

Review

Circulating tumor cells as emerging tumor biomarkers in breast cancer

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Abstract

Circulating tumor cells (CTCs) provide unique information for the management of breast cancer patients, since their detection and monitoring is useful for prognosis, prediction of response to therapy, or monitoring clinical course in patients with localized or metastatic disease. Currently, the most practical application of CTCs is monitoring of patients with metastatic disease. Elevated CTC levels prior to initiation of a new systemic therapy are associated with a worse prognosis while persistently elevated CTC levels strongly suggest that the therapeutic regimen with which the patient is being treated is not working. New areas of research are directed toward developing novel sensitive assays for CTC molecular characterization. Molecular characterization of CTCs is very important for the future use of CTCs as targets of novel therapies. This review has focused on the presentation of recent data showing that CTCs are emerging as novel tumor biomarkers for prognostic and predictive purposes in breast cancer.

Keywords: breast cancer; circulating tumor cells; liquid biopsy; micrometastasis; minimal residual disease; tumor biomarkers.

Introduction

In 1869 Thomas Ashworth, an Australian physician, after observing microscopically circulating tumor cells (CTCs) in blood of a man with metastatic cancer, postulated that "...cells identical with those of the cancer itself being seen in the blood may tend to throw some light upon the mode of origin of multiple tumours existing in the same person..." (1). Nowadays, cancer research has indeed demonstrated the critical role CTCs play in the metastatic spread of carcinomas (2).

Disseminating tumor cells (DTCs) present in bone marrow (BM) and CTCs present in the peripheral blood are highly relevant to the study of the biology of early metastatic spread and provide a diagnostic source in patients with overt metastases. Data from European groups have sustained the prognostic impact of DTCs in the BM of breast cancer patients (3). CTCs detection and enumeration in breast cancer has been established in several clinical studies, showing a correlation with decreased progression-free survival (PFS) and overall survival (OS) in operable (4–8) and advanced breast cancer (9). Detection of CTCs in peripheral blood of early breast cancer patients after chemotherapy is of prognostic significance as well (10).

Novel technologies with the required sensitivity and reproducibility have been and are continually developed to further exploit the diagnostic potential of CTCs (11–14). CTC analysis could play a role as a "liquid biopsy," which will allow physicians to follow cancer changes over time and tailor treatment (15, 16) and represents a promising new diagnostic field for advanced-stage patients; the sensitive CTC detection platforms allow monitoring of disease and treatment efficacy (15–19). Current research on CTCs is focusing on the identification of novel diagnostic and therapeutic biomarkers expressed by these cells. This review has focused on the presentation of recent data showing that CTCs can be used as novel tumor biomarkers for prognostic and predictive purposes in breast cancer.

CTCs and micrometastatic spread

Metastasis remains the leading cause of death among cancer patients because few effective treatment options are available. It is now clear that the metastatic cascade is a series of complex biological processes that enable the movement of tumor cells from the primary site to a distant location and the establishment of a new cancer growth (Figure 1). Metastasis results from a multi-step cascading process that includes: 1) vascularization of the primary tumor; 2) detachment and invasion of cancer cells; 3) intravasation into lymphatic and blood vessels; 4) survival and arrest in the circulation; 5) extravasation into distant organs; and 6) colonization and growth of metastatic tumors (2).

Patients with breast cancer can develop recurrent metastatic disease with latency periods that range from years even to decades. This pause can be explained by cancer dormancy, a stage in cancer progression in which residual disease is present but remains asymptomatic (20). CTCs have a crucial role in tumor dissemination, since they are present in patients

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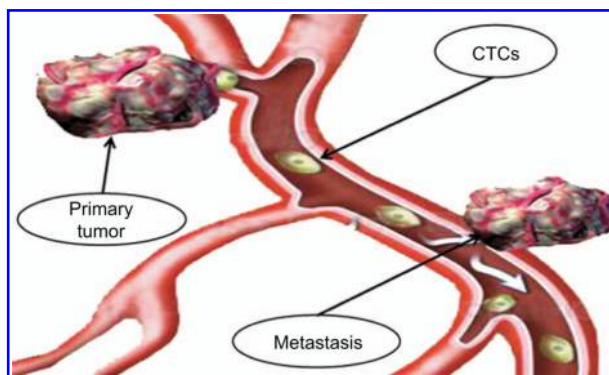


Figure 1 Circulating tumor cells and metastasis.

many years after mastectomy without evidence of disease and are shed from persisting tumor in patients with breast cancer dormancy. Meng et al. have shown that CTCs in patients whose primary breast cancer was just removed had a half-life measured in 1 h–2.4 h and that there appears to be a balance between tumor replication and cell death for as long as 22 years in dormancy candidates (21). Massague's group has recently shown that CTCs can return to the primary tumor, a process termed tumor self-seeding or cross-seeding, and that this helps breeding tumor cells that give rise to aggressive metastatic variants (22, 23).

The prevailing systemic cancer progression model places the engine of cancer progression within the primary tumor before metastatic dissemination of fully malignant cells. The second model, recently proposed by Klein posits parallel, independent progression of metastases arising from early DTCs (24). Data from disease courses, tumor growth rates, autopsy studies, clinical trials and molecular genetic analyses of primary and DTCs are in support of the parallel progression model, urging review of current diagnostic and therapeutic routines (24). Recent data of the same group are questioning the use of primary tumors as surrogate for the genetics of systemic cancer and call for a direct diagnostic

pathology of systemic cancer (25). Release from dormancy of early DTCs may frequently account for metachronous metastasis (26). Since only few selected cancer cells drive tumor progression and are responsible for therapy resistance, high-resolution genome analysis of single metastatic precursor cells that is now possible may be used for the identification of novel therapy target genes (27).

Recent developments have led the field of cancer metastasis to important/historical crossroads (28). New concepts have emerged, such as metastasis suppression and tumor dormancy. Recent researches on the post-transcriptional regulation of metastasis by microRNAs (miRNAs) and epigenetic controls, on the elucidation of the mechanisms underlying the induction of epithelial-to-mesenchymal transition (EMT) and on the stem cell-like phenotype in cancer cells, has opened new horizons in the field. The development of recent tools for imaging of metastasis and current technology platforms play also a very critical role (28). Moreover, metastasis suppressor genes leading to metastatic cell dormancy, such as breast cancer metastasis suppressor 1 (BRMS1) have been extensively studied (29–31). Understanding of the molecular mechanisms regulating EMT in solid tumors will provide new insights into mechanisms of cancer progression, detection of metastases, and development of effective and mechanism-based agents for improved therapeutic intervention (32). Especially microRNAs (miRNAs) are now emerging as critical players in the multi-step metastatic process both promoting and suppressing metastasis (33–35).

Analytical methodology for the isolation and detection of CTCs

Currently, there is a plethora of analytical methodologies for isolating and detecting CTCs. However, there are still a lot of analytical challenges to be solved (Figure 2). Since CTCs are very rare (1 CTC in 10^6 – 10^7 leukocytes) (36), in most cases they are specifically detected by using a combination

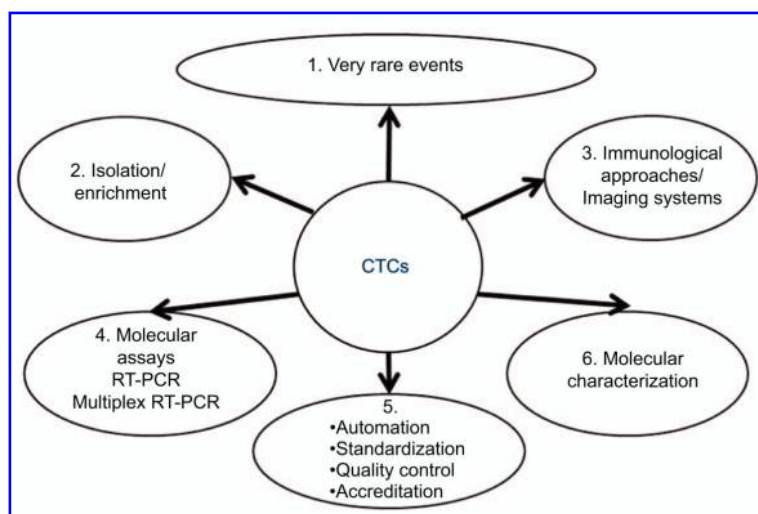


Figure 2 Analytical challenges for the isolation and detection of CTCs.

of two steps: 1) isolation-enrichment; and 2) detection. Detection strategies include detection at the cellular and protein level through immunological approaches and imaging systems, and molecular assays like RT-PCR and multiplex RT-PCR through the detection of gene expression in CTCs. There is still a lot to be done for the automation, standardization, quality control and accreditation of analytical methodologies used for CTC detection and molecular characterization.

Isolation-enrichment

The most widely used approaches for the isolation and enrichment of CTCs are based on the different density of CTCs such as centrifugation in the presence of ficol, immunomagnetic isolation of CTCs, positive or negative through antibodies specific for epithelial markers, such as epithelial cell adhesion molecule (EpCAM) or leukocytes (CD45), respectively, or the most recently developed filtration by size devices. A combination of enrichment methods is also used, e.g., filtration devices in combination with EpCAM positive isolation, ficol enrichment followed by positive immunomagnetic isolation. However, based on many studies showing the heterogeneous nature of CTCs we could say that all enrichment methods are biased by the fact that not all CTCs express the same cell surface antigens, such as EpCAM.

EpCAM based immunomagnetic enrichment assays are perhaps the most common (37), however, EpCAM independent enrichment technologies seem to be superior to detect the entire CTC population since some tumor cells express low or no EpCAM (38, 39). Combinations of other Abs for CTCs positive enrichment, such as anti-CD146 (40) and the Thomsen-Friedenreich (TF) antigen (CD 176) antibodies has been shown to enable the analysis of larger sample volumes and increase tumor cell detection rate (41). Enrichment with anti-CK alone or combined with anti-EpCAM antibodies significantly enhances assay sensitivity (42). Ficoll enrichment followed by positive immunomagnetic isolation through anti-EpCAM immunomagnetic isolation has been also described (43).

Microfluidic filtration devices have also been developed for CTCs analysis (44). The ISET (isolation by size of epithelial tumor cells) system allows the counting and the immunomorphological and molecular characterization of CTCs and is based on the individual isolation of epithelial tumor cells by filtration because of their larger size when compared to peripheral blood leukocytes (45, 46). A filter-based microdevice that is both a capture and analysis platform, capable of multiplexed imaging and genetic analysis and has the potential to enable routine CTC analysis in the clinical setting for the effective management of cancer patients has been developed (47–49). Another microfluidic device, called “the CTC-chip”, has been recently developed for capturing EpCAM-expressing cells in peripheral blood, using antibody-coated microposts (50), while lately, the same group has developed a high-throughput microfluidic mixing device, the herringbone-chip, or “HB-Chip,” which provides an enhanced platform for CTC isolation (51). Another novel microfluidic device can selectively and specifically isolate exceedingly small numbers of CTCs through a mono-

clonal antibody (mAb) mediated process by sampling large input volumes of whole blood directly in short time periods (52).

Detection

CTC detection and characterization techniques include: 1) cytological approaches based on imaging analysis; 2) molecular assays, based on the nucleic acid analysis in CTCs; and 3) protein assays (53).

Detection of CTCs by classical immunocytochemistry is time-consuming, since CTC identification is typically done by trained pathologists through visual observation of stained cyokeratin-positive epithelial CTC. A combination of fluorimetric detection and immunohistochemistry has resulted to the development of the MAINTRAC system which is a fast and quantitative automated microscopic procedure for screening up to 5×10^4 cells in suitable time (54, 55). The technology that produced the largest amount of clinical data on the prognostic relevance of CTCs in breast cancer is the CellSearch system (Veridex, USA) (9). This system is based on a combination of immunocytochemistry and immunofluorescence, by using specific markers for CTCs such as cyokeratins (mainly CK-19), leukocytes, such as CD45 and cell viability as 4'6-diamidino-2-phenylindole (nuclear stain) positive (DAPI) and up to now is the only FDA cleared device for the enumeration of CTC in whole blood. The significance of the CellSearch assay results has been amply demonstrated, first in an extensive pilot study program and then in a series of prospective, well-controlled pivotal clinical trials (56–59). DyLight Technology has been recently applied for multi-marker image analysis of CTCs based on the use of multiple antibodies labeled with fluorochromes of different colors and spectral image analysis to separate different color spectra (60).

Molecular methods for CTCs detection and enumeration are based on the isolation of total RNA from viable CTCs, and subsequent RT-PCR amplification of tumor or epithelial specific targets. These assays take advantage of the extreme sensitivity and specificity that can be achieved through PCR. Moreover they are easy to perform, and especially quantitative RT-PCR assays can be easily automated and subjected to internal and external quality control systems. A major advantage of molecular methods is the flexibility they offer, especially to multiplex these assays and thus reduce the amount of sample required, time and cost of analysis. RT-PCR methodology for the detection of micrometastases in patients with breast cancer was firstly based on the estimation of the number of CK-19 transcripts (61, 62). Quantitative real-time reverse transcription-PCR (RT-qPCR) assays for CK19-mRNA were then developed and evaluated in terms of sensitivity, specificity and clinical potential (6–8, 10, 63, 64).

Nowadays many targets beyond CK-19 are of interest to be evaluated in CTC. However, the fact that CTC are very rare and the amount of available sample is very limited presents a tremendous analytical and technical challenge (65–67). The most important limitation of most available

methodologies for CTC analysis is the small number of gene targets that can be analyzed.

Multi-marker RT-PCR assay for CTC analysis can overcome these problems. The AdnaTest BreastCancer (AdnaGen AG, Germany) kit was developed for the enrichment of CTCs from peripheral blood of breast cancer patients followed by identification of CTC-associated marker transcripts by reverse transcription and multiplex PCR (68, 69). Several mRNA markers may be useful for RT-PCR-based detection of CTCs. Quantification of these mRNAs is essential to distinguish normal expression in blood from that due to the presence of CTCs. Few markers provide adequate sensitivity individually, but combinations of markers may produce good sensitivity for CTC detection (70, 71). A highly sensitive and specific multiplexed PCR-coupled liquid bead array that detects the expression of six gene targets in CTCs has been recently developed and evaluated in clinical samples of early and metastatic breast cancer (MBC) patients (43). CTCs have been also detected through tumor-specific proteins released by CTCs using the EPISPOT (EPithelial ImmunoSPOT) assay (72).

The detection, enumeration and isolation of CTCs have considerable potential to influence the clinical management of patients with breast cancer. There is, however, substantial variability in the rates of positive samples using existing detection techniques. Different detection methods lead to different results as shown by the comparative analysis of the same patient samples with different technologies. Thus, the clinical results largely depend on the technology used to detect CTCs. Despite the fact that most of these methods are highly specific and sensitive, there are not so far extensive studies especially designed to compare their efficacy when using the same clinical samples. This is an important issue for their clinical use, since especially in early disease, differences in analytical sensitivity between these methods can play a very critical role. The lack of standardization of technology hampers the implementation of CTC measurement in clinical routine practice. Various recent studies address this, by comparing different methodologies (73–80).

CTCs as novel biomarkers in breast cancer

CTCs have been identified and characterized in blood of patients with many solid tumors, particularly breast cancer, where the prognostic significance of the presence of micro-metastasis in the BM at the time of diagnosis has been clearly shown (3). Since early blood-borne dissemination of tumor cells plays an important role in breast cancer, a lot of work has been done in this type of cancer in both BM and peripheral blood samples. As repeat sampling of peripheral blood is more acceptable to patients, CTCs analysis can be used as a liquid biopsy that allows for serial monitoring of minimal residual disease and enables clinicians to have prior warning of impending overt metastatic disease. The challenge now is to integrate minimal residual disease as a prognostic and pre-

dictive tool in the management of breast cancer. The number of publications on the prognostic significance of CTCs in breast cancer is constantly rising since 2002 (Figure 3).

Between 10% and 30% of patients with stage I–III breast cancer and 50%–70% of women with MBC have detectable CTCs. In both cases, presence and elevation of CTCs are associated with worse prognosis (81). In the metastatic setting, persistent CTC after three to five weeks of a new therapy seem to indicate lack of activity of that regimen, and an ongoing prospective randomized clinical trial is addressing the relative worth of changing to an alternative treatment rather than waiting for classic clinical and radiologic evidence of progression (SWOG S0500, clinicaltrials.gov, identifier: NCT00382018). In Table 1 the findings on the clinical significance of CTCs in early and advanced breast cancer are summarized.

Early breast cancer

Molecular detection of CK-19 mRNA-positive cells by RT-PCR in the peripheral blood of patients with stages I and II breast cancer before initiation of adjuvant therapy was firstly shown to have an independent prognostic value as a marker of poor clinical outcome by Stathopoulou et al. (4). In the same group, by using RT-qPCR Xenidis et al. confirmed these findings (6). Moreover, the detection of peripheral blood CK19mRNA+ and mammaglobin (MGB1)mRNA+ cells before adjuvant chemotherapy predicts poor disease-free survival (DFS) (7). When the association between detection of CK-19 mRNA-positive CTCs and clinical outcome was analyzed for early breast cancer patients with ER-positive, ER-negative, triple-negative, HER2-positive, and ER-positive/HER2-negative tumors, it was found that the detection of CK-19 mRNA-positive CTCs before adjuvant chemotherapy predicts poor clinical outcome mainly in patients with ER-negative, triple-negative, and HER2-positive early-stage breast cancer (8). When the presence of CK-19 mRNA-positive CTCs was assessed by RT-qPCR in blood samples obtained from 437 patients with early breast cancer before the start and after the completion of adjuvant chemotherapy, it was found that the detection of CK-19 mRNA-

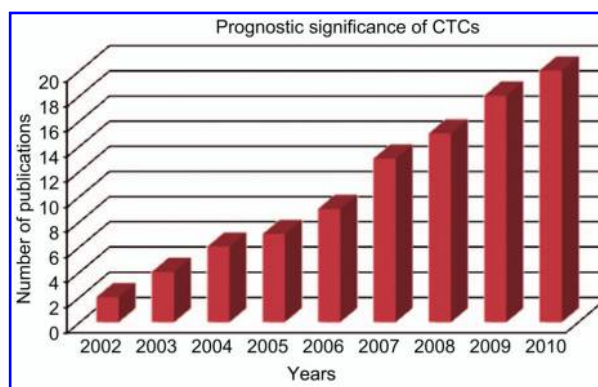


Figure 3 Publications on the prognostic significance of CTCs in breast cancer (PubMed, keywords: prognosis, circulating tumor cells, breast cancer).

Table 1 Clinical significance of CTCs detection in early and advanced breast cancer.

Method	Marker	CTC detection rate	Clinical significance	Ref.
Early breast cancer				
Nested RT-PCR	CK-19	44 of 148 (30%)	DFI: p=0.001, OS: p=0.014	(4)
RT-qPCR	CK-19	Node negative 36 of 167 (21.6%)	DFI: p<0.001, OS: p=0.008	(6)
RT-qPCR	CK-19, mammaglobin HER-12	CK-19: 72 of 145 (41%) Mammaglobin: 14 of 175 (8%) HER-2: 50 of 175 (29%)	DFI: CK-19 (p<0.001), OS: CK-19 (p=0.044) DFI: mammaglobin (p=0.011), OS: mammaglobin (p=0.034) DFI: HER-2 (p<0.001)	(7)
RT-qPCR	CK-19, ER	181 of 444 (41%)	DFI: CK-19 and ER (p=0.001) OS: CK-19 and ER (p=0.001)	(8)
RT-qPCR	CK-19	After adjuvant therapy: 179 of 437 (41%)	DFI: p<0.001, OS: p=0.003	(10)
RT-qPCR	CK-19	Before adjuvant therapy: 91 of 165 (55.2%) After adjuvant therapy: 79 of 162 (48.8%)	Before adjuvant therapy: DFI: p=0.081, OS: p=0.024 After adjuvant therapy: DFI: p=0.057, OS: p=0.128	(82)
CellSearch	Pan-CK	Before and/or after neoadjuvant chemotherapy: 32 of 118 (27%)	DFI: p=0.013	(83)
CellSearch	Pan-CK	Before chemotherapy therapy: 95 of 115 (82.6%) After chemotherapy: 85 of 115 (73.9%)	Before chemotherapy: DFI: p=0.007, OS: p=0.0006 After chemotherapy: DFI: p=0.04, OS: p=0.02	(84)
CellSearch	Pan-CK	Before chemotherapy therapy: 140 of 1489 (9.4%) After chemotherapy: 129 of 1489 (8.7%)	Before chemotherapy: DFI: p<0.0001, OS: p=0.023 After chemotherapy: DFI: p=0.054, OS: p=0.154	(85)
ICC	CK	47 of 71 (66%)	OS: p=0.071, DFI: p=0.052	(86)
RT-PCR	CK-19, HER-2, P1B, PS2, epithelial glycoprotein 2	43 of 72 (60%)	DFI: p=0.031, OS: p=0.03	(87)
ICC	CK and HER-2	17 of 35 (49%)	DFI: p<0.005, OS: p<0.05	(88)
Nested RT-PCR	Mammaglobin	14 of 101 (13.9%)	DFI: p=0.020, OS: p=0.009	(89)
Metastatic breast cancer				
CellSearch	Pan-CK	87 of 177 (49%)	DFI: p<0.001, OS: p<0.001	(9)
CellSearch	Pan-CK	43 of 83 (52%)	DFI: p=0.0014, OS: p=0.0048	(90)
CellSearch	Pan-CK	92 of 195 (47.2%)	DFI: p=0.0122, OS: p=0.0007	(91)
CellSearch	Pan-CK	35 of 138 (25%)	OS: p<0.0001	(56)

DFI, disease-free interval; OS, overall survival.

positive CTCs in the blood after adjuvant chemotherapy is an independent risk factor indicating the presence of chemotherapy-resistant residual disease (9). These findings were confirmed by other groups as well using CK-19 as a marker (87, 88, 92), and mammaglobin (89). The detection of CK-19 mRNA-positive CTCs using RT-PCR both before and after chemotherapy was correlated with the detection of CK-19 mRNA-positive DTCs in patients with early-stage breast cancer. It was found that the determination of the CTC status by RT-PCR conveys clinically relevant information that is not inferior to DTC status and, owing to the ease of sam-

pling, warrants further evaluation as a tool for monitoring minimal residual disease (82, 93). Taken together these findings, may change the clinical management of non-MBC and indicate that the metastatic efficiency of CTC could be higher than previously reported.

By using the CellSearch system in early breast cancer, CTCs did not correlate with the standard prognostic indicators that were considered, thus implicating a lower sensitivity of this system in the adjuvant setting (94). However, according to a study by Pierga et al. that determined whether CTCs are present in the blood of patients with large operable or

locally advanced breast cancer before neoadjuvant chemotherapy and after neoadjuvant chemotherapy before surgery, by using the CellSearch system, CTC detection was not correlated to the primary tumor response but was an independent prognostic factor for early relapse (83). In 115 non-metastatic patients diagnosed with large operable or locally advanced breast cancer, Bidard et al. detected CTC using the CellSearch system before and after neoadjuvant chemotherapy. According to their findings, detection of one or more CTC/7.5 mL before neoadjuvant chemotherapy can accurately predict overall survival (OS) (84). CTCs are detectable and quantifiable in breast surgery patients. When changes in CTCs detection in patients with localized breast cancer before and after surgery were evaluated by using the CellSearch system, the presence of CTCs was confirmed in approximately 30% of patients with localized breast cancer both before and after surgery, with change from positive to negative and vice versa in 40% of cases (95). In patients undergoing surgical resection of clinically localized primary breast cancer CTCs were more likely to be found in hormone receptor negative patients (96). In the German Success trial, detection of at least 2CTCs/23 mL of peripheral blood before or after adjuvant chemotherapy was independently associated with worse DFS in a cohort of 1489 patients with early breast cancer (85).

Metastatic breast cancer

Metastatic breast cancer is incurable; its treatment is palliative. Cristofanilli et al. investigated for the first time whether the presence of CTCs predicts treatment efficacy, PFS, and OS (9). Especially in patients with newly diagnosed MBC who were about to start first-line therapy the same group has shown that detection of CTCs by using the CellSearch system before initiation of first-line therapy in patients with MBC is highly predictive of PFS and OS (90). The same group has recently evaluated the relationship between the detection and prognostic significance of CTCs and sites of metastases in patients with MBC and found that presence of extensive bone metastases is associated with increased CTC numbers (91). The presence of CTCs in MBC patients is associated with increased risk of venous thromboembolism (97), while in inflammatory breast cancer (IBC), metastatic IBC patients had a lower prevalence and fewer CTCs in comparison to metastatic non-IBC patients (98). Liu et al. conducted a prospective study and demonstrated a strong correlation between CTC results and radiographic disease progression in patients receiving chemotherapy or endocrine therapy for MBC (99). However, the value of CTC detection by CellSearch in MBC may depend on the clinical setting and regimen used. In a retrospective analysis of 516 patients with MBC, CTC detection did not predict clinical outcome in chemo-naïve women with HER2-positive MBC treated with anti-HER2 therapy, but had prognostic value in other breast cancer subtypes (100). Similarly, changes in CTCs during treatment did not predict outcome in 67 women treated with first-line bevacizumab/chemotherapy (101). Taken together these findings support the role of CTC enumeration

as an adjunct to standard methods of monitoring disease status in MBC.

CTCs as predictive markers for therapy response

CTCs may identify patients most likely to be cured with aggressive therapy, as well as patients with a propensity for systemic failure. This information may be used to match patients with the most appropriate treatment strategy including combinations of local and systemic therapy.

Monitoring of CTCs during and after systemic adjuvant therapy might provide unique information for the clinical management of the individual cancer patient and allow for a timely change in therapy, years before the appearance of overt metastases signals incurability. Moreover, there is an urgent need for biomarkers for real-time personalized monitoring of the efficacy of systemic adjuvant therapy. At present, the success or failure of anti-cancer therapies is only assessed retrospectively by the absence or presence of overt metastases during the post-operative follow-up period. However, overt metastases are, in general, incurable by most current therapies. Monitoring of CTCs will provide new insights into the clonal selection of resistant tumor cells under biological therapies.

The prognostic value associated with the detection of CTCs in MBC raise additional issues regarding the biological value of this information. A drug-resistance profile of CTCs, which is predictive of response to chemotherapy, independent of tumor type and stage of disease was recently identified and therefore could be used for patient selection (102). Drug-resistant CTCs have predictive value in MBC and possibly retain stem-like properties. Very recently it was found that in MBC, the presence of CTCs expressing multidrug-resistance-related proteins (MRPs), and aldehyde dehydrogenase 1 (ALDH1), is predictive of response to chemotherapy (103). In MBC the change in the number of CTCs was highly correlated with results from imaging before and after therapy, CTCs can be considered a biomarker that may predict the effect of treatment earlier than imaging modalities (86). The differential prognostic and OS showed between patients with and without elevated CTCs before and at the end of chemotherapy, is of special interest in patients without clinical evidence of metastasis (104). According to the GeparQuattro trial, aimed at detecting and characterizing CTCs before and after neoadjuvant therapy (NT) in the peripheral blood of patients with breast cancer, information on the HER2 status of CTC might be helpful for stratification and monitoring of HER2-directed therapies (105).

CTCs and personalized medicine

CTCs analysis has the great advantage of monitoring changes during the course of treatment, serving as a real-time biopsy that can guide to tailored therapies in the near future. The possibility of collecting sequential blood samples for real-time monitoring of systemic-therapy efficacy presents new possibilities to evaluate targeted therapies based on the genomic profiling of CTCs and to improve the clinical management of patients by personalized therapy. This approach

could help to identify novel targets for biological therapies aimed to prevent metastatic relapse. In addition, understanding tumor ‘dormancy’ and identifying metastatic stem cells might result in the development of new therapeutic concepts (106). The role of CTCs in treatment failure and disease progression can be explained by their relation to biological processes, including the EMT and ‘self seeding’, defined as re-infiltration of the primary tumor or established metastasis by more aggressive CTCs (22). Interruption of the metastatic cascade via the targeting of CTCs might be a promising therapeutic strategy (107). Molecular CTC analysis will provide insights into the selection of tumor cells and resistance mechanisms in patients undergoing systemic therapies. This information might support assessing individual prognosis, stratifying patients at risk to systemic therapies, and monitoring therapeutic efficacy (108, 109). Molecular profiling of CTC may offer superior prognostic information with regard to risk assessment for recurrence and predictive judgement of therapeutic regimens (110).

Molecular characterization of CTCs can provide valuable information on the expression of specific receptors of therapeutic interest like HER2 (105, 111–117), activating pathways like angiogenesis (118), EGFR (119, 120), ER/PR (69). The expression of stemness and EMT markers in CTCs were associated with resistance to conventional anti-cancer therapies and treatment failure, highlighting the urgency of improving tools for detecting and eliminating minimal residual disease (121). Though the relationships between EMT and CTCs remains largely unexplored, data validating the implication of EMT processes in CTC formation and animal models with transplantable human breast tumor cells to help characterizing EMT/CTC relationships have been recently reviewed (122).

Expert opinion

Detection of CTCs has been lately associated with prognosis in a variety of human cancers such as breast, lung, colon and prostate. Especially in breast cancer, CTC detection and enumeration has been established in several clinical studies, showing a correlation with decreased progression-free survival and OS. Monitoring of CTC during and after systemic adjuvant therapy provides unique information for the clinical management of the individual breast cancer patient.

Novel technologies with the required sensitivity and reproducibility have been and are continually developed to further exploit the diagnostic potential of CTCs. Recently developed sensitive CTC detection platforms allow monitoring of disease and treatment efficacy and will play a critical role in the clinical laboratory in the near future.

The ability to reliably detect and analyze CTCs at the molecular level, could lead to the development of individualized treatments, where instead of waiting for secondary tumors to appear, their monitoring could give early on information for drug efficacy. Also motivating the interest in CTCs has been the recent development of molecularly targeted cancer therapies, that work best on patients whose tumors have particular characteristics.

Current research is focusing on the identification of novel diagnostic and therapeutic biomarkers expressed in these cells. CTCs hold the promise of playing a role of ‘liquid biopsy,’ which may allow physicians to follow cancer changes over time and tailor treatment.

Outlook

Nowadays the most practical application of CTCs is monitoring of patients with metastatic disease. During the next years CTCs detection and molecular characterization will be established in the Clinical Laboratory as a routine test that will provide unique information for the management of breast cancer patients. CTC analysis has the great advantage of monitoring changes during the course of treatment, serving as a real-time biopsy that can guide to tailored therapies in the near future. The possibility of collecting sequential blood samples for real-time monitoring of systemic-therapy efficacy presents new possibilities to evaluate targeted therapies based on the genomic profiling of CTCs and to improve the clinical management of patients by personalized therapy.

Highlights

1. Detection of CTC in early breast cancer, may provide independent prognostic information.
2. CTC analyses may improve stratification of patients in need of (neo) adjuvant therapies, in early breast cancer.
3. Monitoring of CTC in advanced breast cancer is a promising biomarker for prediction of therapeutic efficacy.
4. Molecular characterization of CTCs will enable the identification of novel therapeutics that will target micrometastatic spread and elucidate CTC connection to cancer stem cells.
5. The outcome of ongoing trials testing new drugs in breast cancer and evaluating the CTC status of the patients will determine whether CTCs will become valuable predictive biomarkers for therapy response or failure.
6. An external quality control system and the development of international standards for CTC enumeration and characterization is urgently needed.
7. A combination of advanced imaging systems and molecular characterization of CTCs will be very useful to further refine prognosis, define treatment strategies and eliminate or reduce the risk of metastasis.
8. The use of modern powerful technologies, such as next generation sequencing will enable the elucidation of molecular pathways in CTCs and lead to the design of novel molecular therapies targeting specifically CTCs.
9. CTCs are emerging as promising tumor biomarkers in breast cancer and their clinical use as a ‘liquid biopsy’ for stratification of patients and real-time monitoring of therapies will have a major impact in personalized medicine.

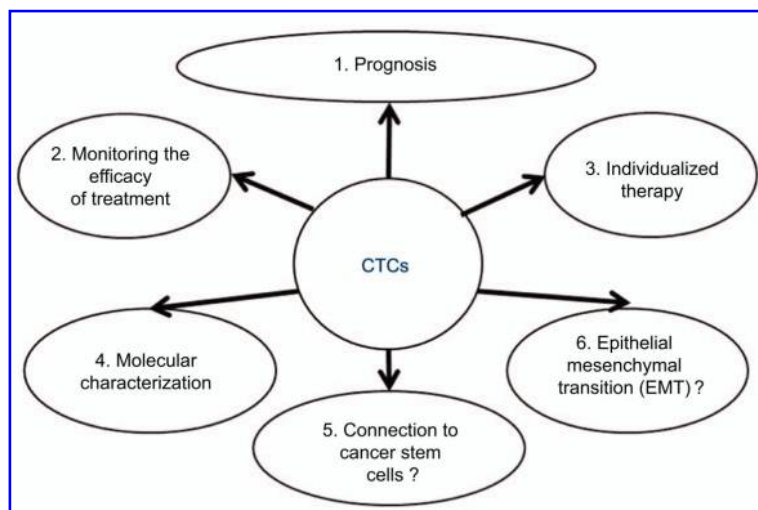


Figure 4 CTCs as novel tumor biomarkers.

Conclusions – future prospects

The cancer circulation problem has received considerable attention in the research community nowadays (123). Detection of CTC may provide: 1) independent prognostic information in early breast cancer; 2) monitoring of CTC in advanced breast cancer is a promising biomarker for prediction of therapeutic efficacy; 3) CTC analyses may improve stratification of patients in need of (neo) adjuvant therapies; 4) molecular characterization of CTCs can provide valuable information on the expression of specific receptors of therapeutic interest; 5) studying the expression of stemness and EMT markers in CTCs is one of the hottest topics in cancer research nowadays (Figure 4).

The main clinical issue that is currently being addressed in CTCs is to evaluate whether CTC detection can lead to a change in the management of cancer patients and can result in improved clinical outcome. So far CTCs have only proven to bear prognostic significance and not to influence management decisions, even not in the metastatic setting. The challenge of using CTCs as novel tumor biomarkers is currently evaluated in clinical trials. The outcome of these ongoing trials testing new drugs in breast cancer and evaluating the CTC status of the patients will determine whether CTCs will become valuable predictive biomarkers for therapy response or failure.

However, there is a clear need for an external quality control system for CTC enumeration and validation of findings for the same samples by participating laboratory centers. The development of international standards for CTC enumeration and characterization is of utmost importance in this case. Cross-validation of findings between different laboratories, using the same or different detection and enumeration platforms is urgently needed.

Another major issue that is currently being addressed in CTCs is their molecular characterization that will enable the identification of novel therapeutics that will target micro-metastatic spread and elucidate their connection to cancer

stem cells. A combination of advanced imaging systems and molecular characterization of CTCs will be very useful to further refine prognosis, define treatment strategies and eliminate or reduce the risk of metastasis. The use of modern powerful technologies, such as next generation sequencing will enable the elucidation of molecular pathways in CTCs and lead to the design of novel molecular therapies targeting specifically CTCs.

In conclusion, CTCs are emerging as promising tumor biomarkers in breast cancer and their clinical use as a ‘liquid biopsy’ for stratification of patients and real-time monitoring of therapies will have a major impact in personalized medicine.

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