Biology, detection, and clinical implications of circulating tumor cells

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Abstract

Cancer metastasis is the main cause of cancer-related death, and dissemination of tumor cells through the blood circulation is an important intermediate step that also exemplifies the switch from localized to systemic disease. Early detection and characterization of circulating tumor cells (CTCs) is therefore important as a general strategy to monitor and prevent the development of overt metastatic disease. Furthermore, sequential analysis of CTCs can provide clinically relevant information on the effectiveness and progression of systemic therapies (e.g., chemo-, hormonal, or targeted therapies with antibodies or small inhibitors). Although many advances have been made regarding the detection and molecular characterization of CTCs, several challenges still exist that limit the current use of this important diagnostic approach. In this review, we discuss the biology of tumor cell dissemination, technical advances, as well as the challenges and potential clinical implications of CTC detection and characterization.

Keywords Disseminating tumor cells (DTC); EMT; metastasis; tumor cell dormancy; tumor cell plasticity

CTC migration: mobility and motility

Cancer metastasis has been correlated with specific genetic biomarkers such as mutations, chromosomal aberrations, and gene expression patterns (Nguyen & Massague, 2007; Wragge et al, 2009; Wikman et al, 2012; Hohensee et al, 2013). Nevertheless, whether CTCs enter the blood circulation through an active migration process, by passive means, or both, remains clinically and scientifically an unresolved question (Joosse & Pantel, 2013). In this review, we employ the terms motile and mobile cells to describe the differences between active and passive migration of the tumor cells.

Motile cancer cells are cells that are able to move on their own accord and that has thus gained the ability to move through the extracellular matrix and penetrate basement membranes and endothelial walls upon intravasation and extravasation. These active migration mechanisms imply modification of cell morphology, position, and surrounding tissue (Friedl & Alexander, 2011). Furthermore, cancer cells may infiltrate as single entities, in clusters, in strands, or in single (Indian) files as observed in lobular breast carcinoma. Single cells must weaken or completely lose their adhesive bonds with neighboring tumor cells for infiltration, whereas collective migration requires stable cell-cell adherence and multicellular coordinated movement (Friedl & Gilmour, 2009). These clusters frequently comprise of different cell morphologies.
that is, both epithelial- and mesenchymal-like. Collective migration may require a leader cell with mesenchymal features, able to create a path for the trailing tumor cells through the surrounding tissue (Friedl & Wolf, 2009).

Mobile cancer cells are moved by external forces such as growth of the tumor, mechanical forces, or friction which cause them to be dragged or pushed out of place (Camara et al, 2006; Fornvik et al, 2013). Although a vast amount of literature is available about the processes involved in active migration of cancer cells through the extracellular matrix, less is known concerning the passive dissemination of cancer cells and how they may be forced into the blood circulation. Carcinomas induce the formation of new blood vessels by the secretion of the vascular endothelial growth factor (VEGF) to facilitate the supply of nutrients and oxygen for growth. This process called angiogenesis, often results in leaky vessels caused by weak interconnections of the vessel’s endothelial cells and intercellular openings (McDonald & Baluk, 2002). Due to outward pushing of the tumor during growth, single or clusters of tumor cells can be forced through the leaky vessels, thus ending up in the blood circulation as ‘accidental CTCs’. Furthermore, tumor cells may be passively moved through the micro-tracks created by other tumor cells by proteolysis or through other pre-existing tissue structures (Friedl & Wolf, 2009).

Because the ‘accidental CTCs’ from epithelial malignancies are more likely to have retained their original phenotype, detection with epithelial-specific markers such as the epithelial cell adhesion molecule (EpCAM) would be feasible. On the other hand, tumor cells that have transformed to a more mesenchymal-like phenotype, and thus exhibit greater plasticity, may prove to be less easily detected by conventional EpCAM-based detection methods (Brabletz, 2012; Joosse et al, 2013). Nevertheless, new evidence shows that a phenotypical transformation is not always required for tumor cell motility (Aceto et al, 2014; Godinho et al, 2014).

**Epithelial–mesenchymal plasticity**

Epithelial cells adhere to their neighboring cells through, among others, adherens junctions via binding of cadherins. Carcinoma cells are of epithelial origin and experience the same cell–cell interaction through adhesion molecules such as cadherins, claudins, or plakoglobin; these interactions prevent them from spreading in the first place (Pantel et al, 1998).

Normal epithelial cells show remarkable plasticity as they are able to reprogram and undergo dynamic and reversible transitions between the epithelial and mesenchymal cell phenotype (Tam & Weinberg, 2013). This de-differentiation process known as epithelial-to-mesenchymal transition (EMT) occurs frequently throughout embryogenesis because cells continuously migrate to form tissues and organs. EMT also plays an important role in wound healing and tissue regeneration (Nieto, 2013). Not surprisingly, carcinoma cells also make use of EMT as they become invasive. Indeed, they typically feature loss of cell–cell adherence proteins such as cadherin, followed by loss of apico-basal polarity, and finally, gaining the ability to migrate and invade (Thiery, 2002). EMT can be triggered by paracrine signaling of TGF-beta, WNT, platelet-derived growth factors, or interleukin-6 (IL-6) but can also be induced by nicotine, alcohol, and ultraviolet light (Thiery et al, 2009; Tam & Weinberg, 2013; Kishi et al, 2014). These triggers activate transcription factors such as Snail, Twist, and Zeb that maintain the mesenchymal phenotype by autocrine signaling (Tam & Weinberg, 2013). Because of the loss of tight and adherens junctions, as well as cytoskeletal changes, typical epithelial markers such as EpCAM and E-cadherin are down-regulated, keratin expression is altered, and finally, up-regulation of mesenchymal markers such as vimentin is observed during EMT (Joosse et al, 2012). Consequently, mesenchymal-like CTC subpopulations are difficult to identify in the hematopoietic cell environment which is also of mesenchymal origin (Joosse & Pantel, 2013). Of note, single tumor cells found in the blood of breast cancer patients exhibit EMT-associated changes, while cell clusters appear to require a partial EMT so that these cells possess the migratory abilities of a mesenchymal cell but retain the cell–cell interaction profile of an epithelial cell (Yu et al, 2013).

**Non-EMT-associated motility**

Centrosome amplification has also been linked to de-differentiation and recently been shown to induce invasion in cancer (Ghadimi et al, 2000; Lingle et al, 2002; Godinho et al, 2014). The centrosomes form the microtubule organizing center in mammalian cells and facilitate chromosomal separation during mitosis via the mitotic spindle apparatus but also participate in the organization of flagella and cilia. EMT is thought not to play a role in the invasion of tumors
with centrosome amplification since expression of E-cadherin is retained. Cell–cell adhesion appears instead reduced downstream of Rac1 signaling by increased Arp2/3-dependent actin polymerization (Godinho et al., 2014). Another form of non-EMT-associated dissemination, which is thought to substantially contribute to metastasis, is the release of circulating tumor cell clusters (Aceto et al., 2014). These clusters are constituted of 2–50 tumor cells held together by plakoglobin-dependent intercellular adhesion. Clustered cells are less likely to undergo anoikis and have an increased likelihood of being trapped in narrow blood vessels, thus favoring extravasation into distant organs.

Therefore, since not all metastatic tumor cells seem to lose their epithelial-like characteristics by EMT, it is important to consider CTCs with epithelial properties too in order to detect the metastasis-initiating cells (MICs).

CTCs in circulation

Once in the bloodstream, CTCs face several natural obstacles that hinder the metastatic process. First are the enormous shearing forces and collisions with blood cells, generated by blood flow. Although shear stresses decrease the number of viable cancer cells dramatically, tumor cells that underwent EMT seem to be more resistant against these forces compared to epithelial tumor cells (Mitchell & King, 2013). Second, CTCs must survive in the bloodstream without their cell–matrix interactions, an occurrence that would normally trigger apoptosis through a process called anoikis. Resistance against anoikis, however, is made possible in CTCs by activated tropomyosin-related kinase B (TrkB) that suppresses caspase-associated apoptosis and enables the cells to survive in liquid suspension (Douma et al., 2004). The third obstacle that CTCs face in the blood is the activity of the immune system. In colorectal cancer, immune escape is obtained by up-regulation of CD47 that prevents CTCs from macrophage and dendritic cell attack (Steinert et al., 2014). Finally, cancer cells must eventually leave the blood circulation, which requires binding to the endothelium lining the vessels. Because platelets enhance this binding, inhibition of platelet aggregation by for instance aspirin can decrease stable tumor cell binding to activated platelets (Uppal et al., 2014).

Tumor cell homing and dormancy

Extravasation starts when CTCs slow down in small capillaries, attach to the endothelium, and finally undergo transendothelial migration (Reymond et al., 2013). One of the most frequent CTCs homing sites are the bones, including for primary malignancies such as colorectal and lung cancer that less typically metastasize to the bone (Pantel & Brakenhoff, 2004; Braun et al., 2005; Riethdorf et al., 2008). It is therefore thought that the bone marrow functions as a reservoir for disseminated tumor cells (DTCs) (Wikman et al., 2008; Kang & Pantel, 2013). In breast cancer, it may take many years up to decades after surgery of the primary tumor until metastases become evident (Goss & Chambers, 2010; Uhr & Pantel, 2011). During this time, bone marrow DTCs (Janni et al., 2011) as well as CTCs derived from the DTCs (Meng et al., 2004) may be found in these patients. DTCs in bone marrow may linger in a dormant state, thus evading systemic therapy while waiting for the appropriate trigger to resume proliferation (Bragado et al., 2013; El Touny et al., 2014). The basis for tumor cell dormancy may be the initial EMT process itself. In squamous cell carcinoma, spatiotemporal regulation of the epithelial–mesenchymal transition is essential for the dissemination and eventual metastasis (Tsai et al., 2012). The mesenchymal phenotype of CTCs that underwent EMT promotes motility but does not favor growth (Celia-Terrassa et al., 2012). Indeed, cancer cells must undergo a reverse mesenchymal-to-epithelial transition (MET) to acquire the ability to proliferate and thus form a metastatic tumor. It has therefore been suggested that tumor cells with an intermediate phenotype can most efficiently disseminate and grow at the distant sites (Bednarz-Knoll & Weinberg, 2012; Tam & Weinberg, 2013).

Figure 1. Metastatic cascade.

Tumor cells may enter the bloodstream passively or actively via biological events such as EMT or centrosomes amplification. Disseminating tumor cells must overcome several hurdles including anoikis, shear stress in the bloodstream, and the immune system in- and outside of the blood circulation. Once at a distant site, tumor cells may extravasate, undergo MET, and grow locally to become a metastasis or remain in dormancy.
Implications of CTCs

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Figure 2.
Enrichment and detection of CTCs

Enrichment of CTCs

CTCs are infrequent and appear at an estimated level of one against the background of millions ($10^6$-$10^7$) of surrounding normal peripheral mononuclear blood cells (PBMCs) (Alix-Panabieres et al., 2012). The occurrence rate might even be lower in cancer patients without obvious metastases (Rink et al., 2012; Rack et al., 2014). Hence, selective enrichment of tumor cells and/or systematic removal of PBMCs and red blood cells (RBCs) (Ozkumur et al., 2013) is required to detect CTCs in the blood of a cancer patient. To date, more than 40 techniques have been developed for CTC detection (Parkinson et al., 2012) and novel strategies are published continuously. CTC enrichment and detection methods have been classified based on whether they exploit the physical or biological properties of the cells (Alix-Panabieres & Pantel, 2014). However, as many enrichment strategies rely on positive selection (usually targeting EpCAM), CTC assays are more commonly grouped into label-dependent and label-independent approaches (Fig 2). Among the numerous EpCAM-based CTC detection technologies, the semi-automated CellSearch system is the most frequently used system and at present the only one cleared by the U.S. FDA. Through this approach, CTC counts have been associated with an independent prognostic power on progression-free survival (PFS) and overall survival (OS) in primary and metastatic disease (Cristofanilli et al., 2004; de Bono et al., 2008; Cohen et al., 2009; Bidard et al., 2014; Rack et al., 2014). Due to its clinical relevance, high reproducibility, and FDA clearance, CellSearch can be considered as a benchmark for all other CTC detection methods appearing on the market. The AdnaTest is another epithelial marker-based research tool that positively enriches CTCs from a blood sample. Using this approach, the presence or disappearance of CTCs was shown to be a prognostic and predictive marker in a study on metastatic breast cancer (Tewes et al., 2009). Microfluidic device platforms like the CTC- or the Herringbone (HB)-Chip seem to be promising alternatives to selectively capture EpCAM-positive CTCs in cancer patients (Nagrath et al., 2007; Maheswaran et al., 2008). A combination of anti-EpCAM and anti-MUC1 capture in a single microfluidic device may further result in an improved capture performance of CTCs (Thege et al., 2014). Another chip-based platform combines a size-based filtration with an affinity-based enrichment strategy, thus enhancing the chance of systematic removal of PBMCs and RBCs (CTC-iChip) (Ozkumur et al., 2013). Fluxion Biosciences has lately introduced a commercially available microfluidic technology (IsoFlux) with magnetic isolation zones to isolate EpCAM-positive tumor cells from biological samples. Using the IsoFlux, recovery rates between 74 and 85% of EpCAMlow- (MDA-MB-231) and EpCAMhigh- (SKBR3)-expressing tumor cells could be obtained (Harb et al., 2013). To circumvent sample volume limitations, GILUPI GmbH has designed an EpCAM-coated wire (CellCollector®) to capture CTCs in vivo (Saucedo-Zeni et al., 2012). This device is positioned through a cannula into the vein of a cancer patient. It is estimated that during the 30-min application time, up to 1.5 l of blood flows over the detector, thus increasing the yield of detectable CTCs.

However, a major caveat is that metastasis formation might require EMT, which in turn might cause loss of CTC detection due to the down-regulation of EpCAM. EMT-related changes such as the down-regulation of EpCAM and Keratins or the up-regulation of EGFR and vimentin have already been described on CTCs and DTCs (Bartkowiak et al., 2009; Armstrong et al., 2011; Gorges et al., 2012; Joosse et al., 2012; Kasimir-Bauer et al., 2012; Gasch et al., 2013). Hence, EpCAM-based enrichment technologies should be re-worked for the comprehensive detection of epithelial (epithelial+/mesenchymal−), complete EMT (epithelial−/mesenchymal−), and intermediate EMT (epithelial+/mesenchymal+) CTC phenotypes.

Depletion of CD45-positive leukocytes (negative selection) is a preferred approach to capture CTCs lacking adequate expression of the EpCAM protein. Using this strategy, viable tumor cells have already been isolated, characterized, and cultured (Deneve et al., 2013). CD45 depletion can be combined with other label-independent methodologies (e.g., red blood cell lysis or density gradient centrifugation) to improve the yield and to displace unwanted erythrocytes (Liu et al., 2011). However, red blood cell lysis or density gradient centrifugation may lead to a loss of tumor cells resulting in false-negative results. The novel CTC-iChip can deplete hematopoietic cells combining this strategy with a size-based enrichment step (Ozkumur et al., 2013). In the antigen-independent mode, this system was able to isolate CTCs from several types of cancers that had lost or never had the epithelial marker characteristics (triple-negative breast cancer and melanoma). A combination of red blood cell lysis, depletion of CD45+ leukocytes, and size-based filtration has also been shown to be feasible in non-small cell lung, breast, colon, and esophageal cancer patients (Wu et al., 2014). Membranous filter devices like the ISET system or the ScreenCell approach appear to enrich CTCs with a high recovery rate as well. However, in particular, EMT-related CTCs may not be stiffer than leukocytes and CTCs are not always larger than leukocytes and might therefore not be retained by these filters. Moreover, even if tumor cells are captured on the membrane, they may be difficult to detach for further molecular examination. The Parsortix system is a novel separation device that captures viable tumor cells based on their physical properties. This system allows a size-based identification of cancer cells within the capture cassette device or harvesting of the enriched tumor cells outside the cassette. The harvested tumor cells are easily accessible and ready for downstream analysis, such as, next-generation sequencing (NGS), qPCR, FISH, ICC, or other cancer cell-related analytical methodologies. Another newly designed microfluidic technology called the JETTA™ microfluidic chip, uses a size- and deformability-based capture scheme to characterize CTCs (Riahi et al., 2014). The novelty of this
Identification and characterization of CTCs

Identification, enumeration, and molecular characterization of CTCs are likely to provide important insights into disease progression and might allow adaptation of therapeutic strategies. Differential CTC detection assays apply (or can be combined with) various strategies for the identification of tumor cells. Morphologic investigation together with fluorescence immunocytochemistry (ICC) is a common procedure for the identification and enumeration of CTCs after enrichment. The CellSearch system classifies a CTC as positive if the cell is ≥ 4 μm, DAPI− (4,6-diamino-2-phenylindole), pan-keratin+, and CD45−. The additional 4th fluorescence channel is accessible for a user-defined detection of, for example, therapy-relevant markers such as the androgen receptor (AR), the prostate-specific antigen (PSA), the human epidermal growth factor receptor 2 (ERBB2), the epidermal growth factor receptor (EGFR) (Riethdorf et al., 2007; Gasch et al., 2013), or EMT-associated molecules (such as N-cadherin or vimentin) (Armstrong et al., 2011). Identification of CTCs by ICC is also applicable when using other enrichment strategies (microfluidic or size-based platforms, the Gilupi CeliCollector™, red blood cell lysis, or density centrifugation-based procedures) (Nagrath et al., 2007; Maheswaran et al., 2008; Saucedo-Zeni et al., 2012; Babayan et al., 2013). However, conventional microscopic examination by a researcher is cost- and time-consuming. The Ariol system (Genetix USA) might be used for automated cell image capturing and analysis if the enriched cells are transferrable onto glass slides (Deng et al., 2008). Investigator microscopic fillers set the limits for CTC enumeration and characterization by ICC; typically, the four different fluorescence channels are used for CTC characterization (two markers of interest), CD45 staining (to exclude false-positive findings), and nuclear counterstaining (DAPI or Hoechst33342). Identification of CTCs by multiplex PCR targeting the numerous tumor-associated mRNA transcripts overcomes filter set limitations. CTC enrichment combined with a RT–PCR technology could already be used for the identification of tumor-related markers (EpCAM, MUC1, and ERBB2), EMT-associated transcripts (PI3Ka (phosphatidylinositol 3-kinase alpha), Akt-2, or Twist1), or stem cell markers such as ALDH1 (aldehyde dehydrogenase 1) (Kasimir-Bauer et al., 2012). Additional transcripts such as KRT-19, MAGE-A3, and PBCD have also been used for the detection and molecular characterization of circulating tumor cells (Strati et al., 2013). Hence, quantitative RT–PCR confers high sensitivity to CTC identification, although uncertainties are foreseeable with respect to specific primer and target gene selection. Additionally, accurate quantification of CTCs is difficult because CTCs are heterogeneous and may express different levels of the genes considered for identification. Furthermore, precise cut-off levels between mRNA amounts of normal blood cells and tumor cells must be determined and validated since tumor-associated targets such as KRT-19 mRNA have also been found in a small number of healthy individuals (Stathopoulou et al., 2003). Hence, a combination of different genes should be used to improve the specificity of RT–PCR for reliable CTC detection.

Another strategy for the identification of CTCs is to enumerate and analyze proteins specifically secreted by viable tumor cells (EPI-SPOT, Epithelial Immuno SPOT). CTCs are enriched by negative depletion and subsequently cultured on a membrane coated with antibodies that capture the secreted proteins. Afterward, the proteins are readily identifiable by immunofluorescence microscopy using fluorochrome-labeled secondary antibodies targeting the protein of interest. Using this strategy, viable CTCs and DTCs have been already identified by the detection of secreted KRT-19, MUC1, PSA, or FGF-2 (Alix-Panabiéres et al., 2007; Alix-Panabiéres, 2012).

Taken together, different strategies can be used for CTC identification and characterization. Frequently, protein- (ICC), RNA-, and/or DNA-based (PCR) approaches are implemented. Each strategy has advantages and drawbacks. A combination of different analytical methodologies is likely to be beneficial and may encompass the heterogeneity of CTCs and help to understand their role in metastasis formation.

Clinical implications of circulating tumor cells

DTCs in the bone marrow have been detected for all solid tumor types, suggesting that this compartment might be a preferred reservoir for blood-borne DTC from where they may re-disseminate to other distant organs such as liver or lungs where better growth conditions may exist. For example, DTCs are prognostic markers in colorectal cancer even though bone metastases are very rare in this cancer type (Lindemann et al., 1992; Pantel et al., 1993). In breast cancer, where bone metastases are more frequent, DTCs in the bone marrow may predict metastatic relapse in other organs as well (Braun et al., 2000, 2005). However, it cannot be excluded that DTCs may home equally well to other organs such as lung or liver, where however they may not be so easily detected as in bone marrow. The observed correlation between DTCs in bone marrow and local relapse in breast cancer (Bidard et al., 2008) is consistent with the recent experimental findings in mice that these cells might even re-infiltrate their primary organs and promote tumor progression (Kim et al., 2009). If the bone marrow is a preferred reservoir for DTCs, drugs targeting the BM–tumor interactions...
(e.g., bisphosphonates or antibodies to the RANK ligand (Lewiecki, 2011; Gronich & Rennert, 2013)) might help to prevent metastatic or even local relapse.

Sequential peripheral blood drawings in particular for real-time monitoring of minimal residual disease in cancer patients undergoing systemic therapies are clearly more acceptable than repeated bone marrow aspirations. Indeed, many research groups are currently assessing the clinical value of CTC analyses, which so far has been proven to provide significant prognostic information in metastatic breast cancer (Zhang et al., 2012; Bidard et al., 2014), and other solid tumors such as of the prostate (de Bono et al., 2008), colorectal (Cohen et al., 2008), and lung (Krebs et al., 2011; Hou et al., 2012) cancer, and seems to be superior over conventional imaging methods for response evaluation (Budd et al., 2006). However, the real challenge of CTC technologies is to monitor minimal residual disease in patients without signs of overt metastasis, as the CTC counts are very low in these patients. Promising results indicating that even such low CTC counts may have prognostic relevance have been recently published for several tumor entities such as breast cancer (Lucci et al., 2012; Rack et al., 2014), bladder cancer (Rink et al., 2011; Gazzaniga et al., 2014), and colorectal cancer (Deneve et al., 2013). Nevertheless, more sensitive methods and/or the analysis of larger amounts of blood (Saucedo-Zeni et al., 2012; Fischer et al., 2013) might be required to increase the robustness of CTC measurements in these patients. The implementation of leukapheresis or other innovative approaches for CTC enrichment (such as the CellCollector™) enables the screening for CTCs in blood volumes of 1.5-25 liters. The analysis of higher blood volumes may significantly improve the capturing rates of CTCs, which might help to predict therapy response and monitoring of the disease especially in early-stage disease.

Furthermore, molecular characterization of CTCs might be essential to identify therapeutic targets and contribute to more ‘tailored’ and personalized anti-metastatic therapies. In current clinical practice, the decision on targeted therapies is solely based on the analysis of the primary tumor although the therapy is directed against metastatic cells (Wan et al., 2013). However, metastatic relapse may occur many years after primary tumor diagnosis and surgical resection (Uhr & Pantel, 2011). Thus, selection pressure during the complex metastatic process might have originated a particular subclone of cancer cells that is underrepresented in the primary tumor and may therefore be easily missed using next-generation sequencing of single CTCs (Heitzer et al., 2013; Lohr et al., 2014). In particular, the microenvironment at the distant metastatic site can alter expression patterns as compared to their ascendants in the primary tumor (Korkaya & Wicha, 2013). All considered, the analysis of metastatic patterns as compared to their ascendants in the primary tumor microenvironment at the distant metastatic site can alter expression of prominent targets for systemic therapy (Wan et al., 2013). Currently, all patients are stratified to trastuzumab (or other anti-ERBB2 therapies) by primary tumor tissue analysis only. Recent reports, however, have shown that ERBB2-positive CTCs can be detected also in patients with ERBB2-negative primary tumors (Riethdorf et al., 2010; Ignatiadis et al., 2011; Hartkopf et al., 2012), suggesting that there might exist additional patients that could benefit from ERBB2-directed therapies. Ongoing clinical studies (e.g., DETECT III (NCT01619111) trial in Germany and CTC-TREAT (NCT01548677) trial in Europe) will reveal whether the ERBB2 status of CTCs may predict response to ERBB2-directed therapies (Bidard et al., 2013a, b).

Another example for the use of CTCs as predictive biomarkers is the presence of ER-negative CTCs in breast cancer patients with ER-positive primary tumors (Babayan et al., 2013). ER is the most common therapeutic target in breast cancer, and 70–80% of patients have ER-positive primary tumors. However, ER-negative CTCs that may have escaped hormonal therapy to block ER-mediated growth frequently occur in these patients.

Genomic CTC analysis can also reveal gene mutations relevant for therapy resistance. For example, KRAS mutations are known to block the effect of therapeutic EGFR inhibition by antibodies or small inhibitors in colorectal cancer patients (Wan et al., 2013). The analysis of individual CTCs has shown a remarkable intra-patient KRAS mutation heterogeneity (i.e., KRASWT and KRASSMT CTCs are present in the same patient) (Gasch et al., 2013). Indeed, the presence of KRAS-mutated CTCs in patients with KRAS wild-type primary colon carcinomas might be one explanation for failure of drug-mediated EGFR inhibition in these patients (Douillard et al., 2013; Peeters et al., 2013). At present, however, only an arbitrary section of the primary tumor is analyzed for KRAS mutations in colorectal cancer patients and the genomic heterogeneity of metastatic cells—the actual targets of systemic therapy—is not taken into consideration for therapy decisions.

In conclusion, the characterization of CTCs may have an important impact as companion diagnostics in future clinical trials testing new targeted therapies (Wan et al., 2013).

Conclusions and outlook

Metastasis starts with cancer cell invasion from the primary tumor through the surrounding tissue into the bloodstream. Tumor cell motility may be acquired by different processes of which epithelial-to-mesenchymal transition (EMT) and amplification of centrosomes are currently considered to be the main ones. EMT leads to (partial) loss of the epithelial characteristics of cancer cells, whereas centrosome amplification-associated spread may retain the expression of epithelial markers. Because of these different dissemination processes, what the true features of the metastasis-initiating cells are and whether these cells can be found among the CTCs that can currently be detected in the blood of cancer patients remain to be fully established. CTC enrichment methods can broadly be divided into EpCAM-dependent cell selection that allows the identification of epithelial tumor cells and on the other hand the EpCAM-independent cell selection methods that may help to identify additional mesenchymal-like CTC subpopulations. Interestingly, the first report on grafting human-derived breast cancer CTCs in xenograft assays indicated that the putative metastasis-initiating cells
pending issues

Do the currently used CTC enrichment and detection techniques allow us to identify bona fide metastasis-initiating cells (MICs)? Are EMT and MET required for tumor cell dissemination and metastatic outgrowth or are non-EMT events more effective in causing metastatic dissemination? Can CTCs be used to investigate the effectiveness of cancer treatment and are CTCs furthermore reliable targets to predict personalized treatment strategies based on a blood test (fluid biopsy)?

expressed EpCAM, although at a reduced level (Baccelli et al., 2013). CTC detection approaches are usually based on the expression or secretion of specific proteins, DNA mutations, or RNA profiling. The development of new enrichment technologies are currently aimed at increased sensitivity and investigating increased blood volume using ‘in vivo strategies’ to gain purity and enumeration of higher CTC counts. Clinically, quantification of CTCs is of high value as these cancer cells generally represent the tumor (metastases) and facilitate real-time monitoring during systemic therapies by sequential peripheral blood sampling. Furthermore, molecular characterization of CTCs might enable the identification of therapeutic targets and contribute to personalized anti-metastatic therapies. Proof of the clinical relevance of the detection and characterization of CTCs has been substantially accumulating during the past decades. The use of xenograft models is a promising approach to gain further insights into the biology of tumor cell dissemination and may further help to test responses to newly designed therapies (Baccelli et al., 2013; Hodgkinson et al., 2014; Yu et al., 2014).

In conclusion, analysis of CTCs in the peripheral blood (liquid biopsy) has a clear potential to further our understanding of the biology of tumor cell dissemination and to improve the management and possibly the prevention of metastatic disease in the near future.

Conflict of interest
The authors declare that they have no conflict of interest.

References

Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H et al. (2014) Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 158: 1110–1122
Alix-Panabieres C, Vendrell JP, Pelle O, Rebillard X, Riedthold S, Muller V, Fabbro M, Pantel K (2007) Detection and characterization of putative metastatic precursor cells in cancer patients. Clin Chem 53: 537–539
Alix-Panabieres C (2012) EPISPOST assay: detection of viable DTCs/CTCs in solid tumor patients. Recent Results Cancer Res 195: 69–76
Alix-Panabieres C, Schwarzenbach H, Pantel K (2012) Circulating tumor cells and circulating tumor DNA. Annu Rev Med 63: 199–215
Alix-Panabieres C, Pantel K (2013) Circulating tumor cells: liquid biopsy of cancer. Clin Chem 59: 110–118
Alix-Panabieres C, Pantel K (2014) Technologies for detection of circulating tumor cells: facts and vision. Lab Chip 14: 57–62
Armstrong AJ, Marenso MS, Oltean S, Kemeny G, Bitting RL, Turnbull JD, Herald CI, Marcom PK, George DJ, Garcia-Blanco MA (2011) Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers. Mol Cancer Res 9: 997–1007
Babayan A, Hannemann J, Spotter J, Muller V, Pantel K, Joosse SA (2013) Heterogeneity of estrogen receptor expression in circulating tumor cells from metastatic breast cancer patients. PLoS One 8: e75038
Baccelli I, Schneeweiss A, Riethdorf S, Stenzinger A, Schillert A, Vogel V, Klein C, Saini M, Bauerle T, Wallwiener M et al. (2013) Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. Nat Biotechnol 31: 539–544
Bartkowiak K, Wieczorek M, Buck F, Harder S, Moldenhauer J, Effenerger KE, Pantel K, Peter-Katalinic J, Brandt BH (2009) Two-dimensional differential gel electrophoresis of a cell line derived from a breast cancer micrometastasis revealed a stem/progenitor cell protein profile. J Proteome Res 8: 2004–2014
Bednarz-Knoll N, Alix-Panabieres C, Pantel K (2012) Plasticity of disseminating cancer cells in patients with epithelial malignancies. Cancer Metastasis Rev 31: 673–687
Bidard FC, Vincent-Salomon A, Gomme S, Nos C, de RY, Thiery JP, Sigal-Zafrani B, Mignot L, Sastre-Garau X, Piega JY (2008) Disseminated tumor cells of breast cancer patients: a strong prognostic factor for distant and local relapse. Clin Cancer Res, 14: 3306–3311
Bidard FC, Fehm T, Ignatiadis M, Smerage JB, Alix-Panabieres C, Janni W, Messina C, Paolotti C, Muller V, Hayes DF et al. (2013a) Clinical application of circulating tumor cells in breast cancer: overview of the current interventional trials. Cancer Metastasis Rev 32: 179–188
Bidard FC, Piega JY, Sonc JC, Thiery JP (2013b) Translating metastasis-related biomarkers to the clinic—progress and pitfalls. Nat Rev Clin Oncol 10: 169–179
Bidard FC, Peeters DJ, Fehm T, Nole F, Gisbert-Criado R, Mavroudis D, Grisanti S, Generali D, Garcia-Saenz JA, Stebbing J et al. (2014) Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. Lancet Oncol 15: 406–414
de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, Doyle GV, Terstappen LW, Pienta KJ, Raghavan D (2008) Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 14: 6302–6309
Brabletz T (2012) To differentiate or not—routes towards metastasis. Nat Rev Cancer 12: 425–436
Bragado P, Estrada Y, Parikh F, Krause S, Capobianco C, Farina HG, Schwie DM, Aguirre-Ghiso JA (2013) TGF-beta2 dictates disseminated tumor cell fate in target organs through TGF-beta-RII and p53alpha/beta signaling. Nat Cell Biol 15: 1351–1361
Braun S, Pantel K, Muller P, Janni W, Hepp F, Kentenich CR, Gastrous S, Wischisch A, Dimpfl T, Kindermann G et al. (2000) Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. N Engl J Med 342: 525–533
Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombe RS, Schlimok G, Diel J, Gerber B, Gebauer G et al. (2005) A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 353: 793–802
Budd GT, Cristofanilli M, Ellis MJ, Stopeck A, Borden E, Miller MC, Matura J, Repollet M, Doyle GV, Terstappen LW et al. (2006) Circulating tumor cells versus imaging—predicting overall survival in metastatic breast cancer. Clin Cancer Res 12: 6403–6409
Camara O, Kavallaris M, Noschel H, Rengsberger M, Jonke C, Pachmann K (2006) Seeding of epithelial cells into circulation during surgery for breast
cancer: the fate of malignant and benign mobilized cells. World J Surg Oncol 4: 67
Celia-Terrassa T, Meca-Cortes O, Mateo F, de Paz AM, Rubio N, Arnal-Estape A, Ell BJ, Bermudo R, Diaz A, Guerra-Rebollo M et al (2012) Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells. J Clin Invest 122: 1849–1868
Chambers AF, Groom AC, MacDonald IC (2002) Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2: 563–572
Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath K, Gabrail NY, Picus J, Morse MA, Mitchell E, Miller MC et al (2009) Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. Ann Oncol 20: 1223–1229
Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LWV et al (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351: 781–791
Deneve E, Riethdorf S, Ramos J, Nocca D, Coffy A, Daures JP, Maudelonde T, Friedl P, Wolf K et al (2010) Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 26: 3213–3221
Deng G, Bauernhofer T, Pichler M, Langer-Freitag S, Seifert AM, Mauermann O, Izbicki JR, Pantel K, Riethdorf S (2013) Heterogeneity of epidermal growth factor receptor status and mutations of KRAS/PIK3CA in circulating tumor cells of patients with colorectal cancer. Clin Chem 59: 252–260
Gazaniga P, de BE, Raimondi C, Gradilone A, Busetto GM, De FE, Nicolazzo C, Giovannone R, Gentile V, Cortesi E et al (2014) Circulating tumor cells detection has independent prognostic impact in high-risk non-muscle invasive bladder cancer. Int J Cancer 135: 1978–1982
Ghadimi BM, Sackett DL, Dillipanipontio MJ, Schrock E, Neumann T, Jauho A, Auer G, Ried T (2000) Centrosome amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations. Genes Chromosom Cancer 27: 183–190
Godinho SA, Picone R, Burage M, Dagher R, Su Y, Leung CT, Polyak K, Brugge JS, Thery M, Pellman D (2014) Oncogene-like induction of cellular invasion from centrosome amplification. Nature, S10: 167–171
Gorges TM, Tinhofer I, Drosch M, Rose L, Zollner TM, Krahm T, von AO et al (2013) Circulating tumour cells escape from EpCAM-based detection due to epithelial-to-mesenchymal transition. BMC Cancer 13: 178
Goss PE, Chambers AF (2010) Does tumour dormancy offer a therapeutic target? Nat Rev Cancer 10: 871–877
Gronich N, Rennert G (2013) Beyond aspirin–cancer prevention with statins, metformin and bisphosphonates. Nat Rev Clin Oncol 10: 625–642
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646–674
Harb W, Fan A, Tran T, Danila DC, Keys D, Schwartz M, Ionescu-Zanetti C (2013) Mutational Analysis of Circulating Tumor Cells Using a Novel Microfluidic Collection Device and qPCR Assay. Transl Oncol 6: 528–538
Hartkopf AD, Banys M, Fehm T (2012) HER2-positive DTCs/CTCs in breast cancer. Recent Results Cancer Res 195: 203–215
Heitzer E, Auer M, Gasch C, Pichler M, Ulz P, Hoffmann EM, lax S, Waldspuehl-Geigl J, Mauermann O, Lackner C et al (2013) Complex tumor genomes inferred from single circulating tumor cells by array-CGH and next-generation sequencing. Cancer Res 73: 2965–2975
Hodgkinson CL, Morrow CJ, Li Y, Metcalf RL, Rothwell DG, Trapani F, Polanski R, Burt DJ, Simpson KL, Morris K et al (2014) Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. Nat Med 20: 897–903
Hohensee I, Lamszus K, Riethdorf S, Meyer-Staeckling S, Glatzel M, Matschke J, Witzel I, Westphal M, Brandt B, Muller V et al (2013) Frequent genetic alterations in EGFR- and HER2-driven pathways in breast cancer brain metastases. Am J Pathol 183: 83–95
Hou JM, Krebs MG, Lancashire L, Sloane R, Backen A, Swain RK, Priest L, Greystoke A, Zhou C, Morris K et al (2012) Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. J Clin Oncol 30: 525–532
Hou HW, Warkiani ME, Khoo BL, Li ZR, Soo RA, Tan DS, Lim WT, Han J, Bhagat AA, Lim CT (2013) Isolation and retrieval of circulating tumor cells using centrifugal forces. Sci Rep 3: 1259
Ignatiadis M, Rothe F, Chaboteaux C, Durbecq V, Rouas G, Criscitiello C, Metallo J, Kheddoumi N, Singhal SK, Michiels S et al (2011) HER2-positive circulating tumor cells in breast cancer. PLoS One 6: e15624
Jann J, Vogl FD, Wiedswang C, Synnestvedt M, Fehm T, Juckstock J, Borgen E, Rack B, Braun S, Sommer H et al (2011) Persistence of disseminated tumor cells in the bone marrow of breast cancer patients predicts increased risk for relapse—a European pooled analysis. Clin Cancer Res 17: 2967–2976
Implications of CTCs

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Joosse SA, Hannemann J, Spotter J, Bauche A, Andreas A, Muller V, Pantel K (2012) Changes in keratin expression during metastatic progression of breast cancer: impact on the detection of circulating tumor cells. Clin Cancer Res 18: 993–1003

Joosse SA, Pantel K (2013) Biologic challenges in the detection of circulating tumor cells. Cancer Res 73: 8–11

Kang Y, Pantel K (2013) Tumor cell dissemination: emerging biological insights from animal models and cancer patients. Cancer Cell 23: S73–S81

Kasimir-Bauer S, Hoffmann O, Wallwiener D, Kimmig R, Fehm T (2012) Expression of stem cell and epithelial-mesenchymal transition markers in primary breast cancer patients with circulating tumor cells. Breast Cancer Res 14: R15

Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XH, Norton L, Massague J (2009) Tumor self-seeding by circulating cancer cells. Cell 139: 1315–1326

Kishi S, Bayliss PE, Hanai JI (2014) A prospective epigenetic paradigm between cellular senescence and epithelial-mesenchymal transition in organismal development and aging. Transl Res 165: 241–249

Korkaya H, Wicha MS (2013) HER2 and breast cancer stem cells: more than meets the eye. Cancer Res 73: 3489–3493

Krebs MG, Sloane R, Priest L, Lancashire L, Hou JM, Greystoke A, Ward TH, Ferraldeschi R, Hughes A, Clack G et al (2011) Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. J Clin Oncol 29: 1556–1563

Lewiecki EM (2011) New targets for intervention in the treatment of postmenopausal osteoporosis. Nat Rev Rheumatol 7: 631–638

Lindemann F, Schlimok G, Dirschd P, Witte J, Riethmuller G (1992) Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet 340: 685–689

Lingle WL, Barrett SL, Negron VC, D Serif J (1992) Changes in keratin expression during metastatic progression of breast cancer: impact on the detection of circulating tumor cells. Clin Cancer Res 18: 993–1003

Longo WL, Barrett SL, Negron VC, D Serif J (1992) Changes in keratin expression during metastatic progression of breast cancer: impact on the detection of circulating tumor cells. Clin Cancer Res 18: 993–1003

Liu Z, Fusi A, Klopcieki E, Schmitter A, Tinhofer I, Nonnenmacher A, Keilholz U (2011) Negative enrichment by immunomagnetic nanobeads for unbiased characterization of circulating tumor cells from peripheral blood of cancer patients. J Transl Med 9: 70

Lohr JC, Adalsteinsson VA, Cibulsksis K, Choudhury AD, Rosenberg M, Cruz-Gordillo P, Francis JM, Zhang CZ, Shalek AK, Satija R et al (2014) Whole-exome sequencing of circulating tumor cells provides a window into metastatic prostate cancer. Nat Biotechnol 32: 479–484

Lu J, Fan T, Zhao Q, Zeng W, Zaslavsky E, Chen JJ, Frohman MA, Golightly MG, Madajewicz S, Chen WT (2010) Isolation of circulating epithelial and tumor progenitor cells with an invasive phenotype from breast cancer patients. Int J Cancer 126: 669–683

Lu X, Mu E, Wei Y, Riethoven S, Yang Q, Yuan M, Yan J, Hua Y, Tiede BJ, Lu X et al (2011) VCAM-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging alpha4beta1-positive osteoclast progenitors. Cancer Cell 20: 701–714

Lucci A, Hall CS, Lodging B, Bhattacharya A, Anderson AE, Xiao L, Bedrosian I, Kuerer HM, Krishnamurthy S (2012) Circulating tumor cells in non-metastatic breast cancer: a prospective study. Lancet Oncol 13: 688–695

Mayeswaran S, Sequist LV, Nagraj S, Ulkus L, Brannigan B, Collura CV, Inserra E, Diederichs S, lafrate AJ, Bell DW et al (2008) Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med 359: 366–377

McDonald DM, Baluk P (2002) Significance of blood vessel leakiness in cancer. Cancer Res 62: 5381–5385

Meng S, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, Beitsch PD, Leitch M, Hoover S, Euhus D et al (2004) Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res 10: 8152–8162

Mitchell MJ, King MR (2013) Computational and experimental models of cancer cell response to fluid shear stress. Front Oncol 3: 44

Nagraith S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, Smith MR, Kwak EL, Digumarthy S, Muzikansky A et al (2007) Isolation of rare circulating tumor cells in cancer patients by microchip technology. Nature 450: 1235–1239

Nguyen DX, Massague J (2007) Genetic determinants of cancer metastasis. Nat Rev Genet 8: 341–352

Nieto MA (2013) Epithelial plasticity: a common theme in embryonic and cancer cells. Science 342: 1234850

Ozkumur E, Shah AM, Ciciliano JC, Emmink BL, Miyamoto DT, Brachtel E, Yu M, Chen PI, Morgan B, Trautwein J et al (2013) Inertial focusing for tumor antigen-dependent and -independent sorting of rare circulating tumor cells. Sci Transl Med, 5: 179ra47

Pantel K, Schlimok G, Braun S, Kutter D, Lindemann F, Schaller G, Funke I, Izbicki JR, Riethmuller G (1999) Differential expression of proliferation-associated molecules in individual micrometastatic carcinoma cells. J Natl Cancer Inst 85: 1419–1424

Pantel K, Passlick B, Vogt J, Stosiek P, Angstwurm M, Seen-Hibler R, Haussinger K, Thetter O, Izbicki JR, Riethmuller G (1998) Reduced expression of plakoglobin indicates an unfavorable prognosis in subsets of patients with non-small-cell lung cancer. J Clin Oncol 16: 1407–1413

Pantel K, Brakenhoff RH (2004) Dissecting the metastatic cascade. Nat Rev Cancer 4: 448–456

Parkinson DR, Dracopoli N, Petty BG, Compton C, Cristofanilli M, Deisseroth A, Hayes DF, Kapke G, Kumar P, Lee JS et al (2012) Considerations in the development of circulating tumor cell technology for clinical use. J Transl Med 10: 138

Peeters M, Douillard JY, Van CE, Siena S, Zhang K, Williams R, Wiezorek J (2013) Mutant KRAS codon 12 and 13 alleles in patients with metastatic colorectal cancer: assessment as prognostic and predictive biomarkers of response to panitumumab. J Clin Oncol 31: 759–765

Rack B, Schindlbeck C, Juckstock J, Andergassen U, Hepp P, Zwingers T, Friedl TW, Lorenz R, Tesch H, Fasching PA et al (2014) Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. J Natl Cancer Inst 106: dju066

Reymond N, d’Aguil EA, Ridley AJ (2013) Crossing the endothelial barrier during metastasis. Nat Rev Cancer 13: 858–870

Riahi R, Gogoi P, Sepehri S, Zhou Y, Handique K, Godsey J, Wang Y (2014) A novel microchannel-based device to capture and analyze circulating tumor cells (CTCs) of breast cancer. Int J Oncol 44: 1870–1878

Riethoven S, Fritsch J, Muller V, Rau T, Schindlbeck C, Rack B, Janni W, Cothi C, Beck K, Janicke F et al (2007) Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the Cell Search system. Clin Cancer Res 13: 920–928

Riethoven S, Wikman H, Pantel K (2008) Review: biological relevance of disseminated tumor cells in cancer patients. Int J Cancer 123: 1991–2006

Riethoven S, Muller V, Zhang L, Rau T, Loibl S, Komor M, Roller M, Huober J, Fehm T, Schrader I et al (2010) Detection and HER2 expression of circulating tumor cells: prospective monitoring in breast cancer patients treated in the neoadjuvant GeparQuattro trial. Clin Cancer Res 16: 2634–2645

Rink M, Chun FK, Minser S, Friedrich M, Mauermann O, Heinzer H, Huland H, Fisch M, Pantel K, Riethoven S (2011) Detection of circulating tumour cells in peripheral blood of patients with advanced non-metastatic bladder cancer. BJU Int 107: 1668–1675
Rink M, Chun FK, Dahlem R, Soave A, Minner S, Hansen J, Stoupiec M, Coith C, Kluth LA, Ahyai SA et al (2012) Prognostic role and HER2 expression of circulating tumor cells in peripheral blood of patients prior to radical cystectomy: a prospective study. *Eur Urol* 61: 810–817

Saucedo-Zeni N, Mewes S, Niestroj R, Gasiorowski L, Murawa D, Nowaczyk P, Tomasi T, Weber E, Dworacki G, Morgenthaler NG et al (2012) A novel method for the in vivo isolation of circulating tumor cells from peripheral blood of cancer patients using a functionalized and structured medical wire. *Int J Oncol* 41: 1241–1250

Stathopoulou A, Gizi A, Perraki M, Apostolaki S, Malamos N, Mavroudis D, Georgoulias V, Lianidou ES (2003) Real-time quantification of CK-19 mRNA-positive cells in peripheral blood of breast cancer patients using the lightcycler system. *Clin Cancer Res* 9: 5145–5151

Steinert G, Scholch S, Niemietz T, Iwata N, Garcia SA, Behrens B, Voigt A, Kloos M, Benner A, Bork U et al (2014) Immune escape and survival mechanisms in circulating tumor cells of colorectal cancer. *Cancer Res* 74: 1694–1704

Strati A, Kasimir-Bauer S, Markou A, Parisi C, Lianidou ES (2013) Comparison of three molecular assays for the detection and molecular characterization of circulating tumor cells in breast cancer. *Breast Cancer Res* 15: R20

Tam WL, Weinberg RA (2013) The epigenetics of epithelial-mesenchymal plasticity in cancer. *Nat Med* 19: 1438–1449

Tewes M, Aktas B, Welt A, Mueller S, Hauch S, Kimmig R, Kasimir-Bauer S (2009) Molecular profiling and predictive value of circulating tumor cells in patients with metastatic breast cancer: an option for monitoring response to breast cancer related therapies. *Breast Cancer Res Treat* 115: 581–590

Thege Fl, Lannin TB, Saha TN, Tsai S, Kochman ML, Hollingsworth MA, Rhim AD, Kirby BJ (2014) Microfluidic immunocapture of circulating pancreatic cells using parallel EpCAM and MUC1 capture: characterization, optimization and downstream analysis. *Lab Chip* 14: 1775–1784

Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* 2: 442–454

Thiery JP, Acloque H, Huang RY, Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. *Cell* 139: 871–890

Tsai JH, Donaher JL, Murphy DA, Chau S, Yang J (2012) Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* 22: 725–736

Uhr JW, Pantel K (2011) Controversies in clinical cancer dormancy. *Proc Natl Acad Sci USA* 108: 12396–12400

Uppal A, Wightman SC, Ganai S, Weichselbaum RR, An C (2014) Investigation of the essential role of platelet-tumor cell interactions in metastasis progression using an agent-based model. *Theor Biol Med Model* 11: 17

Wan L, Pantel K, Kang Y (2013) Tumor metastasis: moving new biological insights into the clinic. *Nat Med* 19: 1450–1464

Wikman H, Vessella R, Pantel K (2008) Cancer micrometastasis and tumour dormancy. *APMIS* 116: 754–770

Wikman H, Laszszus K, Detels N, Uslar L, Wrage M, Benner C, Hohensee I, Ylstra B, Eijlmann K, Zapatak M et al (2012) Relevance of PTEN loss in brain metastasis formation in breast cancer patients. *Breast Cancer Res* 14: R49

Wrage M, Ruosaari S, Eijk JP, Kaifi JT, Hollmen J, Yekebas EF, Izbicki JR, Brakenhoff RH, Streichert T, Riethdorf S et al (2009) Genomic profiles associated with early micrometastasis in lung cancer: relevance of 4q deletion. *Clin Cancer Res* 15: 1566–1574

Wu S, Liu Z, Liu S, Lin L, Yang W, Xu J (2014) Enrichment and enumeration of circulating tumor cells by efficient depletion of leukocyte fractions. *Clin Chem Lab Med* 52: 243–251

Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM et al (2013) Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* 339: 580–584

Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, Desai R, Zhu H, Comailis V, Zheng Z et al (2014) Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science* 345: 216–220

Zhang L, Riethdorf S, Wu G, Wang T, Yang K, Peng G, Liu J, Pantel K (2012) Meta-analysis of the prognostic value of circulating tumor cells in breast cancer. *Clin Cancer Res* 18: 5701–5710

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