et al.*[39] recorded CTC counts using the CellSearch® system in 122 mCRPC patient samples during docetaxel chemotherapy at baseline and after one, four, and ten chemotherapy cycles. Categorical CTC counts (<5 per 7.5 ml blood *vs* ≥5 per 7.5 ml blood) were not only prognostic for overall survival, but high values after only one cycle of chemotherapy were independent prognostic markers of poor progression-free survival (PFS) and predictors of progressive disease after 4 cycles of chemotherapy. It is not necessarily quantification of CTCs/ctDNA, but instead more qualitative analysis that has shown the most efficacy in predicting disease response. Baseline tumor genomic changes including point mutations, copy number variations, oncogene overexpression, and even alternative splice variants have been correlated with response to particular therapeutics.[40–43]

CTC/ctDNA genomic analysis correlating with tumor genomic profiling

As demonstrated by Robinson *et al.*[10] patients with mCRPC often possess a variety of discrete genomic alterations in their metastatic tumors at any one time. Further analysis by Kumar *et al.*[44] in 176 tumor samples from 63 men with mCRPC, including several tumor sites from each patient, revealed similar recurrent aberrations in AR, ETS, TP53, RB1, and copy number variation across the series. In addition, comparison of mutations and copy number aberrations (CNA) across tumor locations from within individual patients showed significant concordance across the intra-patient tumors. That said, intra-individual genomic diversity did occur, and it is known that genomic changes develop over the clinical course of progressive metastatic cancer. In order for genomic analyses of CTCs or ctDNA fragments to provide clinically meaningful substitutes for serial biopsy of solid lesions, it must be assumed that these assays are representative of the patient-wide genomic landscape of the cancer. Several studies across multiple platforms have attempted to validate this belief.

A variety of small studies have shown reasonable concordance between CTCs and tumor tissue for genomic aberrations including copy number alterations, mutations, and rearrangements.[5,45,46] Similarly, evaluation of a series of 165 samples from patients with metastatic tumors (including prostate cancer) comparing tissue biopsy to ctDNA analysis with the Guardant 360™ platform revealed a clinical sensitivity of 85.0% for ctDNA analysis detecting mutated oncogenes noted in tissue biopsy, in conjunction with specificity of 99.6%.[28] Further extensive investigation of an association of different platforms of CTC and ctDNA analysis with tumor tissue analysis across different malignancies and following different lines of therapy is required.

The genomic landscape derived from ctDNA profiling and correlation with clinical outcomes

Having presumably established the relative validity of ctDNA analysis, researchers have now started to examine the association of the genomic landscape identified with this technology with responses to therapy. One large retrospective series evaluated the results of ctDNA genomic profiling using Guardant 360™ assays that had been obtained from 514 men with progressive mCRPC before switch to a new line of systemic therapy. In this study, 94% of patients had at least one ctDNA alteration

detected, and the alterations appeared similar to those previously derived from tumor tissue. The most common recurrent somatic mutations were observed in TP53 (36% of patients); AR (22%); APC (10%); NF1 (9%); EGFR, CTNNB1, and ARID1A (6% each); and BRCA1, BRCA2, and PIK3CA (5% each). The most common genes with increased copy numbers were AR (30%), MYC (20%), and BRAF (18%). Using data from the 163 patients receiving chemotherapy or androgen inhibitors (enzalutamide and abiraterone acetate) in whom clinical outcomes were available, higher number of ctDNA alterations was associated with shorter time to treatment failure (TTF; hazard ratio [HR]: 1.05, $P = 0.026$). Serial profiling in a proportion of patients also showed a trend for developing new mutations particularly in AR and BRCA.[47]

Association of ctDNA and CTC profiling with response to therapy

In a series of 65 patients with mCRPC initiated on enzalutamide, Wyatt *et al.*[48] collected serial cfDNA samples at baseline, 12 weeks, and end of treatment. The samples were interrogated with copy number variation (CNV) profiling and deep androgen receptor sequencing. AR mutations and/or CNV were detected in 48% of baseline samples and 60% of samples at progression. Heavily mutated AR, defined as >2 mutations, presence of AR amplification, and RB1 loss were all associated with decreased PFS on enzalutamide with HRs for progression of 3.94, 2.92, and 4.46, respectively. Correspondingly, Romanel *et al.*[49] analyzed 274 ctDNA samples at baseline and progression for AR copy number and mutational status from 97 patients with mCRPC treated with abiraterone. Patients with baseline AR amplification or mutation, particularly at T878A and L702H, were 4.9 times and 7.8 times less likely to have PSA responses to abiraterone of ≥50% or ≥90%, respectively, coupled with significantly worse survival. These data regarding the deleterious impact of AR mutations and copy number gain on outcomes with enzalutamide or abiraterone have also been shown in other studies.[50,51] At this time, clinicians might be justified in using this kind of genomic information obtained from circulating tumor components to select docetaxel as compared to a novel antiandrogen for a patient's next therapy. Pending randomized trials will need to help clarify whether such genomically driven sequencing decisions improve overall survival.

In addition to point mutations and amplifications of AR, novel AR splice variants have also been demonstrated to predict poor response to abiraterone and enzalutamide in some series. The AR isoform encoded by splice variant V7 (AR-V7) lacks a functional ligand binding domain facilitating androgen independent AR signaling in mCRPC.[52] Antonarakis *et al.*[42] isolated CTCs from 202 men with mCRPC beginning abiraterone or enzalutamide. The CTCs were isolated using a commercially available AdnaTest® platform (Qiagen), and then subsequent mRNA expression analysis was performed to evaluate for the presence of AR-V7 mRNA transcript in the CTCs. There were 3 prognostic categories with best outcomes observed for CTC− patients, while CTC+/AR-V7+ patients exhibited the worst outcomes, and CTC+/AR-V7− patients exhibited intermediate outcomes.[43] Unlike hormonal therapies, subsequent analyses have shown that AR-V7 positivity in CTCs does not necessarily confer resistance to docetaxel or cabazitaxel.[53,54]

Detection of AR-V7 protein in CTCs has also been used as an alternative means to predict lack of treatment response. Using the previously described Epic Sciences™ platform, Scher *et al.*[55] prospectively isolated CTCs from 193 blood samples derived from 161 men with mCRPC initiating a new line of systemic treatment. The researchers identified AR-V7 protein in the CTCs using an immunofluorescence assay employing an anti-AR-V7 rabbit monoclonal antibody. After adjusting for other clinical factors associated with survival, patients with baseline CTC AR-V7 protein positivity had superior overall survival when treated with taxanes relative to AR inhibitors (HR for death: 0.24, 95% confidential interval [CI]: 0.10–0.57; $P = 0.35$). In an additional analysis, AR-V7 protein detection was further delineated to nuclear-specific (18% of samples) versus agnostic to location (nuclear, cytoplasmic, etc., in 29% of samples). On multivariate analysis, nuclear-specific AR-V7 protein positivity was again associated with lower risk of death when treated with taxanes as compared to AR inhibitors (HR: 0.24, 95% CI: 0.078–0.79; $P = 0.019$), but interestingly, location-agnostic AR-V7 protein positivity lost significant association with survival.

Androgen receptor splice variant analysis is not limited to CTCs but can also be applied to circulating tumor nucleic acids. Del Re *et al.*[56] developed a digital droplet polymerase chain reaction (ddPCR) technique for detecting AR-V7 in plasma-derived exosomal mRNA. Thirty-six patients with mCRPC had this analysis performed on their plasma before initiating either abiraterone or enzalutamide. Thirty-nine percent of patients were AR-V7+ at baseline, and analogous to results derived from CTCs, median PFS was significantly longer in this series in AR-V7-negative versus AR-V7-positive patients (20 *vs* 3 months; $P < 0.001$). Similar analysis has been performed using PCR-based assays of whole blood to detect circulating RNA capable of expressing AR-V7 and correlating this finding with lack of response to abiraterone.[57]

Despite studies linking detection of AR-V7 splice variant, whether in CTCs or blood/serum, with inferior response to second-generation hormonal therapy, it is not clear that the presence of AR-V7 is entirely exclusive of response to these drugs or should preclude trial of these medications in such patients. Bernemann *et al.*[58] retrospectively analyzed a group of 21 men with mCRPC who had baseline AR-V7 positivity determined through CTC mRNA analysis and then received abiraterone or enzalutamide. Six of these patients (28.6%) had either PSA stabilization or response with this therapy, suggesting a subgroup of patients with AR-V7 positivity who might benefit from second-generation androgen-directed therapy. In the aforementioned analysis of 202 men with mCRPC, in which Antonarakis *et al.*[42] help establish the prognostic value of AR-V7 positivity, the authors acknowledge that PSA decreases >50% from baseline were noted in 26.7% of CTC+/AR-V7. The authors note that patients who developed responses had lower full-length androgen receptor transcript (AR-FL) levels and lower AR-V7/AR-FL ratios. They postulate that CTCs are heterogeneous in terms of AR-V7 expression and this likely contributes to disease response. These data along with other series of CTC analyses implicating a variety of pathways including noncanonical Wnt signaling in antiandrogen therapy resistance suggest that predicting patient response to these therapeutics has not been perfected.[59] As attempts to commercialize AR-V7 detection progress, attention will have to be focused on the optimal means of analysis, and further prospective randomized trials will need to incorporate this technology into evaluation of new therapeutics.[60]

Examination of circulating tumor components to identify novel actionable therapeutic targets

Beyond predicting response to existing therapies, a way forward in advancing metastatic prostate-cancer management is to exploit serial assessment of circulating tumor components to efficiently locate individualized therapeutic targets. A challenge is that longitudinal monitoring of ctDNA suggests substantial tumor heterogeneity and the presence of separate clones behaving discordantly.[61] Multiple tissue-biopsy-based and ctDNA-based series have identified

DNA-repair defects in a percentage of patients with metastatic prostate cancer.[10,47,48] In a provocative phase 2 trial, Mateo *et al.*[62] treated 50 men with mCRPC, the majority of whom had progressed on docetaxel, AR inhibitors, and cabazitaxel, with the poly adenosine diphosphate (ADP)-ribose polymerase (PARP) inhibitor olaparib. Overall, 16 patients of 49 evaluable (33%) had Response Evaluation Criteria In Solid Tumors (RECIST) or PSA response. NGS of tumor tissue identified DNA-repair defects including abnormalities in BRCA 1/2, ATM, and CHEK2 in 16 patients, and 14 (88%) of them displayed a response to olaparib. In a subsequent series of 319 patients with mCRPC, 5% of patients had germline BRCA mutations identified. Ten out of 11 evaluable patients with germline BRCA2 mutations had somatic deletion of the intact allele in ctDNA.[63] The logical step forward will be designing randomized trials with ctDNA or CTC-based assays for particular genomic abnormalities as screening tools to enrich the patient population for response to therapy.

Detecting changes in histology

Beyond refining prostate cancer diagnosis, circulating tumor components may also play a more nuanced role in identifying changes in histology, which may have therapeutic ramifications. For example, neuroendocrine differentiation may respond to platinum-based chemotherapy.[64] Beltran *et al.*[65] prospectively analyzed metastatic tumor biopsies, serum biomarkers, and CTCs using the Epic Sciences™ platform from 27 patients with mCRPC including 12 patients with neuroendocrine prostate cancer and 5 with clinical features suggestive of neuroendocrine transition. CTCs from neuroendocrine prostate cancers demonstrated unique morphologic characteristics as compared to typical mCRPC with low or absent AR expression, low CK expression, and smaller size, suggesting a potential means for diagnosing this histology without an invasive tissue biopsy. Additional analyses have identified genomic alterations that correlate with neuroendocrine variant prostate cancer including combined alterations in RB1, TP53, and PTEN raising the possibility of ctDNA or CTC-based genomic analysis as an additional means of screening for patients who may have developed this aggressive variant prostate cancer.[66]

CTCS/CTDNA IN THE SETTING OF LOCALIZED PROSTATE CANCER

Although CTC and ctDNA analysis has been most robust in the setting of advanced disease, both of these technologies are potentially applicable to localized prostate cancer as well.

Association of CTCs and ctDNA with outcomes in the setting of localized disease

In one series of 86 patients with high-risk localized prostate cancer (defined as PSA ≥20 ng ml^{-1} and/or Gleason Score ≥8 and/or clinical tumor stage ≥2c), three platforms, CellSearch®, CellCollector® (GILUPI), and EPISPOT were used to attempt to detect CTCs in blood from each patient before and after radical prostatectomy.[17,67,68] CTCs were detected preoperatively in 37%, 54.9%, and 58.7% of patients with each of these respective platforms.[69] Analysis of 30 samples revealed a significant decrease in CTCs by the CellCollector platform from 66% before surgery to 34% after surgery, although the clinical implications are unclear. These data suggest the potential to identify those with residual microscopic disease, which may help select the highest risk patients for adjuvant therapy. CTC detection by EPISPOT before prostatectomy was associated with PSA levels and stage. Additional series of patients with localized prostate cancer have identified a significant proportion of patients with CTCs and ctDNA detectable in the blood.[70–72] Postprostatectomy studies have been inconclusive as to the ability of CTCs to detect biochemical recurrence with some series showing only a minority of such patients having detectable CTCs.[73] One prospective analysis performed by Murray *et al.*[74] in 321 men with resected localized prostate cancer examined patient blood at 90 days postsurgery for the presence of PSA-positive, CD45-negative, circulating prostate cells (CPCs). CPC testing was considered positive when at least one cell was detected per 8 ml of blood. In addition, clinical and pathologic surgical features were used to calculate a Cancer of the Prostate Risk Assessment (CAPRA-S) score as previously described.[75] The results showed that the incorporation of CPCs augmented the discriminative ability of CAPRA-S for predicting biochemical recurrence.[74] It remains to be determined whether perioperative CTC or ctDNA analysis might predict patients who benefit from adjuvant or early salvage radiation or androgen deprivation therapy.

Preoperative serum cfDNA concentration appeared prognostic in 192 men undergoing radical prostatectomy.[72] In this study, 29% of men eventually developed PSA recurrence, and in multivariate analysis, baseline serum cfDNA concentration was significantly associated with PSA recurrence. The mean serum DNA concentrations were 13.7 ng ml^{-1} and 3.8 ng ml^{-1} ($P = 0.001$) in patients who did and did not recur, respectively. The optimal cut-point for determining increased risk for PSA recurrence within 2 years of radical prostatectomy was postulated as >5.75 ng ml^{-1}.

Diagnostic role for circulating tumor components

As the role of screening PSA levels and prostate biopsies has become more controversial, alternative strategies have been sought to aid in diagnosis of prostate cancer.[76,77] In the Prostate Cancer Prevention Trial, among patients with PSA <4.0 ng ml^{-1}, prostate biopsies revealed 15.2% with prostate cancer including 14.9% of those with Gleason score 7–9 disease. Indeed, among those with PSA ≥4 ng ml^{-1}, biopsy appears to have a meager 20.5% sensitivity.[78,79] A recent meta-analysis of 19 trials revealed that quantitative ctDNA levels had collective sensitivity of 73% and specificity of 80% to diagnose prostate cancer.[80] In addition to ctDNA levels, epigenetic modifications such as hypermethylation of glutathione S-transferase 1 (GSTP1) can be investigated.[81] Pooled analysis of studies incorporating qualitative ctDNA characterization including GSTP1 methylation status into the process of diagnosis displayed a sensitivity of 34% and specificity of 99% (95% CI: 0.97–1.00).[80]

EXPERT COMMENTARY

Technologies for detecting CTCs and ctDNA are rapidly being refined, and their everyday usage in treating patients with prostate cancer is likely going to increase (**Figure 1**). Baseline quantification of CTCs and ctDNA levels has been shown to have prognostic value in the setting of metastatic prostate cancer, and ctDNA levels have shown some promise in this regard in the setting of localized disease.[34,35,38,72] Postprostatectomy analyses of CTC and ctDNA levels have shown mixed success in prognosticating biochemical recurrence, but if further development of such techniques could allow earlier detection of minimal residual or high-risk disease after prostatectomy, it could become easier to select patients who require adjuvant or salvage therapies.[73,74,82]

Phase II trials investigating new agents for mCRPC are plagued by limitations of bone metastases, which are not measurable, and the flaws of PSA changes as a surrogate endpoint.[83] Given these problems of detecting early signals of activity when evaluating novel agents with new mechanisms of activity, serial CTC enumeration may serve the purpose of detecting activity as a correlative endpoint. The caveat is that

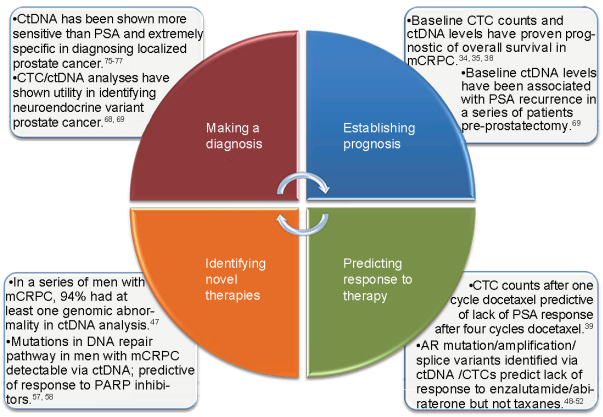

Figure 1: CTCs and ctDNA analysis in prostate cancer proof of concept. CTC: circulating tumor cell; ctDNA: cell-free circulating tumor DNA; mCRPC: metastatic castration-resistant prostate cancer; PARP: poly adenosine diphosphate (ADP)-ribose polymerase; PSA: prostate-specific antigen.

even CTC changes need validation in settings other than chemotherapy and androgen inhibitors. In addition, CTC numbers may be useful as a stratification factor in randomized clinical trials, given the prognostic impact. However, the role of measuring CTCs in routine clinical care is unclear. Switching to a different chemotherapy based on CTC changes after 3 weeks did not lead to better clinical outcomes in women with metastatic breast cancer.[84] Additionally, a significant fraction of patients does not have detectable CTCs at least when using the CellSearch platform, which diminishes its utility for discrimination. Indeed, the small quantities of CTCs and ctDNA detectable in blood are a major barrier to overcome.

Patients with metastatic prostate cancer possess unique combinations of tumoral genomic abnormalities as evidenced by series that looked at tissue biopsy as well as CTC and ctDNA genomic interrogation.[10,46,47] Baseline alterations of AR detected by both CTC and ctDNA analyses have shown prognostic ability in the setting of abiraterone or enzalutamide for mCRPC, although taxanes appear active regardless of AR alterations. However, further validation of these findings is required to enable use in the clinic.[42,43,48,49] It remains to be seen how these genetic abnormalities evolve or become extinct over the course of therapy. Evolving alterations may provide critical insights regarding mechanisms of resistance and inform drug development and rational combinations. Limited data suggest that taxane chemotherapy may reverse some resistance to androgen inhibitors prompting the question of whether such a change could be detected in circulating tumor components.[85] In this context, taxane chemotherapy has been reported to eradicate AR-V7 expressing CTCs, although it is unclear if this restores sensitivity to enzalutamide or abiraterone.[86]

As the treatment of progressive prostate cancer moves toward more individualized therapy based on a unique set of genetic abnormalities in each patient, rapid analysis of the genomic landscape through examination of CTCs and ctDNA is likely to be invaluable. It has been shown that novel clinical targets including new defects in AR, the DNA repair pathways, PTEN/AKT, BRAF, and many others emerge over the course of therapy and can be detected via analysis of circulating tumor components.[47,87] It has also been shown in limited fashion that treatment selection based on the presence of genomic changes in tumor tissue can increase response rates to novel targeted therapies, such as PARP inhibitors.[62] Clearly, these data need to be replicated in the context of CTCs and ctDNA. Immunotherapy has not yet been refined for advanced prostate cancer, but potential biomarkers for response to immunotherapies such as programmed death 1 (PD-L1) expression levels using CTCs or ctDNA-based techniques have already been demonstrated.[88,89] True validation of the role of studying circulating tumor components will occur as new prostate cancer studies designed to incorporate baseline and subsequent analyses of CTCs and ctDNA into predicting response to therapies and ultimately selecting novel therapies that improve overall survival.

Finally, the costs of these assays and presence of multiple platforms to assay CTCs and ctDNA introduce challenges in their development for clinical use. It is unclear if specific platforms may be more robust in certain settings. Thus, these platforms should ideally be compared or standardized in some fashion to allow comparison of data across platforms. An example of one such effort is a study that will enroll 120 predocetaxel mCRPC patients beginning enzalutamide or abiraterone to perform serial AR-V7 profiling employing 3 platforms for each patient: the AdnaTest, Rosettesep, and EPIC Sciences. Moreover, the complementary role, if any, of other noninvasive modes of genomic assays such as urinary DNA and whole blood or peripheral blood monoculear cell (PBMC) RNA profiling requires study.

AUTHOR CONTRIBUTIONS

Both authors participated in the concept, design, and writing of this review. Both authors read and approved the final manuscript.

COMPETING INTERESTS

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