Using circulating tumor cells to guide therapy in breast cancer: could this replace biopsies?

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Keywords: breast cancer • circulating tumor cells • liquid biopsy • tumor heterogeneity

Over the last two decades we have seen a number of important advances in the biological underpinnings of breast cancer that have had a significant impact either directly or indirectly on the management and ultimately on the prognostic outcome of this disease. First, we now know that breast cancer is a heterogeneous disease made up of a number of unique subtypes each with its own natural history and associated prognostic outcome [1]. Second, some of these subtypes have specific targets (including ER and HER2) that have associated unique targeted therapeutic agents that significantly impact the natural history of this disease [2]. Third, data indicate that these targets are not static with approximately 30% of tumors showing discordance in the expression of hormone receptor or HER2 between the primary and associated metastatic tumor [3]. The therapeutic implications of such discordance have understandably resulted in most oncologists seeking to biopsy metastatic sites whenever possible. Despite these advances we have a long way to go with current research focusing on looking for biomarkers that have a dual prognostic and predictive function. An ideal biomarker being one able to accurately predict for early recurrence or progression of disease, provide information to guide thera-

peutic decisions, reliably predict response to specific treatment, provide an easy platform for testing of markers such as ER and HER2 at various time points along a cancer history continuum, and allow for the identification of unique targets that would help specific drug development. Circulating tumor cells (CTCs) is one such biomarker that has been actively investigated over the last decade and has shown promising results in a number of malignancies including breast cancer.

The presence of epithelial cells in the circulation that are similar in appearance to primary tumor cells was first described by TR Ashworth about 150 years ago in a woman with metastatic breast cancer [4]. These detectable cancer cells are CTCs that represent a rare cell population in the circulation usually representing less than 10 cells/ml. These cells can originate from either the primary or metastatic tumor tissue and require special enrichment techniques for their detection [5]. These techniques are based either on the biological properties of the CTCs such as protein secretion or cell surface antigen expression or are based on the physical characteristics of these cells such as presence of electric charges, size of the cell, density or deformability. Currently the only FDA approved CTC enumeration

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technology is the CellSearch system that involves first immunomagnetic capturing of the CTCs using cell surface expression of EpCAM (epithelial cell adhesion molecule) followed by staining of the captured cells for epithelial keratins [6]. Over the last decade we have seen data reported from a number of important studies that looked at the prognostic and predictive role of CTCs in both early stage and metastatic breast cancer.

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The prognostic role of CTC in metastatic breast cancer was first defined in the pivotal trial by Cristofanilli and colleagues [6] in which women with metastatic breast cancer and an elevated CTC level (≥ 5 cells/7.5 ml of blood) were found to have a worse progression free and overall survival compared with women with CTC levels below this cut off threshold. The same group went on to show that baseline CTC levels prior to starting first-line therapy were highly predictive of prognostic outcome with elevated CTC levels at any time point during treatment being a good indicator of rapid disease progression [7,8]. Individual patient data pooled analysis of 1944 patients enrolled across 17 European centers has essentially confirmed the prognostic role of CTCs with the authors reporting that patients whose baseline CTC count was $\geq 5/7.5$ ml of blood having a worse progression free (HR: 1.92, $p < 0.0001$) and overall survival (HR: 2.78, $p < 0.0001$) compared with those with a CTC count less than 5/7.5 ml [9]. The authors further reported that CTC levels at 3–5 and 6–8 weeks following start of treatment was also predictive of progression free and overall survival. The addition of CTC count to the clinicopathological models increased the predictive power of these models, however, interestingly the addition of tumor markers such CA 15–3 and CEA did not add significantly add to the models. In the early stage setting CTC levels have also been shown to be associated with prognostic outcome [10,11]. Rack and colleagues [11] recently reported on a large prospective trial that enrolled over 2000 patients with early stage breast cancer. The authors observed that at baseline prior to starting adjuvant chemotherapy 21.5% of patients had CTCs detected with patients having at least 5 CTCs/30 ml of blood having worse prognostic outcome. The results of this study provided evidence

for CTC levels being a prognostic marker in the early stage breast cancer setting.

Having established the prognostic power of CTCs in both the metastatic and early stage setting the next question was to try and look at the clinical utility of CTC as a predictor of treatment efficacy thereby essentially guiding therapeutic management. In the metastatic setting changes in the levels of CTC counts have been shown in several studies to indicate treatment responses which could be as early as after the first cycle of treatment well before any change is detected on standard imaging modalities [12,13]. In the metastatic breast cancer setting several modalities have been investigated to try and individualize treatment management. Such modalities include molecular profiling that use next generation sequencing to identify targets associated with sensitivity to specific therapeutic agents [14]. Recently oncotype DX has also been investigated in the metastatic breast cancer setting to identify patients who would perhaps benefit from chemotherapy rather endocrine therapy [15]. The problem with such techniques is that it involves using tissue biopsies taken at each time point of progression of disease as one would assume that targets within the tumor tissue do not remain static but are continuously evolving along the cancer progression continuum. The concept of the use of CTCs to guide changes in therapeutic management is an interesting one. The ease with which CTCs can be measured, the fact that it can be measured at various time points during treatment and at the time of progression of disease and the fact that one could test several targets on the CTC itself make it a unique test that is easier to use than having to attain several tissue biopsies and as such several studies have attempted to study its predictive power. Smerage and colleagues [16] recently reported on the final results of the SWOG S0500 study the aim of which was to look at whether changes in chemotherapeutic regimens among women with metastatic breast cancer based on changes in the levels of CTCs measured 21 days after commencing first-line therapy could improve prognostic outcome in this cohort. Unfortunately the authors observed early changing of therapy did not impact either progression-free or overall survival in this cohort. Whether an early change in chemotherapy regimen based on CTC levels in the third line or later setting would impact prognostic outcome is currently being investigated in the CirCe01 trial.

Technology is rapidly evolving and indeed technologies with CTC analytic capabilities have progressed beyond the currently available CellSearch technology [17,18]. The ideal technology would not only improve detection of CTCs over various time points during the course of treatment but would also allow for real time

detection of changes in biological tumor characteristics thereby enabling dynamic genotype directed therapeutic approaches. Key CTC markers that are likely to impact therapeutic decisions include expression of estrogen receptor, mutations in the estrogen receptor gene ESR1, changes in expression of or gene copy number of HER2 and mutations in the PIK3CA gene. Furthermore, one could envision that molecular characterization of CTC would also allow for identification of actionable mutations as well as enabling serial tumor monitoring for detection of acquisition of mutations that would render tumors resistant to certain therapeutic agents [19]. Taking this concept one step further Paoletti and colleagues [20] using an assay based both on CTC enumeration (using CellSearch) and measuring of protein expression developed the Circulating Tumor Cell-Endocrine Therapy Index (CTC-ETI) to help identify patients with endocrine resistant tumors earlier. CTCs were assessed for four endocrine therapy specific markers including estrogen receptor, Bcl-2, HER2 and Ki67 with low expression of these markers hypothesized to be associated with endocrine therapy resistance. The CTC-ETI was categorized into three groups based on a derived score (low was 0.3, intermediate was 4–6 and high was >14) with a high score indicating resistance to endocrine therapy. The investigators established the analytic validity of this test on a small cohort of 50 patients with metastatic breast cancer. Interestingly they identified that 39% of the population studied had a high CTC-ETI score suggesting that a significant subset of patients had endocrine therapy resistant tumors. Such information, if formally validated, would allow oncologists to effectively individualize therapeutic strategies among patients with hormone receptor positive metastatic breast cancer at various time points during the natural course of the disease. Clinical validation of the CTC-ETI test is currently being conducted through two randomized clinical trials (COMETI P2 and SWOG S1222).

References

- 1 Sørlie T, Perou CM, Tibshirani R *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl Acad. Sci. USA* 98(19), 10869–10874 (2001).
- 2 Dawood S1, Broglio K, Buzdar AU, Hortobagyi GN, Giordano SH. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. *J. Clin. Oncol.* 28(1), 92–98 (2010).
- 3 Dieci MV, Barbieri E, Piacentini F *et al.* Discordance in receptor status between primary and recurrent breast cancer

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So where do we go from here? As we formally enter an era where the majority of the treatment for patients with breast cancer will be based on specific targets real time identification of these targets would certainly be facilitated by genotyping of CTCs allowing for a personalized approach to management. Trials such as the SAFIR-01 and the I-SPY studies have used technologies that require tissue biopsies to identify evolving targets and personalizing treatment based on these targets among patients with metastatic breast cancer. Attaining such biopsies are cumbersome and may not always be feasible. CTC measurements at various time points functions as a sort of a real time ‘liquid biopsy’ that allows for rapid assessment at multiple time points. The information derived from these ‘liquid biopsies’ would not only service as a powerful prognostic marker that could help in risk stratification but would also play a major role in the application and monitoring of personalized therapeutic approaches to the management of patients with metastatic breast cancer. However, is it ready for prime time use? Can we effectively replace tissue biopsies with analysis of CTCs? As of now the simple answer would be no. We will need to wait for confirmatory studies to clearly elucidate its clinical utility and define the role of CTCs both as a predictive and prognostic tool.

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has a prognostic impact: a single-institution analysis. *Ann Oncol.* 24(1), 101–108 (2013).

- 4 Ashworth TR. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. *Aust. Med. J.* 14, 146–149 (1869).
- 5 Alix-Panabieres C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. *Clin. Chem.* 59, 110–118 (2013).
- 6 Cristofanilli M, Budd GT, Ellis MJ *et al.* Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N. Engl. J. Med.* 351(8), 781–791 (2004).
- 7 Cristofanilli M, Hayes DF, Budd GT *et al.* Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *J. Clin. Oncol.* 23, 1420–1430 (2005).

- 8 Hayes DF, Cristofanilli M, Budd GT *et al.* Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin. Cancer Res.* 12, 4218–4224 (2006).
- 9 Bidard FC, Peeters DJ, Fehm T *et al.* Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. *Lancet Oncol.* 15, 406–414 (2014).
- 10 Lucci A, Hall CS, Lodhi AK *et al.* Circulating tumour cells in non-metastatic breast cancer: a prospective study. *Lancet Oncol.* 13, 688–695 (2012).
- 11 Rack B, Schindlbeck C, Jückstock J *et al.* Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. *J. Natl Cancer Inst.* 106(5). pii: dju066 (2014).
- 12 Smith BM, Slade MJ, English J *et al.* Response of circulating tumor cells to systemic therapy in patients with metastatic breast cancer: comparison of quantitative polymerase chain reaction and immunocytochemical techniques. *J. Clin. Oncol.* 18, 1432–1439 (2000).
- 13 Liu MC, Shields PG, Warren RD *et al.* Circulating tumor cells: a useful predictor of treatment efficacy in metastatic breast cancer. *J. Clin. Oncol.* 27, 5153–5159 (2009).
- 14 Von Hoff DD, Stephenson JJ Jr, Rosen P *et al.* Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *J. Clin. Oncol.* 28(33), 4877–4883 (2010).
- 15 King TA, Lyman JP, Gonen M *et al.* Prognostic impact of the 21-gene recurrence score in patients presenting with Stage IV breast cancer. Presented at: *49th Annual Meeting of the American Society of Clinical Oncology*, , Chicago, IL, USA, 31 May–4 June 4 2013 (Abstract 507).
- 16 Smerage JB, Barlow WE, Hortobagyi GN *et al.* Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. *J. Clin. Oncol.* 32, 3483–3489 (2014).
- 17 Yu M, Stott S, Toner M *et al.* Circulating tumor cells: approaches to isolation and characterization. *J. Cell. Biol.* 192, 373–382 (2011).
- 18 Pantel K. Alix-Panabie' res C: real-time liquid biopsy in cancer patients: fact or fiction? *Cancer Res.* 73, 6384–6388 (2013).
- 19 Sarangi S, Mosolpuria K, Higgins MJ *et al.* The evolving role of circulating tumor cells in the personalized management of breast cancer: from enumeration to molecular characterization. *Curr. Breast Cancer Rep.* 6(3), 146–153 (2014).
- 20 Paoletti C, Muniz MC, Thomas DG *et al.* Development of circulating tumor cell-endocrine therapy index in patients with hormone receptor positive breast cancer. *Clin. Cancer Res.* pii: clincanres.1913.2014. (2014) (Epub ahead of print).