Abstract

Circulating tumor cells (CTC) have substantial promise for multipurpose biomarker studies in prostate cancer. The IMMC-38 trial conducted by de Bono and colleagues, which was published in the October 1, 2008, issue of Clinical Cancer Research, demonstrated for the first time that CTCs are the most accurate and independent predictor of overall survival in metastatic prostate cancer. Since the publication of prospective trials demonstrating prognostic utility, CTCs have been utilized for nucleic acid analyses, for protein analyses, and in intermediate endpoint studies. CTC studies are also now facilitating the analysis of intrapatient heterogeneity.

Introduction

Approximately 140 years since the first documented observation in 1869 of circulating tumor cells (CTC) by Thomas Ashworth, the FDA cleared the first analytical CTC enumeration assay following three pivotal trials in metastatic breast, colorectal, and prostate cancer patients. In prostate cancer, this key trial (IMMC-38; NCT00133900) was published on October 1, 2008, in this journal (1). Since then, the CTC field has expanded hugely; prior to the publication in 2008, there were only 16 referenced papers on CTC enumeration. Today, there are more than 200 such publications. Here, we summarize and discuss the progress in this field since this landmark study.

The IMMC-38 Trial

The IMMC-38 trial prospectively evaluated, in 276 patients with castration-resistant prostate cancer (CRPC), the association of CTC counts with overall survival (OS) at baseline and after initiation of treatment with cytotoxic chemotherapy. This study met its primary endpoint; an unfavorable posttreatment CTC count, defined as a CTC count of \( \geq 5 \) cells/7.5 mL, was associated with shorter median overall survival at all predefined time points (6.7–9.5 months vs. 19.6–20.7 months; HR, 3.6–6.5; \( P < 0.0001 \)). At baseline, 57% of patients had an unfavorable CTC count with a decreased median survival of 11.5 months; this finding compared with 21.7 months for patients with a favorable CTC count (defined as a CTC count of \(< 5 \) cells/7.5 mL). Patients converting from unfavorable CTC numbers at baseline to favorable CTC counts after treatment had a corresponding improvement in median OS (from 6.8 months to 21.3 months). The CTC count prior to and following initiation of treatment was the strongest prognostic factor, superior to prostate-specific antigen (PSA) falls and many established prognostic variables.

CTCs Fulfill the Surrogacy Criteria for Overall Survival

The results of IMMC-38 have been confirmed in several single-center series (2), as well as in the preplanned analyses of the COU-301 (3), SWOG-S0421 (4) and AFFIRM (5) clinical trials (prognostic studies on CTC enumeration are summarized in Table 1). Other classifications (CTCs \(< 5, 5–50, \) or \(>50\); refs. 2, 6) or the absolute number of CTCs at baseline (7) have also been found to correlate with outcome.

Following the IMMC-38 trial, a posttreatment change in CTC count emerged as a potential biomarker of response, or lack of response, to treatment. Different definitions of response have been investigated with these studies supporting the use of CTC as an intermediate endpoint of benefit for treatment (1, 4, 7). To fulfill the criteria of surrogacy for OS, the posttreatment CTC count has to be prognostic, needs to be affected by an effective treatment, and needs to capture the full effect of the treatment on the outcome measure. These criteria for surrogacy were investigated in the COU-301 abiraterone trial, in which a prospectively planned secondary objective of the trial was to demonstrate that the posttreatment CTC counts were a surrogate for OS. A composite biomarker panel, comprising CTC counts (favorable vs. unfavorable) and lactate dehydrogenase (LDH; \( >250 \) IU/L vs. \( \leq 250 \) IU/L) 12 weeks after treatment initiation, stratified patients into good (CTC \( <5 \) and LDH \( \leq 250 \)), intermediate (CTC \( <5 \) and LDH \( >250 \)), and poor (CTC \( \geq 5 \)) risk groups, and satisfied the Prentice criteria for surrogacy at the individual level (3).

Similar to criteria used in RECIST or PSA responses, the percentage of decline in CTCs as a measure of response to treatment in patients with unfavorable CTC counts at baseline has also been explored. Olmos and colleagues (2), in an initial cohort of patients treated at the Royal Marsden Hospital, observed that patients who achieved a 30% decrease in CTC counts at 4, 8, and...
Table 1. Summary of the prognostic and predictive characteristics of CTCs referenced in this commentary; all CTC counts mentioned are per 7.5 mL of blood

<table>
<thead>
<tr>
<th>Study</th>
<th>Author (ref.)</th>
<th>Details</th>
<th>Patients</th>
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<th>CTC</th>
<th>Summary</th>
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<tbody>
<tr>
<td>IMMC-38 trial</td>
<td>de Bono et al. (1)</td>
<td>Retrospective single center</td>
<td>231</td>
<td>First-, second-, and third-line chemotherapy</td>
<td>Categorical variable&lt;sup&gt;a&lt;/sup&gt;</td>
<td>This study led to FDA clearance of CTC enumeration using the CellSearch platform in patients with CRPC; an unfavorable baseline CTC and unfavorable posttreatment CTC count at 2-4, 6-8, 9-12, and 13-20 weeks' time associated with shorter OS. Patients with CTC conversions, from unfavorable to favorable, or from favorable to unfavorable, showed improved or worsened prognosis, respectively. CTCs predicted OS better than PSA decrements.</td>
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<tr>
<td>IMMC-38 trial</td>
<td>Scher et al. (7)</td>
<td>Retrospective single center</td>
<td>156</td>
<td>First-line chemotherapy</td>
<td>Continuous variable&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Reanalysis of the IMMC-38 data for first-line chemotherapy and CTCs as continuous variable. Three variables consisting of baseline CTC count, baseline LDH concentration (both without a threshold effect), and CTC fold change at 4, 8, and 12 weeks best predicted OS.</td>
</tr>
<tr>
<td>Danila et al. (6)</td>
<td>Retrospective single center</td>
<td>120</td>
<td>Prior to chemotherapy regimens</td>
<td>Continuous variable&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Higher CTCs in patients with bone metastasis (with or without soft tissue involvement) compared with soft tissue-only disease. A higher CTC number (without a threshold effect), PSA level, and lower albumin level were independently associated with a shorter survival.</td>
<td></td>
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<tr>
<td>Olmos et al. (2)</td>
<td>Retrospective single center</td>
<td>119</td>
<td>Phase I and II</td>
<td>Categorical and continuous variable&lt;sup&gt;d&lt;/sup&gt;</td>
<td>A higher baseline count (categorical; without a threshold effect) associated with shorter OS. Group&lt;sup&gt;e&lt;/sup&gt; with favorable CTC conversion posttreatment associated with improved OS compared with those who did not. A CTC decline, as continuous variable, also associated with an improved survival.</td>
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<tr>
<td>SWOG-5042</td>
<td>Goldkorn et al. (4)</td>
<td>Prospective phase III</td>
<td>283</td>
<td>Docetaxel ± atrasentan</td>
<td>Categorical variable&lt;sup&gt;f&lt;/sup&gt;</td>
<td>An unfavorable baseline CTC count associated with a shorter OS; an exploratory analysis demonstrated that an expansion of CTC cutoff points (0, 1–5, 6–53, and &gt;53) predicted survival time without a threshold effect. A conversion to unfavorable CTC count at 3 weeks after treatment was associated with a shorter OS.</td>
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<tr>
<td>AFFIRM</td>
<td>Fleshner et al. (5)</td>
<td>Prospective multicenter phase III</td>
<td>382&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Enzalutamide</td>
<td>Categorical variable&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Unfavorable baseline CTC counts associated with decreased OS in enzalutamide and placebo arms. Conversion from unfavorable to posttreatment favorable CTC counts associated with OS benefit to enzalutamide.</td>
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<tr>
<td>COU-301 and IMMC-38</td>
<td>Lorente et al. (8)</td>
<td>Mixed&lt;sup&gt;i&lt;/sup&gt;</td>
<td>439</td>
<td>Abiraterone acetate and docetaxel</td>
<td>Continuous variable&lt;sup&gt;j&lt;/sup&gt;</td>
<td>Baseline CTC and 30% CTC falls at 4 weeks independently associated with OS in patients treated with abiraterone and chemotherapy. The addition of CTC falls at 4 weeks improved a multivariate model predicting OS with established prognostic covariates.</td>
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<tr>
<td>COU-301</td>
<td>Scher et al. (3)</td>
<td>Prospective multicenter phase III</td>
<td>711</td>
<td>Abiraterone acetate</td>
<td>Categorical variable&lt;sup&gt;k&lt;/sup&gt;</td>
<td>First prospective study to demonstrate a surrogate biomarker for OS in CRPC, satisfying all four Prentice criteria of surrogacy at the individual patient level. The biomarker panel contained CTC plus LDH to define three risk categories&lt;sup&gt;l&lt;/sup&gt;.</td>
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<sup>a</sup>Favorable counts defined as CTC <5 and unfavorable counts as CTC ≥5.<br><sup>b</sup>CTCs analyzed as a continuous variable (log-transformed) and CTC fold change posttreatment compared with baseline.<br><sup>c</sup>CTCs categorized into three groups: group 1, CTCs <5; group 2, CTCs 5–50; group 3, CTCs >50. CTCs with favorable counts were defined as <5 cells.<br><sup>d</sup>Four different groups of patients were compared: group 1, patients with CTCs <5 at all blood-drawn time points; group 2, patients had CTCs ≥5 before the initiation of therapy and whose CTCs decreased to <5 after therapy; group 3, patients with CTCs <5 whose CTCs increased to ≥5 after treatment; and group 4, patients with CTCs ≥5 at all blood-drawn time points.<br><sup>e</sup>Preplanned analysis of favorable CTC counts, <5, and unfavorable counts, CTC ≥5 cells. Exploratory analyses of four categories of CTCs: CTC of 0, CTCs of 1–5, CTCs of 6–53, and CTCs >54.<br><sup>f</sup>447 patients with baseline CTC count, and 382 with both baseline and posttreatment CTC counts.<br><sup>g</sup>Pooled CTC data from retrospective single-center IMMC-38 study and exploratory analysis from the phase III COU-301 trial.<br><sup>h</sup>Continuous variable defined as CTC <5 and unfavorable counts as CTC ≥5.<br><sup>i</sup>Biomarker panel with three risk categories: low (CTCs <5 with any LDH), intermediate (CTCs ≥5 and LDL <250 U/L), and high risk (CTCs ≥5 and LDH ≥250 U/L).
12 weeks had improved outcomes compared with those that did not. These findings were confirmed in a combined analysis of the COU-301 and IMMC-38 trials (8) presented at the American Society of Clinical Oncology in 2015.

**Decision Making on Posttreatment CTC Count**

Following the IMMC-38 trial and confirmatory prospective studies, phase II clinical trials have started to integrate a posttreatment CTC count as a primary endpoint; we envision that the use of CTC as an intermediate endpoint could accelerate drug approval for advanced prostate cancer. Within the next decade, additional large phase III trials have to address whether earlier therapeutic switches to the next line of treatment based on early additional large phase III trials have to address whether earlier treatment CTC count as a primary endpoint; we envision that the studies, phase II clinical trials have started to integrate a post-treatment. An investigator-initiated international multicenter phase III study, supported by the Movember Foundation, CTC-STOP, will start recruitment in 2015, and will evaluate the utilization of CTC counts to guide earlier treatment switch from first-line docetaxel to second-line cabazitaxel. This study will build further on the findings of the IMMC-38 study to ascertain whether earlier CTC-guided discontinuation can deliver more precise care in metastatic CRPC (mCRPC). Over 1,200 patients with unfavorable CTC counts, and predominantly bone metastatic disease, will be randomized between two arms for a comparison of standard-of-care therapeutic decision making with CTC-guided drug discontinuation using OS as the primary endpoint. Unlike the recently published SWOG-S0500 study in breast cancer, this trial is sufficiently powered to detect a meaningful survival benefit.

**Other Advances**

Moving beyond enumeration, molecular characterization of CTCs can allow the molecular stratification of patients for novel androgen-directed therapies, taxanes, and targeted treatments. These genomic analyses in so-called liquid biopsies can also be used for the identification of primary or acquired resistance mechanisms. Parallel to the development of the CellSearch platform, more than 40 other technologies have been established to detect and isolate CTCs, using distinct biological or physical properties to distinguish them from the millions of surrounding blood cells (9). The CellSearch platform enriches for CTCs by separating epithelial cells from blood by means of an anti-EPCAM antibody bound to ferrofluids (10) and has become the gold standard for all subsequent techniques. This system, however, only detects CTCs expressing both EPCAM and cytokeratins 4, 5, 6, 8, 10, 13, 18, and 19. CTCs not displaying this immunophenotype will be missed, and future studies will need to confirm the frequency of such CTCs (e.g., EPCAM-negative cells) and their relationship with clinical outcome. Molecular characterization of CTCs using DNA-based techniques is now feasible with the CellSearch system. Androgen receptor (AR) mutations can be detected by next-generation sequencing, and chromosomal rearrangements, such as ERG translocations, PTEN loss, AR, and MYC amplification, can be interrogated by FISH; gene copy number can also be analyzed by comparative genomic hybridization. In addition, a complementary antibody can be added to the standard CellSearch antibody cocktail allowing immunocytochemical evaluation of an extra epitope; such studies have shown that high Ki67 expression, cellular localization of AR, or bundling of microtubules is associated with response to treatment and drug–target engagement. The preservative in the blood-draw tube (CellSave) used to run samples on the CellSearch system precludes the analysis of RNA or the ability to culture CTC. This limitation can be overcome by the use of EDTA blood-draw tubes in combination with CellSearch Profile kits or any of the innovative alternative platforms, such as the MagSweep, Verifast, RosetteSep, and Parsortix. CTCs can now be cultured from some patients; they can be xenographed into nude mice and established into patient-specific cell lines, providing a pharmacologic testing ground to putatively individualized patient care. One of the constraints of all CTC systems is the limited numbers of available cells present in the small volume of blood. This obstacle may be overcome by using leukapheresis product with CellSearch or other platforms to increase the yield of enriched CTCs; an alternative assay might involve inserting an antibody-coated wire into a vein for 30 minutes to directly capture CTCs from the bloodstream in an attempt to isolate CTCs from a blood volume of 1.5 L.

Concerns that antibody-based applications can alter cell behavior and confound biochemical or transcriptome investigation have driven negative selection approaches, allowing for enrichment of CTCs with a broader range of phenotypes. The CTC-Chip can perform both positive and negative selection depending on the type of antibodies used, whereas the Microfluidic cell concentrator is solely based on negative selection. In addition, enrichment strategies exploiting such physical properties as cell size, cell density, and deformability have been reported, as well as electrical and acoustic methods that may bypass the necessity of antibody use. Finally, CTC detection methods have been developed focusing on the detection of viable CTCs, for example, by assessing their ability to secrete PSA or cytokeratins (EPISPOT), the activity levels of telomerase, and their metastatic potential by their ability to infiltrate collagenous matrices. Clinical trials that implement qualitative assessment of CTC to adaptively stratify patients into different treatment arms are warranted, and should comprise the arsenal of treatments available in mCRPC.

**Discussion**

An increasing understanding of the landscape of somatic genomic aberrations in primary and metastatic disease promises to lead to the delivery of more precise care for patients with prostate cancer. One of the key issues that have hampered advancement has been obtaining adequate tissue from mCRPC patients with predominantly bone or nonbiopsiable nodal disease for molecular characterization to guide patient stratification. The acquisition of CTCs from the bloodstream can overcome this issue in many mCRPC patients with detectable CTC counts, making these “liquid biopsies” a rapid and reliable assessment to assist with therapeutic decision making and stratification of prostate cancer patients on a case-to-case basis in adaptive genomically driven trials. Especially in those patients not deriving benefit from treatment, such as nonresponders with high posttreatment CTC...
counts, a real-time molecular characterization of CTC may identify informative biological alterations that could include clonal heterogeneity to support therapeutic decisions in overcoming primary resistance by hormonal or chemotherapeutic manipulation. Stratification into clinical trials can be based on AR amplification or aberration; ERG translocations; or alterations in PI3K, Wnt, or RAS/RAF pathways and DNA damage repair genes. Integrative clinical sequencing recently identified these potentially actionable aberrations in approximately 90% of patients with mCRPC (11).

In oncology, there is still a major reliance on PSA and imaging to support decisions in instances when the clinical picture is ambiguous; however, there are limitations to the use of the composite endpoints recommended by the Prostate Cancer Working Group (PCWG) in PCWG2. PSA fall algorithms are not robust surrogates for OS, and the earliest time point progressive disease can be demonstrated is commonly with a confirmatory bone scan 18 weeks after treatment initiation. To date, CTCs have been isolated, quantified, or qualitatively assessed in approximately 2,000 patients with prostate cancer. These studies explicitly show the potential of CTCs to identify patients with primary resistance as early as 4 to 8 weeks after treatment initiation, to monitor treatment efficacy, study drug-target interaction, and identify mechanisms of resistance at an individual level. These features make CTCs one of the most promising and versatile biomarkers in translational oncology. In addition to the international phase III multicenter CTC-STOP trial, other phase III trials will need to determine whether CTC-guided discontinuation is beneficial in prostate cancer. Another key issue to be addressed in the following years is to determine physician and patient acceptance in utilizing CTCs to direct treatment; the CTC-STOP trial will investigate these questions by implementing questionnaires and assessing physician adherence to CTC-directed discontinuation.

In conclusion, advancements in the field on enumeration, isolation, and molecular characterization have established CTCs as an efficient and promising all-around translational biomarker that is furthering the individualization of patient care and can change the daily management of patients with prostate cancer within the next decade.

Disclosure of Potential Conflicts of Interest

L.W.M.M. Tenstaapen reports receiving a commercial research grant from Janssen Diagnostics; is listed as an inventor on U.S. patents (No: 5,985,153; No: 5,993,665; No: 6,013,188; No: 6,136,182; No: 6,361,749; No: 6,365,362; No: 6,551,843 B1; No: 6,623,982 B1; No: 6,620,627 B1; No: 6,623,983 B1; No: 6,645,731 B2; No: 6,660,159 B1; No: 6,790,366 B2; No: 6,890,426 B2; No: 7,056,657 B2; No: 7,332,288 B2; 7,863,012 B2; No: 8,329,422 B2) related to the CellSearch system, the rights of which are assigned to Johnson & Johnson; and is the chairman of the department of Medical Cell BioPhysics at the University of Twente, which receives research funding related to the CellSearch system from Johnson & Johnson. J.S. de Bono reports receiving speakers bureau honoraria from Janssen. No potential conflicts of interest were disclosed by the other authors.

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References


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