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Clinical Utility of Circulating Tumor Cells in Advanced Prostate Cancer

Tian Zhang[1] · **Andrew J. Armstrong**[1,2]

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Abstract Men with metastatic castration-resistant prostate cancer (mCRPC) frequently have circulating tumor cells (CTCs) that are detectable in their peripheral blood. The CellSearch® method of enumerating CTCs is presently the only FDA-cleared CTC test available clinically for men with mCRPC and has been shown to have prognostic significance in this setting, both before and during systemic therapy. Clinical utility, reflecting the ability of this test to favorably change outcomes, is a more controversial and higher bar. The CellSearch® CTC assay can provide updated prognostic and potentially surrogate information in specific clinical scenarios and in clinical trials, but formal randomized trials of clinical utility remain an unmet clinical need. Recent data suggest that CTCs may harbor genetic information (such as the androgen receptor splice variant 7, AR-V7) relevant to changing clinical management and predicting treatment sensitivity or resistance to cancer therapies such as enzalutamide, abiraterone, and taxane chemotherapies. Further molecular characterization of CTCs, cell-free DNA, or RNA can also provide additional information that may have clinical utility. Thus, CTC research is moving toward predictive medicine, based on the biologic characterization and improvements in clinical outcomes associated with heterogeneous cell types both within and between patients.

Keywords Circulating tumor cells · Clinical utility · Castration-resistant prostate cancer · Metastatic prostate cancer · AR-V7 · Clinical biomarkers

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✉ Andrew J. Armstrong
andrew.armstrong@dm.duke.edu

Tian Zhang
tian.zhang2@dm.duke.edu

[1] Division of Medical Oncology, Department of Medicine, Duke Cancer Institute, Duke University Medical Center DUMC 103861, Durham, NC 27710, USA

[2] Division of Urology, Department of Surgery, Duke Cancer Institute, Duke University Medical Center DUMC 103861, Durham, NC 27710, USA

Introduction

Circulating tumor cells (CTCs) were initially described in 1869 from a 38-year-old patient who had multiple subcutaneous tumors and an unknown primary site of disease [1]. The morphology of cells isolated from his peripheral blood was similar to that of cells seen on tumor biopsy. Ashworth concluded that "cells identical with those of the cancer itself… may tend to throw some light upon the mode of origin of multiple tumours existing in the same person," [1] thus commenting on the process of metastatic disease. Indeed, our current understanding of CTCs is that they are shed from primary or metastatic tumors into the circulation and lodge in distant sites to propagate metastases over time [2•].

More than a century and a half later, there are now more than 30 technologies available to isolate CTCs, separating CTCs based on size, density, electric charge, and cell surface markers [2•, 3•]. We now know that CTCs can express both epithelial and mesenchymal cell surface markers [4, 5], that CTCs can be molecularly characterized at the single or pooled cell level, and that RNA, protein, and DNA within CTCs can be measured and connected to clinical outcomes. In addition, emerging technologies are now able to isolate cell-free DNA or RNA to measure such biomarkers without the requirement for CTCs [2•, 6]. Finally, there is clear recognition that CTCs and their biomarkers are dynamic and can change over time

depending on treatment and selection pressures in the cancer and cancer micro- or macroenvironment.

For purposes of this concise clinical review, we will focus on the FDA-approved CellSearch® CTC assay and its clinical utility based on the present state of the science, and highlight key emerging CTC-based technologies as examples that are moving rapidly toward the demonstration of clinical utility in men with advanced prostate cancer.

Defining Clinical Utility

Clinical utility for a diagnostic test has a formal definition through the US Department of Health and Human Services Agency for Healthcare Research and Quality. In this document, criteria for analytic validity are provided as are criteria for Clinical Laboratories Improvement Amendment (CLIA) Act certification, which are beyond the scope of this clinical review (see http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm124208.htm). Clinical validity is defined as being a test that is clinically usable [7], based on reliability, accuracy, and needed sensitivity/specificity and predictive value to impact patient care. In contrast, clinical utility refers to the ability of a test to be useful to medical practice, through improved benefits or reductions in harms or costs above and beyond the best available tests. This risk/benefit assessment can be applied at the individual level, to groups of patients, and at a societal level. CTCs could impact clinical utility in a number of ways, ranging from changing treatment decisions (stopping a therapy when it is no longer working or continuing a therapy when there is ongoing benefit), improving tolerability of a systemic regimen (through early treatment cessation or appropriate treatment selection, including no treatment), improving survival (through improved treatment selection and reduction in toxicity), and improving cost-effectiveness (through reductions in ineffective drug exposure times). To date, however, most of the existing data around CTCs as measured in men with metastatic castration-resistant prostate cancer (mCRPC) has focused on prognosis, which while important, has not led to changes in management or formal testing of treatment switching strategies. Thus, we will next focus this review on the existing data around CTCs as a prognostic and potentially surrogate biomarker.

CTCs as a Prognostic Biomarker

CTC-derived PSA by rtPCR was one of the first assays to demonstrated clinically relevant prognostic information in men with mCRPC. However, further development of this assay was not pursued despite its relative ease of use and measurement and despite the independent prognostic information provided beyond serum PSA protein measurements [8, 9]. However, these data led to the concept in CRPC of circulating tumor cell-based biomarkers of treatment resistance and outcome.

The first analytically and clinically validated CTC detection platform was the CellSearch® assay, which captures cells expressing epithelial cell adhesion molecule (EpCAM) with an anti-EpCAM antibody conjugated to a ferromagnetic bead. Cells are isolated and then stained for cytokeratins (CKs) as well as CD45, a common leukocyte marker. CTCs are selected as captured cells positive for CK and negative for CD45. The initial clinical study using the CellSearch technology assessed 964 patients with malignant disease, 123 of whom had metastatic prostate cancer. These patients contributed 188 samples (each with 7.5 mL of whole blood), with 77 (41 %) of the samples having more than 5 CTCs [10]. In 344 healthy volunteer or benign disease samples, 5 % had 1 CTC per 7.5 mL of blood and none had more than 2 CTCs in 7.5 mL of blood [10]. CellSearch was thus a sensitive platform for CTC detection in prostate cancer patients. In addition, the analytic validity of the test was ascertained through repeatability assays, spiking/recovery assays, and comparisons of local vs. central laboratory assays. Based on these results, sensitivity down to a single cell was demonstrated, with a level of variability ranging from 8 to 10 % in repeat sampling. Based on the absence of CTCs in healthy controls, a high specificity (>99 %) using a cutoff of a single CTC was observed. In metastatic prostate cancer, the number of CTCs detected per 7.5 mL of whole blood can range widely depending on the context.

CTCs detected by the CellSearch assay were shown to be prognostic for survival in patients with metastatic castration-resistant prostate cancer (mCRPC) [11, 12]. In one prospective study, the median CTC count was 16 but detection ranged from 0 to 847 cells in a single 7.5-mL tube of blood. Reproducibility was high (>99 %), and additional molecular analyses of these cells confirmed AR amplification or expression of other PC-specific biomarkers, confirming their malignant origin [13]. In an additional prospective study of the prognostic impact of CTCs, de Bono et al. found worse overall survival for patients who had ≥5 CTCs compared to those who had <5 CTCs prior to starting a new cytotoxic therapy. Patients who had ≥5 CTCs had a median overall survival of 11.5 months, compared to 21.7 months for those who had <5 CTCs (HR 3.3, 95 % CI 2.2–5.1, p value <0.0001) [11]. Therefore, the cutoff of ≥5 CTCs at baseline was found to be a prognostic marker in indicating worse survival on treatment. Furthermore, de Bono et al. found that enumerating CTCs at 9–12 weeks after starting a new therapy (mostly docetaxel) had more accuracy of predicting survival than 30 % PSA decline (0.82 vs. 0.68, respectively, in the area under the curve for the receiver operating characteristic) [11]. Men with mCRPC who had unfavorable to favorable CTC conversions demonstrated similar survival to those who had favorable CTCs at baseline,

indicating the ability of CTCs to provide updated prognostic information depending on the response of a given patient. Finally, baseline CTC enumeration was independently prognostic after adjusting for other known prognostic factors such as functional status, hemoglobin, LDH, and alkaline phosphatase and was one of the strongest prognostic factors for survival. These data suggested that CTCs can provide independent prognostic information on overall survival over time above and beyond what would be anticipated based on standard clinical and biomarker data.

A recent single-center clinical validation analysis of CellSearch® CTCs in 89 patients with metastatic prostate cancer confirmed that CellSearch® CTC enumeration was associated with poor survival (CTCs <5 had median overall survival of 16.6 months compared to CTCs >5 with median overall survival of 8.9 months, HR 0.43, 95 % CI 0.24–0.77) [14]. CTCs provided independent prognostic information on both overall and progression-free survival in these men with mCRPC and provided prognostic information even in subgroups defined by visceral metastatic disease. CTC levels were not well predicted by standard blood-based biomarkers such as PSA, hemoglobin, LDH, and alkaline phosphatase, suggesting that the CTC dissemination phenotype is an independent poor-risk phenotype distinct from other clinical phenotypes [14, 15].

Pre-treatment CTCs were recently demonstrated to provide prognostic information in a large phase 3 validation trial (SWOG S0421), in which over 263 evaluable men with mCRPC were followed over time during docetaxel-based chemotherapy [16•]. In this study, median CellSearch CTCs were 5 cells/7.5 mL whole blood, and patients with high CTCs were more likely to have higher PSA, liver metastases, anemia, and higher alkaline phosphatase and bone pain. PSA responses to chemotherapy were seen in 63 % of the high (≥5) CTC men as compared to 44 % of the low (<5) CTC men ($p = 0.01$), and RECIST responses were also less commonly observed in high vs. low CTC men (14 vs. 31 %, respectively). Survival was also independently linked to high CTCs at baseline, and in this study, CTCs were characterized by 0, 1–5, 6–53, and >54 cells, with corresponding median overall survival (OS) estimates of 28, 23, 14, and 11 months, respectively (Fig. 1) [16•]. Collectively, these data illustrate that CTCs are associated with poor outcomes as a continuous variable, and provide independent prognostic information around survival, above and beyond radiographic and PSA responses.

Post-treatment CTC monitoring for response or progression can provide updated survival prognostication in combination with additional biomarkers. For example, in men with mCRPC who have a persistently elevated LDH and CTC levels at 12 weeks following docetaxel chemotherapy, survival outcomes are inferior to those in men with normalized CTCs and LDH [17]. In this study, CTCs were highest in men with bone metastases as compared with lymph node metastases, illustrating that CTC dissemination may reflect the hematogenous vs. lymphangitic spread of tumor cells, and this information carries prognostic importance over time [17].

The CTC-LDH biomarker panel was validated in a recent study, which verified the prognostic significance of these two blood-based assays, but also analyzed their surrogate value. A surrogate biomarker is an intermediate outcome that fully captures the effect of treatment on overall survival. Presently, no surrogates exist in trials of men with mCRPC, and this represents an unmet need for drug development. Scher et al. examined the biomarkers taken at 12 weeks on a large prospective phase 3 trial, COU-AA-301, in which 711 patients were treated with abiraterone acetate plus prednisone vs. prednisone alone in the past-docetaxel setting and had evaluable CTCs for analysis [18••]. The combination of CTCs and LDH levels at 12 weeks fulfilled all four Prentice surrogacy criteria [19] for predicting overall survival, while CTCs alone failed to meet all surrogacy criteria. In this study, treatment was associated with improved survival (criterion 1), treatment had a significant impact on CTCs and the CTC/LDH biomarker panel (criterion 2), and the CTC and CTC/LDH biomarker

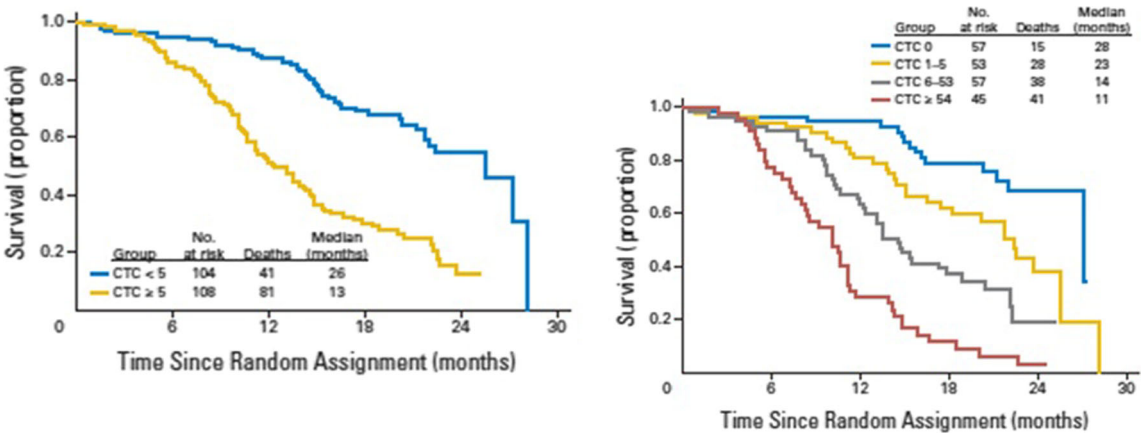

Fig. 1 Kaplan-Meier survival curves of overall survival with baseline CTC cutoff of <5 or >5 (**a**) or baseline CTCs of 0, 1–5, 6–53, and >54 (**b**). From [16•]. Reprinted with permission. ©2014 American Society of Clinical Oncology. All rights reserved

outcomes were associated with survival (criterion 3). However, equivalency of survival outcomes after adjusting for CTCs could not be demonstrated. Moreover, this equivalency was demonstrated for the CTC/LDH biomarker panel, in which the impact of treatment on survival lost significance when adjusting for the surrogate. Patients who had CTCs ≥5 cells and high LDH >250 U/L at 12 weeks had a 2-year overall survival rate of 2 % compared to 46 % for those patients who had CTCs <5 cells and low LDH <250 U/L [18••]. Median OS in the high CTC/LDH group was 8.7 months as compared to a median OS of 22.2 months in the low CTC/LDH group, while the intermediate group of high CTC and low LDH had an intermediate median OS of 12 months ($p < 0.001$) (Fig. 2) [18••]. While this is just a single study and requires external validation in other positive phase 3 trials in men with mCRPC, these data suggest that CTCs can be useful as part of a biomarker panel, to estimate survival outcomes long term after just 3 months of therapy with abiraterone acetate in the post-docetaxel treatment space. Whether validation data can be generated in the chemotherapy-naïve treatment space (where abiraterone acetate and enzalutamide are now used) is unknown, given that many men in these earlier disease states have low CTCs and LDH levels. Finally, these data do not specify which treatment to use in these persistently poor-risk patients, and thus the clinical utility of such a biomarker panel is not firmly established. Presently, it is unclear how management should change based on the CTC/LDH biomarker panel in isolation and much more impact and clinical utility the combination of CTCs and LDH provides over standard radiographic, clinical, and PSA-based assessments.

As yet, the detection and enumeration of CTCs in patients with mCRPC have not been found to predict treatment response for any therapy and have yet to be successfully used to guide treatment and result in improved clinical outcomes,

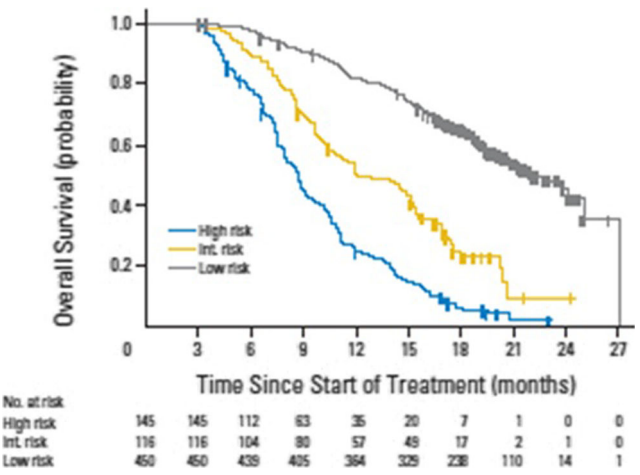

Fig. 2 Kaplan-Meier survival curve of overall survival for patients separated into high, intermediate, and low risk based on CTC enumeration and LDH level. From [18••]. Reprinted with permission. ©2015 American Society of Clinical Oncology. All rights reserved

whether hormone based or chemotherapy based. In women with metastatic breast cancer, switching therapy early based on unfavorable CTC declines did not result in improved survival over standard clinical management [20], possibly due to the lack of effective systemic agents against the metastatic dissemination process in chemorefractory disease. While CTCs are adversely prognostic in metastatic breast cancer, their enumeration was not predictive of improved survival through early changes in therapy. Thus, clinical utility requires both CTCs to be measured reliably, to be adversely prognostic, but also therapies that are effective in these poor-risk patients need to be available. We and others have recently reviewed such approaches to using CTCs for molecular profiling, tumor phenotyping, and developing targets for therapy [2•, 3•, 21]. Recent data suggests that in some contexts and under specific conditions, CTCs can be cultured and drug susceptibility testing can be performed, which may provide novel information to help guide therapy [22–24]. However, presently, the clinical utility of CellSearch® is limited to indicating prognosis over time in the context of an overall clinical assessment.

One limitation of the CellSearch®-defined CTC assay is its dependency on EpCAM, a biomarker of epithelial cell differentiation. Our group and others have shown that CTCs often display both epithelial and mesenchymal cell surface markers [4, 22, 25, 26]. In addition, different cell phenotypes, such as stem-like or de-differentiated cells, may not be detected using the CellSearch® assay due to lack of EpCAM expression. Moreover, CTCs are often not reliably detected until a patient has widely metastatic disease, with disease refractory to chemotherapy and limited therapeutic options. Therefore, in these clinical scenarios, CellSearch® CTCs do not have clinical utility to change clinical management and thus are often not ordered as part of standard clinical care. For this reason, additional approaches are under development using negative selection or physiologic properties of CTCs to enrich for important CTC subsets, including CTC clusters, EpCAM-negative CTCs, and other phenotypes [3•, 27, 28].

AR-V7, a Predictive Biomarker

Several splice variants of the androgen receptor (AR), including AR splice variant 7 (AR-V7), lack the ligand-binding domain [29]. This leads to androgen-independent AR signaling and prostate cancer growth. The ability to detect such variants in peripheral blood has the potential to be a CTC-based test with clinical utility, given its potential negative predictive value for the benefit of novel hormonal therapies.

A recent study by Antonarakis et al. examined the clinical outcomes of men with mCRPC based on the presence of AR-V7 RNA detected in CTC-enriched blood by reverse transcription PCR (RT-PCR) [30••]. They isolated CTCs via the

commercially available AlereTM CTC AdnaTest and used RT-PCR to evaluate for AR-V7 expression. They found that patients who had AR-V7 invariably had resistance to both enzalutamide and abiraterone acetate. In 31 patients who received abiraterone acetate and who had sufficient CTCs for analysis, PSA response was 0 % for patients with detectable AR-V7 and 68 % in patients without AR-V7 ($p = 0.004$) [30••]. In the patients treated with abiraterone acetate, PSA progression-free survival (PFS) was 1.3 months for patients with AR-V7, vs. 5.3 months for patients without AR-V7 (HR 16.1, $p < 0.001$) [30••]. In 31 patients who received enzalutamide and had sufficient CTCs for analysis, PSA response was 0 % for those patients with AR-V7 and 53 % for those without AR-V7 ($p = 0.004$) [30••]. For these patients, median PSA PFS was 1.4 months in patients with AR-V7 and 6.0 months in patients without AR-V7 (HR 7.4, $p < 0.001$) [30••]. Thus, patients with detectable AR-V7 had clear resistance to novel hormonal agents of enzalutamide and abiraterone acetate. The presence of AR-V7 may therefore be a predictive biomarker for lack of clinical benefit from these agents. However, these data require external validation and validation of clinical utility in a larger sample size before they can be used to guide standard treatment. While now CLIA approved, further steps are needed in clinical validation before this test can be incorporated into medical practice and treatment guidelines. These efforts are underway in several clinical trials assessing AR-V7 in a range of assays and in the context of broader CTC molecular profiling and enumeration (e.g., NCT02269982). Other trials are examining the ability of AR-V7 to identify patients more likely to benefit from alternative approaches, such as the AR antagonist and degrader galeterone (NCT02438007) or the taxane cabazitaxel (NCT02379390). These trials together with additional validation trials will provide data around the clinical utility of CTC AR-V7 status and how this impacts clinical management and outcomes of men with mCRPC.

Taking their studies of AR-V7 further, the same group examined serial AR-V7 in a small cohort of patients receiving taxane chemotherapy. They found that 3 patients had AR-V7 expression during their entire course of treatment, 8 patients converted from AR-V7 negative to positive during treatment with either AR-targeting agents or taxanes, and 6 patients reverted from AR-V7 positive to negative during taxane therapy only [31•]. The authors concluded that AR-V7 could be monitored serially to reflect tumor responses, and these data suggest that some treatments may select for V7-driven clones while others may be selectively toxic to such clones.

Additionally, a different group looked at AR-V7 by RT-PCR of CellSearch CTC-enriched blood and sensitivity to cabazitaxel in patients with mCRPC. They initially enumerated CTCs in patients with mCRPC treated with cabazitaxel and performed AR-V7 RT-PCR analysis on those with >10 CTCs at baseline [32]. Of 29 patients with >10 CTCs, 16 had AR-V7. AR-V7 was found in 100 % (5/5) of patients treated previously with abiraterone and in only 35 % (7/20) of untreated patients [32]. The detection of AR-V7 did not affect PSA response, PFS (HR 0.8, 95 % CI 0.4–1.8, $p = 0.6$) or OS (HR 1.6, 95 % CI 0.6–4.4, $p = 0.4$) [32]. With this study, the investigators showed that cabazitaxel has clinical efficacy in mCRPC patients regardless of AR-V7 status. However, the number of men enrolled on this study was extremely small, and the estimates of response, PFS, and OS were wide, and thus these data can be considered exploratory and not definitive.

Finally, the Hopkins investigators prospectively analyzed the presence of AR-V7 and clinical outcomes to taxanes (both docetaxel and cabazitaxel) compared to clinical outcomes to AR-targeting treatments (abiraterone and enzalutamide). Both AR-V7-positive and AR-V7-negative patients had PSA response to taxane therapy (41 vs. 65 %, respectively, $p = 0.19$) [33••]. AR-V7-positive patients were also more likely to respond to taxanes than to AR-directed treatments (41 vs. 0 %, $p < 0.001$) [33••]. Again, the sample size for this study was small, and a relative treatment resistance for taxanes in the presence of AR-V7 of up to 60 % could not be excluded. Thus, larger confirmatory studies are needed.

Through the above four studies of AR-V7 in patients with mCRPC, AR-V7 has emerged as a negative predictive biomarker indicating resistance to AR-targeting agents such as abiraterone or enzalutamide, and preserved potential sensitivity to taxanes such as docetaxel or cabazitaxel. In addition, in these early studies, taxane therapy (and not AR-directed treatment) was associated with reversion from AR-V7 positivity to AR-V7 negativity. These data suggest potential clinical utility in informing on clinical decision-making in men with mCRPC. For example, if AR-V7 is detected prior to abiraterone acetate or enzalutamide administration, is there still benefit with these agents, or should treatment be chemotherapy? Does AR-V7 emergence explain de novo resistance or acquired resistance to AR-directed therapies, or both? And is the AR-V7 test necessary in men with mCRPC who are failing either abiraterone acetate or enzalutamide, given clear evidence of cross-resistance to these oral agents even without knowledge of V7 status [34–41]? Finally, these clinical validation studies are small with sample sizes around 30 patients each and therefore will require external validation from other trials and other datasets, as well as prospective validation around clinical decision-making and utility.

Moving Toward Clinical Utility of CTCs

Blood-based biomarkers have the potential for clinical utility if they have direct impact on the real-time clinical

management of a patient. An ideal CTC test would therefore be noninvasive, reproducible, sensitive and specific, nondisruptive, and inexpensive. In addition to these qualities, a CTC test with high clinical utility would alter clinical management depending on the result. As CTCs can reflect evolving tumor characteristics in real time, CTCs have real potential to change the management based on real-time CTC findings.

Juxtaposed, the two current technologies with CellSearch® CTCs and AR-V7 from CTCs showcase different clinical utility. Without the characteristic of predicting for treatment response or resistance, CellSearch® CTCs are limited as a prognostic biomarker only, although surrogacy outcomes are emerging as a potential measure of clinical utility in some contexts. As described above, fulfilling surrogacy may inform on clinical decision-making both at the individual level (stopping an ineffective therapy or continuing an effective one) and at a trial level (earlier endpoints for drug approval or screening for activity of agents in phase 2–3 trials). In comparison, the detection of AR-V7 in CTCs may indicate treatment resistance to AR-directed therapies such as abiraterone acetate and enzalutamide. The presence or absence of AR-V7 can also be detected in serial collections, and the changing nature of AR-V7 may potentially prove to be a predictive marker of response to treatment (although this has yet to be shown in a prospective external fashion).

A prognostic biomarker alone (e.g., CTC enumeration) may have clinical utility in select circumstances, where existing diagnostic information (scans, symptoms, PSA, and other biomarkers) provides conflicting information, such as during bone scan flare or nonspecific changes in pain level or PSA drift [42]. However, CTCs have not been formally evaluated for this purpose, and the present emphasis has been on developing CTC enumeration by CellSearch® as an efficacy/response surrogate, rather than as a predictive biomarker. Presently, other assays using CTCs, cell-free RNA, or cell-free DNA are moving forward as predictive tools that have a greater potential on informing treatment selection. In order to be approved as a predictive biomarker by the FDA, CTCs as biomarkers will need to be incorporated as correlative endpoints in multiple prospective phase 3 trials. The initial study (COU-AA-301) utilizing CTCs as biomarkers to treatment with abiraterone vs. placebo in the post-chemotherapy setting showed its surrogacy as a predictive biomarker only in conjunction with LDH [18••]. CTCs as biomarkers in the AFFIRM study examining the use of enzalutamide in the post-chemotherapy setting has yet to be fully analyzed. These and other large prospective studies of CTCs performed in conjunction with the treatment studies will need to be incorporated into the final FDA analysis and approval for the clinical utility of CTCs [43].

Conclusions and Future Directions

In conclusion, CellSearch® CTCs are useful in providing prognostic information about a patient's disease progression [11]. Moreover, CellSearch® CTC enumeration in combination with other biomarkers (such as LDH) may provide some useful information on prognosis and surrogacy in monitoring patients on novel hormonal agents such as abiraterone acetate [18••]. Validation of such surrogacy measures in clinical scenarios that mirror current clinical use (i.e., in chemotherapy-naïve men) and across multiple mechanisms of drug action (cytotoxic, AR-directed, immunologic, etc.) is needed before CTCs can be incorporated into registrational strategies. Patients with detected AR-V7 in CTCs have resistance to AR-targeting therapies [30••] but sensitivity to chemotherapy such as taxanes [32, 33••]. AR-V7 can also be detected serially during treatment, and status can change based on treatment pressures [31•], suggesting clinical utility of measuring this over time.

Circulating biomarkers such as AR-V7 in CTCs may eventually have the clinical utility of preventing the use of ineffective drugs or the cost of such therapies. Further clinical trials with prospective biomarker studies, including AR-V7, are needed in order to validate predictive biomarkers to predict for treatment response and resistance.

Ongoing research into the molecular characterization of CTCs, of novel isolation platforms to identify novel CTC phenotypes, and in trials testing the clinical utility of such measures is critical to success for CTCs in the future. This research has already encompassed multiple new CTC platforms [3•], which, once validated, may prove their clinical utility in changing clinical management of prostate cancer. While beyond the scope of this concise review, additional platforms such as the EPIC platform and the CTC iCHIP using negative selection, have the potential to capture cells and measure such potential predictive biomarkers [44]. Epithelial plasticity may also "hide" mesenchymal or stem-like cells not captured by EpCAM. Moreover, the disease setting and treatment pressures from novel therapies may change the CTCs that are evaluable in the peripheral blood stream. There are active clinical studies in place to profile CTCs in the blood of patients with advanced prostate cancer, at different time points, and to study CTC changes under treatment pressure (e.g., development of circulating molecular predictors of chemotherapy and novel hormonal therapy benefit in men with metastatic castration-resistant prostate cancer, NCT02269982). The ultimate clinical utility for CTCs and their molecular characteristics will be demonstrated by their ability to inform on clinical management and allow clinical oncologists to practice real-time, predictive, personalized medicine tailored to a patient's disease.

Compliance with Ethical Standards

Conflict of Interest Tian Zhang has received research support through a grant from Janssen, has received compensation from Bayer for service

as a consultant and on an advisory board(s), and has a patent for the detection of circulating tumor cells by novel method pending.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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