

Circulating tumor cells and their clinical significance

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Metastases to other organs and the formation of secondary tumors are responsible for 90% of cancer-related deaths. However, even in the early stages of cancer, about 30–40% of patients with localized disease may have latent metastasis, which are likely derived from circulating tumor cells (CTCs) involved in disease progression. Therefore, detection and analysis of CTCs can play an important role in the diagnosis and decision-making of adjuvant treatment that aims to prevent metastasis. At present, patients' selection of treatment is based on the statistical risk of recurrence of metastatic disease, without considering whether the tumor cells have spread from the primary tumor. This may lead to unnecessary treatment of non-metastatic disease patients. Therefore, early detection of CTCs in the blood is critically important, and should allow for a more accurate assessment of disease severity. Here, we provide an overview of CTC phenotypes, including plasticity of CTCs, and their clinical significance.

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Introduction

Circulating tumor cells (CTCs) have been detected in blood, and first described in 1896 by Thomas Ashworth [1]. CTCs are defined as circulating cells whose antigenic or genetic characteristics correspond to the characteristics of a specific tumor type. The presence of CTCs in the blood stream reflects the process of metastasis, as described by the theory of 'seed and soil' [2]. Under this definition, metastasis occurs when cancer cells are detached from the primary tumor and enter the bloodstream, moving in the direction of distant organs where they can settle and create a new focus i.e., a metastasis [2].

Together, metastasis and the formation of secondary tumors are responsible for 90% of deaths in cancer patients. However, even in the early stages of cancer, about 30–40% of patients with localized disease may have latent metastases, potentially derived from CTCs involved in the progression of the disease [3, 4]. Therefore, detecting and analyzing CTCs may play an important role in the diagnosis and decision to provide adjuvant therapy. Patients have been hitherto selected based on the statistical risk of metastatic relapse, without due consideration of whether cancer

cells have spread from the primary tumor. This may result in unnecessary therapeutic treatment of patients without metastases and, consequently, serious side effects of such treatment. For this reason, early detection of CTCs is critically important, as this would allow for a more accurate assessment of the disease stage, and for monitoring effects of treatment. Compared to metastatic tissue biopsy, there are many advantages of isolating CTCs. For example, blood collection is easier, cheaper, less invasive for the patient, and can be repeated several times while tracking cancer treatment. A blood sample containing CTCs is often referred to as a 'liquid biopsy', and may serve as a proxy for metastatic cancer [5]. In the future, liquid biopsy could be introduced for diagnostic screening, thus allowing for cancer to be monitored more easily via blood testing.

The biology of metastasis

Metastatic colonization by CTCs is a complex process involving many steps, such as tissue invasion and intravasation, survival in the bloodstream, extravasation, organ infiltration, colonization of a metastatic niche, and formation of macrometastasis (Fig. 1).

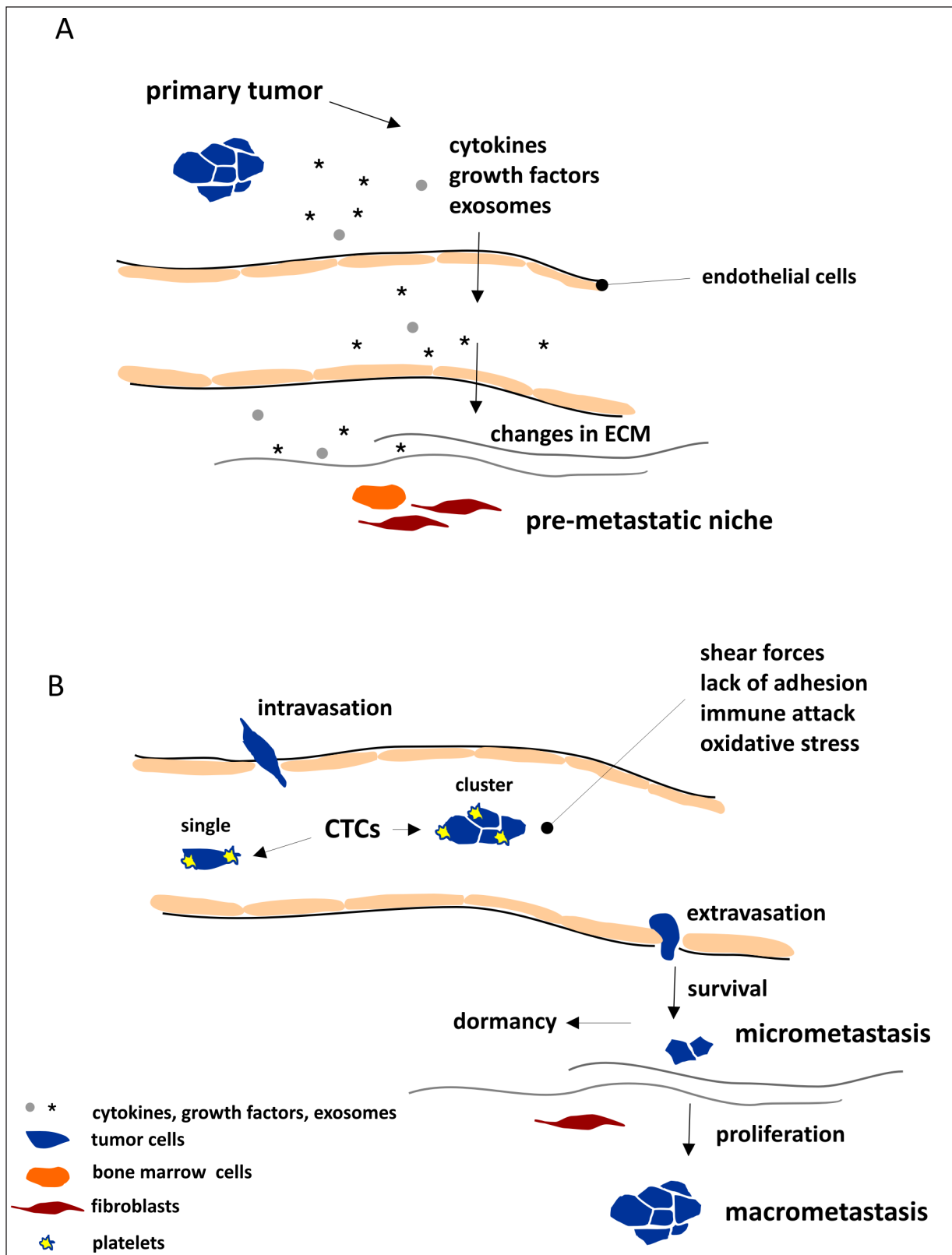


Figure 1. Metastatic cascade. **A.** Primary tumor secretes factors responsible for pre-metastatic niche formation at the distant site. This step facilitates subsequent tumor cell seeding by remodeling the composition of the extracellular matrix. **B.** Motile tumor cells (single cells and/or cell clusters) intravasate into circulation, where they become subjected to severe stress factors, including shear forces, lack of adhesion, immune reaction and oxidative stress. The influence of these factors can be modulated by platelets, which shield tumor cells from shear stress and immune attack. The lack of adhesion is compensated by either EMT or intracellular adhesion in case of CTC clusters. Subsequent extravasation into pre-metastatic niche allows for tumor cell survival and leads to either dormancy or proliferation and macrometastasis

Parallel progression

Even small tumors at an early stage of advancement can shed cancer cells, which contradicts the conventional, linear model of cancer progression and dissemination [6]. In breast cancer, disseminated cancer cells (DCCs, also called DTCs, disseminated tumor cells) have been shown to contain fewer genetic alternations than primary cells, suggesting that they were seeded very early on and progressed separately. This phenomenon is known as the 'theory of parallel progression', and poses important therapeutic problems, since cell dissemination occurs even before diagnosis [7]. Previous studies have shown that early DCCs are not as effective as late DCCs in the formation of primary tumors after implantation in the mammary fat pad. However, early DCCs are more metastasis-competent, and form more metastases and at a faster rate than late DCCs [6].

Early dissemination was demonstrated to depend on progesterone hormone signaling and abnormalities in the WNT signaling pathway [7]. As shown by Hosseini et al. [6], early DCCs have a different molecular signature than cells of the primary tumor. Further knowledge is needed to unveil the trigger mechanisms of early dissemination, which will be critical to future schemes of prevention and treatment.

Routes of invasion

For early or late cancer cells to disseminate, several vital problems need to be resolved; one of these problems involves efficient migration into the circulatory system.

The first step in the metastatic cascade is an invasion of surrounding tissues, which usually coincides with degradation of the stroma, activation of the immune system, and generation of cancer associated fibroblasts (CAFs) and the reactive stroma. These create a microenvironment that supports migration and cancer cell invasion. Cell migration can take many forms, such as the migration of single cells secreting extracellular-matrix (ECM) degrading enzymes, migration along collagen fibers [8] or nerve fibres [9], or collective migration in strands, sheets, or clusters. Most tumors are of epithelial origin, and epithelial cells are typically non-motile and do not secrete ECM-degrading enzymes, which is the hallmark of invasive cells. Thus, to acquire the ability to migrate and degrade ECM, cells can undergo an epithelial-mesenchymal transition (EMT). Epithelial cells that convert to a mesenchymal phenotype lose their apical-basal polarization, and thus their ability to adhere to neighboring cells, acquire a spindle shape, and potential to secrete metalloproteinases [10]. EMT is triggered by transcription factors, such as SNAIL, SLUG, ZEB, KLF8, or Twist. During the process of EMT, the expression of proteins specific for epithelial cells (e.g., E-cadherin, cytokeratins and EpCAM) decreases, whereas expression of proteins characteristic for mesenchymal cells (e.g., vimentin and N-cadherin) increases. The ability to penetrate the basement membrane

and extracellular matrix also increases. These are widely regarded as metastasis-promoting factors. However, the exact role of EMT in cancer dissemination is currently under debate. This is because, although EMT undeniably renders cells more motile and invasive, supports their survival in the bloodstream and protects them from anoikis (a form of apoptosis triggered by the lack of adhesion), several recent reports suggest that it is dispensable for metastasis — especially in breast and pancreatic cancers [11, 12]. It is also worth recalling that, to efficiently colonize a new metastatic site, cancer cells must reverse EMT and undergo the mesenchymal-epithelial transition (MET). This reversal further complicates the process.

Other than the mesenchymal and collective mode of migration used by cancer cells to spread, there is also amoeboid cell migration, which is faster than the other types of migration and is adhesion-independent. Amoeboid cells do not degrade ECM, but rather, squeeze through the ECM structures or cracks in cell layers. Amoeboid migration also requires substantial phenotypic changes, usually in the form of a mesenchymal-amoeboid transition (MAT).

In contrast to such modes of migration, collective movement of cancer cell clusters do not require extensive phenotype changes, since they retain the principal property of epithelial cells, which is the presence of strong cell-cell contacts. Presumably, this gives cancer cells an advantage in efficient seeding, given that they have been shown to have higher metastatic potential than single CTCs [13]. Indeed, polyclonal metastases have been documented in prostate cancer, and in mouse models of breast cancer [13, 14]. Although CTC clusters retain cell-cell contacts, studies have shown that they are not fully epithelial, but rather, display a hybrid epithelial-mesenchymal phenotype [13, 15]. This hybrid phenotype may be crucial to their metastatic efficiency.

CTC mobilization by treatment

A comprehensive review by Martin et al. [16] summarizes the current knowledge on the effects of anti-cancer treatment on mobilization of CTCs into the circulation. It was shown that different types of treatment applied to cure locoregional disease (i.e., radiotherapy, surgery, systemic therapies) may actually contribute to the increase in the number of CTCs in patients' blood, or an increase in proliferative capacity of CTCs. One possible explanation of this finding is the detrimental effect of various anti-tumor therapies on tumor vasculature, which may facilitate the entry of cancer cells into the circulation. So far, no studies have proved that this effect impacts metastasis in patients, but certainly such an opportunity exists. Indeed, there are studies of metastasis in irradiated versus non-irradiated mice that point to this conclusion [17, 18]. As addressed later in this review, such effects may interfere with assessing the prognostic powers of CTC count in patients, which should

be taken into consideration. Further understanding of this topic may be critically important for deciding on treatment options, especially adjuvant therapies.

In the circulation

Hematogenous dissemination encompasses intravasation of cancer cells and their survival in the bloodstream. Intravasation is promoted by a number of factors, including crosstalk in secretion between tumor cells and perivascular macrophages, TGF- β signaling, and the expression of Twist [19]. Following intravasation, cancer cells expose themselves to an extremely hostile environment and are exposed to many potentially damaging agents, such as shear forces, lack of adhesion, the innate immune response, and oxidative stress. As such, it is not surprising that survival of cancer cells in the bloodstream (or lymphatic system) is minimal. However, cancer cells have devised some forms of protection, including EMT and association with platelets, which shields them from both immune attack and hemodynamic shear forces. Platelets release TGF- β , inducing EMT [20], and they also secrete adenine nucleotides which relax endothelial cell junctions, which supports extravasation [21]. CTCs also undergo reversible metabolic changes, which helps them to withstand oxidative stress [22].

CTC flow is determined by patterns of blood circulation. This may help to explain why the lungs and liver are some of the most common target sites of metastasis, due to the venous circulation from most organs and the gut. Extravasation is thought to occur by mechanical entrapment of CTCs in capillaries and rupture of these vessels [23]. This mechanism could be especially effective for CTC clusters, when considering their size. CTC clusters were recently shown, however, to traverse capillary vessels, thereby reversibly re-organizing into a single-chain structure [24]. These data demonstrate that not all CTCs will end up being lodged within a capillary vessel. Rather, CTCs can extravasate by adhering to the vascular wall, and transmigrating to the other side of the endothelial cell layer.

Seeding

Infiltrating new tissue is the next and very inefficient step in cancer cell dissemination. Although CTCs extravasate and become CTCs, their chance of survival depends on the supportive metastatic niche in which they can proliferate, or at least survive and stay dormant. The creation of a pre-metastatic niche, even before the arrival of tumor cells, is promoted by factors secreted by the primary tumor (i.e., cytokines, growth factors, exosome components). These travel via the circulation to distant organs, and initiate structural and functional changes at a new site, which primarily encompass modifications to ECM components (i.e., fibronectin, tenascin C, collagens, galectins, periostin, osteopontin, versican, lysyl oxidase, MMPs) and the recruitment

of bone marrow-derived cells [25]. Pre-metastatic changes create a permissive microenvironment, and allow some of the tumor cells to survive after extravasation. Nevertheless, a substantial proportion of tumor cells remain dormant. Further changes (e.g., immune, hormonal, or growth factor activation) may induce proliferation of these cells and produce macrometastasis.

Clinical application

To date, the only test approved by the FDA for measuring CTCs is the CellSearch system (Veridex, USA). Defined criteria for measuring CTCs and clinical study outcomes have led to the inclusion of the CellSearch test as a prognostic tool for breast cancer [26, 27], colorectal cancer [28], and prostate cancer [29] in advanced stages of metastatic cancer. The number of preclinical and clinical studies on the possible applications of CTCs continues to grow, and studies have been conducted on both early stage disease and advanced cancer. These studies have focused primarily on using the CellSearch platform with RT-PCR. However, to date, no system based on CTCs has been approved to guide the appropriate choice of treatment and to monitor treatment efficacy. This may be because studies evaluating CTCs are often difficult to interpret, which may be due to various factors, including: different techniques for measuring CTCs, different criteria for defining CTCs (expression of various tumor markers), heterogeneity among enrolled patients, or a limited number of patients meeting required enrollment criteria. At present, the only FDA-approved clinical application of CellSearch is in estimating the chances of disease progression and/or survival. Indeed, the majority of research studies, including those reviewed by the FDA, confirm the prognostic value of CTCs for estimating clinical parameters, such as disease-free survival (DFS), overall survival (OS), and progression-free survival (PFS).

CTCs as a prognostic factor: measurement of CTCs prior to treatment, and monitoring CTC levels after initiation of therapy

Breast cancer

A landmark study by Cristofanilli et al. demonstrated that the amount of blood CTCs (measured by CellSearch) in women with metastatic breast cancer prior to the start of a new therapy differentiated patients at high vs low risk of rapid progression [26]. The cut-off point between the two groups was set at 5 CTC per 7.5 mL of peripheral blood. Other studies, with 75 patients have shown that this established cut-off point is also predictive of PFS and OS. Further studies have confirmed the prognostic value of CTCs, suggesting that it is possible to assess progression rates prior to undertaking antitumor therapy in patients with metastasis to distant organs [27, 30, 31]. Taken together, these encouraging results provide the basis of further research for using CTCs in qualifying patients to more invasive therapies.

Prostate cancer

A study on 231 patients with prostate cancer resistant to castration (CRPC) with distant metastases has shown the prognostic value of CTCs, according to OS rates [32]. This study contributed to the acceptance of CellSearch by the FDA for this group of patients [32]. In this study, elevated CTC levels (i.e. ≥ 5 CTCs/7.5 mL blood) measured by an assay prior to chemotherapy was associated with poorer OS than in patients who had CTC levels < 5 . This association has also been observed after the initiation of therapy. Survival was significantly longer among patients with CTC levels below the cut-off level, relative to patients with persistently high CTCs. For patients with CTC levels that increased in the follow-up to ≥ 5 CTCs, OS was poorer compared to patients with persistently low levels (< 5 CTCs). This may be due to the resistance of the subpopulation of cancer cells to the applied treatment. Taken together, these results indicate that continual assessment of CTCs during therapy may allow for closer evaluation of treatment efficacy and/or a better survival profile than a single assessment of CTCs prior to treatment.

To confirm these assumptions, two clinical trials are currently underway through the Stand Up to Cancer and the Prostate Cancer Foundations [33]. These studies aim to evaluate the potential for changing levels of CTCs in the blood during therapy to predict disease course among patients with prostate cancer.

Colorectal cancer

One study used the CellSearch system to confirm distant metastases in 430 colorectal cancer patients undergoing chemotherapy [28]. Using an estimated cut-off point of 3 CTCs/7.5 mL blood, the authors found that CTCs showed prognostic value for both PFS and OS. As with the study on prostate cancer [32], a decrease in CTCs below the cut-off point after chemotherapy was associated with significantly longer PFS and OS, compared with rates observed in patients with persistently high CTCs. A study by Molnar et al. in colorectal cancer patients treated with chemotherapy reported a significantly higher number of single CTCs, doublets, and CTC clusters in treatment non-responders relative to responders [34]. Moreover, in the study by Molnar et al., higher levels of CTCs correlated with macroscopic progression [34].

CTC as a substitute endpoint in clinical trials

When planning clinical trials, endpoints are defined. Endpoints are variables that determine patient health status for those taking part in the trial, and may include: survival, death, quality of life (rating in scores), or myocardial infarction. Instead of endpoints, surrogate endpoints may be used. Surrogate endpoints may be indicators of physiological or pathological processes (e.g., blood pressure levels), and are

also called 'biomarkers' [35]. CTCs as a potential surrogate endpoint may be beneficial may be useful for predicting disease progression in early and late stages of cancer, monitoring response to treatment, or minimizing molecular residual disease after therapy. Several clinical studies on the efficacy of anti-cancer therapy have evaluated CTCs as a potential surrogate endpoint. For example, one study found that measuring CTCs via the CellSearch system was more sensitive than radiological imaging as a prognostic factor among patients with advanced breast cancer [36]. Further, the authors found an association between high and persistent levels of CTCs and a shorter OS, whereas radiological scans showed no disease progression [36]. Based on these results, the authors concluded that CTCs may indeed be a superior surrogate endpoint measure, as they are highly reproducible and correlate more closely with OS than do changes determined via traditional radiology.

In another study, CTC levels reportedly predicted disease progression at 9–12 weeks after the initiation of therapy among patients with advanced breast cancer [37]. This technique is therefore more precise than FDG-PET and CT, and predicted disease progression when radiographic imaging had little prognostic value for patients with CTC levels below the threshold level of 5/7.5 mL blood. Yet another study found that CTC levels measured early, prior to diagnostics (i.e., at 7–9 weeks) were strongly correlated with subsequent radiographic images [31]. Taken together, these results suggest that CTCs may be used together with radiographic imaging to evaluate response to therapy. However, for CTCs to be considered a reliable endpoint in clinical trials, further research is needed in this area. There is also a need for more accurate validation of the selected method for measuring these cells in a particular patient test group.

CTCs for assessing effectiveness of the applied therapy

Prognostic properties of CTCs measured by the CellSearch method have been used to further study how specific therapies may be selected. For example, therapies may be selected based on baseline levels of CTCs in the blood prior to therapy, or based on changes in CTC numbers during therapy.

These possibilities are currently being studied on a large scale in patients with metastatic breast cancer, through the SWOG S0500 trial (American South West Oncology Group) [38]. The purpose of the SWOG S0500 trial is to confirm whether persistently high CTC numbers ($\geq 5/7.5$ mL blood) after the first chemotherapy cycle are an indicator of disease progression and/or for a change in treatment strategy. The assumption is that unchanged blood levels of CTCs reflect cancer cell resistance to the adopted therapy. Therefore, patients with persistently high CTCs should shift to another treatment, whereas patients with changing levels

should continue the current therapy. This strategy thus allows for assessment of whether early referral to alternative therapy improves OS and PFS. Unfortunately, however, preliminary phase III results are not as promising as expected. Although results have confirmed the prognostic value of CTCs after the first cycle of chemotherapy in predicting OS, they did not show clinical value for evaluating the effectiveness of therapy. Given that patients continued their therapy that was initiated prior to qualifying for the SWOG S0500 study, it was not possible to exclude the effect of therapy on CTCs in the blood. The effect of various therapies on CTCs has not yet been defined, and the available literature data are not consistent in this regard [31]. It is possible that after the first treatment cycle, it is too early to assess CTC fluctuations concerning treatment efficacy.

Another recently initiated study in this group of patients is the CirCe01 study, which is evaluating changes in CTCs (measured by CellSearch) as early resistance markers after the initiation of therapy [39]. Upon observing persistently high CTC levels after the third cycle of chemotherapy, some patients will be directed to an alternative treatment. After the first cycle of an alternative therapy, CTCs will again be monitored, and upon reaching ≥ 5 CTCs/7.5 mL of blood, patients will be redirected to yet another therapy. The premise of this study is to establish whether early detection of chemoresistance is possible by measuring CTCs in the blood. There is however, a risk that a too rapid interruption of targeted anti-cancer therapy will stimulate the biological activity of some cancer cell subpopulations, and that introducing alternative therapies will not yield the expected benefits.

Additionally, to establish an effective antineoplastic therapy, clonal evolution of cancer cells should be characterized by means of CTC phenotype features. Expression of tumor markers (e.g., ER, PG, HER2) may vary between primary tumor cells and distant metastases in breast cancer [40, 41]. As a result, circulating blood cells with metastatic potential may provide a better genetic model for guiding appropriate therapy for a particular patient. The purpose of these studies is not only to identify patients who may benefit from a certain therapy, but also to discover the mechanisms responsible for cancer cells developing treatment resistance and identifying appropriate therapeutic targets. One such study is the DETECT III study, which includes patients with breast cancer without HER2 expression in the primary tumor, and at least one detected CTC cell expressing this receptor. The purpose of the DETECT III study is to determine the efficacy of lapatinib (an inhibitor of the tyrosine kinase receptors HER2 and EGFR) in a select group of patients [41].

Another strategy for choosing a therapy for advanced breast cancer is based on the CTC-Endocrine Therapy Index (CTC-ETI) test, which simultaneously measures CTCs in the blood and the expression of selected tumor markers (i.e.,

ER, BCL-2, HER2, Ki-67) that are predictors of resistance and sensitivity to endocrine therapy [42]. The CTC-ETI test aims to identify patients with estrogen receptor (ER) expression, for whom endocrine therapy may be ineffective. Validating the CTC-ETI test could in the future be used as a criterion for choosing an appropriate therapy (i.e., endocrine vs chemotherapy) among patients with ER-positive breast cancer with distant metastases.

Several studies have found a correlation between the presence of CTCs and disease stage, among patients with primary colorectal cancer as well as patients with distant metastasis. In these studies, CTCs were measured via CellSearch after tumor removal but before chemotherapy, and the cut-off point was ≥ 2 CTCs/7.5 mL of blood. The proportion of patients with CTCs ranged from 5% to 25% at stages I–III, and 62% at distant metastasis stage [43, 44]. One study showed no CTCs after surgery among patients at stages I–III using the same cut-off point [45]. An interesting correlation was found in a homogeneous but small group of 37 patients at stage II and III, such that CTC levels were higher in patients at high risk relative to lower risk patients of the same stage [46]. These results highlight the potential use of CTCs among patients with metastatic colorectal cancer. Specifically, CTCs may be useful for guiding the application of more or less aggressive therapy for stage III, and introducing adjuvant chemotherapy for high risk stage II patients.

CTCs in localized tumors without distant metastases

Thus far, the study outcomes when using CTCs in patients with stage I–III breast cancer have not been clear in terms of the number of detectable CTCs. However, these studies do suggest that measuring CTCs before and during therapy may be an additional factor predicting risk of local disease relapse, and can be measured in addition to more commonly assessed biomarkers (i.e., tumor size, lymph node involvement, HER2 amplification). Consequently, CTCs may be of clinical relevance in the early detection of residual disease. Consistent with this notion, one study demonstrated that CTCs with CK19 expression (as detected by RT-PCR measured mRNA levels) are an independent predictor of PFS and OS among patients with both involved and clear lymph nodes [47]. These results were subsequently confirmed in an independent study of 167 patients with uninvolved lymph nodes undergoing chemotherapy [48]. CTCs were monitored in 23 mL of peripheral blood in the German SUCCESS study, which includes 1,500 patients with metastatic and non-metastatic breast cancer at high risk and treated with chemotherapy. In that study, they found a correlation between the presence of ≥ 1 CTC/23 mL of blood before chemotherapy and the number of lymph nodes involved, and detection of ≥ 1 CTC during chemotherapy subsequently predicted DFS and OS [32]. In another study of 404 patients

undergoing tumor resection, the presence of CTCs prior to surgical intervention was associated with a slightly higher likelihood of local recurrence (78.9% for a 4-year period without recurrence) compared to patients without CTCs (88.4%) [49]. However, the small difference in outcomes between groups and the occurrence of metastases among patients without CTCs do raise some concerns about whether the CellSearch method can be used for determining the risk of recurrence for this group of patients.

Clinical application of CTCs: Summary

The lack of prospective studies on a homogenous and large group of patients together with the lack of knowledge about the effects of different therapies on CTC rates, preclude us from assessing whether a clinical application of CTCs is possible. When examining these applications among breast cancer patients, it appears to be particularly important that patients are adequately stratified according to their cancer subtypes. In 2017, the first European statistical analysis of the potential predictive value by CellSearch was published [50]. The analysis included 1,944 patients with metastatic breast cancer, from 51 centers. Using the cut-off threshold of 5 CTCs/7.5 mL blood, results clearly indicated that detection of CTCs — both before and during treatment — was associated with worse prognosis. Furthermore, CTCs measured prior to any new treatment constitute independent prognostic markers that could supplement the currently used clinical predictive biomarkers of breast cancer. It is also noteworthy that predicted disease progression was more accurate when based on measurement of CTC levels after the initiation of therapy, compared to predictions based on levels of tumor markers recommended for monitoring metastatic breast cancer according to the international guidelines (e.g., CEA, CA15-3) [51]. This is the first analysis carried out in a large group of patients in Europe, and demonstrates the utility of CTCs in monitoring the progression of distant metastatic breast cancer, and accurately determining the risk of disease progression in a given patient.

Perspective

CTCs are expected to be routinely used in the next ten years alongside other clinical methods and markers (i.e., imaging techniques, serum markers) for tracking tumor growth over time [52]. We expect that fluid biopsy outcomes from a patient's blood obtained via modern and automated methods will enable the clinician to detect the cancer, determine its severity, and establish therapeutic goals. These outcomes will also allow for more personalized and modifiable treatments, based on the qualitative and quantitative monitoring of CTCs [53]. Moreover, more in-depth molecular research on CTCs broadens the knowledge-base on the biology of metastasis, which also has important implications for treating cancer patients.

Abbreviations

CAFs — cancer associated fibroblasts
CEA — carcinoembryonic antigen
CK — cytokeratins
CRPC — cancer resistant to castration
CTC — circulating tumor cells
DCCs — disseminated cancer cells
DTCs — disseminated tumor cells
ECM — extracellular-matrix
EMT — epithelial-mesenchymal transition
EpCAM — epithelial adhesion molecule
EpCAM — epithelial cell adhesion molecule
FDA — Food and Drug Administration
FU — follow up
HER2 — human epidermal growth factor
IF — immunofluorescence,
MAT — mesenchymal-ameboid transition
MBC — metastatic breast cancer
mCRPC — metastatic castration-resistant prostate cancer
MET — mesenchymal-epithelial transition
OS — overall survival
PFS — progression-free survival
RT-PCR — reverse transcription-PCR
TGF- β — transforming growth factor beta

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