

Solidifying Liquid Biopsies: Can Circulating Tumor Cell Monitoring Guide Treatment Selection in Breast Cancer?

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Almost 150 years ago, T.R. Ashworth first described the presence of epithelial cells in the blood of a woman with metastatic breast cancer that were similar in appearance to her primary tumor cells.¹ Indeed, many patients with a variety of solid tumors, including breast cancer, have detectable cancer cells circulating in the bloodstream, so-called circulating tumor cells (CTCs). CTCs represent a rare cell population in the blood, typically less than 10 cells/mL compared with 1 million WBCs/mL. However, the detection of CTCs within a routine blood specimen provides an opportunity to monitor cancer noninvasively, in essence a liquid biopsy.^{2,3} While dramatic technological advances have recently transformed the analytic capabilities of both CTCs and free plasma DNA analyses, clinical trials based on the first-generation technologies are now providing important insight into how such blood-based monitoring might be integrated into clinical management.

In 2004, a seminal study by Christofanilli et al⁴ evaluated the prognostic significance of CTCs in metastatic breast cancer, utilizing the Veridex CellSearch assay. The CellSearch assay involves fixation of cells within a blood specimen, followed by immunomagnetic capture of CTCs on the basis of cell surface expression of the epithelial cell adhesion molecule (EpCAM) and staining and scoring of captured cells for expression of epithelial keratins.⁵ Women with metastatic breast cancer and elevated CTCs (≥ 5 cells per 7.5 mL of blood) were found to have a worse progression-free survival (PFS) and overall survival (OS) than women with CTCs below this detection threshold. The prognostic significance of elevated CTCs was subsequently further confirmed in breast⁶⁻⁹ as well as prostate¹⁰ and colon¹¹ cancer. However, expert guidelines from national organizations do not currently support the routine use of CTCs for prognostic assessment, given the unproven clinical benefit of such measurements.

In the article that accompanies this editorial, Smerage et al¹² present results from the SWOG S0500 clinical trial, testing whether adjustment of chemotherapeutic regimens based on CTC measures of treatment response can lead to improved clinical outcome in women with metastatic breast cancer. CTCs were measured by CellSearch assay before the start of first-line chemotherapy in women with metastatic breast cancer (N = 595). Of these, 319 (54%) women had elevated CTCs (≥ 5 cells per 7.5 mL of blood) at baseline. The 123 (20.7%) women who had detectable CTCs at baseline and continued to have elevated CTCs despite one cycle of

chemotherapy were randomly assigned to either continuation of the same treatment or to an alternative second-line chemotherapy. Both the first-line and second-line therapies were at the discretion of the treating physician, and neither were informed in any way by CTC results. Thus, the study tested the hypothesis that switching among chemotherapy regimens based on early CTC measures of nonresponse might improve outcome, compared with waiting for more traditional radiological evidence of disease progression. The two arms of the randomized study were well balanced with respect to baseline demographic and tumor characteristics and powered to detect a 70% increase in OS (8 to 13.6 months).

The study confirmed the prognostic significance of CTC measurements: women who had elevated CTCs at baseline and did not have a decline in CTC numbers following first-line chemotherapy had an OS of only 13 months, compared with 23 months for women whose baseline CTCs declined following first-line chemotherapy and 35 months for women without detectable CTCs at baseline. However, for women in the worst prognostic group (ie, those who continued to have elevated CTCs following a cycle of first-line chemotherapy), switching to an alternative chemotherapy regimen did not change either OS (10.7 v 12.5 months; $P = .98$) or PFS (3.5 v 4.6 months; $P = .64$). The trial included patients with diverse breast cancer subtypes, including those who had received endocrine or biologic therapies before the first-line chemotherapy, thus constituting a heterogeneous population with metastatic breast cancer. In addition, CTC change was only assessed as a binary categorical variable (patients were either positive or negative for CTCs, based on the detectability threshold of 5 CTCs per 7.5 mL of blood). Thus, major changes in CTC numbers in either direction were not scored unless they crossed the threshold of detectability. Whether any of these issues could have modified the magnitude or significance of the results is unclear, but they are important considerations as we move into the next generation of CTC-driven clinical trials.

The SWOG S0500 CTC trial is an important study for several reasons. First, it was a well-designed biomarker trial addressing an important clinical question related to clinical utility of CTCs in metastatic breast cancer. Extending beyond past observational studies, this trial involved random assignment based on CTC measurements and sets up a good model of conducting a biomarker trial using standardized technology across multiple institutions. Second, the trial results strongly validate the

prognostic utility of CTCs, both at baseline and especially at 1 month after treatment initiation. This is consistent with a recent pooled analysis of 20 studies of patients with metastatic breast cancer (N = 1,944) reporting an almost two-fold decrease in PFS and OS in women with elevated CTCs at baseline and at 3 to 4 weeks after treatment.¹³ Thus, as suggested by the authors, CTC enumeration may serve as a prognostic biomarker to guide patient stratification in clinical trials, even if it is not indicated to direct switching among current standard chemotherapy regimens. Finally, and perhaps most importantly, the trial highlights the importance of moving beyond simple CTC enumeration toward applying molecular measurements in CTC to assess tumor biology and therapeutic response. It is uncommon for breast cancer that has acquired resistance to one chemotherapy regimen to exhibit a high degree of sensitivity to a randomly selected alternative chemotherapy regimen, presumably explaining the failure of SWOG S0500 to demonstrate a benefit to such an approach in women whose CTC count fails to decline. In contrast, the emerging application of CTC diagnostics to genotype-directed clinical trials, including those with PIK3CA and HER2 inhibitors, holds considerable promise. The availability of such molecular CTC readouts was limited during the SWOG 0500 accrual period (2006-2012), but they are expected to predominate in future clinical studies.

An exceptional feature of CTC analyses in cancer is that multiple developing technologies are coevolving with equally rapidly emerging therapeutic applications. Indeed, it is worth considering both the new analytic capabilities of CTC technologies, as well as new genotype-directed therapeutic approaches that rely on accurate and real-time measurements of tumor characteristics. Newer CTC isolation platforms under development appear to be more sensitive than the currently available CellSearch technology,^{14,15} and it is expected that the fraction of patients with detectable CTCs, as well as the dynamic range of CTC measurements during a course of therapy, may be considerably improved. Most importantly, newer isolation technologies that do not rely on antibodies against epithelial markers to capture CTCs are able to identify cancer cells that have undergone epithelial-to-mesenchymal transition and constitute significant fractions of the CTC load in some patients with breast cancer.^{16,17} Among the key CTC markers that are likely to inform therapeutic decisions in breast cancer are persistent expression of the estrogen receptor and recently discovered mutations in the estrogen receptor gene *ESR1*,¹⁸ alterations in *HER2* expression and gene copy number, and mutations in the *PIK3CA* gene. Each of these biomarkers could constitute a compelling rationale for a specific targeted therapy, and clinical trials to assess the therapeutic impact of such measurements in CTCs would likely have a major impact on clinical practice. Indeed, as we enter the era of targeted therapies in breast cancer, molecular diagnostic studies of CTCs may facilitate the identification of actionable mutations, measurement of pharmacodynamic response, and serial tumor monitoring for acquisition of drug resistance-associated mutations.¹⁹ For many women with metastatic breast cancer, these treatment choices relating to hormonal and targeted therapies are likely to arise before indications for cytotoxic chemotherapy, and their predictive value will be tied to signaling pathways that have been well characterized.

In conclusion, the information derived from CTCs as liquid biopsies is likely to play a major role in the application and monitoring of therapies

that are linked to powerful predictive biomarkers of response in metastatic breast cancer. Although the SWOG 0500 trial failed to demonstrate a benefit to CTC-directed changes among chemotherapy regimens, it has helped set the stage for a new generation of CTC trials aimed at genotype-driven personalized breast oncology.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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