

Circulating Tumor Cells in Breast Cancer: Are They Indicative of the State of Disease?

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Introduction

Despite latest advances in the ability to predict recurrence of breast cancer by assays which utilize mRNA transcripts of genes related to proliferation capacity at the molecular level, questions still remain regarding a tumor's likelihood to metastasize at the whole cellular level, and whether this potential is based upon genetic propensity and/or interaction with the environment? The 21-gene Oncotype DX assay (Genomic Health, Redwood, CA), the 76-genomic-grade Mammprint assay (Veridex, Rotterdam, Netherlands), and the 2-gene ratio of HOXB13 to IL17R in the Theros assay (Biotheranostics), have been very useful in clinical practice to help determine whether chemotherapy in the adjuvant setting would have a significant impact in decreasing the likelihood of breast tumor recurrence or distant metastases from breast cancer [1]. The question is posed as to whether following the detection of circulating tumor cells (CTCs) at the single-cell level after adjuvant treatment may be useful and feasible in determining breast cancer recurrence after treatment, as evidenced in the arena of metastatic breast cancer, as an indicator determining the full response to treatment.

The process of breast cancer metastasis is a fascinating mechanism actively being elucidated by virtue of understanding the various necessary components of basement membrane invasion, circulation into the bloodstream or local migration via the lymphatic system adhesion to substrate and propagation or sustaining of a clonal proliferation at a secondary site away from the primary tumor. The last decade has shown the emergence of evidence that CTCs specifically related to breast cancer have been detected in the bone marrow, but then clinical significance is unknown in regards to impact upon prognosis in early stage breast cancer.

Landmark studies by Cristofanilli et al. [2-6] have demonstrated that CTCs detected after treatment in metastatic breast cancer can indicate early progression of disease and help clinician decide on changing in therapeutic regimens sooner rather than later when disease is detected on scans. In their landmark paper in the New England Journal, they showed that in patients undergoing chemotherapy treatment for metastatic breast cancer, those with ≥ 5 CTCs/7.5 cc whole blood had a shorter median progression-free survival (PFS) time and shorter overall survival (OS) than patients having < 5 CTCs/7.5 cc blood (PFS, 2.7 months versus 7.0 months, $p < 0.001$; OS, 10.1 months versus > 18 months, $p < 0.001$). In addition, a decrease in the number of CTCs to < 5 from baseline to first followup (4-5 weeks after initiation of new therapy) was also found to be predictive of PFS and OS [2]. But how do these CTCs truly translate into potential to trigger tumor colonization and growth? Norton and Massague [7] have defined the concept of tumor self-seeding or self-metastasis as a mechanism of tumor propagation: Their hypothesis is that in addition to cell proliferation outweighing the rate of cell death in cancer, "inappropriate cell movement", migration and thus invasion and metastases is responsible for generating a cancer. In their research group, Kim et al. [8] showed that aggressive CTCs from early tumors can overcome tight vascular wall barriers and

colonize other organs, and [potentially enhance chemical signaling in the microenvironment, thus stimulating an attractive environment for cancer cell growth. These signals include tumor-derived cytokines IL-6 and IL-8, CXCL1, and cytoskeleton proteins MMP1/collagenase-1, and fascin-1, thus stimulating infiltration .

CTCs have been specifically studied in breast cancer, but a growing body of data stems from CTCs also also being studied in other tumor types such as small cell lung cancer [9,10], castration-resistant metastatic prostate cancer [11,12], and colorectal cancer [13,14], and this concept of genetic mechanism and stimulation of the environment in order to promote growth may certainly apply in a general sense.

Multiple studies have shown that following CTCs in breast cancer may predict tumor response to primary treatment. Not only did Cristofanilli [2] landmark work show that ≥ 5 CTCs in the peripheral circulation directly related to increase rate of disease progression, Gradilone et al. [15] have shown specifically that in CTCs evaluated for MDR-related proteins (MRPs), ALDH1, Estrogen Receptor alpha(a), and HER2-neu, that CTC-positive patients had shorter progression-free survival (PFS), as did those whose CTCs had ≥ 2 MRPs. In addition, Pierga et al. [16] and in Clinical Oncology News, have shown that CTCs could be directly compared with elevations in serum tumor markers (i.e., CA 15.3, carcinoembryonic antigen [CEA], and lactate dehydrogenase [LDH]); in 267 pts treated with first-line therapy for metastatic breast cancer and followed for median 16 months, 65% of patients had at least one CTC, 44% had ≥ 5 CTCs; 64% had elevated CA-15.3, 51% had elevated CEA, 45% had elevated LDH. The presence of CTC levels predicted poor progression-free survival and OS, independent of the tumor markers, however; elevated levels of CA 15-3, CEA and LDH were prognostic for PFS, but only LDH remained prognostic for OS [16]. Gao et al. [17] showed CTCs in MBC prior to SCT were predictive of shorter PFS.

In the neoadjuvant treatment setting, studies done by Pierga et al. [18] in the BEVERLY 1 and BEVERLY2 [19] trials, which showed a significant drop of CTCs after 4 cycles of chemotherapy treatment including bevacizumab for HER-2-negative inflammatory breast cancer, with correlation to complete response still needed as a future endpoint. Liu [20] presented from 6 pooled studies of metastatic breast

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cancer that persistently elevated CTC levels detected at baseline, and 3-week-intervals after treatment, were highly associated with treatment failure irrespective of clinicopathologic variables, disease subtype, and type of treatment. The question remains as to whether CTC after adjuvant treatment are helpful when post-operative therapy has been given in order to enhance the chances of cure rate.

In the adjuvant setting, studies done by the German SUCCESS trial (Simultaneous Study of Gemcitabine-Docetaxel Combination Adjuvant treatment, as well as Extended Bisphosphonate and Surveillance) showed that the presence of 1 or more CTC nearly doubled the risk for relapse and death in pts with early stage breast cancer [21]. In this study, 2026 pts with primary breast cancer, stages I through III, were sampled for CTC and tumor markers using CellSearch technology (Veridex, LLC) at four different time points: before chemotherapy, after chemotherapy, after 2 years of endocrine/zoledronic acid treatment and after 5 years of endocrine/zoledronic acid treatment [21], with at least one CTC considered positive. At median followup of 35 months, patients who were CTC-positive before treatment was initiated had a significantly worse disease-free survival than those who were CTC-negative, with DFS 38.5 months versus 41.4 months respectively. OS was significantly worse for those who were CTC-positive prior to treatment, and presence of CRCs before treatment was independent. Interestingly, those patients who were CTC-positive before treatment were more likely to be node-positive, but no correlation was observed between CTC status and tumor size, grade, or hormone-receptor status. Furthermore, Janni [22] found and reported at the 2010 San Antonio Breast Cancer Symposium that also from the SUCCESS, CTCs appearing 2 years after adjuvant chemotherapy were dependent upon the type of endocrine treatment, with a trend toward higher CTCs immediately after the completion of chemotherapy in the anastrozole group as compared to the tamoxifen group, but similar in both groups at 2 years after primary diagnosis. Hepp [23] then reported at the 2011 American Society of Clinical Oncology (ASCO) meeting that a relationship between CA 27-29 and CTCs occurred.

What tools and technology are available for validated clinical use? The technique described in Cristofanilli [2] landmark paper reports cells positive for EPCAM with antibody-coated magnetic beads with fluorescently labeled monoclonal antibodies to distinguish epithelial cells (CK 8,18,19) from leukocytes (CD 45, allophycocyan) with CTCs as nucleated cells lacking CD45 but expressing CK. These were correlated with standard imaging studies. They stress that "our results may not be valid for patients who do not have measurable disease or those starting a new regimen of hormone treatment, immunotherapy, or both". In Gradilone [15] paper, CTCs were isolated by CELLection Dynabeads coated with moAb to EpCAM and RT-PCR run for CD45,8,20, with CTCs defined as EpCAM+, negative for CD45, marker for leukocytes, + for CK8,CK20. CellSearch is now FDA approved for use in monitoring metastatic breast cancer, and has been available since December 2004 in the US. CellSearch by Quest Diagnostics uses immunomagnetic cell enrichment with antibodies targeting EpCAM and nucleus labeling with fluorescent dye and offers CPT code for billing purposes (Veridex LLC) [24]. The MD Anderson Cancer Center is endeavoring to see if CTCs can be purged by giving a high-dose chemotherapy regimen with carboplatin/cyclophosphamide/thiotepa with "rescue" by autologous stem cell transplant to metastatic breast cancer patients (excluding those with brain metastases) who have persistent CTCs by Veridex Technology after completion of standard treatment [25].

So, which tools are currently feasible in the day-to-day clinic setting? While logistically feasible as a peripheral phlebotomy, which tests are covered by insurance, thus obviating the need for other invasive biopsies, etc. In a very beneficial review geared toward clinicians by Sabbath and Schwartzberg [26], as well as in Quest Diagnostics (Veridex LLC) package insert, insurance CPT codes are even suggested for billing purposes in private practice; these are based on AMA Guidelines (Quest Diagnostics website) [27,28].

A scientific caveat to this, however, is whether these cells are truly the same as the original tumor, and are they indicative of the behavior [4,29,30]? Further studies will need to be done on CTCs to classify those associated with worsened prognosis, including study of epithelial cell markers and genetic analysis [4]. Previously, bone marrow metastases have been evaluated as to their presence and their clinical significance in early stage breast cancer [31]. Qi X [32] reported that in operable breast cancer cases, there was seen a significant difference in CTC rates at different TNM stages and between those with different IHC subtypes, and were higher in HER2+ versus triple-negative tumors as compared with luminal tumors. Schmitt et al. [33] have reported an RT-PCR based test as AdnaTest BC kit and assessed for transcripts for EpCAM, MUC1, HER-2, correlated to bone marrow cells and to ER status, and did find a confirmed difference in detection rates based on the phenotype of the breast cancer. Bischoff [34] reported at ASCO the development of a specific antibody cocktail in order to very individually target and follow by HER-2-positive CTCs as surveillance; this "stealth" maneuver will be most important in clarifying whether CTCs are from the original tumor and are biologically significant in the setting up of a new viable recurrence [35]. Certainly, this begs the question as to if they are detected, when and at what level of number of cells detected will a patient benefit from cytotoxic treatment or even biologic antibody-driven treatment given the attendant side effects of treatment, including infection risk from myelosuppression, gastrointestinal effects, thromboembolic risk, cardiovascular risk, including hypertension and/or cardiomyopathy.

While determinations can be made at the molecular level in terms of the likelihood of recurrence of a primary breast cancer, methods for further validation of the recurrence of breast cancer at the cellular level need to be developed. While there is solid evidence that CTCs may be followed for treatment response in metastatic breast cancer, as illustrated by thought leaders who have developed the assays and tracked these during patient treatment, the critical question remains as to whether these CTCs may be reliable to follow after primary treatment in order to determine likelihood of recurrence or development of distant metastases. While there is data that early detection of recurrent disease by intensive surveillance CT/PET scanning in asymptomatic individuals may not afford a gain in OS [36], more study would be needed to understand the impact of detecting CTCs in surveillance. A limitation to this statement is, of course, that if disease is detectable at a minimum of 7-9mm in size, corresponding to several logs of cellular burden, would a microscopic CTC make an impact [37]? As discussed by Gradilone [15], and Cristofanilli [2], it is posed that the count of CTCs is superior in prognostic value to PET's metabolic imaging.

How are the American Society of Clinical Oncology (ASCO) and national guidelines incorporating this? The ASCO has included tumor markers in surveillance recommendations after primary treatment for breast cancer, however, "there is as yet insufficient evidence to recommend following CTCs" [38], nor is there recommendations on their use by the National Comprehensive Cancer Network [39]. Oncologists need to settle this and assimilate these data into their

practices in order to make decisions toward changing therapy; many will do so in conjunction with other pieces of data including scans, and enrolling patients onto clinical trials will help guide this endeavor. The Southwest Oncology Group (SWOG), [40] for example, is conducting a randomized phase III trial (0500) to test the strategy of changing therapy versus maintaining therapy for metastatic breast cancer patients who have elevated circulating tumor cell levels at first followup assessment in order to help decide on therapeutic strategy. The patients are stratified to HER2-positive disease requiring continued trastuzumab versus HER-2-negative, and ER-positive disease requiring continued endocrine therapy versus those who do not; the end point include time to progression. At this writing, other surrogate markers regarding potential for recurrence are not being compared or amplified in these CTCs, particularly in those patients whose disease definitely shows signs of progression. For example, could other transcripts related to recurrence expression i.e., in the Oncotype DX assay 21-gene array, be assessed in order to determine to the exact behavior of a CTC in the microenvironment? As discussed by Danila et al. [11], surrogate biomarkers of survival may be further elucidated by studying CTCs, and identifying drug sensitivity markers within CTCs, potentially allowing for specific therapeutic targeting in cancer treatment [41,42].

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