Circulating tumor cells: biology and clinical significance

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Circulating tumor cells (CTCs) are tumor cells that have sloughed off the primary tumor and extravasate into and circulate in the blood. Understanding of the metastatic cascade of CTCs has tremendous potential for the identification of targets against cancer metastasis. Detecting these very rare CTCs among the massive blood cells is challenging. However, emerging technologies for CTCs detection have profoundly contributed to deepening investigation into the biology of CTCs and have facilitated their clinical application. Current technologies for the detection of CTCs are summarized herein, together with their advantages and disadvantages. The detection of CTCs is usually dependent on molecular markers, with the epithelial cell adhesion molecule being the most widely used, although molecular markers vary between different types of cancer. Properties associated with epithelial-to-mesenchymal transition and stemness have been identified in CTCs, indicating their increased metastatic capacity. Only a small proportion of CTCs can survive and eventually initiate metastases, suggesting that an interaction and modulation between CTCs and the hostile blood microenvironment is essential for CTC metastasis. Single-cell sequencing of CTCs has been extensively investigated, and has enabled researchers to reveal the genome and transcriptome of CTCs. Herein, we also review the clinical applications of CTCs, especially for monitoring response to cancer treatment and in evaluating prognosis. Hence, CTCs have and will continue to provide significant insights into metastatic processes and open new avenues for useful clinical applications.

INTRODUCTION

Metastasis is the most lethal feature of cancer1. Despite significant developments in cancer diagnosis and treatment over the past centuries, metastasis remains a major obstacle to improving clinical outcomes of cancer patients2. Nevertheless, we have witnessed significant progress over the past two hundred years in revealing fundamental concepts underlying the development of metastasis and in creating new technologies to facilitate cancer metastasis research. Fig 1 highlights the key discoveries and milestones in the study of cancer metastasis. The ‘seed and soil’ hypothesis first described by Ashworth in 1869 by Ashworth who observed “some cells” in the blood of a metastatic cancer patient with an appearance similar to tumor cells in the primary tumors3. CTCs have been assumed to be tumor cells that have been sloughed from the primary tumor and are swept away by the circulatory or lymphatic systems. To date, most CTC research has focused on CTCs in the blood circulation. CTCs were first described in 1896 by Paget in the 1890s clearly clarifying the progress of cancer metastasis. With the advances of science and technologies, particularly since the 2000s, a plethora of new technologies have been advanced, such as high-throughput sequencing4,5, transgenic mouse models6, CRISPER/Cas9 editing tools7, and single-cell sequencing8. With these powerful technologies, the biological phenomena underlying metastasis, such as epithelial-mesenchymal transition (EMT) of tumor cells9, the role of exosomes in supporting metastasis10, circulating tumor cells (CTCs) and CTC clusters in seeding metastatic colonies11, and complex interactions between tumor cells and microenvironment12, have gradually been unmasked, along with the discovery of numerous metastasis-related driver genes. The ‘black box’ of metastasis is gradually being unveiled, and effective metastasis-targeting agents are believed to be on the horizon in the near future.

Cancer metastasis is a complex multistep process involving cancer cell invasion in the primary site, intravasation into circulation, survival in the circulation, extravasation from the circulation, and attachment to and colonization of the metastatic site (Fig. 2). CTCs are defined as tumor cells that have been sloughed from the primary tumor and are swept away by the circulatory or lymphatic systems. To date, most CTC research has focused on CTCs in the blood circulation. CTCs were first described in 1869 by Ashworth who observed “some cells” in the blood of a metastatic cancer patient with an appearance similar to tumor cells in the primary tumors13. CTCs have been assumed to be the substrate of metastasis. Although CTCs originate from the primary tumor, they are distinct from primary tumor cells14, with EMT transition properties that help them break free from the primary tumor and facilitate intravasation into the bloodstream, dissemination in clusters of CTCs to increase metastatic potential, and exhibit stemness features that enhance their ability to initiate metastasis (Fig. 2). However, most CTCs perish in the circulation, and only limited CTCs survive and infiltrate distant organs. Interactions between CTCs and the blood environment (Fig. 2), including how CTCs escape immune surveillance in the blood, have been widely implicated in the metastatic mechanisms of CTCs. It has taken more than a century for researchers to recognize the critical role of CTCs in cancer metastasis, due to the unique technical challenges required to isolate these very rare CTCs from...
the massive pool of circulating blood cells. However, in the past two decades, emerging technologies for CTC isolation have allowed research on the biology of CTCs and have facilitated the clinical applications of CTCs in cancer screening, treatment response monitoring, and prognosis evaluation.

In this review, the biology of CTCs, as well as their interaction with the blood microenvironment, is fully reviewed. In addition, the growing number of highly sophisticated CTC enrichment and isolation technologies will be summarized. Finally, we also discuss the tremendous potential of CTCs in clinical applications.

**THE BIOLOGY OF CTCs**

**Molecular characterization of CTCs**

Experimental evidence has supported the notion that tumor cells can spread even during the early stages of tumor evolution. However, in the past two decades, emerging technologies for CTC isolation have allowed research on the biology of CTCs and have facilitated the clinical applications of CTCs in cancer screening, treatment response monitoring, and prognosis evaluation.

In this review, the biology of CTCs, as well as their interaction with the blood microenvironment, is fully reviewed. In addition, the growing number of highly sophisticated CTC enrichment and isolation technologies will be summarized. Finally, we also discuss the tremendous potential of CTCs in clinical applications.

**Molecular markers of CTCs**

A panel of molecular markers has been used to detect CTCs in various cancers. CTC-associated markers used for different cancers are summarized in Table 1. As most cancers are of epithelial origin, the most common marker used for CTCs is EpCAM, a "universal" epithelial marker of cancers. EpCAM expression varies among different cancer types, and EpCAM-based CTC detection technologies are widely applied for cancers that strongly express EpCAM, such as breast and prostate cancer. Many studies have shown that CTCs in breast and prostate cancer are EpCAM-positive, and have validated their prognostic value in either early or metastatic stage cases. Other epithelial-derived cancer types, such as pancreatic, colorectal, and hepatocellular cancers, also have a considerable detection rate of EpCAM-positive CTCs. Similarly, the presence of these EpCAM-positive CTCs predicts early distant metastasis and poorer survival of patients. However, using EpCAM as a CTC marker has limitations. It cannot be used in tumors that are EpCAM-negative or with low expression, such as neurogenic cancers. CTCs can undergo EMT, and epithelial markers, including EpCAM, are down-regulated during EMT, which affects the detection rate of EpCAM-positive CTCs. Although there are doubts as to whether EpCAM-based technologies are appropriate to detect all CTCs, numerous studies have illustrated the potential value of EpCAM-positive CTCs in clinical applications.

To some extent, EpCAM-positive CTCs are a substantial subgroup of all CTCs, thus EpCAM-positive CTCs still could be a reliable biomarker if the cancer prognosis and therapeutic efficacy is relevant to EpCAM-positive CTCs.

Due to the EMT activity of some epithelial cancer cells, detecting only EpCAM-positive CTCs probably underestimates the actual total CTC population and misses important biological information of EpCAM-negative CTCs. In some cancer types, such as non-small-cell lung cancer (NSCLC) patients, it was even found that the quantity of EpCAM-negative CTCs was significantly larger than EpCAM-positive CTCs. Nevertheless, the poor isolation of CTCs by EpCAM-based technologies can be rescued by using both epithelial and mesenchymal cancer markers, as well as by marker-independent detection methods. For example, in breast cancer, the use of fluorescent-magnetic nanoparticles consisting of a dual-antibody interface targeting both EpCAM and N-cadherin has contributed to high-efficiency isolation and rapid identification of CTCs.

In biliary tract cancer, a single-cell assay for detecting CTCs allowed identification of both epithelial CTCs and non-conventional CTCs which lacked epithelial and leukocyte markers, and therefore led to an increase of CTC positivity rate. The EMT program of cancer cells exhibits molecular alterations, including decreased expression of epithelial markers (E-cadherin, ZO-1, claudins, and occludins) and increased expression of mesenchymal markers (vimentin, N-cadherin, fibroblast-specific protein 1, and fibronectin). EMT is executed by EMT-related transcription factors, mainly belonging to the SNAIL, TWIST, and ZEB families.

All these EMT-related molecules can theoretically be used for EMT-CTCs targeting methods. However, many EMT-related molecules are cytoplasmic or nuclear proteins, precluding their usage in the currently available membrane molecular-based technologies of CTC detection. Proteins such as E-cadherin, vimentin, and twist were most often used in the past (Table 1), probably because of their accessibility of detection in traditional CTC detection technologies including flow cytometry sorting, immunostaining, and fluorescence in situ hybridization (FISH) staining. However, the emergence of single-cell CTC sequencing technologies will make it possible to unmask the EMT status of CTCs more...
comprehensively, and can cover all the EMT-related molecular alternations at the RNA level.

Other biomarkers, such as human epidermal growth factor receptor-2 (HER2)\(^3\)\(^{-}\)\(^4\), estrogen receptor\(^3\)\(^9\)\(^{-}\)\(^1\)\(^0\), prostate-specific membrane antigen\(^5\)\(^1\)\(^{-}\)\(^5\)\(^3\), folate receptor\(^5\)\(^4\)\(^{-}\)\(^5\)\(^6\), and survivin\(^5\)\(^7\), have been described as CTCs markers in different cancers, with different clinical significance. These cancer-specific CTC markers are listed in Table 1. Most of these cancer-specific CTC markers are in accordance with the specific molecular markers of the primary tumor. However, there is discordance in the expression of specific markers between the primary tumor and CTCs. For example, the rates of discordance of HER2 gene amplification between CTCs and primary breast tumor were around 15\%\(^5\)\(^8\), suggesting a clonal selection of CTCs or clonal acquisition, probably due to genetic instability. It should be mentioned that for melanoma, a skin cancer that begins in melanocytes, the detection technologies of CTCs are based on several melanoma cell adhesion molecules, such as HMW-MAA\(^5\)\(^9\)\(^{-}\)\(^6\)\(^1\), MART-1\(^6\)\(^2\)\(^{-}\)\(^6\)\(^4\), CD146\(^6\)\(^1\)\(^,6\)\(^5\), and MAGE A\(^3\)\(^5\)\(^2\)\(^,6\)\(^3\), which are very specific molecular markers for melanoma.

The variety of CTCs markers indicates the heterogeneity of CTCs among different cancer types. Even in one patient, CTCs are spatio-temporally heterogenous, which may be the result of a spatially different microenvironment in the blood and temporal changes in therapy response. Thus, it is difficult to define the entire CTC population using the very limited molecular markers currently available. In addition, CTC markers should not be constant among different stages of cancer and treatment periods.

Genome analysis of CTCs. Genomic instability contributes to tumor evolution and the emergence of resistant tumor subclones. Monitoring tumor genomic instability, especially in terms of tumor resistance and metastases, greatly contributes to the evaluation of treatment response and precision medicine. The evaluation of CTCs assessment using noninvasive liquid biopsy is accessible for serial sampling to detect the genomic instability of the tumor.

Determining the status of EGFR and KRAS mutations is crucial for guiding treatment in NSCLC patients receiving EGFR tyrosine kinase inhibitors and colorectal cancer patients treated with anti-EGFR therapy respectively. The concordance of mutations between CTCs and matched primary or metastatic tumor tissue has attracted much attention. Using a microfluidic technique to capture CTC, Maheshvaran et al. found that only two of 31 patients with mutations were overlooked from their detection assay\(^6\)\(^7\). They identified the EGFR activating mutation in CTCs in 92\% of metastatic patients with NSCLC and detected the drug-resistant mutation T790M in CTCs of 33\% of patients who responded to tyrosine kinase inhibitor therapy and in 64\% of patients who exhibited clinical progression\(^6\)\(^7\). For the analysis of the KRAS gene mutation, the mutational concordance rate between CTCs and matched primary tumors ranged from 37\% to 90\% in colorectal cancer cases\(^5\)\(^8\)\(^{-}\)\(^7\)\(^1\). This difference in the concordance rate may be due to the different CTC selection protocols used in these studies. KRAS mutations are also common in pancreatic ductal adenocarcinoma (PDAC), present in 90\% of PDAC cases. However, Kulemann et al. found that the discordance rate of KRAS mutation in CTCs and corresponding PDAC tumors was 42\%\(^7\)\(^2\). Studies of
| Cancer types               | Epithelial markers          | Mesenchymal markers       | Specific markers |
|---------------------------|-----------------------------|---------------------------|-----------------|
| Breast cancer             | EpCAM/CK8,18,19              | Vimentin 280–283          | HER2 37–46      |
|                           | CK 5/7/8/18/19              | Twist 253,282,284         | ER 49–50        |
|                           | E-Cadherin 9,280,281        | Fibronectin 9,280         | AR 235          |
|                           |                             | N-Cadherin 9,280,286      | MRP 88          |
| Prostate cancer           | EpCAM/CK8,18,19              | Vimentin 102,289–291      | PSMA 51–53      |
|                           |                             | Twist 90,291              | PSA 239         |
|                           |                             |                            | EGFR 51         |
|                           |                             |                            | ARV7 256–292–294|
|                           |                             |                            | PIM1 320        |
| Kidney cancer             | EpCAM 240                   |                            | CD147 296       |
| Bladder cancer            | EpCAM/CK8,18,19              | Vimentin 252,303–305      | PI3K α 126      |
|                           |                             | Twist 252,303,305         | CEA 307–309     |
|                           |                             | N-Cadherin 303,305        | PRL 325         |
|                           |                             | AKT2 303,305,306          |                |
|                           |                             | LOXL3 310                |                |
|                           |                             | Plastin 311               |                |
|                          | Non-small-cell lung cancer  | EpCAM/CK8,18,19            | Folate receptor 54–56 |
|                           | CK7/8/18/19 312,313          | Vimentin 109,313,314      | Telomerase activity 329 |
|                           | EpCAM/CK8,18,19              | Twist 313                 |                |
|                           |                             | N-Cadherin 314            |                |
|                           |                             | AXL 313                   |                |
| Small-cell lung cancer    | EpCAM/CK8,18,19              | Vimentin 242,327          | DLL3 242       |
| Pancreatic cancer         | EpCAM/CK8,18,19              | Twist 257,333–335         |                |
|                           |                             | Twist 34                   |                |
|                           |                             | KLF8 335                  |                |
| Hepatocellular carcinoma  | EpCAM/CK8,18,19              | Vimentin 336,341–343      | GPC3 344,345    |
|                           | EpCAM,CK19                   | Twist 336,341–343,347     | ASGPR 344       |
|                           | EpCAM 346                   |                            |                |
| Gastric cancer            | EpCAM/CK8,18,19              | Vimentin 352              | XAF1 353       |
|                           | CK19/CK20 344,349–351        | N-Cadherin 355            | MT1-MMP 356     |
|                           |                             |                            | Survivin 17     |
|                           |                             |                            | HER2 17         |
| Esophageal cancer         | EpCAM/CK8,18,19              |                            | MART-1 162,63   |
|                           |                             |                            | HMMW-MAA 19,61,65,359|
|                           |                             |                            | CD146 11,65,359 |
| Cervical cancer           | EpCAM/CK8,18,19              |                            | MAGE A3 52,63,66|
| Melanoma                  |                             |                            | GalNAc-T 93     |
|                           |                             |                            | MAGE A1–6 360   |
|                           |                             |                            | hTERT 360       |
|                           |                             |                            | MLANA 46        |
|                           |                             |                            | B4GALNT1 96     |
|                           |                             |                            | PAX3 62,66      |
|                           |                             |                            | DCT 65          |

CTC markers mainly includes the epithelial markers, the mesenchymal markers, and the cancer specific CTC markers.

ARV7: androgen-receptor splice variant 7, ASGPR: asialoglycoprotein receptor, CEA: carcinoembryonic antigen, EGFR: epidermal growth factor receptor, GPC3: glypican 3, MAGE A1–6: melanoma antigen-encoding gene family member A1-family) member A6, MRP: multidrug resistance-related proteins, PI3K α: phosphatidylinositol 3-kinase α, PIM1: proviral integration site for the Moloney murine leukemia virus-1, PRL: phosphatase of regenerating liver-3, PSA: prostate specific antigen, PSMA: prostate-specific membrane antigen, SERPINE1/PAI1: serpin peptidase inhibitor clade E, XAF1: XIAP-associated factor 1.
gene mutation analysis in CTCs were also conducted in many cancers such as prostate cancer, breast cancer, hepatocellular carcinomas, and a mutational discordance between CTCs and corresponding tumors were often found. The discordance rate was probably attributed to the different detection efficiency of CTC mutations, or the heterogeneity between CTCs and primary tumor cells. Genomic assessment of tumor tissue and CTCs can be complementary. So, a combination of mutational testing of CTCs and tumor specimens would guide treatment more precisely.

Determining copy number alternations (CNA) of CTCs helps analyze and track cancer profiles as tumors evolve. In lung cancer, Ni et al. found that CTCs exhibit reproducible CNAs patterns, similar to those of metastatic tumors, and different patients shared similar CNAs patterns. In small-cell lung cancer, a CNA-based classifier for CTCs correctly assigned 83.3% of patients as chemoresistant or chemosensitive. In breast cancer, the assessment of CNAs in archived CTCs is feasible. Paolelli et al. found that the CNAs of CTCs and paired metastatic tumor tissue in breast cancer patients were highly concordant, although CTCs and matched tumor tissue harbored several discordant copy number alterations, suggesting that CTCs were the subclone cells of tumor tissues. In triple-negative breast cancer, CTCs with chromosome 10 and 21q CNAs are predictive of clinical progression, and their network analysis presented connected modules including HER/ phosphatidylinositol-4,5-bisphosphate 3-kinase/RAS/JAK signaling. In prostate cancer, Lambro et al. revealed that CNAs of CTCs were interdependent and intercell heterogeneous, and could be missed in bulk biopsy analyses. In metastatic castration-resistant prostate cancer, whole genomic copy number analysis of CTCs showed that common genomic gains in CTCs involved genes such as androgen receptor (AR), mesenchymal-to-epithelial transition (MET), ERG, and cyclin-dependent kinase 12, while common genomic losses were observed in genes such as phosphatase and tensin homolog (PTEN), RAF1, and GATA2. Similarly, Malih et al. also observed that CNAs in genes including PTEN, RB1, TP53, and AR closely associated with genomic instability and survival in aggressive variant prostate cancer.

Other genome analyses have also been conducted in CTCs. FISH testing was adopted in CTCs to detect biomarkers for treatment sensitivity, such as ALK FISH testing in CTCs of NSCLC patients and HER2 FISH testing in CTCs of breast cancer patients. Recently, based on the technique of single-cell resolution DNA methylation analysis, the DNA methylome of single CTCs and CTC clusters was revealed for breast cancer patients, and indicated that the CTC cluster hypomethylation profile obtained was associated with a poor prognosis and that treatment with Na+/K+ ATPase inhibitors to dissociate CTC clusters could revert the methylation profile of CTC clusters and suppress metastasis.

**Transcriptome analysis of CTCs.** Single-cell sequencing has developed rapidly in recent years and has been applied to investigate the CTC transcriptomes. Single-cell expression profiles can distinguish CTCs from mesothelial cells and blood cells in lung adenocarcinoma, with representative markers including EpCAM for CTCs. Single-cell sequencing-based transcriptome analysis revealed heterogeneity in the CTC subpopulation. By testing the expression of proliferation-associated genes such as the Ki-67 proliferation marker, Magbanua et al. found that 65% of CTCs in patients with metastatic breast cancer had low proliferation Ki-67 and that the 35% of patients with a high proliferation Ki-67 expression had a poor prognosis. Cheng et al. performed a single cell transcriptome analysis of 666 CTCs in patients with metastatic breast cancer. They determined that intra-patient CTCs were heterogeneous with regard to EMT-like and MET-like states, and CTCs were enriched for the stem-like phenotype. Single-cell sequencing of CTCs also greatly helped in discovering driver signaling pathways that contributed to metastasis and treatment failure. RNA-Seq of single prostate CTC indicated the activation of noncanonical Wnt signaling in androgen resistant patients. Further, using mouse models, ectopic expression of Wnt5a attenuated the effects of an AR inhibitor and suppression of Wnt5a could partially restore the sensitivity in drug-resistant prostate cancer cells. CTCs have been associated with a poor prognosis in colorectal cancer. A study of the CTC-specific transcriptome profile of six metastatic colorectal cancer patients characterized 410 CTC-specific genes, which were primarily related to cell movement and adhesion, such as VCL, ITGB5, bone morphogenetic protein 6, transforming growth factor beta 1, and talin 1, and were related to cell death and proliferation, such as amyloid beta precursor protein, clusterin, and TIMP1.

Epithelial-to-mesenchymal transition of CTCs

EMT is the process by which epithelial tumor cells lose their intercellular adhesion and acquire mesenchymal and invasive properties. During dissemination, tumor cells detach themselves from the basement membrane through EMT activation and directly enter the circulation, serving as CTCs traveling to distant sites. When CTCs extravasate, they then undergo a reverse process termed MET and proliferate to form macro-metastases. Herein, metastatic development depends on the delicate balance of the transition between these two phenotypes. The activity of EMT-MET was also proposed to play an important role in the metastatic process of CTCs. Using mouse models, it was found that epithelial-type CTCs with a restricted mesenchymal transition had the strongest lung metastases formation capacity, whereas mesenchymal-type CTCs showed limited metastatic ability. In breast cancer, CTCs exhibit dynamic changes in EMT composition, and mesenchymal CTCs were found to be closely associated with cancer progression. A plethora of studies has shown increased EMT of CTCs rather than primary tumor cells in various cancers. In a study based on the bioinformatics analysis of seven sets of gene chips, Guan et al. showed that compared with primary tumors, the main changes in CTCs involved cell adhesion, EMT, and apoptosis. In a prospective study including 39 patients with invasive breast cancer, Tashireva et al. observed a majority of heterogeneous CTC phenotypes (22 out of 24 detectable samples) exhibiting EMT plasticity. Interestingly, it was found that fluid shear stress can induce the EMT of CTCs via JNK signaling in breast cancer, which further confirmed the relationship between augmented EMT of CTCs and poor patient survival.

Clinically, combining the total CTC count and the proportion of mesenchymal CTCs can be used to monitor therapeutic resistance and predict prognosis in cancer patients due to the significant survival differences of this criterion. For example, since the baseline presence of total CTCs in advanced NSCLC conferred poor prognosis, and the presence of over five EMT-CTCs indicated progressive disease. Different numbers of total CTCs and EMT CTCs were found to play an important role in determining the prognosis of breast cancer patients. Intriguingly, it was emphasized that a better understanding of EMT-CTC subtypes and their interactions with peripheral blood mononuclear cells could help design better anti-metastatic treatments. As CTC EMT-positive patients with neutrophil-to-lymphocyte ratios ≥3 had an 8.6 times increased risk of disease recurrence compared with CTC EMT-negative patients with lower neutrophil levels, inflammation-based scores increased the prognostic value of CTCs in primary breast cancer. Therefore, targeting the EMT pathway may prevent tumor cell spread in early-stage patients and eradicate metastatic cells in advanced stages.

The stemness of CTCs

Many previous studies have indicated a subpopulation of aggressive CTCs with “stemness” traits in different cancers, which
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CTCs AND THE BLOOD MICROENVIRONMENT

When transported in the bloodstream, a major of CTCs are constrained by detrimental shear stress, or die from anoikis, a programmed cell death mechanism due to loss of cell attachment. Only a small fraction of CTCs interact tightly with platelets, neutrophils, macrophages, myeloid-derived suppressor cells (MDSCs), or cancer-associated fibroblasts (CAFs) to escape the immune system and promote their survival. Recently, accumulating studies suggest that the interaction and modulation between CTCs and hostile blood microenvironment is essential for adhesion to endothelial cells, tissue invasion, and tumor metastasis (Fig. 3).

Interaction of CTCs with neutrophils

Neutrophils are the most abundant circulating leukocytes in humans and have recently been studied to support cancer progression. An increased number of neutrophils in circulation is associated with poor prognosis in several types of cancers. The formation of CTC-white blood cells (WBCs) clusters was previously reported within the bloodstream. In 2019, Szczerba et al. determined that CTCs were significantly associated with neutrophils in both mouse models and breast cancer patients, exhibiting more metastatic potential with greater expression of genes that involve cell cycle progression compared to CTCs alone. These observations are consistent with previous findings showing the proliferation role of neutrophils on tumor cells. CTC and neutrophil binding is mediated by the cell–cell junction and possibly requires the vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells.

CTCs undergo considerable levels of fluid shear flow during their dissemination, and the fluid shear flow itself may have an impact on CTCs. Using a model of breast cancer with brain metastasis, it was suggested that hemodynamic shear flow could upregulate the stemness genes of CTCs in surviving under conditions of shear flow. EMT-like transition of CTCs by downregulating ERK and GSK3β signaling could promote the conversion of CTCs into stem-like CTCs with high sphere-forming and tumor-initiating capacity.

It is crucial to understand the mechanisms regulating CTCs stemness, and interfering with the CTCs subpopulation stemness properties may more efficiently suppress cancer progression and relapse. CTCs undergo considerable levels of fluid shear flow in the bloodstream, and their interaction with neutrophils may contribute to stem cell survival and dissemination. In glioblastoma, RNA-seq analysis revealed Wnt activation-induced stemness and chemoresistance in CTCs. In prostate cancer, the stem cell marker CD133 was observed in the majority (> 80%) of CTCs of patients with metastatic castration-resistant prostate cancer, and a stem-like subpopulation of the CTCs was more prevalent in EpCAM-negative CTCs than in EpCAM-positive CTCs.

CTCs in the blood microenvironment, and their interaction with neutrophils, platelets, CAFs and TAMs. CAFs: cancer-associated fibroblasts, TAMs: tumor-associated macrophages

![CTCs in the blood microenvironment](image_url)

**Fig. 3**

- **CTC Extravasation**
  - Blood flow
  - Shear stress
  - CTC-neutrophil cluster
  - Metastatic potential
  - VCAM-1 mediated adhesion
  - Immune escape
  - Neutrophils
  - NK cells
  - Dormant CTCs
  - Mac-1/ICAM-1
  - HMGB1
  - NET
  - P-selectin

- **Endothelium**
  - CTC-platelet clusters
  - CTC-CAF cluster
  - CTC Survival in circulation
  - Recruitment
  - EMT, migration, invasion
  - IL-8
  - TAMs
  - Heterogeneity, metastatic potential, invasiveness

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Neutrophils can also promote metastasis in an indirect manner. Neutrophil extracellular traps (NETs) are web-like structures formed by DNA–histone complexes and proteins released from activated neutrophils, with the ability to impact on CTC biology. Many studies have found that NETs were able to capture CTCs in the circulation and, in doing so, promote metastatic dissemination. In vitro and in vivo experiments have shown that CTC adhesion to NET is mediated by β1-integrin expressed on both NET and cancer cells, while this effect was abrogated following DNase I administration. In a murine model of surgical stress, the NET formation triggered the release of high-mobility group box 1, which activated TLR9-mediated pathways in CTCs and therefore accelerated the progression of liver metastases. In addition, NETs can also awaken dormant cancer cells and promote metastasis. Recently, Albrengues et al. elegantly demonstrated that two NET-associated proteases, neutrophil elastase and matrix metalloproteinase 9 (MMP9), concentrate at laminin, provoke its cleavage, by generating an epitope that induced awakening of dormant cancer cells by integrin activation and FAK/ERK/MLCK/YAP signaling. In turn, tumor-expressed protein, such as protease cathepsin, has been shown to support lung metastasis of breast cancer by promoting NET formation in metastatic niches. Coiled-coil domain containing protein 25, another protein expressed on the cancer cell membrane, could serve as a specific sensor for the DNA component NETs, which induces migration and adhesion of tumor cells. In addition, by forming NETs, circulating neutrophils can help CTCs escape immune surveillance by suppressing the activation of peripheral leukocytes. The function of natural killer (NK) cells, the antitumor response of effector T cells, the function of natural killer group 2, member D (NKG2D) in NK cells by platelet-derived TGF-β, as well as platelet-mediated shedding of NKG2D ligands, which contribute to impaired antitumor cytotoxicity, and (4) platelet-derived glucocorticoid-induced TNF-related ligand which activates GITR in NK cells and reduces their cytotoxicity. Furthermore, NK cells and platelets can also interfere with neutrophils, T cells, and macrophages and modulate their immune function. In addition to safeguard CTCs within the bloodstream, platelets are also involved in the adhesion of endothelial cells. The attachment of platelets and CTCs is mediated by platelet adhesion receptors, such as the integrin αIIbβ3 and P-selectin, thereby supporting the firm adherence of CTCs to the endothelial wall. Furthermore, tumor cell-activated platelets release ATP from dense granules, which then induces the activation of the endothelial P2Y2 receptor and allows transendothelial migration of tumor cells by increasing permeability. One study has also revealed that the interplay between integrin α6β1 on platelets and its receptor, a disintegrin, and metalloprotease 9 on CTCs is necessary for the extravasation process of cancer cells. Platelets could also increase vascular permeability to help tumor cell extravasation. For example, a preclinical lung metastasis model showed that tumor cell-associated CD97, an G protein-coupled receptor, can initiate platelet activation thereby leading to granule secretion, including both ATP and lysophosphatidic acid release. Similarly, the interplay between platelet-specific receptor glycoprotein VI and its ligand galectin-3 expressed on colon and breast cancer cells was revealed to promote platelet activation and ATP secretion. Consequently, these platelet secretions favor a process of tumor metastasis by regulating vascular permeability. Recently, Xu et al. discovered that PtxtAlbSNO can block tumor-specific platelet functions to suppress tumor EMT as well as prevent platelet adhesion around CTCs. PtxtAlbSNO can also inhibit TGF-β secretion and enhance intratumoral immune cell infiltration to reverse the immunosuppressive TME, thereby suppressing distant metastasis. Taken together, the close and complex crosstalk between CTCs and platelets might involve distinct molecule variants and signaling pathways and possibly represent a promising antitumor strategy, particularly attractive for the treatment of several cancers.
Interaction of CTCs with MDSCs

MDSCs are a heterogeneous subset of myeloid cells characterized by immunosuppressive properties that also promote metastatic dissemination. Under a standard protocol for isolating human MDSCs, Cassetta et al. found that polymorphonuclear (PMN)-MDSC were significantly expanded among most cancer types except melanoma compared with infection and inflammation. CTC-MDSC clusters are thought to evade immune surveillance of the T cell response. Indeed, a decrease in circulating MDSCs was associated with an increase in activated OX40+PD-1 T cells in patients with diffuse large B-cell lymphoma. Furthermore, Sprouse et al. found that in vitro co-culture of CTCs derived from melanoma and breast cancer patients and PMN-MDSCs enhanced Notch activation in CTCs through direct interaction between Jagged1 (Notch1 ligands) expressed on MDSCs and the Notch1 receptor expressed on CTCs. The increased production of reactive oxygen species production of MDSCs could upregulate Notch1 receptor expression, therefore, promoting CTC proliferation. The potential mechanisms underlying the interplay between CTCs and MDSCs remains to be determined.

Interaction of CTCs with CAFs

CAFs are one of the most abundant components in the TME and play a prominent role in tumor initiation, angiogenesis, metastasis, and drug resistance. Mechanistically, CAFs remodel the extracellular matrix structure, which allows tumor cells to invade through the stroma and communicate with cancer cells by secreting growth factors, chemokines, and cytokines. However, little is known about the interplay between CAFs and CTCs. Duda et al. first demonstrated that CTCs could carry CAFs from the primary tumor to the metastatic site in mouse models of lung cancer metastasis. These host-derived CAFs directly enhance tumor cell survival and promote the formation of metastasis, while depleting CAFs from lungs significantly reduces the number of macroscopic metastasis and extends survival rate in mice. Moreover, CAFs can protect CTCs from the fluid shear forces during the dissemination process. In a three-dimensional co-culture model, CAFs were found to induce shear resistance to prostate tumor cells through stable intercellular contact, as well as soluble factors (such as CXCL5, CCL2, and CCL7), which are associated with cell survival, invasion, and EMT. In addition to experimental models, circulating CAFs (identified by FAP and α-SMA co-expression) have been detected in the peripheral blood of patients with metastatic breast cancer but not in patients at early stages and exhibit excellent precision in metastatic diagnosis when isolated using a novel acoustic microstreaming platform.

TECHNOLOGIES FOR CTCs ENRICHMENT AND ISOLATION

Over the past few years, many methods have been proposed to capture CTCs. Due to the extremely small proportion of CTCs in patients’ blood, it is still a great challenge to accurately isolate CTCs from the numerous blood cells, and especially to invent applicable methods that can efficiently detect viable CTCs for subsequent in-depth analysis. Here, we will discuss the development of CTC-related technologies over the last two decades, as they have experienced tremendous growth. We will emphasize the most innovative methods associated with nanoscale materials or novel microfluidic chips, hoping to provide a useful framework of CTC-related technologies.

Generally, there are three core strategies of CTCs technologies, which include (1) capture and enrichment, (2) detection and identification, and (3) release. The first strategy of capture and enrichment involves a specific interaction between CTCs and materials through physical interactions or antibody–antigen interactions. The second strategy of detection, which means identifying the CTCs, refers to various methods, such as fluorescence microscopy, fluorescence spectrophotometry, flow cytometry, surface-enhanced Raman scattering, or electrical impedance. In the last strategy, released CTCs are mainly used for downstream analysis, such as genomics, transcriptomics, proteomics, and CTCs culture.

Classic CTC-related technologies based on physical properties

The physical separation enrichment method of CTCs is based on differences between CTCs and blood cells in size, density, deformability, and electrical properties. The isolation by size of epithelial tumor cells system can filter blood samples through an 8-μm diameter polycarbonate TRACK-ETCH-type membrane, but it has low efficiency. An improved method, which consists of a pressure regulating system, the flexible micro spring array covers, reaches a capture efficiency of 90% with a detection of CTCs in 76% of samples. However, there are various trends of CTC counts observed from different samples, making this method not reliable for widespread use. CTCs can also be sorted using the Oncoquick system, a density-dependent technique that allows red blood cells and WBCs to be filtered, or by Apostream that uses dielectric electrophoresis techniques in the microfluidic chamber to capture CTCs. These systems require large volume of blood and cannot collect the CTCs of a similar size as WBCs, which are their main limitations. Overall, the methods based on physical properties are generally inefficient, poor in purity, and lack of specificity, although the vitality is good and the cost is relatively inexpensive.

Classic CTC-related technologies based on biological properties

Biological property-based technology is another important method for CTC detection. Based on antibody–antigen interaction, CTCs are usually positively enriched using epithelial (EpCAM) and mesenchymal (vimentin) markers as well as negatively enriched by using CD45 to deplete unwanted leukocytes. EpCAM-dependent techniques are most commonly used by researchers. The CellSearch system, the only FDA-approved device for clinical use, employs EpCAM antibody-coated ferromagnetic beads to enrich CK+CD45-/DAP+C and remove CK-/CD45+/DAP+WBCs. However, CTCs strongly adhere to the surface of the equipment in antibody interaction-based methods, making them difficult to be released. This deficiency can be resolved in another EpCAM-dependent, MagsWeeper system, which uses a magnetic rod to enrich CTCs and eliminate cells not bound by magnetic beads, allowing the release of CTCs for the following biochemical analysis. The Capantrap is another representative of the EpCAM-dependent technique, which provides morphological, cytological, and genetic characterization of individual CTCs. In summary, techniques based EpCAM are extensively-used. However, because the CTC surface antigen has high heterogeneity, CTCs that have low expression of EpCAM may not be enriched, causing inaccurate results, while methods based on physical properties do not have this limitation. Therefore, combining the advantages of different technologies or looking for CTCs with high sensitivity and specific tumor markers have gradually won the attention of researchers. Recently, with the development of microfluidic chips, nanomaterials, and next-generation sequencing, researchers have many advanced technologies to stimulate progress in CTC-related technologies. Importantly, researchers are trying to reach higher levels of CTC-related technologies in several key parameters: yield, purity, enrichment ratio, throughput, viability, sensitivity, specificity, release rate, accessibility for further analysis, and simplicity of equipment operation.

Recent CTC-related technologies: microfluidic-based and nanotechnology-based techniques

Besides the classic CTC-related technologies discussed above, some newer technologies, such as microfluidic-based and...
nanotechnology-based techniques have been developed. Microfluidic-based cell sorting approaches use “intrinsic” (e.g., fluid dynamic forces) versus “extrinsic” external forces (e.g., magnetic, electric field, acoustic, and optical forces) to separate cells, and then select target cells from a sample of heterogeneous cells through different physical and biological properties. The CTC-chip is a silicon microfluidic platform on which the CTCs are captured on the slides of molecular marker coated posts. The CTC-chip can separate viable CTCs from whole blood without pre-labeling or processing of samples, resulting in increased cell activity and separation purity. A modified chip-based platform using gold nanoparticles on a herringbone chip (NP205) easily detaches viable CTCs and safely releases cells for further analysis by utilizing a chemical ligand-exchange reaction with gold nanoparticles on a herringbone chip. Furthermore, the monolithic CTC-iChip has high-efficiency WBC depletion and allows the characterization of CTCs with epithelial and mesenchymal characteristics. Although these microfluidic chips have greatly contributed to the development of the detection of CTCs (i.e., improved capture efficiency, viability, and depletion of WBCs), they are not widely applied for clinical use due to limitations, which include long set-up time, high initial cost, bulky instrumentation, and limited ability to perform single-cell molecular analysis.

In an attempt to capture CTCs in an automated manner (Table 2), Zhang et al. successfully sorted MCF-7 cells from a 5 mL volume of diluted blood within 23 m with a recovery rate of 85%206. Even more remarkable, Jia et al. developed a less costly self-driving micro-cavity array chip to achieve cell loading, lysing, isothermal amplification, and signal read-out on a single chip. This novel chip can perform genetic analysis at the single-cell level, it has great potential in personalized therapy and efficacy monitoring. Furthermore, another automated and integrated microfluidic system proposed by Wang et al. is reported to achieve CTC capture and identification within 90 m. With the advantages of automation, stability, economy, and user-friendly operation, this system provides broad prospects for cancer screening and prognosis, especially in HCC209. Additionally, Lee et al. invented a microfluidic-based integrated system to achieve simultaneous on-chip isolation and characterization of circulating tumors utilizing differences in magnetic field gradient and immune fluorescence. Furthermore, this novel system can differentiate on-chip eight different subtypes of heterogenous CTCs, guiding the diagnosis and prognosis of breast cancer201. By combining the microfluidic technology and in situ molecular profiling techniques, the On-chip Post-processing Enabling chip platform has the ability to perform molecular analyses of single CTC from metastatic breast cancer and metastatic pancreatic cancer patients without any off-chip processes, suggesting its potential implementation of early molecular detection for cancer metastasis201. Taken together, less costly automated and integrated microfluidic systems that allow easy CTCs detection and cell analysis have great clinical value.

With the progress of nanomaterials, nanotechnology-based methods are becoming promising tools for CTC detection at an early-stage disease and for the monitoring of cancer development, as well as in vivo imaging212. Nanomaterials have a large surface-to-volume ratio and allow CTC isolation at high specificity and CTC detection at high sensitivity by adsorbing numbers of targeting ligands to bind specific molecules on cancer cells. At present, studies have reported many types of nanomaterials (Table 2) for CTC detection, including magnetic nanoparticles213, gold nanoparticles218-226 and quantum dots. For example, studies have shown that the utilization of tannic acid-functionalized magnetic nanoparticles214, CoFe2O4@Ag magnetic nanohybrids215, and peptide-based magnetic nanoparticle216 enhances the capture efficiency of CTCs in breast cancer patients. Among them, peptide-based magnetic nanoparticles can distinguishing epithelial and mesenchymal CTC subgroups and allow analysis at the single-cell level, the detection effects of which are supported by magnetic nanoparticles and microfluidic-based integrated systems218. Regarding gold nanoparticles, there have been significant developments. For example, the cytosensor proposed by Yang et al. showed excellent analytical performance, with a wide linear range, satisfactory CTC release (93.7-97.4%), and good cell viability. Liu et al. reported that gold nanoparticle-modified black phosphorus nanosheets improved the stability in detecting CTC219. Furthermore, the combination of a microfluidic system and gold nanoparticles presents a wider range of applications. Wang et al. synthesize an interferential zinc oxide coating with a nanostructure on the microsphere surface, which increases the specific surface area and thus leads to an improved capturing efficiency of CTCs220. In addition, the utilization of multicolor magnetic surface-enhanced Raman scattering nanotags and chip-based immunomagnetic separation could detect four different surface protein markers on individual tumor cells in a quantitative and simultaneous manner, thus facilitating the separation of CTC subpopulations221.

Although nanotechnology-based techniques can provide broad prospects for CTC research in various tumors in a cost-effective and simple manner, there are limitations and challenges. First, many factors (e.g., binding of nanoparticle probes, aggregation, detection at high sensitivity by adsorbing numbers of targeting ligands to bind specific molecules on cancer cells) can affect nanoparticle-based detections, leading to decreased reliability and reproducibility. Second, most nanoparticle-based assays are prepared for academic studies, and they are still unrealistic for widely clinical translation.Third, there is possible toxicity of nanoparticles.

In the era of precision medicine, CTCs analysis has great clinical value. Tools must be sharpened first if workers are to do their job well. Therefore, CTC-related technologies are the underlying foundation to the application of CTCs in precision medicine. Herein, we reviewed previous CTCs-related technologies based on physical and biological properties, and the most recent development of techniques associated with microfluidic-based and nanoparticle-based approaches. Although there are strengths and weakness between different methods, we believe that an effective combination of these techniques may benefit CTCs research in many ways, especially the in-depth analysis and possibility in clinical applications.

CLINICAL APPLICATIONS OF CTCs

Clinically, CTCs are now used as surrogate biomarkers for many solid cancers. Numerous studies have been carried out, mainly in breast cancer, prostate cancer, lung cancer, liver cancer, pancreatic cancer, gastric cancer, and melanoma. Although the clinical guidelines have not included the clinical use of CTCs, besides the inclusion of CTCs as part of the cM0 tumor classification (i.e., no clinical of overt metastasis but the detection of tumor cells in blood), many studies have predicted the great potential of CTCs in clinical applications. In this section, we will mainly present the role of CTCs as biomarkers for diagnosis, prognostication, and therapy monitoring in different cancers (Fig. 4).

Early diagnosis of cancer

As a non-invasive method, CTC detection is attractive in assisting cancer diagnosis. Studies of CTCs used for early diagnosis of cancer in the past three years are listed in Table 3. A tumor lesion already has more than 10⁹ tumor cells by the time they are detectable in patients using current imaging procedures, such as computed tomography, magnetic resonance imaging, and positron emission tomography. Diagnosis of cancers as early as possible, especially for fast-progressing cancers, is the best way to defeat them. Studies have determined that CTCs are correlated with tumor stage, but the clinical utility of CTCs in cancer detection or even in early cancer diagnosis is still a matter
| Technology                                    | Key features                                                                 | Strengths                                                                 | Weakness                                                                 | Author (year) |
|----------------------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------|---------------|
| Automated microfluidic method                | • A fully integrated microfluidic device and a set of robust fluid-driven    | • Label-free and simple                                                   | • A need of sample dilution to avoid cell–cell interaction              | Zhang et al. 207 |
|                                              | and control units                                                           | • High recovery rate (85%)                                                | • Quantitative design rules are still lacking for channels              |               |
|                                              | • A flow regulatory chip and two cell separation chips                       | • Rapid processing time (23 m)                                            | • Air diffuse across the thin polydimethylsiloxane walls under some      |               |
| Self-driving micro-cavity array chip         | • Integrate sample detection structure and vacuum driving system.           | • Less costly and simple                                                 | •                | Jia et al. 208 |
|                                              | • Use the “film-polydimethylsiloxane chip-film” structure and oil sealing   | • An excellent linear                                                   | •                |               |
|                                              | method during amplification reaction to minimize water loss.                | • Mutational gene profiling of single CTC                                | •                |               |
|                                              | • Achieve cell loading, lysing, isothermal amplification, and signal read-out| • Achieve cell loading, lysing, isothermal amplification, and signal read-out on one chip. | •                |               |
|                                              | on one chip.                                                                |                                                                           | •                |               |
| Automated and integrated microfluidic method | • Integrate a 3D printed off-chip multisource reagent platform, a bubble     | • Achieve CTC capture and identification within 90 m.                     | • Deficiencies in patient recruitment                                    | Wang et al. 209 |
|                                              | retainer, and a single CTC capture microchip.                               | • Decrease immunostaining time and antibody consumption by 90%           | •                |               |
|                                              |                                                                           | • Detect CTCs from various cancers.                                      | •                |               |
|                                              |                                                                           | • Isolate CTCs with an efficiency of >99%.                                | •                |               |
|                                              |                                                                           | • Differentiate eight different subtypes of heterogenic CTCs              | •                |               |
|                                              |                                                                           | • Simultaneous on-chip isolation and discrimination of CTCs               | •                |               |
| Integrated microfluidic method              | • Use magnetic field gradient and immune-fluorescence differences.         |                                                                           | • Limitation of distinguishable fluorescence wavelength light of microscope | Lee et al. 210 |
| Nanotechnology-based methods                 |                                                                           |                                                                           | •                |               |
| Magnetic nanoparticles                       |                                                                           |                                                                           | •                |               |
| Tannic acid-functionalized magnetic          | • Tannic acid interact with glycolayx on cancer cells                       | • Inhibit the nonspecific adhesion of peripheral blood mononuclear cell.  | EpCAM-dependent                                                    | Ding et al. 214 |
| nanoparticles                                |                                                                           | • 95.1% of capture purity for MCF-7 cells                                 | •                |               |
| Peptide-Based Magnetic Nanoparticle          | • Use N-Cadherin recognition peptide-functionalized magnetic nanoparticles   | • High capture efficiency (about 85%) of mesenchymal CTCs from spiked human blood | Unmentioned                                                   | Jia et al. 213 |
| CoFe2O4@Ag magnetic nanohybrids              | • Gold electrodes are modified with MXene nanosheets and an HBS aptamer is immobilized on the MXene layers | • Distinguished epithelial and mesenchymal subgroups.                     | • Ag has some aggregations.                                             | Vajhadin et al. 216 |
|                                              | • CoFe2O4@Ag magnetic nanohybrids is bonded to the HBS.                     | • A wide linear range of 10^2–10^6 cells/mL, low detection limit of 47 cells/mL | The magnetic property of magnetic probes can affect the isolation of magnetic cells. |               |
| Gold nanoparticles                            | • Au nanoparticles-loaded two-dimensional bimetallic PdMo nanocubes assembled with an aptamer composed of a thiol-modified EpCAM | Excellent analytical performance | Long preparation time of the Au@PdMo nanocubes | Yang et al. 218 |
| AuNP-anchored black phosphorus                | • Use electrochemical detection                                             | • A satisfactory CTCs release reaching a range of 93.7–97.4% and good cell viability | Complex detection procedure | Liu et al. 221 |
| phosphorus nanosheets                         | • Use BP@AuNPs@aptamer as a probe combined with immunomagnetic separation   | • Good sensitivity and selectivity in the detection of HER2-positive cells in blood samples | EpCAM-dependent |               |
| Antibody-Functional Microsphere Integrated    | • Consist of a semicircle arc and arrays                                   | • Cost-effective and environmentally friendly                             | •                |               |
| Filter Chip                                  | • Use interfacial zinc oxide coating with nanostructure on the surface of the microsphere to increase specific surface area |                                                                           | •                |               |

Table 2. The CTC detection technologies in recent three years (from 2018 to 2021), mainly including microfluidic chip and nanotechnology-base methods.
of debate. CTCs are considered a surrogate marker of metastatic activity, but whether metastatic dissemination of CTCs in patients occurs early during tumor formation is still controversial. However, in mouse models, early dissemination seeding metastasis has been found in breast and pancreatic carcinogenesis, indicating that CTC circulation is likely to be a very early event in cancer progression. In Barrière et al.’s study CTCs were detected in 41% of T1 stage and 47% of axillary lymph node-negative breast cancer patients, both of which are early-stage breast cancer. In the study by Thery et al. the CTC positivity rate was 21% and 24% in lymph node-negative and positive breast cancer, respectively. Based on this hypothesis, CTCs can be detected earlier before the primary tumor is visible on imaging studies, while the biggest challenge of the application of CTC application in early cancer diagnosis is indeed their scarcity and isolation. The limited sensitivity of CTC detection methods hinders their use as an effective biomarker in early cancer diagnostics.

Evaluation of the cancer prognosis
The prognostic value of CTCs has been extensively studied. CellSearch is the only FDA-approved system for CTC detection used clinically. Based on CellSearch system, CTCs represent an independent prognostic factor. Studies evaluating other CTC detection systems such as CanPatol, CTC-chip obtained similar results. CTC enumeration is the main target of investigation, with a cut-off value of ≥5 for positivity, which usually indicates a worse prognosis. It is generally considered that increased CTC counts are correlated with higher likelihood of metastasis and cancer aggressiveness. In a meta-analysis pooling 2239 breast cancer patients including 21 studies, the CTC count before neoadjuvant chemotherapy had a detrimental and decremental impact on patient survival, and patients with one, two, three to four, and five or more CTCs displayed a HR of death (95% CI) of 1.09 (0.65–1.69), 2.63 (1.42–4.54), 3.83 (2.08–6.66), and 6.25 (4.34–9.09), respectively. Furthermore, elevated baseline CTC levels were associated with inferior survival, the presence of CTC clusters often predicted poor prognosis, and increasing CTC counts or failure to clear CTCs during treatment was also a prognostic factor for worse survival. Many studies have found that the molecular phenotypes of CTCs have strong prognostic value. EMT and stemness are the main molecular phenotypes of CTCs studied clinically. CTCs with expression of mesenchymal or stemness-related markers associated with inferior survival. The expression of other molecular markers, such as HER2, CD47, PD-L1 also have prognostic implications. Most studies investigate the prognostic value of CTCs at a single timepoint, while intriguingly, some studies have taken CTC dynamics into account. Magbanua et al. developed a novel latent mixture model to stratify groups with similar CTC trajectory patterns during the treatment course, and they found that analysis of serial CTCs can further stratify the patients of poor prognosis into distinct prognostic subgroups. The dynamic changes of CTCs may act as a surrogate prognosis biomarker over the long course of cancer progression. Given the rapid advancements in the accessibility and strengthening of sequencing technologies at the single-cell level, we may expect that in the future, genomic/transcriptional profiles of CTCs may serve as an outstanding prognostic marker, representing biological information that is more comprehensive and more closely related to prognosis. Studies of CTCs for predicting prognosis in recent three years are summarized in Table 3.

Monitoring of the therapeutic response
In many clinical trials, CTCs have been used as useful biomarkers for monitoring cancer treatment responses, either combined with imaging examinations, serum biomarkers or alone. Researchers prefer to involve CTCs in the evaluation of therapeutic efficiency, in view of the higher sensitivity of CTCs than imaging examination in some cases. As a non-invasive method, the detection of CTCs may also contribute to avoiding frequent
| Cancer type | Author & year | CTCs utility | Detection methods | CTC marker | Patients | Main findings | Clinical Trial No. |
|-------------|--------------|--------------|-------------------|------------|----------|---------------|-------------------|
| Breast cancer | Paoletti et al.\textsuperscript{232} | prognostic value | CellSearch | EpCAM, CK | 549 mBC | In mBC patients with 1st line chemotherapy, CTC counts was associated with mortality. | NCT00382018 |
| | Mego et al.\textsuperscript{361} | prognostic value | RT-qPCR | TWIST, SNAIL1, SNAIL2, ZEB1 | 427 stage I-III BC | CTC EMT was detected in 18% of patients, which was associated with worse DFS. | NA |
| | Magbanua et al.\textsuperscript{233} | prognostic value | CellSearch | EpCAM, CK | 742 untreated BC | CTC positivity associated with reduced DFS. | NA |
| | Strati et al.\textsuperscript{263} | prognostic value | density-based isolation, RT-qPCR | EpCAM, TWIST1, CD24/CD44, ALDH1 | 100 early BC | Detection of TWIST1 overexpression and stem-cell transcripts in EpCAM+ CTCs provides prognostic information. | NA |
| | Wang et al.\textsuperscript{255} | therapeutic monitoring | CellSearch | EpCAM, CK | 160 early BC | CTC counts were lower in surgical group than trastuzumab group, but the OS rate in surgical group was higher. | NA |
| | Radovich et al.\textsuperscript{279} | prognostic value | microfluidic device | EpCAM | 123 early triple-negative BC | Positive CTC and circulating tumor DNA after neoadjuvant chemotherapy associated with inferior DFS and OS. | NCT02101385 |
| | Zhang et al.\textsuperscript{362} | recurrence monitoring | Cyttel detection | CD133, CEP8 | 135 early Luminal A BC | There were no differences in DFS and OS between CTC monitoring and routine re-examination monitoring group. | NA |
| | Magbanua et al.\textsuperscript{234} | prognostic value, therapeutic monitoring | CellSearch | EpCAM, CK | 294 ER + mBC | CTCs ≥ 5 was detected in 31% of the patients. Letrozole with bevacizumab gain better OS than without bevacizumab in patients with CTC ≥ 5. | NCT00601900 |
| | Wang et al.\textsuperscript{286} | prognostic value, guiding therapy | CellSearch | EpCAM, CK | 105 HER2-advanced BC | HER2 + CTCs ≥ 2 associated with shorter survival and higher risk for disease progression (HR 2.16). Those received anti-HER2 targeted therapies had improved PFS. | NA |
| | Stefanovic et al.\textsuperscript{363} | no prognostic value | CellSearch | EpCAM, CK | 261 mBC | CTCs had no prognostic value in different receptor change pattern subgroups. | NA |
| | Papadaki et al.\textsuperscript{254} | prognostic value | density-based isolation | CK, CD47, PD-L1 | 198 (100 early BC, 98 mBC) | CTCs expressing CD47 and PD-L1 have independent poor prognostic implications in mBC. | NA |
| | Jin et al.\textsuperscript{364} | diagnostic value | CytoSorter | EpCAM, CK | 130 BC | CTC detection rates in BC patients at Tis and T1-4 stages were 50%, 81.67%, 91.07%, 100%, and 100%, respectively. | NA |
| | Silveira et al.\textsuperscript{235} | prognostic value | CellSearch | EpCAM, CK | 198 HER2- mBC | CTC count ≥ 1 and ≥5 was detected in 37% and 22% of the patients at 4 weeks of treatment, respectively. CTCs levels at four weeks had a | NCT01745757 |
| Cancer type          | Author & year   | CTCs utility      | Detection methods     | CTC marker          | Patients | Main findings                                                                 | Clinical Trial No. |
|---------------------|-----------------|-------------------|-----------------------|---------------------|----------|--------------------------------------------------------------------------------|---------------------|
| Renal cell carcinoma| Zhang et al.    | no prognostic value | CanPatrol-ITMCTCs | EpCAM, CK, Beclin vimentin, TWIST, | 199 RCC  | No differences in the OS and DFS of RCC between the different numbers of CTCs and Beclin1 expression. | NCT01942837, NCT01942837 |
|                     | Basso et al.    | prognostic value  | CellSearch           | EpCAM, CK          | 195 RCC  | Patients with $\geq 3$ CTCs had a shorter OS.                                         | NA                  |
|                     | Chemic et al.   | prognostic value  | CellSearch           | EpCAM, CK         | 100 NSCLC|                                                                               | NA                  |
| Prostate cancer     | Siewert et al.  | prognostic value  | CellSearch, RT-qPCR  | EpCAM, CK, AR-Vs   | 118 mCRPC| CTC count was independently associated with PFS and OS in mCRPC patients with cabazitaxel treatment. | NA                  |
|                     | Kruijff et al.  | prognostic value  | CellSearch           | EpCAM, CK         | 120 mCRPC|                                                                               | NA                  |
|                     | Graf et al.     | therapeutic       | Streck tubes         | CK, AR-V7         | 193 mCRPC| Patients with detectable nuclear-localized AR-V7 in CTCs had superior survival with taxanes over ARSIs. | NA                  |
|                     | Cieślikowski et al. | diagnostic value | CellSearch, EPISPOT, GILUPI CellCollector | EpCAM, CK, PSA, FGF2 | 104 PC  | High CTC counts related to high-risk prostate cancer patients with occult metastases at the time of diagnosis. | NA                  |
|                     | Schonhoft et al.| diagnostic value  | Epic Sciences        | CK, AR             | 294 mCRPC| Chromosomal instability in CTCs was associated with poor OS in patients treated with AR signaling inhibitors and taxanes. | NA                  |
|                     | Armstrong et al.| prognostic value  | AdnaTest, Epic Sciences | CK, AR, AR-V7    | 118 mCRPC| AR-V7 in CTCs was independently associated with shorter PFS and OS with abiraterone or enzalutamide. | NA                  |
|                     | Xu et al.       | diagnostic value  | Parsortix            | CK, vimentin       | 155 PC  | Combining the PSA, CTCs and the 12-gene panel, the AUC of clinically significant prostate cancer prediction was 0.927. | NA                  |
|                     | Sperger et al.  | prognostic value  | VERSA                | EpCAM              | 147 mCRPC| A transcriptional profile detectable in CTCs can serve as an independent prognostic marker in mCRPC. | NCT01942837, NCT01942837 |
|                     | Paoletti et al. | prognostic value  | CellSearch           | EpCAM, CK, HER2- mBC | 121 ER + /HER2- mBC | Significant prognostic impact on PFS and OS. CTCs $\geq 5$ at baseline was detected in 36% of patients. Elevated CTC at 1 month was associated with worse PFS. | NCT01701050 |
|                     | Magbanua et al. | prognostic value  | CellSearch           | EpCAM, CK         | 469 mBC | Intermediate or high CTC trajectory pattern was associated with poor prognosis. | NCT00785291 |
|                     | Shliakhutunou et al. | guiding therapy | magnetic isolation, RT-qPCR | EpCAM, Survivin, HER2-neu | 228 BC | CTC-oriented personalized adjuvant chemotherapy (turn to taxanes or add gemcitabine) can 100% eradicate CTCs, and increase 5-year DFS by 7.4% and OS by 11.6% | NA                  |

Circulating tumor cells: biology and clinical significance
Lin et al.
| Cancer type                    | Author & year | CTCs utility | Detection methods            | CTC marker               | Patients | Main findings                                                                 | Clinical Trial No. |
|-------------------------------|---------------|--------------|------------------------------|--------------------------|----------|-------------------------------------------------------------------------------|-------------------|
| **Non-small-cell lung cancer**| Li et al.     | prognostic value | CytoploRare Kit, FR ligand-TaqMan | CD45, CD14                | 347 NSCLC | Pulmonary venous-CTCs were detected in 48% of 100 patients, serving as early predictors of NSCLC recurrence after surgery. The median follow-up time was 38 months. Preoperative CTC concentration was an independent prognostic factor. | NA                |
| Small-cell lung cancer        | Messaritakis et al. | prognostic value | CellSearch, Ficoll-Hypaque | Notch 1–4 receptors, CK CD45, vimentin | 108 SCLC | The detection of DLL3+/CD45- CTCs at baseline and progression was related to decreased PFS and OS, respectively. | NA                |
|                              | Wang et al.   | prognostic value | EpCAM-independent           | EpCAM, CD45, DAPI         | 138 SCLC | The high number of CTC predicted adverse prognosis.                           | NA                |
| Hepatocellular Carcinoma      | Ha et al.     | prognostic value | Tapered slit filter         | CK                        | 105 HCC | Postoperative CTCs was detected in 23.8% of HCC patients and it may serve as an independent predictor of recurrence. | NA                |
|                              | Chen et al.   | no prognostic value | Canpatrol                 | EpCAM, CK, vimentin, TWIST | 256 HCC | CTC count and EMT status were not correlated with clinical stages or predictive of HCC recurrence. | NA                |
|                              | Cheng et al.  | diagnostic value | CanPatrol                  | EpCAM, CK, vimentin, TWIST | 113 HCC | Mesenchymal CTCs were increased in late-stage HCC patients. The cut-off value CTCs ≥ 1 was for the diagnosis of HCC. | NA                |
|                              | Sun et al.    | prognostic value | CellSearch                 | EpCAM, CK                 | 197 HCC | CTC count ≥ 3 was associated with higher risk of postoperative extrahepatic metastases. | NA                |
|                              | Lei et al.    | prognostic value | CanPatrol                  | EpCAM, CK, vimentin, TWIST | 160 HCC | The numbers of EpCAM mRNA+ CTCs and Nanog mRNA+ CTCs were correlated with postoperative HCC recurrence, with Nanog > 6.7 (HR = 2.33) being the most crucial marker. | NA                |
| Pancreatic cancer             | Wei et al.    | diagnostic value, treatment monitoring | CytoQuest, microfluidic chip | vimentin, EpCAM, CK      | 100 PDAC | Vimentin+ CTCs were detected in 76% of patients with PDAC. Preoperatively higher counts was correlated with shortened RFS. | NA                |
|                              | Zhao et al.   | prognostic value | CanPatrol                  | EpCAM, vimentin, TWIST    | 107 PDAC | CTCs were detected in 78.5% of PDAC patients. Patients with ≥ 6 total CTCs had significantly decreased OS and PFS. | NA                |
|                              | Hugenschmidt et al. | guiding therapy, prognostic value | CellSearch               | EpCAM                     | 209 patients | CTC-positive (≥1 CTC/7.5 mL) preoperatively showed a detrimental outcome despite successful tumor resections. | NCT01919151      |
| Gastric cancer                | Szczepanik et al. | prognostic value | flow cytometry             | CD45, CD44, CK            | 228 GC | CK+/CD44+ cells were significantly more common among patients with distant metastases. | NA                |
| Cancer type | Author & year | CTCs utility | Detection methods | CTC marker | Patients | Main findings | Clinical Trial No. |
|-------------|---------------|--------------|-------------------|------------|----------|---------------|-------------------|
| Colorectal cancer | Miki et al.\textsuperscript{379} | prognostic value | Ficoll | EpCAM, CEA, CK | 150 GC | The number of EpCAM −/CEA + cells was higher in patients with stage II–III and IV than in patients with stage I. A lower number of these cells indicated a higher 3-year RFS. | NA |
| | Kuroda et al.\textsuperscript{380} | prognostic value | Ficoll | EpCAM, FGFR2, CD45 | 100 GC | FGFR2 + CTCs (≥ 5 cells/10 mL blood) showed poorer RFS. | NA |
| | Nevisi et al.\textsuperscript{244} | prognostic value | CellSearch | EpCAM, HER2 | 105 GC | HER2-expression on CTCs was an independent prognostic factor for both overall and progression-free survival. | NA |
| Colorectal cancer | Wang et al.\textsuperscript{381} | prognostic value | magnetic isolation | chromosome enumeration probe | 130 CRC with stage II-III | Postoperative CTCs were significantly correlated with poor RFS. | NA |
| | Bidard et al.\textsuperscript{382} | prognostic value | CellSearch | EpCAM, CK | 153 CRC with liver metastasis | Baseline CTCs ≥3 was detected in 19% of the patients. CTC ≥ 3 at baseline and 4 weeks after therapy showed shorter OS. | NCT01442935 |
| | Wang et al.\textsuperscript{383} | prognostic value | Cyttel | Chromosomes 8 and 17 H1 | 121 CRC | CTCs were detected in 58.7% of CRC patients. Advanced CRC patients with CTC-positive had worse PFS and OS. | NA |
| | Messaritaki et al.\textsuperscript{384} | prognostic value | Density gradient isolation, RT-qPCR | CEACAMS, EpCAM | 198 advanced CRC | CEACAMS was a dynamic adverse prognostic CTC biomarker in patients with metastatic CRC. | NA |
| | Su et al.\textsuperscript{252} | prognostic value | CellSearch | EpCAM, CK, vimentin, twist, PRL-3 | 156 CRC | CTCs were detected in 100% of CRC patients. The count of mesenchymal and PRL-3 + CTCs ≥ 12 was significantly associated with recurrence and shorter DFS. | NA |
| | Sastre et al.\textsuperscript{245} | prognostic value | CellSearch | EpCAM, CK | 1202 metastatic CRC | Baseline CTCs ≥3 was detected in 41% of the patients. | NCT01640405, NCT01640444 |
| | Pan et al.\textsuperscript{385} | prognostic value | magnetic isolation | CK19 | 149 CRC | CTC counts were associated with TNM stages. The change escalated more rapidly in the CTC-positive group. | NA |

\textit{AR} androgen receptor, \textit{BC} breast cancer, \textit{CK} cytokeratin, \textit{CTC} circulating tumor cell, \textit{CRC} colorectal cancer, \textit{DAPI} 4',6-diamidino-2-phenylindole, \textit{DFS} disease free survival, \textit{DRFS} distant recurrence-free survival, \textit{EMT} epithelial-mesenchymal transition, \textit{GC} gastric cancer, \textit{HCC} hepatocellular carcinoma, \textit{HER2} human epidermal growth factor receptor-2, \textit{mBC} metastatic breast cancer, \textit{mCRPC} metastatic castration-resistant prostate cancer, \textit{NA} not applicable, \textit{NSCLC} non-small-cell lung cancer, \textit{OS} overall survival, \textit{PC} prostate cancer, \textit{PDAC} pancreatic ductal adenocarcinoma, \textit{PD-L1} programmed cell death ligand-1, \textit{PFS} progression free survival, \textit{qRT-PCR} quantitative real time polymerase chain reaction, \textit{RCC} renal cell carcinoma, \textit{RFS} recurrence free survival.
radiation exposure from imaging studies during the evaluation of treatment response. Most studies found a decrease or clearance in CTC counts was associated with a good therapeutic response, while the increase of CTC counts signified the opposite. The Response Evaluation Criteria in Solid Tumors (RECIST) guidelines are the most often used standard for evaluating therapeutic response in solid tumors. However, in some studies, changes in CTC following therapy were not correlated with RECIST responses in cancer patients. Indeed, CTCs assessment has not been included in the RECIST guidelines. Some CTCs measurement technologies have been recently developed to achieve genotyping for CTCs, which can also detect crucial gene mutations, such as ER, HER2, EGFR, KRAS, and TP53, thus helping clinicians in treatment personalization and resistance options at the time of tumor progression. Studies using CTCs to monitor treatment response in recent three years are summarized in Table 3.

The great potential for CTCs in the clinical application of cancer diagnostics has emerged, although clinically, its use as a surrogate biomarker for cancer screening, treatment monitoring, and prognosis predicting is still limited. Once metastasis occurs, repeat biopsies of metastatic lesions are usually difficult to obtain, and different metastases are heterogenous even in the same patients. CTCs testing using peripheral blood samples is convenient, and may be more representative of the traits of metastatic cells, which are derived from different metastatic lesions in patients. Nevertheless, there is still a lack of guidelines for the clinical use of CTCs, such as a standardized CTCs detecting assay for different cancers, a combination diagnostic scheme with other clinical examinations, and indications for the appropriate timepoints for blood sampling.

DISCUSSION
Studies investigating CTCs have the great potential to reveal the fundamental processes of metastases, including the mechanisms involved in extravasation of CTCs from the primary tumor, how CTCs interact with blood cells to survive in the circulatory microenvironment, and how CTCs intravasate into the distant metastatic site to initiate new lesions. Significant molecular traits of CTCs can greatly contribute to identify targets for anti-metastatic therapies. Only a small proportion of CTCs can finally generate metastases, thus studies focusing on these strongly metastatic CTCs may provide deeper insights into CTCs-related therapeutic targets.

Various CTCs detecting technologies have emerged, however, the sensitivity and specificity of these technologies still need to be further improved. Epithelial marker-based CTC detection technology, such as the CellSearch system has opened a new era for CTC analysis and clinical applications, but their drawbacks are rapidly being acknowledged and appreciated by researchers. EMT is a crucial trait of metastatic cancer cells, indicating insufficient capture efficiency of epithelial marker-based CTC detection technology. However, mesenchymal marker-based detection technologies may also be contaminated by non-CTCs, such as tumor-associated fibroblasts and endothelial clusters, which induce the risk of false positivity. Nevertheless, it is intriguing that recent studies have reported that those non-cancerous tumor-derived cells presented in cancer patients are also important surrogate biomarkers for cancer patients. Cancer-type-specific molecular markers for CTCs are likely another option, as CTCs of different cancer types possess different molecular markers. However, the sensitivity and specificity of known cancer-type-specific CTC markers are not satisfactory. Physical-property-based CTC detection technologies also have the problem of contamination by non-CTCs, especially for those with similar physical properties as CTCs. Microfluidic-based and nanotechnology-based CTC detection technologies have become popular in recent years, while the efficiency of these technologies still needs further large-scale clinical validation. High cell detection efficiency and contamination removal capability are the two key strengths of a successful CTC detection technology, while substantial technical optimization of CTC detection is urgently needed to achieve these requirements.

Comprehensive characterization of CTCs is lacking. The limited amount of genomic DNA, RNA, and protein content of CTCs is a bottleneck for exploring their genome, transcriptome, epigenome, and proteome properties. Nonetheless, the emerging genome and transcriptome studies of CTCs have recently profited from the fast-evolving technology of single-cell sequencing, while the proteome studies of CTCs are still elusive due to the very limited technologies for proteome exploring at a single-cell level. However, the study of the CTC proteome is imminent, not only because it can provide a picture of the biological characterization of CTCs, but also because it can help to discover CTC-specific membrane proteins which may help optimize CTC detection.

As for the solid tumor microenvironment, the blood microenvironment around CTCs also plays a significant role in tumor survival and invasion capacity. However, knowledge of the underlying mechanisms behind the survival of CTCs is still limited, as it is a complex process that involves not only shear forces and fluid mechanics but also soluble factors and tumor-associated extracellular vesicles, which are not detailed here. Furthermore, it remains to be confirmed whether CTC clusters are more suitable for interacting with other blood components or adapting to shear forces than single migratory CTCs. If combined with specific biomarkers for strategically detecting CTCs and the interaction of CTCs with associated peripheral blood cells, we could improve the clinical practicability and monitoring power of CTCs by obtaining more comprehensive information on tumor burden and immune status of patients.

Although CTCs have shown initial promise in clinical applications, many challenges must still be overcome before CTC analysis can be widely applied in the clinic. Today, the clinical application of CTCs mainly depends on the analysis of CTC cell enumeration and molecular phenotypes. A more comprehensive characterization of CTCs based on their genome, transcriptome, and proteome with high-throughput sequencing will further benefit clinical application, but also add to the complexity and difficulty of data analysis. CTCs will be a crucial component of “Precision medicine” in the future, as they can provide the genetic, phenotypic, genotypic, and functional characterization and provide an opportunity to study drug susceptibility that is related to metastasis. The genome and transcriptome analysis of CTCs can unveil potential drug targets. Viable CTCs for drug sensitivity/resistance testing over the therapy course can guide precision medication. However, the culture of CTCs is very challenging: (1) limited methods are available to isolate viable CTCs, which also yield low numbers of CTCs and (2) a favorable circulatory microenvironment for CTC survival is difficult to mimic. Very limited CTC-derived cell lines from cancer patients have been established. Optimization of CTC culture conditions will be needed.

Furthermore, CTCs, circulating tumor DNA (ctDNA), and exosomes are all present in liquid biopsy samples. An exploration of the advantages and disadvantages of each substrate present in the liquid biopsies, and how better incorporate them into clinical application is needed to achieve more precise diagnoses. Among liquid biopsy methods, CTCs have tremendous advantages, as isolated CTCs can be viable, which can optimize CTC-derived explants or three-dimensional organoid cultures for functional testing or for drug-screening assays. The study of CTCs is attractive, and CTC detection may likely become an essential component of cancer management in the future. As the picture becomes clearer, we are fully confident about the promising potentials of CTCs.
REFERENCES

1. Sethi, N. & Kang, Y. Unravelling the complexity of metastasis-molecular understand- ing and targeted therapies. Nat. Rev. Cancer 11, 735–748 (2011).
2. Lambert, A. W., Pattabiraman, D. R. & Weinberg, R. A. Emerging biological principles of metastasis. Cell 168, 679–691 (2017).
3. Paget, S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 8, 98–101 (1989).
4. Jones, S. et al. Comparative lesion sequencing provides insights into tumor evolution. Proc. Natl Acad. Sci. USA 105, 4283–4288 (2008).
5. Ley, T. J. et al. DNA sequencing of a cytogenetically normal acute myeloid leukemia genome. Nature 456, 66–72 (2008).
6. Rosinol, H. et al. Early dissemination seeds metastasis in breast cancer. Nature 540, 552–558 (2016).
7. Chen, S. et al. Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis. Cell 160, 1246–1260 (2015).
8. Navin, N. et al. Tumour evolution inferred by single-cell sequencing. Nature 472, 90–96 (2011).
9. Yu, M. et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 339, 580–584 (2013).
10. Peinado, H. et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat. Med. 18, 883–891 (2012).
11. Aceto, N. et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Proc. Natl Acad. Sci. USA 110, 1216–1221 (2013).
12. Qual, D. F. & Joyce, J. A. Microenvironmental regulation of tumor progression and metastasis. Nat. Med. 19, 1423–1437 (2013).
13. Ashworth, T. R. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Australas. Med. J. 14, 146–149 (1869).
14. Pantel, K. & Speicher, M. R. The biology of circulating tumor cells. Oncogene 35, 1216–1224 (2016).
15. Aliv-Panabieres, C. & Pantel, K. Challenges in circulating tumor cell research. Nat. Rev. Cancer 14, 623–631 (2014).
16. Harper, K. L. et al. Mechanism of early dissemination and metastasis in Her2+ mammary cancer. Nature 540, 588–592 (2016).
17. Rosinol, H. et al. Early dissemination seeds metastasis in breast cancer. Nature 540, 552–558 (2016).
18. Landou, E. S. & Markou, A. Circulating tumor cells in breast cancer: detection systems, molecular characterization, and future challenges. Clin. Chem. 57, 1242–1255 (2011).
19. Thanh Huong, P. et al. Emerging role of circulating tumor cells in gastric cancer. Cancers 12, 695–716 (2020).
20. Gires, O., Pan, M., Schinke, H., Canis, M. & Baeuerle, P. A. Expression and function of epithelial cell adhesion molecule EpCAM: where are we after 40 years? Cancer Metastasis Rev. 39, 969–987 (2020).
21. Popoviv, D., Vucic, D. & Dikic, I. Ubiquitination in disease pathogenesis and treatment. Nat. Med. 20, 1242–1253 (2014).
22. Ciriello, C., Sotiriou, C. & Ignatiadis, M. Circulating tumor cells and emerging blood biomarkers in breast cancer. Curr. Opin. Oncol. 22, 552–558 (2010).
23. Gori, M. A. et al. Circulating tumor cells as biomarkers of prostate, bladder, and kidney cancer. Nat. Rev. Urol. 14, 90–97 (2017).
24. Varillas, J. I. et al. Microfluidic isolation of circulating tumor cells and cancer stem-like cells from patients with pancreatic ductal adenocarcinoma. Theranostics 9, 1417–1425 (2019).
25. Marcello, M. et al. Circulating biomarkers for early detection and clinical management of colorectal cancer. Mol. Asp. Med. 69, 107–122 (2019).
26. Xia, W. et al. In vivo cointaneous identification of hepatocellular carcinoma circulating tumor cells by dual-targeting magnetic-fluorescent nanobeads. Nano Lett. 21, 634–641 (2021).
27. Ye, Q., Ling, S., Zheng, S. & Xu, X. Liquid biopsy in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. Mol. cancer 18, 114 (2019).
28. Gall, T. M. H., Belete, S., Khandelia, E., Frampton, A. E. & Jiao, L. R. Circulating tumor cells and circulating tumor DNA in pancreatic ductal adenocarcinoma. Am. J. Pathol. 189, 71–81 (2019).
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55. Chen, X. et al. Folate receptor-positive circulating tumor cells as a predictive biomarker for the efficacy of first-line pemetrexed-based chemotherapy in patients with non-squamous non-small cell lung cancer. Ann. Transl. Med. 8, 631 (2020).

56. Wei, S. et al. Effect of vein-first vs artery-first surgical technique on circulating tumor cells and survival in patients with nonsmall cell lung cancer: a randomized clinical trial and registry-based propensity score matching analysis. JAMA Surg. 154, e190972 (2019).

57. Cao, W. et al. Using detection of survivin-expressing circulating tumor cells in peripheral blood to predict tumor recurrence following curative resection of gastric cancer. J. Surg. Oncol. 103, 110–115 (2011).

58. Krishnamurthy, S. et al. Discordance in HER2 gene amplification in circulating and disseminated tumor cells in patients with工作者 breast cancer. Cancer Med. 2, 226–233 (2013).

59. Bidard, F.-C. et al. Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastasiceval melanoma. Int. J. Cancer 134, 1207–1213 (2014).

60. Bettegowda, C. et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci. Transl. Med. 6, 224ra224 (2014).

61. Lucci, A. et al. Circulating tumor cells and early relapse in node-positive melanoma. J. Am. Coll. Surg. 227, 116–124 (2018).

62. Lin, S. Y. et al. Prospective molecular profiling of circulating tumor cells from patients with melanoma receiving combinatorial immunotherapy. Clin. Chem. 66, 169–177 (2020).

63. Maheswaran, S. et al. Detection of mutations in EGFR in circulating lung-cancer cells. N. Engl. J. Med. 359, 366–377 (2008).

64. Fabbari, F. et al. Detection and recovery of circulating colon cancer cells using a dielectrophoresis-based device: KRAS mutation status in pure CTCs. Cancer Lett. 335, 225–231 (2013).

65. Buim, M. E. et al. Detection of KRAS mutations in circulating tumor cells from patients with metastatic colorectal cancer. Cancer Biol. Ther. 16, 1289–1295 (2015).

66. Kalikaki, A. et al. KRAS genotypic changes of circulating tumor cells during treatment of patients with metastatic colorectal cancer. PloS One 9, e104902 (2014).

67. Kondo, Y. et al. KRAS mutation analysis of single circulating tumor cells from patients with metastatic colorectal cancer. BMC Cancer 17, 311 (2017).

68. Kulemann, B. et al. Pancreatic cancer: circulating tumor cells and primary tumors show heterogeneous KRAS mutations. Sci. Rep. 7, 4510 (2017).

69. Lohr, J. G. et al. Whole-exome sequencing of circulating tumor cells provides a window into metastatic prostate cancer. Nat. Biotechnol. 32, 479–484 (2014).

70. Faugeroux, V. et al. An accessible and unique insight into metastasis mutational content through whole-exome sequencing of circulating tumor cells in metastatic prostate cancer. Eur. Urol. Oncol. 3, 498–508 (2020).

71. Deng, G. et al. Single cell mutational analysis of PROS1 in circulating tumor cells and metastases in breast cancer reveals heterogeneity, discordance, and mutation persistence in disseminated tumor cells from bone marrow. BMC Cancer 14, 456–464 (2014).

72. Fernandez, V. et al. TP53 mutations detected in circulating tumor cells present in the blood of metastatic triple negative breast cancer patients. Breast Cancer Res. 16, 445 (2014).

73. Kelley, R. K. et al. Circulating tumor cells in hepatocellular carcinoma: a pilot study of detection, enumeration, and nextgenerationsequencing in cases and controls. BMC Cancer 15, 206 (2015).

74. Ni, X. et al. Reproducible copy number variation patterns among single circulating tumor cells of lung cancer patients. Proc. Natl Acad. Sci. USA 110, 21083–21088 (2013).

75. Carter, L. et al. Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemotherapy and chemorefractory small-cell lung cancer. Nat. Med. 23, 114–119 (2017).

76. Paolotti, C. et al. Comprehensive mutation and copy number profiling in archived circulating breast cancer tumor cellsdocuments heterogeneous resistance mechanisms. Cancer Res. 78, 1110–1122 (2018).

81. Ortolan, E. et al. Blood-based genomics of triple-negative breast cancer progression in patients treated with neoadjuvantchemotherapy. ESMO Open 6, 100086 (2021).

82. Lambros, M. B. et al. Single-cell analyses of prostate cancer liquid biopsies acquired by apheresis. Clin. Cancer Res.: J. Am. Assoc. Cancer Res. 24, 5635–5644 (2018).

83. Gupta, S. et al. Whole genomic copy number alterations in circulating tumor cells from men with abiraterone enzalutamide-resistant metastatic castration-resistant prostate cancer. Clin. Cancer Res.: J. Am. Assoc. Cancer Res. 23, 1346–1357 (2017).

84. Malhij, P. D. et al. Single-cell circulating tumor cell analysis reveals genomic instability as a distinctive feature of aggressiveprostate cancer. Clin. Cancer Res.: J. Am. Assoc. Cancer Res. 26, 1413–14153 (2020).

85. Paillet, E. et al. Detection of circulating tumor cells harboring a unique ALK rearrangement in ALK-positive non-small-cell lung cancer. J. Clin. Oncol. 31, 2273–2281 (2013).

86. Mayer, J. A. et al. FISH-based determination of HER2 status in circulating tumor cells isolated with the microfluidic CEEPplatform. Cancer Genet. 204, 589–595 (2011).

87. Frithiof, H., Aaltonen, K. & Ryden, L. A FISH-based method for assessment of HER-2 amplification status in breast cancer circulating tumor cells following CellSearch isolation. OncoTargets Ther. 9, 7095–7103 (2016).

88. Gokuneta, S. et al. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. Cell 176, 98–112.e114 (2019).

89. Dong, Y., Wang, Z. & Shi, G. Liquid biopsy based single-cell transcriptome profiling characterizes heterogeneity of disseminated tumor cells from lung adenocarcinoma. Proteomics 20, e1900224 (2020).

90. Magbanua, M. J. M. et al. Expanded genomic profiling of circulating tumor cells in metastatic breast cancer patients to assess biomarker status and biology over time (CALGB 40502 and CALGB 40503, Alliance). Clin. Cancer Res.: J. Am. Assoc. Cancer Res. 24, 1486–1499 (2018).

91. Cheng, Y.-H. et al. Hydro-Seq enables contamination-free high-throughput single-cell RNA-sequencing for circulating tumor cells. Nat. Commun. 10, 2163–2173 (2019).

92. Miyamoto, D. et al. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. Science 349, 1351–1356 (2015).

93. Barbazan, J. et al. Molecular characterization of circulating tumor cells in human metastatic colorectal cancer. PLoS One 7, e40476 (2012).

94. Kallergi, G. et al. Epithelial to mesenchymal transition markers expressed in circulating tumour cells of early and metastatic breast cancer patients. Breast Cancer Res. 13, R59 (2011).

95. Balasubramanian, P. et al. Multiparameter analysis, including EMT markers, on negatively enriched blood samples from patients with squamous cell carcinoma of the head and neck. PLoS One 7, e24084 (2012).

96. Theodoropoulos, P. A. et al. Circulating tumor cells with a putative stem cell phenotype in peripheral blood of patients with breastcancer. Cancer Lett. 288, 99–106 (2010).

97. Armstrong, A. J. et al. Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelialmesenchymal markers. Mol. Cancer Res. 9, 997–1007 (2011).

98. Liu, X. et al. Epithelial-type systemic breast carcinoma cells with a restricted mesenchymal transition are a major source ofmetastasis. Sci. Adv. 5, eaav4275 (2019).

99. Thiery, J. P. & Lim, C. T. Tumor dissemination: an EMT affair. Cancer Cell 23, 272–273 (2013).

100. Pecot, C. V. et al. A novel platform for detection of CK+ and CK- CTCs. Cancer Discov. 1, 580–586 (2011).

101. Sun, Y. F. et al. Circulating tumor cells from different vascular sites exhibit spatial heterogeneity in epithelial andmesenchymal composition and distinct clinical significance in hepatocellular carcinoma. Clin. Cancer Res.: J. Am. Assoc. Cancer Res. 24, 547–559 (2018).

102. Xu, L. et al. The novel association of circulating tumor cells and circulating megakaryocytes with prostate cancer prognosis. Clin. Cancer Res. 23, 5112–5122 (2017).

103. Dongre, A. & Weinberg, R. A. New insights into the mechanisms of epithelial-mesenchymal transition and implications forcancer. Nat. Rev. Mol. Cell Bio 20, 69–84 (2019).

104. Guan, Y. et al. Identification of key genes and functions of circulating tumor cells in multiple cancers through bioinformaticanalysis. BMC Med. Genom. 13, 140 (2020).

105. Tashireha, L. A. et al. Heterogeneous manifestations of epithelial-mesenchymal plasticity of circulating tumor cells in breast cancer patients. Int. J. Mol. Sci. 22, 2504–2521 (2021).
106. Xin, Y., Li, K., Yang, M. & Tan, Y. Fluid shear stress induces EMT of circulating tumor cells via jnk signaling in favor of their survival during hematogenous dissemination. *Int. J. Mol. Sci.* 21, 8115–8130 (2020).

107. Horimoto, Y. et al. Analysis of circulating tumor cell and the epithelial mesenchymal transition (EMT) status during eribulin-based treatment in 22 patients with metastatic breast cancer: a pilot study. *J. Transl. Med.* 16, 287 (2018).

108. Guan, X. W. et al. The prognostic and therapeutic implications of circulating tumor cell phenotype detection based on epithelial-mesenchymal transition markers in the first-line chemotherapy of HER2-negative metastatic breast cancer. *CancerCommun* 39, 1–10 (2019).

109. Lindsay, C. R. et al. A prospective examination of circulating tumor cell profiles in non-small-cell lung cancer molecularsubgroups. *Ann. Oncol.* 28, 1523–1531 (2017).

110. Satelli, A. et al. Epithelial-mesenchymal transitioned circulating tumor cells capture for detecting tumor progression. *Clin. Cancer Res.* 21, 899–906 (2015).

111. Brechbuhl, H. M. et al. Analysis of circulating breast cancer cell heterogeneity and interactions with peripheral bloodmononuclear cells. *Mol. Cancer* 39, 1129–1139 (2020).

112. Miklikova, S. et al. Inflammation-based scores increase the prognostic value of circulating tumor cells in primary breast cancer. *Cancers* 12, 1134–1148 (2020).

113. Wu, Y. et al. SUMOylation represses Nanog expression via modulating transcription factors Oct4 and Sox2. *PLoS One* 7, e39606 (2012).

114. Zhang, R. et al. Co-expression of stem cell and epithelial mesenchymal transition markers in circulating tumor cells ofbladder cancer patients. *OncoTargets Ther.* 13, 10739–10748 (2020).

115. Zhao, P. et al. Establishment and characterization of a CTC cell line from peripheral blood of breast cancer patient. *Breast Cancer* 10, 6095–6104 (2019).

116. War, S. et al. New lymphbin microfluidic device detects circulating tumor cells expressing cancer stem cell marker and circulating tumor microemboli in hepatocellular carcinoma. *Sci. Rep.* 9, 18575 (2019).

117. Liu, T. et al. Circulating glioma cells exhibit stem cell-like properties. *Cancer Res.* 78, 6632–6642 (2018).

118. Yin, J. et al. Circulating tumor cells enriched by the depletion of leukocytes with bi-antibodies in non-small cell lung cancer:potential clinical application. *PLoS One* 10, e0137076 (2015).

119. Jin, J., Tang, K., Xin, Y., Zhang, T. & Tan, Y. Hemodynamic shear flow regulates biophysical characteristics and functionsof circulating breast tumor cells remi-niscient of brain metastasis. *Soft Matter* 14, 9528–9533 (2018).

120. Choi, H. et al. Hydrodynamic shear stress promotes epithelial-mesenchymal transition by downregulating ERK and GSK3βactivities. *Breast Cancer Res.* 21, 6 (2019).

121. Follain, G. et al. Fluids and their mechanics in tumor transit: shifting metabolism. *Nat. Rev. Cancer* 20, 107–124 (2020).

122. Strick, B. & Offermanns, S. Intravascular survival and extravasation of tumor cells. *Cancer cell* 32, 282–293 (2017).

123. Rejniak, K. A. Circulating tumor cells: when a solid tumor meets a fluid micro-environment. *Adv. Exp. Med. Biol.* 936, 93–106 (2016).

124. Garrido-Nava, C. et al. Cooperative and escaping mechanisms between circu-lating tumor cells and blood constituents. *Cells* 8, 1382–1391 (2019).

125. Shaul, M. E. & Fridlender, Z. G. Tumour-associated neutrophils in patients with cancer. *Nat. Rev. Clin. Oncol.* 16, 601–620 (2019).

126. Aliausto, M. et al. The effect of peripheral blood values on prognosis of patients with locally advanced gastric cancer before treatment. *Med. Oncol.* 27, 1060–1065 (2010).

127. Hu, S. et al. The preoperative peripheral blood monocyte count is associated with liver metastasis and overall survival incolorectal cancer patients. *PLoS One* 11, e0157486 (2016).

128. Wang, Y. et al. Circulating neutrophils predict poor survival for HCC and pro-mote HCC progression through p53 andSTAT3 signaling pathway. *J. Cancer* 11, 3736–3744 (2020).

129. Stott, S. L. et al. Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. *Proc. Natl Acad. Sci.* USA 107, 18392–18397 (2010).

130. Szczepa~za, B. M. et al. Neutrophils escort circulating tumor cells to enable cell cycle progression. *Nature* 566, 553–557 (2019).

131. Hattar, K. et al. Interactions between neutrophils and non-small cell lung cancer cells: enhancement of tumor proliferationand inflammatory mediator synthesis. *Cancer Immunol., Immunother.* 63, 1297–1306 (2014).

132. Spica, J. D. et al. Neutrophils promote liver metastasis via Mac-1-mediated interactions with circulating tumor cells. *Cancer Res.* 72, 3919–3927 (2012).

133. Strell, C., Lang, K., Niggemann, B., Zanenk, K. S. & Entschladen, F. Surface molecules regulating rolling and adhesion toendothelium of neutrophil gran-ulocytes and MDA-MB-468 breast carcinoma cells and their interaction. *Cell. Mol. Life Sci.* 64, 3306–3316 (2007).
20

162. Stark, K. et al. Distinct pathogenesis of pancreatic cancer microvesicle-associated venous thrombosis identifies newanthrombogenic targets in vivo. 
Arteriosclerosis, thrombosis, Vasc. Biol. 38, 772–786 (2018).

163. Labelle, M., Begum, S. & Hynes, R. O. Direct signaling between platelets and cancer cells induces an epithelialmesenchymal-like transition and promotes metastasis. 
Cancer Cell 20, 576–590 (2011).

164. Guo, Y., Cui, W., Pei, Y. & Xu, D. Platelets promote invasion and induce epithelial to mesenchymal transition in ovarian cancer cells by TGF-β signaling pathway. 
Gynecol. Oncol. 153, 639–650 (2019).

165. Xiong, G. et al. Hsp47 promotes cancer metastasis by enhancing collagen-dependent cancer cell-platelet interaction. 
Proc. Natl. Acad. Sci. USA 117, 3748–3758 (2020).

166. Egan, K., Cooke, N. & Kenny, D. Living in shear: platelets protect cancer cells from shear induced damage. 
Clin. Exp. Metastasis 31, 697–704 (2014).

167. Haemmerle, M. et al. Platelets reduce anoikis and promote metastasis by activating YAP1 signaling. 
Nat. Commun. 8, 310 (2017).

168. Nieswandt, B., Hafner, M., Echtenacher, B. & Männel, D. N. Lysis of tumor cells by natural killer cells in mice is impedebly platelets. 
Cancer Res. 59, 1295–1300 (1999).

169. Placke, T. et al. Platelet-derived MHC class I confers a pseudonormal phenotype to cancer cells that subverts the antitumourreactivity of natural killer immune cells. 
Cancer Res. 72, 440–448 (2012).

170. Kopp, H. G., Placke, T. & Salih, H. R. Platelet-derived transforming growth factor-beta down-regulates NKG2D thereby inhibiting natural killer cell antitumor reactivity. 
Cancer Res. 69, 7775–7783 (2009).

171. Maurer, S. et al. Platelet-mediated shedding of NKG2D ligands impairs NK cell immune-surveillance of tumor cells. 
Oncoimmunology 7, e1364827 (2018).

172. Placke, T., Salih, H. R. & Kopp, H. G. GITR ligand provided by thrombopoietic cells inhibits NK cell antitumor activity. 
J. Immunol. 189, 154–160 (2012).

173. Ren, J. et al. Platelet TRAIL-ERK5 axis facilitates net-mediated capturing of circulating tumor cells and distant metastasis after surgical stress. 
Cancer Res. 81, 2373–2385 (2021).

174. Rachidi, S. et al. Platelets subvert T cell immunity against cancer via GARP-TGFβ axis. 
Sci. Immunol. 2, 7911–7917 (2013).

175. Xiang, B. et al. Platelets protect from septic shock by inhibiting macrophage-dependent inflammation via thecyclooxygenase 1 signalling pathway. 
Nat. Commun. 4, 2657 (2013).

176. Benders, G. & Borsig, L. Cancer cell adhesion and metastasis: selectins, integrins, and the inhibitory potential of heparins. 
Int. J. Cancer. 2012, 676731 (2012).

177. Lonsdorf, A. S. et al. Engagement of αIIbβ3 (GPIIb/IIIa) with αvβ3 integrin mediates interaction of melanoma cells with platelets: a connection to hema-toxygenous metastasis. 
J. Biol. Chem. 287, 2168–2178 (2012).

178. Coupland, L. A., Chong, B. H. & Panheart, C. R. Platelets and P-selectin control tumor cell metastasis in an organ-specificmanner and independently of NK cells. 
Cancer Res. 72, 4662–4671 (2012).

179. Schumacher, D., Strilic, B., Sivarak, K. K., Wettscbureck, N. & Offermans, S. Platelet-derived nucleotides promote tumorcelltransendothelial migration and metastasis via P2Y2 receptor. 
Cancer Cell 24, 130–137 (2013).

180. Mammadova-Bach, E. et al. Platelet integrin 617T controls lung metastasis through direct binding to cancer cell-derivedADAM9. 
JCI Insight 1, e88248 (2016).

181. Ward, Y. et al. Platelets promote metastasis via binding tumor CD97 leading to bidirectional signaling that coordinatestransendothelial migration. 
Cell Rep. 23, 808–822 (2018).

182. Mammadova-Bach, E. et al. Platelet glycoprotein VI promotes metastasis through interaction with cancer cell-derivedgelatin-3. 
Blood 135, 1146–1160 (2020).

183. Xu, Y. et al. Blockade of platelets using tumor-specific NO-releasing nanoparticles prevents tumor metastasis and reverses tumor immunosuppression. 
ACS Nano 14, 9780–9795 (2020).

184. Cassetta, L. et al. Differential expansion of circulating human MDSC subsets in patients with cancer, infection and inflammation. 
J. Immunother. Cancer 8, 1232–1237 (2020).

185. Liu, Q., Liao, Q. & Zhao, Y. Myeloid-derived suppressor cells (MDSC) facilitate distant metastasis of malignancies byshielding circulating tumor cells (CTC) from immune surveillance. 
Med. Hypotheses 87, 34–39 (2016).

186. Jiménez-Cortegana, C. et al. Circulating myeloid-derived suppressor cells and regulatory T cells as immunological biomarkers in refractory/relapsed diffused large B-cell lymphoma: translational results from the R2-GDP-GOTEL trial. 
J. Immunother. Cancer 9, 2332–2334 (2021).

187. Sprouse, M. L. et al. PMN-MDSCs enhance CTC metastatic properties through reciprocal interactions via ROS/Notch/nodal signaling. 
Int. J. Mol. Sci. 20, 1916–1935 (2019).

188. Chen, X. & Song, E. Tumor foos to friends: targeting cancer-associated fibrob-lasts. Nat. Rev. Drug Discov. 18, 99–115 (2019).
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Lin et al.

245. Sastre, J. et al. Association between baseline circulating tumor cells, molecular tumor profiling, and clinical characteristics in a large cohort of chemo-naive metastatic colorectal cancer patients prospectively collected. Clin. Colorectal Cancer 19, e110–e116 (2020).

246. Bidad, F. C. et al. Circulating tumor cells in breast cancer patients treated by neoadjuvant chemotherapy: a meta-analysis. J. Natl Cancer Inst. 110, 560–567 (2018).

247. Murlidhar, V. et al. Poor prognosis indicated by venous circulating tumor cell clusters in early-stage lung cancers. CancerRes 77, 5194–5206 (2017).

248. Janssens, S., Bendahl, P. O., Larsson, A. M., Aaltosen, K. E. & Ryden, L. Prognostic impact of circulating tumor cell capillary and clusters in serial blood samples from patients with metastatic breast cancer in a prospective observational cohort. BMC Cancer 16, 433 (2016).

249. Wang, C. et al. Longitudinally collected CTCs and CTC-clusters and clinical outcomes of metastatic breast cancer. BreastCancer Res. Treat. 161, 83–94 (2017).

250. Wang, C. et al. Improved prognostic stratification using circulating tumor cell clusters in patients with metastatic castration-resistant prostate cancer. Cancers 13, 268–280 (2021).

251. Lozano, R. et al. Value of early circulating tumor cells dynamics to estimate docetaxel benefit in metastatic castration-resistant prostate cancer (mCRPC) patients. Cancers 13, 2334–2344 (2021).

252. Su, P. et al. Mesenchymal and phosphatase of regenerating liver-3 status in circulating tumor cells may serve as a crucial prognostic marker for assessing relapse or metastasis in postoperative patients with colorectal cancer. Clin. Transl. Gastrointest. Tumors. Cells, 10, 80265 (2020).

253. Strati, A., Nikolou, M., Georgoulias, V. & Lianidou, E. S. Prognostic significance of TWIST1, CD24, CD44, and ALDH1 transcript quantification in EpCAM-positive circulating tumor cells from early stage breast cancer patients. Cells 8, 652–667 (2019).

254. Papadaki, M. A. et al. Clinical relevance of immune checkpoints on circulating tumor cells in breast cancer. Cancers 12, 376–396 (2020).

255. Wang, X. Q., Liu, B., Li, B. Y., Wang, T. & Chen, D. O. Effect of CTGs and INHBA level on the effect and prognosis of different treatment methods for patients with early breast cancer. Eur. Rev. Med. Pharmacol. Sci. 24, 12735–12740 (2020).

256. Graf, R. P. et al. Clinical utility of the nuclear-localized AR-V7 biomarker in circulating tumor cells in improving patient treatment choice in castration-resistant prostate cancer. Eur. Urol. 77, 170–177 (2020).

257. Wei, T. et al. Vimentin-positive circulating tumor cells as a biomarker for diagnosis and treatment monitoring in patients with pancreatic cancer. Cancer Lett. 452, 237–243 (2019).

258. Economos, C., Morrissey, C. & Vessella, R. L. Circulating tumor cells as a marker of response: implications for determining treatment efficacy and evaluating new agents. Curr. Opin. Urol. 22, 190–196 (2012).

259. Li, Y., Wu, S. & Bai, F. Molecular characterization of circulating tumor cells from bench to bedside. Semin. Cell. Dev. Biol. 75, 88–97 (2018).

260. Massard, C. et al. RECIST response and variation of circulating tumor cells in phase 1 trials: a prospective multicentric study. Eur. J. Cancer 83, 185–193 (2017).

261. Ntzifa, A., Kotsakis, A., Georgoulias, V. & Lianidou, E. Detection of EGFR mutations in plasma cfDNA and paired CTCs of NSCLC patients before and after osi-Cancer 7, 112 (2020).

262. Pan, X. & Zhang, X. Utility of circulating tumor cells and DNA in the management of advanced colorectal cancer. Future Oncol 16, 1289–1299 (2020).

263. Garrido-Navas, M. C. et al. The polemic diagnostic role of tp53 mutations in liquid biopsies from breast, colon and lung cancers. Cancers 12, 3343–3359 (2020).

264. de Kruijff, J. E. et al. Circulating tumor cell enumeration and characterization in metastatic castration-resistant prostate cancer patients treated with cabazitaxel. Cancers 11, 1212–1225 (2019).

265. Ceslikowski, W. A. et al. Circulating tumor cells as a marker of disseminated disease in patients with newly diagnosed high-risk prostate cancer. Cancers 12, 160–175 (2020).

266. Basso, U. et al. Prognostic role of circulating tumor cells in metastatic renal cell carcinoma: a large, multicenter, prospective, observational. Oncologist 26, 740–750 (2021).

267. Chemi, F. et al. Pulmonary venous circulating tumor cell dissemination before tumor resection and disease relapse. Nat. Med. 25, 1534–1539 (2019).

268. Messiartakis, I. et al. Characterization of DLL3-positive circulating tumor cells (CTCs) in patients with small cell lungcancer (SCLC) and evaluation of their clinical relevance for front-line treatment. Lung Cancer 135, 33–39 (2019).

269. Sun, Y. F. et al. Postoperative circulating tumor cells: an early predictor of extrahepatic metastases in patients with hepatocellular carcinoma undergoing curative surgical resection. Cancer Cytopathol. 128, 733–745 (2020).

270. Matsushita, D. et al. Clinical significance of circulating tumor cells in the response to trastuzumab for HER2-negative metastatic gastric cancer. Cancer Chemother. Pharm. 87, 789–797 (2021).
triple-negative (BCSCG04A): a multicenter, double-blind, prospective trial. Ann. Oncol. 24, 2766–2772 (2013).

271. Pierga, J. Y. et al. Circulating tumor cells and brain metastasis outcome in patients with HER2-positive breast cancer: the LANDSCAPE trial. Ann. Oncol. 24, 2999–3004 (2013).

272. Smerage, J. B. et al. Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0900. J. Clin. Oncol. 32, 3483–3489 (2014).

273. Hall, C. et al. Circulating tumor cells after neoadjuvant chemotherapy in stage I-III triple-negative breast cancer. Ann. Surg. Oncol. 22, 5552–5558 (2015). Suppl 3.

274. Hall, C. S. et al. Prognostic value of circulating tumor cells identified before surgical resection in nonmetastatic breast cancer patients. J. Am. Coll. Surg. 223, 260–269 (2016).

275. Riethdorf, S. et al. Prognostic impact of circulating tumor cells for breast cancer patients treated in the neoadjuvant “geparquattro” trial. Clin. Cancer Res. 23, 5384–5393 (2017).

276. Sparano, J. et al. Association of circulating tumor cells with late recurrence of estrogen receptor-positive breast cancer: a secondary analysis of a randomized clinical trial. JAMA Oncol. 4, 1700–1706 (2018).

277. Rossi, G. et al. Cell-free DNA and circulating tumor cells: comprehensive liquid biopsy analysis in advanced breast cancer. Clin. Cancer Res. 24, 560–568 (2018).

278. Trapp, E. et al. Presence of circulating tumor cells in high-risk early breast cancer during follow-up and prognosis. J. Natl Cancer Inst. 111, 380–387 (2019).

279. Radovich, M. et al. Association of circulating tumor DNA and circulating tumor cells after neoadjuvant chemotherapy with disease recurrence in patients with triple-negative breast cancer: preplanned secondary analysis of the BRET1-158 randomized clinical trial. JAMA Oncol. 6, 1410–1415 (2020).

280. Zhou, J. et al. Epithelial-mesenchymal transition status of circulating tumor cells in breast cancer and its clinical relevance. Cancer Biol. Med. 17, 169–180 (2020).

281. Le, Duf. et al. EpCAM-independent isolation of circulating tumor cells with epithelial-to-mesenchymal transition and cancer stem cell phenotypes using ApoStream(R) in patients with breast cancer treated with primary systemic therapy. PLoS One 15, e0229903 (2020).

282. Zhang, S. R. et al. Mesenchymal phenotype of circulating tumor cells is associated with distant metastasis in breast cancer patients. Cancer Manag. Res. 9, 691–700 (2017).

283. Bulloni, M. et al. In patients with metastatic breast cancer the identification of circulating tumor cells in epithelial-tomesenchymal transition is associated with a poor prognosis. Breast Cancer Res. 18, 30 (2016).

284. Papadaki, M. A. et al. Circulating tumor cells with stemness and epithelial-to-mesenchymal transition features are chemoresistant and predictive of poor outcome in metastatic breast cancer. Mol. Cancer Ther. 18, 437–447 (2019).

285. de Krijff, I. E. et al. Androgen receptor expression in circulating tumor cells of patients with metastatic breast cancer. Int. J. Cancer 145, 1083–1089 (2019).

286. Bock, C. et al. Distinct expression of cytokertan, N-cadherin and CD133 in circulating tumor cells of metastatic breast cancer patients. Future Oncol. 10, 1751–1765 (2014).

287. Goodman, O. B. et al. Circulating tumor cells in patients with castration-resistant prostate cancer baseline values and correlation with prognostic factors. Cancer Biomark. 18, 1904–1913 (2019).

288. Goldkorn, A. et al. Circulating tumor cell counts are prognostic of overall survival in SWOG S0421: a phase III trial of docetaxel with or without atrasentan for prostate cancer. J. Clin. Oncol. 33, 1136–1137 (2014).

289. Satelli, A. et al. Potential role of nuclear PD-L1 expression in cell-surface vimentin positive circulating tumor cells as apogeneric marker in patients with cancer. Sci. Rep. 6, 28910 (2016).

290. Chen, J. et al. Metabolic reprogramming-based characterization of circulating tumor cells in prostate cancer. J. Exp. Clin. Cancer Res. 37, 127 (2018).

291. Liu, H., Ding, J., Wu, Y., Wu, D. & Qi, J. Prospective study of the clinical impact of epithelial and mesenchymal circulating tumor cells in localized prostate cancer. Cancer Manag. Res. 12, 4549–4560 (2020).

292. Antonarakis, E. S. et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N. Engl. J. Med. 371, 1028–1038 (2014).

293. Okegawa, T. et al. AR-V7 in circulating tumor cells cluster as a predictive biomarker of abiraterone acetate and enzalutamide treatment in castration-resistant prostate cancer patients. Prostate 78, 576–582 (2018).

294. Tagawa, S. T. et al. Expression of AR-V7 and AR(V667E) in circulating tumor cells correlates with outcomes to taxane therapy in men with metastatic prostate cancer treated in TAXANERGY. Clin. Cancer Res. 25, 1880–1888 (2019).

295. Markou, A. et al. PIM-1 is Overexpressed at a high frequency in circulating tumor cells from metastatic castration-resistant prostate cancer patients. Cancer 12, 1188–1201 (2020).
322. Hou, J. M. et al. Evaluation of circulating tumor cells and serological cell death biomarkers in small cell lung cancer patients undergoing chemotherapy. Am. J. Pathol. 175, 808–816 (2009).
323. Hou, J. M. et al. 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 (2012).
324. Huang, C. H. et al. A multicenter pilot study examining the role of circulating tumor cells as a blood-based tumor marker inpatients with extensive small-cell lung cancer. Front Oncol. 4, 271 (2014).
325. Naito, T. et al. Prognostic impact of circulating tumor marker cells in patients with small cell lung cancer. J. Thorac. Oncol. 7, 512–519 (2012).
326. Normanno, N. et al. Prognostic value of circulating tumor cells’ reduction in patients with extensive small-cell lung cancer. Lung Cancer 85, 314–319 (2014).
327. Messaritakis, I. et al. Phenotypic characterization of circulating tumor cells in the peripheral blood of patients with small cell lung cancer. PLoS One 12, e0181211 (2017).
328. Bidar, F. C. et al. Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. Ann. Oncol. 24, 2057–2061 (2013).
329. Court, C. M. et al. A novel multimarker assay for the phenotypic pro-

330. Gall, T. M. et al. Reduced dissemination of circulating tumor cells with no-touch isolation surgical technique in patients with pancreatic cancer. JAMA Surg. 149, 482–485 (2014).
331. Hugenschmidt, H. et al. Circulating tumor cells are an independent predictor of shorter survival in patients undergoing resection for pancreatic and peri-

332. Okubo, K. et al. Clinical impact of circulating tumor cells and therapy response in pancreatic cancer. Eur. J. Surg. Oncol. 43, 1050–1055 (2017).
333. Dong, X. et al. Spatial heterogeneity in epithelial to mesenchymal transition properties of circulating tumor cells associated with distant recurrence in pancre-

334. Zhao, X. H. et al. Molecular detection of epithelial-mesenchymal transition markers in circulating tumor cells from pancreatic cancer patients: Potential role in clinical practice. World J. Gastroenterol. 25, 138–150 (2019).
335. Zhu, P. et al. Circulating tumor cells expressing Kruppel-like factor 8 and vimentin as predictors of poor prognosis in pancreatic cancer patients. Cancer Control 20, 2057–2061 (2013).
336. Ou, H. et al. Circulating tumor cell phenotype indicates poor survival and recurrence after surgery for hepatocellular carcinoma. Dig. Dis. Sci. 63, 2373–2380 (2018).
337. Wang, P. X. et al. Circulating tumor cells are an indicator for the administration of adjuvant transther mal chemoembolization in hepatocellular carcinoma: a single-

338. Zhang, Q. et al. A prospective study on the changes and clinical signi-

339. Zhou, J. et al. Preoperative circulating tumor cells to predict microvascular invasion and dynamical detection indicate the prognosis of hepatocellular car-

340. Jin, L. et al. Evaluation of the diagnostic value of circulating tumor cells with epithelial-to-mesenchymal transition process in circulating tumor cells in hepatocellular carcinoma patients. Hepatol. Int. 10, 640–646 (2016).
341. Qi, L. N. et al. Circulating tumor cells undergoing EMT provide a metric for diagnosis and prognosis of patients with hepatocellular carcinoma. Cancer Res. 78, 4731–4744 (2018).
342. Wang, Z. et al. Correlation between postoperative early recurrence of hepato-

343. Court, C. M. et al. A novel multimarker assay for the phenotypic profiling of circulating tumor cells in hepatocellular carcinoma. Liver Transpl. 24, 946–960 (2018).
344. Yi, B. et al. The clinical significance of CTC enrichment by GP3C-IML and its genetic analysis in hepatocellular carcinoma. J. Nanobiotechnol. 19, 74 (2021).
345. Sun, Y. F. et al. Dissecting spatial heterogeneity and the immune-evasion mechanism of CTCs by single-cell RNA-seq in hepatocellular carcinoma. Nat. Commun. 12, 4091 (2021).
346. Yin, L. C. et al. Twist expression in circulating hepatocellular carcinoma cells predicts metastasis and prognosis. Biomed. Res. Int. 2018, 3789613 (2018).
347. Sun, Y. F. et al. Circulating stem cell-like epithelial cell adhesion molecule-

348. Lee, S. J. et al. Circulating tumor cells are predictive of poor response to che-

349. Uenosono, Y. et al. Clinical significance of circulating tumor cells in peripheral blood from patients with gastric cancer. Cancer 119, 3984–3991 (2013).
350. Zhang, Q. et al. A prospective study on the changes and clinical signi-

351. Zhang, Q. et al. A prospective study on the changes and clinical signi-

352. Zhang, Y. et al. Circulating tumor cells in advanced cervical cancer: NRG Oncology-Gynecologic Oncology Group study 240 (NCT 00803062). Mol. Cancer Ther. 19, 2363–2370 (2020).
353. Anand, K. et al. Pilot study of circulating tumor cells in early-stage and meta-

354. Kim, D. D., Yang, C. S., Chae, H. D., Kwok, S. G. & Jeon, C. H. Melanoma antigenencoding gene family member A1-6and iHETER in the detection of circulating tumor cells following CD45(-) depletion and RNA extraction. Oncol. Lett. 14, 837–843 (2017).
355. Mego, M. et al. Circulating tumor cells with epithelial-to-mesenchymal transition phenotypes associated with inferior outcomes in primary breast cancer. Anticancer Res. 39, 1829–1837 (2019).
356. Zhang, Y. et al. Utility of circulating tumor cells for detection of early-stage luminal breast cancer. Am. J. Med. Sci. 360, 543–551 (2020).
357. Stefanovic, S. et al. The lack of evidence for an association between cancer biomarker conversion patterns and CTC-status in patients with metastatic breast cancer. Int. J. Mol. Sci. 21, 2161–2171 (2020).
358. Jin, L. et al. Evaluation of the diagnostic value of circulating tumor cells with CytoSorter(R) CTC capture system inpatients with breast cancer. Cancer Med. 9, 1638–1647 (2020).
359. Shilakhhtouno, Y. A. CTCs-oriented adjuvant personalized cytostatic therapy non-

360. Schonhoff, J. D. et al. Morphology-predicted large-scale transition number in circulating tumor cells identifies a chromosomal instability biomarker associated with poor outcome in castration-resistant prostate cancer. Cancer Res. 80, 4892–4903 (2020).
361. Armstrong, A. J. et al. Prospective multicenter study of circulating tumor cell AR-V7 and taxane versus hormonal treatment outcomes in metastatic castrationresistant prostate cancer. JCO Precis. Oncol. 4, 1285–1301 (2020).
362. Xu, L. et al. Noninvasive detection of clinically significant prostate cancer using circulating tumor cells. J. Urol. 203, 73–82 (2020).
363. Sperger, J. M. et al. Prospective evaluation of clinical outcomes using a multiplex liquid biopsy targeting diverse resistance mechanisms in metastatic prostate cancer. J. Clin. Oncol. 39, 2926–2937 (2021).
364. Zhang, P. et al. The significance of detection of circulating tumor cells and BECLIN1 in peripheral blood of patients with renal cell carcinoma. Crit. Rev. Eukaryot. Gene Exp. 30, 483–492 (2020).
365. Liu, J. Z. et al. Circulating tumor cells can predict the prognosis of patients with non-small cell lung cancer after resection: retrospective study. Transl. Lung Cancer Res. 10, 995–1006 (2021).
366. Wang, P. P. et al. Circulating tumor cells as a new predictive and prognostic factor in patients with small cell lung cancer. J. Cancer 11, 2113–2122 (2020).
374. Ha, Y. et al. Circulating tumor cells are associated with poor outcomes in early-stage hepatocellular carcinoma: a prospective study. *Hepatol. Int.* **13**, 726–735 (2019).
375. Chen, Y. et al. Circulating tumor cells undergoing EMT are poorly correlated with clinical stages or predictive of recurrence in hepatocellular carcinoma. *Sci. Rep.* **9**, 7084 (2019).
376. Cheng, Y. et al. Diagnostic value of different phenotype circulating tumor cells in hepatocellular carcinoma. *J. Gastrointest. Surg.* **23**, 2354–2361 (2019).
377. Lei, Y. et al. Association of preoperative NANOG-positive circulating tumor cell levels with recurrence of hepatocellular carcinoma. *Front. Oncol.* **11**, 601668 (2021).
378. Szczepanik, A. et al. CD44(+) cytokeratin-positive tumor cells in blood and bone marrow are associated with poor prognosis of patients with gastric cancer. *Gastroncancer* **22**, 264–272 (2019).
379. Miki, Y. et al. Circulating CEA-positive and EpCAM-negative tumor cells might be a predictive biomarker for recurrence in patients with gastric cancer. *Cancer Med.* **10**, 521–528 (2021).
380. Kuroda, K. et al. Circulating tumor cells with FGFR2 expression might be useful to identify patients with existing FGFR2-overexpressing tumor. *Cancer Sci.* **111**, 4500–4509 (2020).
381. Wang, D. et al. Prognostic models based on postoperative circulating tumor cells can predict poor tumor recurrence-free survival in patients with stage II-III colorectal cancer. *J. Cancer* **10**, 4552–4563 (2019).
382. Bidard, F. C. et al. Circulating tumor cells and circulating tumor DNA detection in potentially resectable metastatic colorectal cancer: a prospective ancillary study to the unicancer Prodige-14 Trial. *Cells* **8**, 516–528 (2019).
383. Wang, L. et al. Circulating tumor cells as an independent prognostic factor in advanced colorectal cancer: a retrospective study in 121 patients. *Int. J. Colorectal Dis.* **34**, 589–597 (2019).
384. Messaritakis, I. et al. Evaluation of the role of circulating tumor cells and microsatellite instability status in predicting outcome of advanced CRC patients. *J. Pers. Med.* **10**, 235–347 (2020).
385. Pan, R. J. et al. Detection and clinical value of circulating tumor cells as an assisted prognostic marker in colorectal cancer patients. *Cancer Manag. Res.* **13**, 4567–4578 (2021).