Review Article DOI: 10.18203/issn.2456-3994.IntJMolImmunoOncol20170052

Circulating tumor cells in breast cancer

Akhil Jain[1], Moushumi Suryavanshi[2], Ullas Batra[1], Pankaj Goyal[1], Dinesh Doval[3]

Departments of [1]Medical Oncology, [2]Consultant, Center for Molecular Diagnostics & Cell Biology, [3]Department of Medical Oncology & Research, Rajiv Gandhi Cancer Institute & Research Centre, New Delhi, India

Correspondence to: Akhil Jain, E-mail: drakhiljain17@gmail.com

ABSTRACT

Breast cancer is a heterogeneous disease. Liquid biopsy is a novel diagnostic tool and may provide answers to many questions related to unevenness in prognosis and ultimate outcome. Different technologies for CTC isolation, enrichment, detection, and characterization are under evaluation. Various clinical trials and meta-analysis have been conducted to define the role of CTC in early and metastatic breast cancer. CTCs are superior to other serum markers for prognostication. Their role as predictive marker remains elusive.

Key words: Circulating tumor cells, Early breast cancer, Metastatic breast cancer, Liquid biopsy

"The cancer cells that crawl"

The last decade has been the era of "revolutionary ideas" and "commendable practice changing" trends in cancer treatment.

Breast cancer has largely been known as a "systemic disease." Paget's "seed and soil" hypothesis is one of the accepted theories that may explain the progression of limited disease to distant sites. Owing to emendation in the adjuvant treatment of early breast cancer (EBC) patients, prognosis of these patients is gratifying. Approximately, 20–30% of appropriately treated EBC patients eventually fail and land up in metastatic stage.[1]

Not only there has been upsurge in the invention of novel targeted therapies and immunotherapies, but also the field of cancer diagnostics has undergone immense elaboration. Presently, prognosis and prediction of treatment outcome are merely defined on the characteristics of either resected tumor or biopsy specimen. At times, this may be fallacious and unsatisfactory. The novel diagnostic tools such as liquid biopsy may provide answers to many questions related to unevenness in prognosis and ultimate outcome. Liquid biopsy is collection and/or extraction of anybody fluid and processing it for separating tumor products. It is a liquid biomarker isolated from body fluid and may be called "real-time cancer detection." The liquid biopsy may yield circulating tumor cells (CTCs), circulating tumor nucleic acids (ctNA, circulating tumor DNA [ctDNA], miRNA, mRNA, and long non-coding RNA), or exosomes (small membrane-derived vesicles, 40–100 nm, containing various molecules such as signal proteins, miRNA, lipids, and exoDNA).[2] These components of tumor are collectively called as circulating tumor products (CTPs) and may be released into the peripheral blood from either primary tumor or metastatic deposits.

Role of Circulating Tumor Cells in Metastatic Progression

Breast cancer is a heterogeneous disease and may possess different types of cancerous clones even in a single tumor mass. During metastatic progression, the tumor cells must first detach from the primary tumor and intravasate into the bloodstream. This is possible owing to the genetic evolution of clone cells and phenotypic changes in the epithelial cells (epithelial-to-mesenchymal transition [EMT]).[3] These cells in circulation called CTCs must evade immune detection and extravasate into microvessels of a target tissue. The successful formation of a micrometastatic or metastatic lesion, however, is dependent on the ultimate ability of these cells to adapt, survive, proliferate, and induce neoangiogenesis in the target tissue.[4]

Studies in the field of do rmant metastasis initially relied on the detection of micrometastasis which as defined by the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) is any tumor focus at any site >0.2 mm diameter and/or >200 confluent tumor cells but none >2.0 mm.[5] Tumor dormancy in these micrometastasis has been reported and accepted as a logical reason for prolonged latency before developing overt metastasis. The prognostic significance of micrometastasis in the case of breast cancer was established in lymph nodes (N1mi) and other sites (M1) as in the case of bone marrow.[6]

With advances in technology focus shifted more toward the detection of isolated tumor cells (ITCs) denoted as N0(i+) and disseminated tumor cells (DTCs) denoted as cM0(i+) by UICC and AJCC and defined as either isolated or clusters of cells <0.2 mm in diameter in lymph nodes as ITCs and any tissue

outside breast and surrounding regional lymph nodes (bone marrow, peritoneal cavity, and blood) in the absence of clinical/radiographic findings of overt metastases. The major difference between DTCs and micrometastasis as defined originally by UICC and AJCC lies in their non-proliferating dormant state and absence of stromal reaction. Since epithelial cells are normally absent in the bone marrow and peripheral circulation, detection of these kinds of cells in the blood or marrow implies the presence of CTC or DTC.[7]

The case of CTCs demands special mention. These CTCs are circulating at very low frequency in the blood, thereby making their concentration minuscule (~1 CTC per 10^5–10^7 leukocytes). Such a low concentration necessitates the development of sensitive and specific approaches for their isolation, enumeration, and molecular characterization. Butler and Gullino estimated that 1 g of tumor tissue (10^9 cells) sheds about 3–4 × 10^6 tumor cells into the circulation per day. Not all of these CTCs are successful in establishing distant metastatic disease. Fidler *et al.* demonstrated that after 24 h of intravenous administration of tumor cells, <0.1% cells remain viable and <0.01% of these viable CTCs can produce metastasis.[8] Fidler named those successful as the "decathlon champions" of CTCs. The predicted half-life of these CTCs is in the range of 1–2.4 h. This low "success rate" could be explained by a model similar to "survival of the fittest."

Methods for Circulating Tumor Cells Isolation and Detection

There are many different technologies for CTC isolation, enrichment, detection, and characterization [Table 1]. These technologies may be divided into the phase of enrichment and isolation, followed by cell detection and cell characterization. Technology for CTC isolation uses either physical separation or immunoaffinity-based methods for CTC enrichment.[9] Physical separation is based on their biophysical and biomechanical properties which distinguish CTCs from normal blood cells, e.g., size (larger; >8 μm), less deformability, density, and electricity. Immunoaffinity-based enrichment is based on a CTC marker usually a cell surface protein. The "perfect" CTC marker for immunoaffinity-based enrichment would be expressed on all CTCs but not on autochthonous blood cells (leukocytes, endothelial cells, hematopoietic stem cells, and mesenchymal stem cells). Such marker should not be repressed or destroyed during the invasion and circulation process. The search for cell surface markers is ongoing, and many including EpCAM and Muc1 have been tried for immunoaffinity-based enrichment. Currently, defined CTC is a cell with nucleus, visible cytoplasm, and the expression of cytokeratin and absence of CD45 expression.[10]

Using above-mentioned differential properties of CTCs, they can be enriched either by positive selection of CTCs, e.g., EpCAM-based CellSearch; negative depletion of non-CTCs, e.g., red blood cells by hypotonic lysis or density gradient-based methods and white blood cells by antibodies directed against leukocytes, e.g., leukocyte common antigen (CD45); or on a combination of both. Examples of commercial tests based on positive selection – CellSearch and CTC chip. Examples of commercial tests based on negative depletion – RosetteSep.[11] Finally, CTC detection and characterization can be done by immunocytological (flow cytometry); molecular (reverse transcription-polymerase chain reaction [RT-PCR]); and functional assays (cell culture – EPISPOT; xenotransplantation).

Hardships in Circulating Tumor Cells Research

Circulating epithelial cells have been reported in patients without malignancy, e.g. inflammatory conditions and benign colon diseases, but these conditions are rare.[12]

Many CTC detection techniques including CellSearch depend solely on positive selection of CTCs based on epithelial protein expression (e.g. EpCAM and cytokeratins). Since these expressions may have intra- and inter-tumoral heterogeneity, this process of positive selection may not be absolutely accurate. Second, detection of epithelial markers alone may miss CTCs bearing mesenchymal signatures. These issues have been addressed by using EpCAM-independent enrichment approaches by antibody-independent and antibody-dependent methods.

"Hitting" the functionally competent CTCs is the most needed and is being tested *in vitro* immunoSpot ELISPOT test, the invasion assay (based on active digestion of a fluorescently labeled cell adhesion matrix), and *in vitro* xenotransplantation assay. While most of these tests are underway their clinical validation, only one test CellSearch, a technology based on EpCAM-positive enrichment, has received US Food and Drug Administration (FDA) approval for CTC detection as an aid in monitoring patients with metastatic breast, colorectal, and prostate cancer.

Circulating Tumor Cells Subpopulation

Work by various researchers has shown that CTC may have different phases/ages in its lifespan. It has been reported to exist as a subpopulation expressing either epithelial markers or mesenchymal predominance or in intermediate state. It may also acquire the characteristics of quiescence and self-renewal, thus behaving as a cancer stem cell (most commonly identified by CD44+/CD24− or ALDH1 expression).[13] Now, DTCs are also recognized as a subtype of CTC.

Applications of Isolated Circulating Tumor Products

CTCs isolated from patient's blood (or other fluids) may be used for a number of applications such as identifying specific markers by immunostain; genomic amplification and translocation by fluorescence *in situ* hybridization. Nucleic acid

Table 1: Various CTC isolation and detection methods

Platform	Vendor/Developer	Methodology
Immunoaffinity assay		
Cell search	Veridex	Epcam-coated beads based positive selection using magnetic beads followed by staining and image analysis. Clinically validated in metastatic breast, colorectal and prostate cancer. Only FDA approved platform
Adna test	Adnagen	Immunomagnetic bead enrichment (EPCAM, MUC-1, mesothelin) followed by nested PCR
Anti-EPCAM, Anti-CK antibody	Glenn Deng, Stanford University	CTC enrichment assay using the combination of anti-CK and anti-EPCAM antibodies
Cell collector	Gilupi	Functionalized structured medical wire coated with anti-EPCAM antibodies placed directly into the blood stream of a patient via an indwelling catheter. Stays in the arm vein for 30 min and thus enables the capture of CTCs *in vivo*
Biofluidica CTC	Biofluidica	Epcam-coated chip to capture EPCAM expressing cells followed by elution and electrical counting
Epispot	Laboratoire de virologie	Initial depletion of CD45 followed by EPCAM expressed selection
Microfluidic devices		
Oncocee	Biocept	Biotin-tagged antibodies that bind selectively to CTCs
Clearcell	Clearbridge	Label-free technology that uses lateral traps to capture tumor cells based on size and deformability
Herringbone-chip	Daniel Haber and Mehmet toner	Microvortices are used to significantly increase the number of interactions between target CTCs and the antibody-coated chip surface
De novo Sciences Jetta 400	Wayne Klohs Sunitha Nagrath Gil Omenn David Parkinson Ken Pienta	CTC isolation is achieved by flowing a sample over a proprietary designed set of 56,320 microfluidic capture chambers. The Systems will then characterize the cells for downstream analysis
Size based devices		
Screencell	Screencell	Microporous membrance filter allows size selective isolation of CTCs
Cellsieve	Creatv microtech	Lithographically fabricated filters with precision pore dimensions
Size and deformability		
Parsortix	Angle	Uses size and deformability using a wier-type step filter
Density		
Oncoquick	Greiner bio one	Porous barrier density gradient centrifugation technology
VI. Immunomagnetic and physical properties		
Magsweeper	Stanford University	Immunomagnetic enrichment of target cells. Individual extraction of isolated cells based on their physical characteristics

CTC: Circulating tumor cell, FDA: Food and Drug Administration, PCR: Polymerase chain reaction

(DNA or RNA) can be extracted and applied for sequencing, quantitative RT-PCR, and expression profile analysis. It may also be possible to obtain cell culture from the isolated viable CTC. Haber *et al*. beautifully narrated the difference between utilities of CTC and ctDNA. ctDNA may be largely utilized for amplification, deletion, translocation, point mutation, and chromosomal abnormalities.[14]

Circulating Tumor Cells in Breast Cancer Patients

There have been various clinical trials and meta-analysis [Table 2] to understand and establish the role of CTC in breast cancer in the last few years. CTCs have been separated in both metastatic breast cancer (MBC) and, later, EBC patients. Below is the brief understanding of the so far gained knowledge regarding CTCs in breast cancer.

Circulating Tumor Cells in Metastatic Breast Cancer

The utmost need to improve the survival of MBC patients led Cristofanilli *et al*. to test the hypothesis that the levels of CTC could predict survival. They conducted a prospective, multicenter study in 177 measurable MBC patients. Levels of CTCs were tested both before the start of treatment and at first follow-up visit, that is, approximately at three to four weeks. As a control, circulating epithelial cells were rare in healthy (mean, 0.1 ± 0.2 per 7.5 ml of whole blood) and benign breast disorders (mean, 0.1 ± 0.9 per 7.5 ml of whole blood). A Cutoff threshold of ≥5 CTC/7.5 ml was reported as binary notation capable of conferring prognostic significance. Patients with CTCs ≥5 per 7.5 ml of whole blood, as compared with the group with fewer than 5 CTCs per 7.5 ml, had a shorter median progression-free survival (PFS) (2.7 vs. 7.0 months, $P < 0.001$) and shorter

Table 2: Trials in CTC breast

Trial	Conclusion	Study
CTC in metastatic breast cancer		
Cristofanilli *et al*.	≥5 CTC/7.5 ml: Cut-off for prognostic significance ≥5 CTC/7.5 ml had shorter median PFS and OS levels at baseline and at first follow-up most significant predictors of PFS and OS	Prospective
Cristofanilli *et al.*	CTCs have superior and independent prognostic value	Prospective
Smearge *et al.*	Confirmed prognostic value of CTCs Early change of therapy was not effective in changing OS	Prospective
Giuliano *et al*.	≥5 CTCs/7.5 ml Increased baseline number of metastatic sites Development of new lesions were significantly greater on treatment failure Significant shorter time to visceral metastasis	Retrospective
Pierga *et al.*	Confirmed independent poor prognostic value associated with increased CTCs in terms of survival	Prospective
Bidard *et al.*	Established CTC as dynamic prognostic marker of PFS and OS	Pooled analysis
CTC in early breast cancer		
Janni *et al.*	Established CTC as a significant independent prognostic factor for DFS, DDFS, BCSS and OS In EBC, at least 1 CTC may be of value	Pooled analysis
Rack *et al.*	CTCs are prognostic for reduced DFS, distant DFS, BCSS, and OS before the start of systemic treatment CTCs are prognostic for DFS after completion of adjuvant chemotherapy CTC clearance does not predict chemotherapy benefit	Pooled analysis
Lucci *et al.*	Detection of one or more circulating tumor cells at start of therapy predicted both decreased PFS and OS	Prospective
Pachmann *et al.*	≥5 CTC/7.5 ml had shorter median OS	Prospective
CTC in inflammatory breast cancer		
Hall et al.	Prognostic significance of one or more CTC in 7.5 ml blood on RFS	Prospective
Piegra et al.	CTC detection at baseline independently predicted poor 3-year DFS and 3-year OS	Pooled analysis

CTC: Circulating tumor cell, DFS: Disease-free survival, Os: Overall survival, Rfs: Relapse-free survival

overall survival (OS) (10.1 vs. >18 months, $P < 0.001$). At the first follow-up visit after the initiation of therapy, this difference between the groups persisted (PFS, 2.1 vs. 7.0 months; $P < 0.001$; OS, 8.2 vs. >18 months; $P < 0.001$). Investigators concluded that this cutoff gave a reliable estimation of disease progression and survival earlier than estimations made with the use of traditional imaging methods (3–4 vs. 8–12 weeks after the initiation of therapy, respectively). The multivariate analysis showed that the levels of CTCs at baseline and at the first follow-up visit were the most significant predictors of PFS and OS of all the variables.[15]

In a similar study at M. D. Anderson Cancer Center, CTCs were counted in 151 patients of MBC. These patients were also evaluated for other prognostic cancer markers such as hormone receptor and Her2 status along with CA 27.29. Cases with 5 or more CTC had a median OS of 13.5 months. The median OS for those with <5 CTC was above 29 months. The research group rested their case stating that CTCs have superior and independent prognostic value.[16]

Based on the background that increased CTCs were associated with poor prognosis in terms of PFS and OS, Smerage *et al.* (SWOG SO500 trial) tested the response of change of chemotherapy after one cycle of first-line chemotherapy in MBC patients. Patients who had increased CTCs after 21 days of therapy were randomized either into continuing same therapy or change of therapy. No difference in survival was reported in these two arms (10.7 months and 12.5 months, respectively, $P = 0.98$). Median survival of patients who did not have increased CTCs at baseline and whose CTCs dropped after first cycle of therapy were 35 and 23 months, respectively. The prognostic value of CTCs was confirmed, and investigators concluded that early change of therapy was not effective in changing OS. They suggested that such findings may be predictive of innate chemotherapy refractoriness of the tumor and strongly recommended the clinical trial participation of patients having increased CTCs after one cycle of therapy.[17] Riethdorf *et al.* suggested the differential responses to treatment between the primary tumor and metastasis and/or CTCs.[18] CirCe01 and STIC CTC are the currently undergoing clinical utility trials based on CTC count.

Any disease has some risk factors that may be either prognostic of the disease behavior or predictive of the treatment response. In advanced breast cancer, traditional prognostic factors do not always adequately predict treatment response. Giuliano *et al.* in their retrospective study explored the role of CTC counts as predictors of disease evolution in breast cancer patients with limited metastatic dissemination. A pre-treatment level ≥5 CTCs/7.5 ml was associated with an increased baseline number of metastatic sites compared with <5 CTCs/7.5 ml ($P = 0.0077$). At the time of treatment failure, development of new lesions was significantly greater and frequent in

patients with ≥5 CTCs/7.5 ml compared with those with <5 CTCs/7.5 ml. Patients with predominantly nonvisceral metastatic sites and single metastatic site, with ≥5 CTCs/7.5 ml had remarkably significant shorter time to visceral metastases and development of new metastatic sites compared with <5 CTCs/7.5 ml. Patients with increased CTCs had worse survival. Thus, they concluded that baseline CTCs counts can be used as an early predictor of metastatic potential in breast cancer patients with limited metastatic dissemination.[19]

An important question that intrigues the mind of oncologist is whether CTC is a best prognostic marker to date. The first prospective observational study in this regard was IC 2006-04. The study confirmed the independent poor prognostic value associated with increased CTCs in terms of survival.[20] Bidard *et al.* reported the prospectively planned secondary objective of the IC 2006-04 study, the comparison of CTC with different serum tumor markers (carcinoembryonic antigen [CEA], cancer antigen 15-3 [CA 15-3], and cytokeratin fragment 21-1) and non-tumor markers (lactate dehydrogenase and alkaline phosphatase).[21] Both the investigators reported no clear prognostic superiority of CTC over serum markers. To establish the clinical validity of CTC quantification and other serum markers, a pooled analysis including 1944 patients over 17 centers was performed. Increased CTC (≥5 CTCs/7.5 ml) at baseline, after 3–5 and 6–8 weeks of therapy conferred significantly poor survival in terms of PFS and OS. Bidard *et al.* reported the superiority of CTC over serum markers (CEA and CA15.3) and defined CTC as "dynamic prognostic marker of PFS and OS which should be interpreted in conjugation with other features; addition of CTC count at baseline and monitoring during treatment significantly increases the prognostic value of the model."[22]

Circulating Tumor Cells in Early Breast Cancer

Over the past few years, integration of multidisciplinary approach for treating breast cancer has boosted the cure rate and survival of breast cancer patients. Still number of appropriately treated EBC patients, approximately 20–30%, ultimately fail and land up in metastatic stage.[3] Pantel *et al.* have reported that occult micrometastasis is out-of-reach of high-resolution technologies.[23] Questioning the traditional prognostic factors and peeping into CTCs notoriety gives many plausible explanations.

In the early phase of CTC era, DTC, a subtype of CTC, was shown to have prognostic value in EBC patients. Braun *et al.* in their pooled analysis of 4703 patients, having DTC in bone marrow, reported poor outcome in these patients before initiation of therapy.[6] Janni *et al*. conducted a pooled analysis of 3173 patients having non-metastatic (Stage I–III) breast cancer from 5 breast cancer institutes. The presence of CTCs was associated with a large tumor size, increased lymph node involvement, unfavorable histological grade, and lobular tumor type, whereas no significant association was identified between CTC presence and menopausal status, hormone-receptor status, or HER2 status. Patients with CTCs more often received neoadjuvant and/or adjuvant chemotherapy than did patients without CTCs. Presence of CTC was a significantly independent prognostic factor for disease-free survival (DFS), distant DFS, breast cancer-specific survival, and OS. CTC was not significantly associated with prognosis for nodal stage N0 disease and also in hormone receptor negative/HER2-positive tumors. They stressed upon that though ≥5 CTCs/7.5 ml is the accepted cutoff in metastatic setting, CTCs prognostic relevance in EBC is independent of cutoff value, and presence of at least 1 CTC may be of value.[24]

Another large prospective, randomized, multicentric study (SUCCESS, Germany) which gives insight into the role of CTC in EBC was reported by Rack *et al*. CTCs were analyzed in 2026 patients with EBC before adjuvant chemotherapy and in 1492 patients after chemotherapy. They reported increased CTCs to be a prognostic marker for reduced DFS, distant DFS, breast cancer-specific survival, and OS before the start of systemic treatment and for DFS after completion of adjuvant chemotherapy. CTC detection was more in node-positive patients as compared to node negative ($P < 0.001$). No association was found with tumor size, grading, or hormone receptor status. Interestingly, the initially CTC-negative patients who subsequently developed CTCs fared better than initially CTC-positive patients whose CTCs disappeared post-treatment, suggesting CTC clearance does not predict chemotherapy benefit.[25]

Lucci *et al.* conducted a prospective study of 302 chemo-naive patients with Stage I to III operable breast cancer undergoing surgery for their primary tumors between February 2005 and December 2010 at MD Anderson Cancer Center and showed that detection of one or more CTCs at start of therapy predicted both decreased PFS and OS.[26]

A German group studied 35 women with EBC and enumerated their CTC before any treatment. 17 tested positive for CTC and the other 18 tested negative. Follow-up data showed that the group that tested negative for CTC had a median OS of 125 months. In contrast, the group with 5 or more CTC/7.5 ml of blood had a median OS of only 61 months.[27]

Circulating Tumor Cells in Inflammatory Breast Cancer

An intermediate between EBC and MBC is IBC (inflammatory breast cancer). Hall *et al.* in their work reported the prognostic significance of one or more CTC in 7.5 ml blood who received neoadjuvant therapy for IBC. Using this cutoff, approximately 15–20% of non-IBC patients have CTC detection.[28] Hall *et al*. reported CTC detection, with same criteria, in 27% of patients. In IBC, CTC detection was not associated with either tumor characters or with pathological complete response. They reported the major prognostic impact on the relapse-free survival.

Piegra *et al.*, recently, in the pooled analysis of two multicentric Phase II trials (BEVERLY 1 and 2) of neoadjuvant therapy combined with bevacizumab showed that CTC detection is an independent prognostic factor in 52 primary HER2+ IBC patients. At baseline, 39% patients had ≥1 detectable CTC. After 4 cycles of CT, a dramatic drop in CTC to a rate of 9% was observed. Pathological complete response rate was 40% and was associated with the absence of hormonal receptor and HER2+ status. CTC detection at baseline independently predicted the significant difference in 3-year DFS (70% vs. 39% for patients with < 1 vs. ≥ 1 CTC/7.5 mL and 3-year OS [92% vs. 56%]).[29]

Circulating Tumor Cells in HER2+ Breast Cancer

Yet, another poor prognostic factor in the outcome of breast cancer patients is HER2 overexpression. Wulfing *et al.* and Hayashi *et al.* have reported the poor prognostic value of HER2+ CTC in comparison to HER2-CTC in patients with EBC and MBC, respectively. Wulfing even reported the HER2 disconcordance rate between primary tumor and CTC in EBC patients.[30]

Currently, treatment decisions at the time of MBC relapse are generally made based on the receptor status of the primary breast cancer. However, discordance in receptor status between primary tumor and disease recurrence has been observed in up to 10% of patients. It is either because of clonal evolution or shedding of the "hidden" HER2+ cells from the primary. How shall those patients be treated who are negative for Her2 amplification at primary site but exhibit amplification in CTCs? As yet, there are minimal data addressing this. Meng *et al.*reported in their retrospective study of 24 patients with MBC and HER2 − primary tumor, that four of nine patients with HER2+ CTCs at the time of metastatic disease received trastuzumab. Of these, one had rapid remission of symptoms and complete response on imaging, two patients had partial responses, and one no response.[31]

The question remains open and continues to beg the answer. DETECT III, randomized phase III trial (NCT01619111), and French CirCe T-DM1, single arm study shall answer this question.

Circulating Tumor Cells as Liquid Biopsy

Imaging allows obtaining a biopsy from most metastatic sites and is relatively cheap and standard of care but fraught with serious morbidity occasionally and a rare fatality. In light of this, analysis of biomarkers on CTC is an attractive option and has been alluded to as liquid biopsy. Serial assessment of biomarker status, therefore, can only be realistically obtained from a less invasive procedure such as harvested CTCs. The barriers to use of liquid biopsy, however, are many such as cost, availability, validated platforms, and clinical validity and utility of test outcomes. However, needless to say that concept is appealing.

Circulating Tumor Cells as Companion to Imaging in Determining Response to Treatment

Investigators have shown that CTCs evaluation may be more accurate than imaging used to evaluate the effectiveness of treatment in MBC. In a pioneering work performed in 2006, MBC patients had imaging tests done before and 10 weeks after they began therapy. CTC was measured 4 weeks after the start of therapy. The group that responded to treatment based on imaging tests but had 5 or more CTCs suffered a poorer outcome than the cohort with CTC counts below 5 but less definite response on imaging. These findings suggest that the levels of CTC were far more important at predicting survival compared to the actual visual changes noted on imaging tests. In addition, there was a 15% disagreement in the interpretation of the imaging tests between the two radiologists, compared to <1% variation in the results of CTC testing. The precision of CTCs enumeration coupled with superior response predictor demonstrates the potential of CTCs *vis-à-vis* radiologic studies and seems to be a more robust predictor of survival than is a radiographic response.[32]

Conclusion

CTCs are the unique and novel way of liquid biopsy. Ease of accessing blood sample or any other fluids do merit over other invasive procedures. CTCs bear prognostic significance in breast cancer patients (early, inflammatory, and metastatic). CTCs are superior to other serum markers for prognostication. Their role as predictive marker remains elusive. CTC evaluation is limited by the availability of current isolation technologies and their cost. Various trials are underway to better understand the validity and utility of CTC in breast cancer.

The increasing use of precision molecules in the treatment of cancer and acquired resistance thereof may propel the use of liquid biopsy to seek secondary mutations. Can the liquid biopsy reliably and accurately mirror the changes in the tumor sites is a question that needs to be answered, however, this seems to be an important potential use. With thousands of targeted molecule in development, an early intermediate end point will be handy in speedy launch of newer drugs.

The methods of enumeration and harvesting CTCs are many but lack analytical validity which in any case is by comparison to cell search system (the only FDA approved system) which itself has received criticism for relying on EPCAM-based positive selection, the expression of which may actually be suboptimal during EMT. Other new systems are under evaluation and may have proven ability to address the heterogeneity of CTC.

There are several challenges to making CTCs as a multifunctional cancer biomarker, but such challenges also provide opportunity for innovative and ingenious discoveries. The limit of science is decided by the ability of the human

mind to think and once the mind is seeded by a new thought the answers will come. History bears testimony to this fact.

References

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Peto R, Davies C, Godwin J, Gray R, Pan HC. Comparisons between different polychemotherapy regimens for early breast cancer: Meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet 2012;379:432-44.
2. Diaz LA, Bardelli A. Liquid biopsies: Genotyping circulating tumor DNA. J Clin Oncol 2014;32:579-86.
3. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420-8.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. cell. 2011;144:646-74.
5. Edge S. AJCC Cancer Staging Manual. 7th ed. New York, NY, USA: Springer; 2010.
6. Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, *et al.* A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 2005;353:793-802.
7. Oakman C, Pestrin M, Bessi S, Galardi F, Di Leo A. Significance of micrometastases: Circulating tumor cells and disseminated tumor cells in early breast cancer. Cancers (Basel) 2010;2:1221-35.
8. Fidler IJ. Metastasis: Quantitative analysis of distribution and fate of tumor emboli labeled with 125I-5-iodo-2′-deoxyuridine. J Natl Cancer Inst 1970;45:773-82.
9. Harouaka R, Kang Z, Zheng SY, Cao L. Circulating tumor cells: Advances in isolation and analysis, and challenges for clinical applications. Pharmacol Ther 2014;141:209-21.
10. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, *et al*. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 2004;10:6897-904.
11. Gabriel MT, Calleja LR, Chalopin A, Ory B, Heymann D. Circulating tumor cells: A review of non-EpCAM-based approaches for cell enrichment and isolation. Clin Chem 2016;62:571-81.
12. Pantel K, Denève E, Nocca D, Coffy A, Vendrell JP, Maudelonde T, *et al.* Circulating epithelial cells in patients with benign colon diseases. Clin Chem 2012;58:936-40.
13. McInnes LM, Jacobson N, Redfern A, Dowling A, Thompson EW, Saunders CM. Clinical implications of circulating tumor cells of breast cancer patients: Role of epithelial–mesenchymal plasticity. Frontiers in Oncology. 2015;5:42. doi:10.3389/fonc.2015.00042.
14. Haber DA, Velculescu VE. Blood-based analyses of cancer: Circulating tumor cells and circulating tumor DNA. Cancer Discov 2014;4:650-61.
15. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, *et al*. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781-91.
16. Cristofanilli M, Broglio KR, Guarneri V, Jackson S, Fritsche HA, Islam R, *et al*. Circulating tumor cells in metastatic breast cancer: Biologic staging beyond tumor burden. Clin Breast Cancer 2007;7:34-42.
17. Smerage JB, Barlow WE, Hortobagyi GN, Winer EP, Leyland-Jones B, Srkalovic G, *et al*. Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. J Clin Oncol 2014;32:3483-9.
18. Riethdorf S, Müller V, Zhang L, Rau T, Loibl S, Komor M, *et al*. Detection and HER2 expression of circulating tumor cells: Prospective monitoring in breast cancer patients treated in the neoadjuvant GeparQuattro trial. Clin Cancer Res 2010;16:2634-45.
19. Giuliano M, Giordano A, Jackson S, De Giorgi U, Mego M, Cohen EN, *et al*. Circulating tumor cells as early predictors of metastatic spread in breast cancer patients with limited metastatic dissemination. Breast Cancer Res 2014;16:1.
20. Pierga JY, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, *et al*. High independent prognostic and predictive value of circulating tumor cells compared with serum tumor markers in a large prospective trial in first-line chemotherapy for metastatic breast cancer patients. Ann Oncol 2012;23:618-24.
21. Bidard FC, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, *et al*. Assessment of circulating tumor cells and serum markers for progression-free survival prediction in metastatic breast cancer: A prospective observational study. Breast Cancer Res 2012;14:1.
22. Bidard FC, Peeters DJ, Fehm T, Nolé F, Gisbert-Criado R, Mavroudis D, *et al*. Clinical validity of circulating tumour cells in patients with metastatic breast cancer: A pooled analysis of individual patient data. Lancet Oncol 2014;15:406-14.
23. Pantel K, Alix-Panabières C, Riethdorf S. Cancer micrometastases. Nat Rev Clin Oncol 2009;6:339-51.
24. Janni WJ, Rack B, Terstappen LW, Pierga JY, Taran FA, Fehm T, *et al*. Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. Clin Cancer Res 2016;22:2583-93.
25. Rack B, Schindlbeck C, Jückstock J, Andergassen U, Hepp P, Zwingers T, *et al*. Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. J Natl Cancer Inst 2014;106:dju066.
26. Lucci A, Hall CS, Lodhi AK, Bhattacharyya A, Anderson AE, Xiao L, *et al*. Circulating tumour cells in non-metastatic breast cancer: A prospective study. Lancet Oncol 2012;13:688-95.
27. Pachmann K, Dengler R, Lobodasch K, Fröhlich F, Kroll T, Rengsberger M, *et al*. An increase in cell number at completion of therapy may develop as an indicator of early relapse. J Cancer Res Clin Oncol 2008;134:59-65.
28. Hall CS, Karhade M, Laubacher BA, Kuerer HM, Krishnamurthy S, DeSnyder S, *et al*. Circulating tumor cells and recurrence after primary systemic therapy in stage III inflammatory breast cancer. J Natl Cancer Inst 2015;107:djv250.
29. Pierga JY, Bidard FC, Autret A, Petit T, Andre F, Dalenc F, *et al*. Circulating tumor cells (CTC) and pathological complete response (pCR) as independent prognostic factors in inflammatory breast cancer (IBC) in a pooled analysis of two multicentre phase II trials (BEVERLY 1 & 2) of neoadjuvant chemotherapy combined with bevacizumab. J Clin Oncol 2015;33:108.
30. Wülfing P, Borchard J, Buerger H, Heidl S, Zänker KS, Kiesel L, *et al*. HER2-positive circulating tumor cells indicate poor clinical outcome in stage I to III breast cancer patients. Clin Cancer Res 2006;12:1715-20.
31. Meng S, Tripathy D, Shete S, Ashfaq R, Haley B, Perkins S, *et al*. HER-2 gene amplification can be acquired as breast cancer progresses. Proc Natl Acad Sci U S A 2004;101:9393-8.
32. Budd GT, Cristofanilli M, Ellis MJ, Stopeck A, Borden E, Miller MC, *et al*. Circulating tumor cells versus imaging--predicting overall survival in metastatic breast cancer. Clin Cancer Res 2006;12:6403-9.

How to cite this article: Jain A, Suryavanshi M, Batra U, Goyal P, Doval D. Circulating tumor cells in breast cancer. Int J Mol Immuno Oncol 2017;2:4-10.

Source of Support: Nil. **Conflict of Interest:** None declared.