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Perspective

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Circulating tumour cells for early detection of clinically relevant cancer

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Abstract

Given that cancer mortality is usually a result of late diagnosis, efforts in the field of early detection are paramount to reducing cancer-related deaths and improving patient outcomes. Increasing evidence indicates that metastasis is an early event in patients with aggressive cancers, often occurring even before primary lesions are clinically detectable. Metastases are usually formed from cancer cells that spread to distant non-malignant tissues via the blood circulation, termed circulating tumour cells (CTCs). CTCs have been detected in patients with early stage cancers and, owing to their association with metastasis, might indicate the presence of aggressive disease, thus providing a possible means to expedite diagnosis and treatment initiation for such patients while avoiding overdiagnosis and overtreatment of those with slow-growing, indolent tumours. The utility of CTCs as an early diagnostic tool has been investigated, although further improvements in the efficiency of CTC detection are required. In this Perspective, we discuss the clinical significance of early haematogenous dissemination of cancer cells, the potential of CTCs to facilitate early detection of clinically relevant cancers, and the technological advances that might improve CTC capture and, thus, diagnostic performance in this setting.

Sections

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Introduction

Globally, cancer is the second most common cause of death[1], driven by mortality rates that increase with disease stage at diagnosis[2]. Detecting cancer at an advanced stage severely limits treatment options and typically results in a poor prognosis. Indeed, the options available for patients with metastatic solid tumours are very rarely curative. Therefore, efforts in the field of early cancer detection and diagnosis are paramount to improving patient outcomes. Many strategic programmes focused on early cancer detection have been launched worldwide, including Europe's Beating Cancer Plan by the European Commission, the UK Research and Innovation Accelerating detection of disease challenge, and the US NIH/National Cancer Institute Cancer Moonshot initiative.

In the UK, advances in early cancer diagnosis over the past 5 years include the implementation of Rapid Diagnostic Clinics (RDCs), which have resulted in the detection of malignancies in 7% of patients referred owing to non-site-specific symptoms in England who would otherwise have experienced substantial delays in diagnosis[3]. The Welsh RDCs have reduced the mean time to a definitive diagnosis from 84.2 days to as low as 5.9 days (or 40.8 days if further investigations were required) for patients with vague symptoms raising suspicion of cancer[4]. These RDCs not only decrease the time to diagnosis for many patients but potentially also offer an ideal setting for the testing and application of novel diagnostic biomarkers of cancer. Of note, however, the COVID-19 pandemic has caused delays in cancer diagnosis for many patients, subsequently increasing morbidity and mortality[5,6]. Better biomarkers and novel approaches that facilitate cancer detection are needed to accelerate diagnosis and mitigate the delays and challenges presented by pandemics such as those caused by COVID-19.

The current gold standard for cancer diagnosis is histopathology, which usually involves an invasive procedure to biopsy solid tumour tissue. Indeed, multiple biopsy samples can be required, yet this approach still provides only limited information on tumour heterogeneity. In addition, biopsy samples cannot be obtained from individuals who have no clinical evidence of cancer, severely limiting the capacity for early detection, or from those who are not fit enough to undergo such invasive procedures. Notably, advanced imaging technologies, such as multi-parametric MRI and PET–CT, can only detect primary tumours and metastases that are already well established, consisting of $>10^9$ cells[7]. Therefore, research on liquid biopsy assays has expanded rapidly, with the exploration of many different biomarkers in distinct bodily fluids for the evaluation of various solid malignancies[8]. Liquid biopsy sampling is minimally invasive or non-invasive, enabling repeat sampling in the same individual to detect cancer as well as to assess treatment response and/or monitor for disease progression[9]. Furthermore, circulating cancer-derived material can originate from both primary and (micro)metastatic sites, potentially providing a better representation of the entire heterogeneous tumour cell population than that afforded by tissue biopsy sampling[10].

Metastasis is the main cause of cancer-related death and can occur at an early stage of tumour development in patients with aggressive cancers[11]. The first step of metastatic dissemination involves cancer cell invasion into the blood circulation via which the cells can spread to other parts of the body. Studies focused on disseminated tumour cells (DTCs) in the bone marrow of patients with breast cancer have revealed early metastatic spread to distant sites even in patients with small, early stage tumours[12]. However, DTCs can enter dormancy such that the metastatic lesion might form and subsequently be detected many years after initial cancer cell dissemination[13,14]. Therefore, circulating tumour cells (CTCs) in the blood might be the first indicators of the early steps of cancer metastasis, might enable monitoring for this process in a minimally invasive manner and have the potential to be applied as a tool for the early detection of aggressive cancers. CTCs can have prognostic utility as evidenced by the FDA approvals of the CELLSEARCH platform for the prediction of progression-free and overall survival in patients with metastatic prostate cancer[15], breast cancer[16,17] or colorectal cancer (CRC)[18,19]. The prognostic value of CTCs has also been demonstrated in patients with bladder cancer[20,21], head and neck cancer[22,23], and pancreatic cancer[24]. However, the research community is increasingly focusing on the potential use of CTCs in early cancer detection and diagnosis. Given that not all cancers are lethal and some remain latent for many years, the diagnosis of slow-growing cancers can lead to overdiagnosis and overtreatment, exposing patients to more harm than potential benefits and increasing the costs incurred by health-care systems for unnecessary diagnostic procedures and treatments[25]. Therefore, for liquid biopsy assays to be effective tools for early cancer diagnosis, they must focus on identifying aggressive cancers that require immediate treatment, and the analysis of captured CTCs might have an advantage in this regard.

We hypothesize that aggressive cancers with metastatic potential will release CTCs very early during tumorigenesis. Herein, we discuss the literature on the metastatic process and CTC analysis with a particular emphasis on their salience at early stages of cancer development, highlighting research advances to support our hypothesis. We also provide our thoughts on future directions for research to facilitate the application of CTCs in early cancer detection, particularly technological improvements to increase the sensitivity of CTC assays. Although CTCs might also be used to detect minimal residual disease, this application is beyond the scope of this Perspective and has been reviewed elsewhere[26].

Cancer metastasis

Metastatic colonization of secondary sites is initiated by cancer cells accumulating alterations affecting genes involved in various processes identified as the hallmarks of cancer[27,28], which support the migration, invasion, survival and eventual outgrowth of these cells beyond their tissue of origin. The metastatic cascade starts with local invasion of the primary tumour cells into their surrounding microenvironment and subsequent migration across the endothelial barrier, intravasating into the blood or lymphatic system[29]. After entering the circulation, CTCs are carried in the blood to other body sites, where they can extravasate, proliferate and establish metastatic lesions (Fig. 1).

Cancer cells can undergo epithelial-to-mesenchymal transition (EMT) to facilitate their detachment from the primary tumour and intravasation into the blood circulation[30]. EMT involves the loss of epithelial characteristics, for example, downregulation of the adhesion molecule E-cadherin, and the acquisition of mesenchymal characteristics, including expression of the cytoskeletal protein vimentin[31]. Vimentin has been found to be overexpressed in numerous malignancies, such as breast cancer[32,33] and extrahepatic cholangiocarcinoma[34], and is associated with cancer cell invasiveness and metastasis[35]. The process of EMT is mediated through TGFβ, Wnt and Notch signalling and can be controlled by the transcription factors SNAI1 (also known as SNAIL), SNAI2 (SLUG), TWIST1 and FOXC2 (ref. 36). This invasion process can involve single cancer cells or clusters of tumour cells. Intravasation of single cells can be mediated through TGFβ signalling[37], whereas the intravasation of cell clusters might be initiated by primary tumour hypoxia[38], although various alternative pathways probably also contribute to this process.

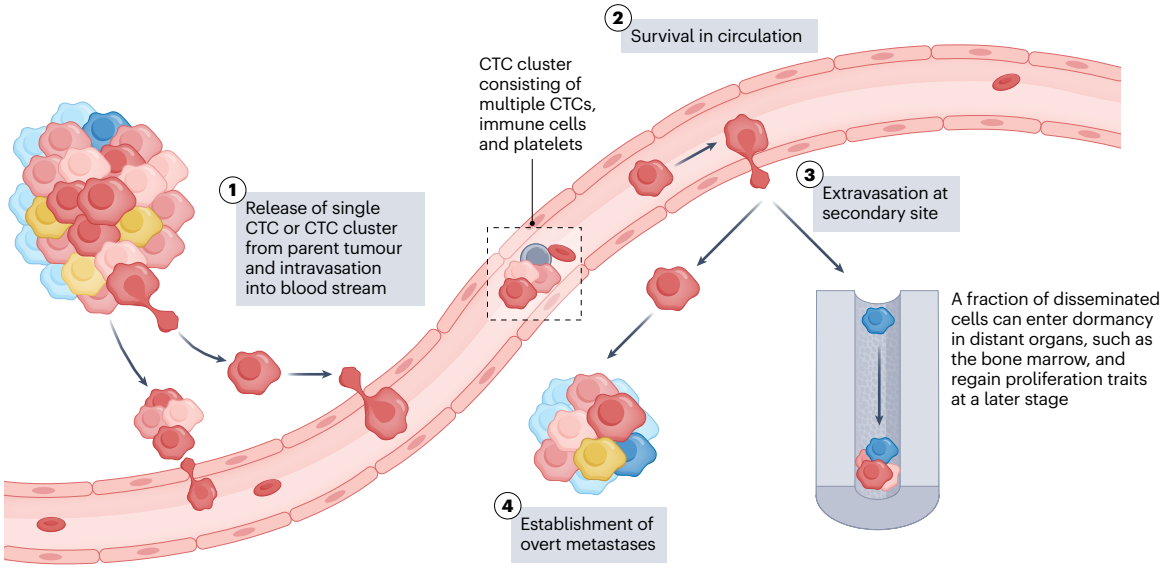

Fig. 1 | Key steps in the formation of metastases by CTCs. Dissemination of cancer cells from the primary tumour into circulation (step 1) can involve either single cells or cell clusters containing multiple circulating tumour cells (CTCs) as well as immune cells and platelets, known as microemboli. CTCs that can survive in circulation (step 2) can exit the bloodstream (step 3) and establish metastatic tumours (step 4), or they can enter dormancy and reside in distant organs such as the bone marrow (inset). Dormant disseminated cancer cells can regain proliferative capacity at a later stage and establish overt metastatic lesions after a long latency of several months or even years, depending on the primary tumour type.

One of the most crucial and under-investigated steps in the metastasis process involves CTC intravasation and survival in the blood[39] prior to their extravasation at a secondary site. After infiltrating the circulation, CTCs are subject to a harsh environment in which they must undergo complex, adaptive processes in order to survive. For example, both hydrodynamic forces[40] and the actions of immune cells can result in CTC death[41]. Nascent CTCs can also undergo anoikis after detachment from the extracellular matrix[42], and sudden changes in cellular oxygen levels after entering the blood can require rapid adaptation of cancer cells[43].

Extravasation of CTCs involves their interaction with endothelial cells lining the surrounding blood vessel, a process mediated through integrin adhesion[44]. Provided that the new niche has a suitable microenvironment for cancer cell survival and growth, the invading CTCs can proliferate and establish overt metastatic lesions (Fig. 1). Therefore, only if the metastatic cascade is successfully completed will patients develop secondary tumours, at which point their treatment options will be limited. By contrast, detection of CTCs in patients with early stage cancers might not necessarily preclude curative therapy as long as they have no signs of overt metastases. However, CTC detection might indicate the presence of an aggressive cancer with high propensity for metastatic dissemination, warranting the addition or intensification of adjuvant systemic therapy with the aim of eradicating any occult micrometastases. Conversely, an absence of CTCs might provide opportunities for treatment de-escalation in some disease settings provided that the detection methods have sufficient sensitivity.

Early dissemination of cancer cells

Cancer cell spread occurs early but is usually detected late

Metastases are usually detected at late stages of cancer development when cancer cells have evolved to form a large tumour burden beyond the primary organ of origin, which typically precludes curative therapy and results in a short survival duration. Nevertheless, many patients with cancer do not die from metastatic disease until years after their initial diagnosis, with several lines of evidence indicating that cancer cell dissemination can be an early event in tumorigenesis. In early work from the 1950s onwards, calculations based on cell proliferation rates suggested that primary tumours as small as 5 mm in diameter can metastasize to multiple sites months or even years before their detection[45–47]. Subsequently, the detection of DTCs has provided direct evidence that the initial dissemination to distant sites often occurs at an early stage of cancer development. Indeed, DTCs have been identified in patients with various early stage cancers[48,49], including the earliest stages of gastric cancer[48], invasive breast cancer[12] and even ductal carcinoma in situ[50–52]. Notably, the detection of DTCs in the bone marrow of patients with CRC, a cancer type in which overt skeletal metastases are very rare, suggests that cancer cell spread and outgrowth can be two distinct biological processes[53]. Patients with breast ductal carcinoma in situ can also have clinically undetectable micrometastases or occult secondary lesions[52]. This phenomenon is perhaps attributable to micro-invasion of tumours (with no invasive foci >1 mm), which are only detectable by immunocytochemistry of bone marrow aspirates[52]. Furthermore, CTCs have been detected in blood samples years before a clinical cancer diagnosis[54]. In mouse models, DTCs were detectable soon after orthotopic implantation of a small number of breast cancer cells[50], and CTCs with mesenchymal features were detected in the blood prior to pancreatic tumour detection by rigorous histological analysis[55]. These findings emphasize that EMT and dissemination of cancer cells can precede tumour discovery.

Studies using next-generation sequencing technologies to measure the genomic divergence between primary and metastatic tumours have suggested that early micrometastasis, before primary tumour detection, is a common feature of many human cancers[56–58]. For example, exome-sequencing data revealed a low level of genomic

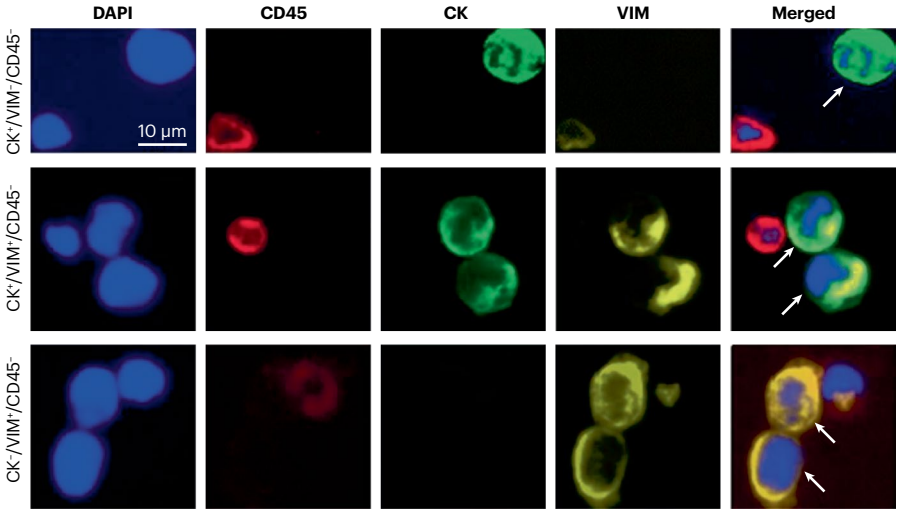

Fig. 2 | Different subtypes of CTCs in association with epithelial-to-mesenchymal transition. The immunofluorescent microscopy images illustrate three subsets of circulating tumour cells (CTCs) with differential expression of characteristic markers isolated from patients with prostate cancer using the cell size-based Parsortix isolation system. The first row shows a representative epithelial CTC, which is positive for the epithelial marker cytokeratin (CK) and negative for the mesenchymal marker vimentin (VIM) and the leukocyte marker CD45. The middle row depicts two CTCs undergoing epithelial-to-mesenchymal transition as determined by positive staining for both CK and VIM. The last row features two mesenchymal CTCs, which are positive for VIM and negative for CK. Reprinted with permission from ref. 76, American Association for Cancer Research.

divergence between paired primary colorectal tumours and brain or liver metastases[57]. In addition, the fact that cancers of unknown primary account for 3–5% of all malignancies[59] provides further hints of early metastatic dissemination while the primary tumour remains undetectable with current diagnostic technologies. Furthermore, for more than 50 years it has been known that cancer can sometimes be inadvertently transmitted to patients transplanted with apparently healthy organs, suggesting that cancer cell dissemination occurred at an early stage in tumour development and remained undetected in the donor organs[49].

Cancer cell dormancy can explain the late detection of metastasis

Dormancy of DTCs might be the main reason for the late detection of metastasis, long after cancer cell dissemination early in primary tumour development. DTC dormancy, during which the cells usually have a low proliferative index (as assessed by Ki67 staining)[53], is described as a state of G0–G1 phase mitotic arrest from which cells have the capacity to regain proliferative traits and establish overt metastatic lesions, often many years later[13]. Exit from the dormant phase can occur even after surgical removal of the primary tumour[60]. For example, ~40% of patients with prostate cancer treated by radical prostatectomy have biochemical recurrence, suggesting the presence of DTCs or undetectable micrometastases at the time of surgery[61,62]. DTCs detected many years after primary tumour resection must have been released into circulation before the apparently successful surgical treatment, forming occult micrometastases at a later stage[14].

In various mouse models, DTCs that are released early in tumorigenesis can exhibit metastatic capacity[63,64] although they often initially enter dormancy[65]. DTCs in a state of cell cycle arrest are hypothesized to be insusceptible to therapies that target the increased proliferative activity of tumour cells[66,67]. DTCs obtained from the bone marrow of patients with breast and gastrointestinal cancers have been shown to have a very low Ki67 index[53]; therefore, these non-cycling cells are likely to be more resistant to cytotoxic therapies such as the typical chemotherapy regimens administered to patients with solid tumours. However, therapeutic agents with activity against DTCs and CTCs that are in G0–G1 phase of the cell cycle (for example, immune-checkpoint inhibitors such as anti-PD-(L)1 antibodies)[68] or senolytic drugs (for example, BCL-2 inhibitors) that can re-activate the apoptotic pathway to eliminate cells under cell cycle arrest might be effective in eradicating micrometastases[69]. In addition to the current limitations in detecting micrometastases, DTC dormancy leads to understaging, and thus undertreatment, of many aggressive tumours, presenting an obstacle to effective eradication of cancer and leading to later metastatic relapse. However, cancer cell dormancy might also provide a therapeutic window of opportunity to cure cancer before the metastases are well established with many diverse subclones and a protective local ecosystem. Nevertheless, research into cancer cell dormancy is limited owing to the invasive and technically challenging nature of sample acquisition, particularly as DTCs are most commonly obtained from bone marrow[70]. Further understanding of the mechanisms underpinning cancer cell dormancy is crucial to predict metastatic potential, and Cancer Research UK and the US National Cancer Institute have jointly proposed a Cancer Grand Challenge to investigate this major issue. Currently, biomarkers for measuring local cancer cell invasion are lacking; however, once invasion into circulation has taken place, CTCs might hold a wealth of information for monitoring micrometastasis and assessing the risk of eventual overt metastasis.

Circulating tumour cells

CTCs are cancer cells that have shed from the solid tumour and entered the circulation. As previously mentioned, they have the capacity to extravasate at a different anatomical site and establish overt metastatic lesions. Although their existence has been known for more than 150 years[71], only in the past few decades has technology advanced to enable experimental investigation of CTCs and evaluation of their biomarker utility[72]. Anti-epithelial cell adhesion molecule (anti-EpCAM) antibody-based CTC capture technologies have played an important part in the initial clinical application of CTCs as a biomarker and one of these systems, the CELLSEARCH platform, was the first CTC detection technology approved by the FDA for clinical use[73]. However, the EMT phenomenon is important to consider when isolating CTCs given that expression of EpCAM is typically reduced during this transition[74]. Epitope-independent isolation strategies might therefore be more effective in isolating all subtypes of CTCs. For example, CTCs with both epithelial and mesenchymal characteristics have been identified in patients with prostate cancer using the cell size-based Parsortix isolation system[75,76] (Fig. 2). Other CTC isolation methods based on cell

size or density, including the Isolation by Size of Epithelial Tumor cells (ISET)[77], FaCTChecker[78] and Oncoquick[79] platforms, also have demonstrated utility for harvesting CTCs independently of epithelial marker expression. Evidence indicates that certain subtypes of CTCs (such as those with partial EMT or mesenchymal phenotypes) have greater potential to seed distant metastases and are associated with a poor prognosis in patients with various cancers[76,80]. In addition, emerging evidence suggests that not only are CTC numbers increased during sleep or rest phases but these CTCs also have an increased ability to metastasize than those generated during active phases[81].

Clusters of CTCs with and without leukocytes might have an important role in seeding distant metastases[82]. These CTC clusters (microemboli) are defined as groups of two or more CTCs and can consist of CTCs alone (homotypic) or can include various stromal cells, such as cancer-associated fibroblasts, and/or platelets and immune cells (heterotypic)[83,84]. The greater metastatic capacity of CTC clusters compared with individual CTCs has been demonstrated in mouse models of breast cancer[85], with one study finding that 97% of metastases originated from CTC clusters[86]. Data indicates that CTC clustering can lead to DNA hypomethylation of binding sites for transcription factors that promote cell stemness and proliferation, thus enhancing their metastatic potential[87]. In addition, a study involving patients with breast cancer found that neutrophils interacting with CTCs induced upregulation of genes involved in cell cycle progression in cancer cells, perhaps leading to more efficient formation of metastases[82]. Similar findings have been reported for platelet-associated CTCs[88]. Furthermore, non-cancer cells present in such heterotypic clusters might protect the CTCs from hydrodynamic shear stress and immune attack[89]. Given that CTCs in clusters still also engage in some degree of cell–cell adhesion, they can provide stimuli to one another during their circulation in the blood, potentially protecting each other from anoikis[83]. Clustering of CTCs (which might each have different phenotypes) is also likely to better enable them to foster a supportive ecosystem after extravasation[87]. Data from mouse models suggest that, owing to their larger size, CTC clusters are more likely than single CTCs to become mechanically trapped in capillaries, increasing their potential to extravasate at secondary sites[90]. This phenomenon has also been noted in patients with metastatic breast and cervical cancer, with CTC retention observed in the lung microvasculature[91]. CTC clusters have also been reported in patients with prostate cancer but at a varying rate depending on the isolation strategy applied[92–94]. These findings suggest that CTC clusters are associated with metastasis development, and this has been confirmed in a cohort of patients with breast cancer[81]. Importantly, CTC clusters might provide clinically relevant information on tumour heterogeneity. For example, one study involving patients with prostate cancer reported varying levels of cytokeratin expression among CTCs within the same cluster[95], and evidence derived from patients with breast cancer indicates that expression of specific types of cytokeratins (such as cytokeratin 16) might affect the biology and metastatic potential of CTCs[96].

A multitude of characteristics can be measured in CTCs, including genetics and epigenetics as well as protein levels, which might help us to understand many processes involved in the formation of metastases (Fig. 3). For example, single-cell whole-exome sequencing of CTCs derived from 10 patients with localized high-risk prostate cancer revealed thousands of single-nucleotide variants, insertions and/or deletions (indels), and copy-number alterations, which were ultimately associated with pathways involved in telomere preservation, DNA damage repair and response to docetaxel chemotherapy[97]. Genetic analysis of CTCs might also provide additional information on tumour mutational burden and intrapatient heterogeneity in mutational profiles[98]. Furthermore, evidence indicates that epigenetic profiling of CTCs might be clinically important, with *SOX17* hypermethylation noted in CTCs from a substantial proportion of patients with breast cancer, including up to 54% of those with early stage disease[99], suggesting silencing of this tumour suppressor gene. Increased hypermethylation of the tumour suppressor genes *CST6* and *BRMS1* has also been noted in CTCs from patients with metastatic breast cancer compared with CTCs derived from those with apparently localized disease[100]. Hence, CTC analysis might help us to better understand EMT and intravasation mechanisms as well as the control of DTC dormancy, and also help us to discriminate between latent and aggressive cancers.

In addition, CTC quantification might be indicative of tumour burden in patients with aggressive cancers as reported in a study involving patients with primary lung adenocarcinoma[101]. However, in malignancies such as prostate cancer, for which the cancer grade group is determined by pathological grade rather than tumour size (T stage), CTC positivity and number are more dependent on tumour aggressiveness than purely on tumour burden[102]. Interestingly, CTCs have been found to pass through the blood–brain barrier and can therefore be detected in patients with primary brain tumours[103]. Although most of the published research on CTCs to date has been focused on their prognostic capacity in patients with advanced-stage cancers, exploration of the utility of CTCs in the field of early cancer detection is now increasing (Fig. 3).

CTCs in early detection of cancer

Early research on CTCs did not explore their utility in the diagnosis of early stage cancers because CTCs were initially thought to be a feature of advanced-stage disease and also owing to the limitations of technologies to detect such scarce cells[104]. However, evidence suggests that the process of local invasion and intravasation of cancer cells can occur quickly, in a timescale of hours[105], and therefore CTC detection might be able to precede a clinical cancer diagnosis. This possibility is supported by data from genetically engineered PLCY mouse models of pancreatic intraepithelial neoplasia and pancreatic ductal adenocarcinoma, in which fluorescently tagged transgenic pancreatic epithelial

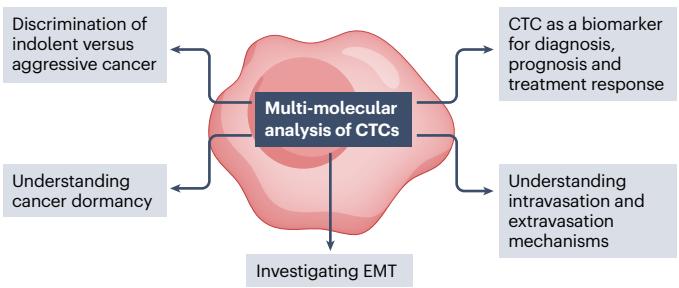

Fig. 3 | Characterization of CTCs to increase understanding of metastasis and improve the management of cancer. Multi-molecular analyses of circulating tumour cells (CTCs) can provide a wealth of information on various processes involved in cancer dissemination and metastasis, including cancer cell intravasation, extravasation, dormancy and epithelial-to-mesenchymal transition (EMT). In addition, successful detection of cancer cell dissemination in the form of CTCs can provide less invasive biomarkers for cancer diagnosis and prognostication – considering that the presence of these cells in the blood inherently differentiates clinically relevant aggressive cancers from indolent tumours – as well as for predicting and monitoring treatment response.

cells were detectable in the circulation and seeded the liver before frank pancreatic tumours could be detected by histopathological and fluorescence imaging[55].

A number of clinical studies have assessed the potential of CTCs for cancer detection using blood samples from patients with a known cancer diagnosis (Table 1). For example, CTCs have been detected in patients with early stage (stage I–IIIA) breast cancer[16,17,106,107], with more than one CTC detected in 20% of patients with stage I disease, 26.8% with stage II disease and 26.7% with stage III disease[108]. CTCs have also been detected in patients with non-metastatic CRC, including stage I and II disease, using the CELLSEARCH technology[19] and the CellMax microfluidic platform (which also involves anti-EpCAM antibody-based cell capture)[109]. In patients with non-metastatic prostate cancer, CTCs have also been detected using the CELLSEARCH technology[110,111]; using the Parsortix isolation system based on cell size and deformability, we detected CTCs in >50% of patients with localized disease[76,102]. In a study using size-based cell filtration followed by morphological characterization, CTCs were detected in 49% of patients with stage I non-small-cell lung cancer (NSCLC), which was not different from the frequency of detection in patients with stage II to IV disease (48%, 48% and 52%, respectively)[77]. Furthermore, a meta-analysis of 18 prospective studies found that CTC positivity is a promising biomarker for predicting unfavourable overall survival in patients with early stage NSCLC (HR 3.53, 95% CI 2.51–4.95; $P < 0.00001$)[112], emphasizing the potential of CTCs to predict aggressive cancers and thus potentially guide the development of novel treatment strategies. In the context of pancreatic cancer, CTCs were detectable using the EpCAM-based NanoVelcro CTC chip in 60% of patients with stage II disease, and CTC positivity discriminated patients with pancreatic ductal adenocarcinoma from those with non-adenocarcinoma pancreatic diseases with a sensitivity of 75% and specificity of 96.3% (AUC 0.867, 95% CI 0.798–0.935; $P < 0.001$)[113].

In a prospective study to predict the biopsy-based diagnosis of 98 individuals with suspected prostate cancer prior to biopsy, CTC detection using the Parsortix isolation system was strongly correlated with clinically significant cancer[102] (Table 1). The study cohort included patients with symptoms suggestive of prostate cancer, concerning serum prostate-specific antigen (PSA) levels and/or an abnormal digital rectal examination. Clinically significant cancer was defined based on serum PSA level, Gleason score and clinical stage[114,115]. CTC positivity was defined as any epithelial CTC ($CK^+vimentin^-CD45^-$), any 'EMTing' CTC ($CK^+/Vimentin^+/CD45^-$) and/or more than three mesenchymal CTCs ($CK^-vimentin^+CD45^-$)[102]. CTC positivity score (as defined above) combined with serum PSA levels predicted the biopsy-based diagnosis of clinically significant prostate cancer with an AUC of 0.869, and additional inclusion of transcriptomic analysis of CTCs using a 12-gene panel increased predictive accuracy, with an AUC of 0.927 (ref. 102). In a study involving 265 asymptomatic individuals without a cancer diagnosis but with risk factors for various malignancies (including a family history of cancer, or lifestyle factors or medication usage associated with increased risk), 132 (49.8%) had detectable CTCs using the ISET technology[116]. Although this high CTC positivity rate requires independent validation, follow-up tests performed within 10 months of CTC detection revealed early cancerous lesions in 20% of CTC-screened individuals; prostate-specific membrane antigen-based PET scans provided evidence of early stage prostate cancer in 50% of men with physiologically normal serum PSA levels but detectable CTCs[116]. In another study using the ISET technology in 168 patients with chronic obstructive pulmonary disease, the 5 patients with detectable CTCs all subsequently had NSCLC diagnosed within 1–4 years through annual CT-based screening[54]. Importantly, all 5 patients had resectable tumours and none had evidence of disease recurrence by CT or ISET at 12 months after surgery. In a larger screening study using the same technology in a cohort of 614 patients with chronic obstructive pulmonary disease, CTCs were identified in 5 patients found to have prevalent lung cancer, although the sensitivity was only 26.3%[117]. Moreover, baseline CTC status did not predict the development of 19 interval lung cancers missed on initial low-dose CT screening or extrapulmonary cancers[117] (Table 1).

Together, these studies show that CTCs have the potential to be used in the early detection and/or diagnosis of clinically relevant cancers, although further investigations and technical improvements are required. Currently, many ongoing clinical trials are investigating the use of CTCs for early cancer diagnosis, including the PROLIPSY trial for prostate cancer (NCT04556916) and similar trials for breast cancer (NCT03511859), NSCLC (NCT02380196), CRC (NCT05127096) and pancreatic cancer (PANCAID). If these clinical trials produce positive results, the use of CTCs in early cancer detection might become a reality.

Future directions

Although the literature to date suggests that CTC analyses can provide viable biomarkers with applications in various aspects of malignancy (from diagnosis to monitoring treatment response), more research is required to develop CTCs as reliable biomarkers for early cancer detection. Despite the remaining challenges, emerging data underscore the promise of liquid biopsy of CTCs in early cancer detection. Most importantly, owing to their integral link with metastasis, and thus aggressive cancers, CTCs have an advantage over many other non-invasive or minimally invasive biomarkers in specifically identifying invasive cancers for early therapeutic intervention at a stage when the disease is still curable. This advantage might also help to avoid overdiagnosis of indolent cancers, which is a major issue in many malignancies such as prostate, breast, lung and thyroid cancers[118]. Here, we share our thoughts on the directions of future developments to accelerate the application of CTCs in early cancer detection (Box 1).

Maximizing CTC detection

Currently, the main issue restricting the use of CTCs for early cancer detection relates to the scarcity of these cells in routine blood sample volumes, which limits their sensitivity in the detection of cancer[117]; therefore, it is important to maximize the number of CTCs available for analysis. Given the inherently low numbers of CTCs released by early stage cancers, however, another obvious approach is to improve the sensitivity of the detection technologies. Capturing all CTCs has proven difficult with epitope-dependent isolation strategies and, subsequently, research has been focused on developing and applying isolation methods based on cell size, density or morphology to increase CTC yield[54,75]. Each of these technologies has its limitations, such as the omission of EpCAM-negative CTCs undergoing EMT with traditional epitope-based isolation platforms or of small CTCs with devices involving selection based on cell size. Thus, a combination of multiple selection methods might be more efficient in gathering the CTCs to study; however, although complex sequential selection strategies are likely to increase CTC purity, they might also be accompanied by an increase in cell loss during each processing step. Continued validation of CTC detection technologies will be key to ultimately obtaining approval of such systems for early cancer detection. This process

Table 1 | Studies of CTCs for early detection and screening of different cancers

Cancer type	Patient cohort	CTC isolation technology	Results	Ref.
CTC detection in patients with known cancer				
CRC, prostate cancer and ovarian cancer	Patients with biopsy-proven cancer	Density-based isolation using Lymphoprep or cell size-based filtration with track-etched membranes	CTCs were detected in 0 of 11 donors without known cancer (0%), 23 of 25 patients with CRC (92%), 10 of 10 patients with prostate cancer (100%) and 4 of 4 patients with ovarian cancer (100%)	170
NSCLC	Newly diagnosed, treatment-naive patients	High-definition CTC assay based on cell morphology and high-throughput counting	CTCs were detected in 57 of 78 patients (73%); no difference in median CTC counts between disease stages	171
NSCLC	250 patients with resectable NSCLC and 59 individuals without cancer	ISET (cell size-based platform)	Malignant, circulating non-haematological cells were detected in 41% of patients with NSCLC and in 0% of individuals without cancer	77
PDAC	Patients evaluated prior to surgical treatment (early disease) or palliative chemotherapy (advanced disease)	ScreenCell (cell size-based isolation platform)	CTCs detected in 3 of 4 patients with early disease (75%) and 5 of 7 with advanced-stage disease (71%); no CTCs detected in 9 donors without known cancer	172
PDAC	Patients evaluated prior to treatment	EpCAM-based NanoVelcro CTC chip	CTCs detected in 54 of 72 patients (75%); CTCs as a biomarker for PDAC had an AUC of 0.867, 75% sensitivity and 96.4% specificity	113
Gastric cancer	Treatment-naive patients, prior to surgery	FAST disc (cell size-based centrifugal microfluidic system; CK^+ or $EpCAM^+$ cells counted)	105 of 116 patients with gastric cancer (91%) and 3 of 31 donors without known cancer (10%) had detectable CTCs	173
CRC	9 patients with CRC and 1 with tubular adenoma	CellMax (microfluidic chip coated with anti-EpCAM antibodies)	8 of 10 patients with cancer or adenoma (80%) and 1 of 5 donors without known cancer (20%) had detectable CTCs	174
CRC	287 patients with CRC evaluated prior to surgery, including 239 with non-metastatic disease	CELLSEARCH (EpCAM-based isolation platform)	44 patients had detectable CTCs (15%) and CTC detection correlated with disease stage	110
Breast cancer	Treatment-naive patients with stage I–IV disease	Magnetic-activated cell sorting (CK^+ cell selection)	CTCs were detected in 0 of 4 (0%), 6 of 22 (27%), 6 of 6 (100%) and 2 of 3 (67%) patients with stage I to IV disease, respectively	175
Breast cancer	Patients with treatment-naive breast cancer (n=102), patients with benign breast disease (n=177) and women without cancer (n=64)	CytoSorter (EpCAM-based detection using a microfluidic-based immune-capture CTC platform)	CTCs detected in 17.2% of women without cancer, 40.7% of patients with benign breast disease and 91.2% of patients with breast cancer; CTC detection had an AUC of 0.889 for early to mid-stage breast cancer, with a specificity of 93.8%	107
Breast cancer	Patients evaluated prior to surgical treatment (curative setting)	Nanostructured titanium oxide-coated slides to capture all cells post erythrolysis	16 of 28 patients (57%) had CTCs; 5 of 28 patients (18%) had detectable clusters; 1 of 30 healthy donors (3%) had detectable CTCs	108
Prostate cancer	Men with newly diagnosed, high-risk, non-metastatic prostate cancer	CELLSEARCH	5 of 36 patients had detectable CTCs (14%), including 1 patient with a circulating tumour microemboli	111
Prostate cancer	155 treatment-naive patients with localized disease	Parsortix (cell size-based platform)	84 of 155 patients (54%) were positive for CTCs, which were associated with a higher Gleason score (P=0.0003), risk group (P<0.0001) and clinically significant prostate cancer (P<0.0001)	102
CTC detection preceding a cancer diagnosis				
Lung cancer	Patients with COPD without clinically diagnosed lung cancer	ISET	5 of 168 patients (3%) had detectable CTCs at baseline assessment and all developed lung nodules within 1–4 years	54
Multiple cancer types	542 individuals, including 277 with and 265 without known cancer but with risk factors	ISET	CTCs were detected in 277 of 277 patients with known cancer (100%) and 132 of 265 individuals without known cancer (50%); standard diagnostic imaging performed within 10 months subsequently revealed early cancers in 24 of 132 individuals with detectable CTCs (20%)	116
CRC	667 participants before colonoscopy, including 235 healthy individuals and 432 patients with either CRC or adenomas	CellMax	AUC for detection of CRC was 0.940, with a sensitivity of 95.2%; AUC for adenoma detection was 0.868; healthy individuals could be distinguished from patients with CRC or adenoma based on CTC count	109

Table 1 (continued) | Studies of CTCs for early detection and screening of different cancers

Cancer type	Patient cohort	CTC isolation technology	Results	Ref.
CTC detection preceding a cancer diagnosis (continued)				
Lung cancer	614 individuals meeting the eligibility criteria for lung cancer screening	ISET	5 of 614 individuals had detectable CTCs (0.8%) and all were found to have lung cancer; sensitivity of CTC positivity for lung cancer detection was 26.3%, with a specificity of 96.2%, a negative predictive value of 97.6% and a positive predictive value of 18.4%	117
Prostate cancer	98 patients with suspected prostate cancer based on high serum PSA levels and/or abnormal digital rectal examination	Parsortix	Positive CTC predicted biopsy outcome and prostate cancer aggressiveness (AUC of CTC positivity was 0.811); the combination of CTC score, a 12-CTC-gene panel and serum PSA level generated an AUC of 0.927	102

Table includes published studies in which CTCs were evaluated as a potential diagnostic biomarker in patients with known cancer or preceding a cancer diagnosis. Prognostic studies are not included unless baseline, pre-treatment CTC data was available. CK, cytokeratin; COPD, chronic obstructive pulmonary disease; CRC, colorectal cancer; CTC, circulating tumour cell; EpCAM, epithelial cell adhesion molecule; ISET, Isolation by Size of Epithelial Tumor cells; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; PSA, prostate-specific antigen.

might involve standardization of technologies with interlaboratory ring trials to assess reproducibility and data comparability as is being performed by the European Liquid Biopsy Society, which could eventually lead to recommendations on minimally required procedures for reporting studies based on CTCs. These recommendations will include requirements regarding pre-analytical and quality-control processes that enable optimal results with the use of CTCs as clinical biomarkers. Pre-analytical factors, such as the time and anatomical site of blood draw, needle size and type of blood collection tube, ambient temperature, and storage conditions, should all be carefully considered and outlined in CTC analysis protocols. Machine learning and other artificial intelligence approaches might be applied to large datasets in wide-scale CTC analyses, and have already been used to investigate CTCs from patients with ovarian cancer[119]. However, these improvements might still be insufficient to realize the potential of CTC analysis for early cancer detection; increasing the absolute number of CTCs to be isolated through blood sampling at the optimal anatomical location and time, and perhaps even increasing the sample volume, might be the key. Besides blood, shed cancer cells can be detected in other body fluids, such as lymph or cerebrospinal fluids, but their isolation requires more invasive sampling procedures.

The use of different blood vessels for sample acquisition has already been explored to increase the chance of detecting CTCs. For example, blood sampling from the pulmonary vein in patients with NSCLC[120], the portal vein in those with pancreatic cancer[121] or the mesenteric vein in those with CRC[122] has been shown to increase the number of CTCs detected compared with peripheral blood sampling. However, these sites are difficult to sample routinely as required for application in cancer detection. As noted previously, data from a study in patients with breast cancer suggests that the time of blood sampling is an important consideration in capturing a sufficient number of CTCs for downstream analysis[81]. A substantially higher number of single CTCs and CTC clusters were detected in samples collected at 4:00 am (rest phase) compared to 10:00 am (active phase), with the data suggesting that 78.3% of CTCs are released during the rest phase at night[81]. This new information might lead to researchers altering the time of blood sampling to enhance CTC collection. Explanations for the differences in day and night time release of CTCs have been proposed[123,124]; further studies to determine the influence of circadian rhythms and other physiological conditions on CTC release for each cancer type should be carried out to enable the development of protocols for maximal CTC capture.

For clinical diagnostic applications, the smaller the volume of blood the better, ideally no more than the volume collected in a single 10-ml blood collection tube. The current routine blood sample volume used for CTC analysis is ≤7.5 ml (refs. 75,125). Nevertheless, the utility of CTCs for early cancer detection will be improved if the blood volume interrogated for these cells can be substantially increased. In this regard, Kim et al.[126] developed a temporary indwelling intravascular aphaeretic system connected to an external anti-EpCAM antibody-based microfluidic herringbone graphene oxide CTC chip that enabled sampling of 1–2% of the whole blood volume in canine models and the isolation of injected MCF-7 breast cancer cells. Apheresis systems have similarly been used in patients with metastatic prostate or breast cancer to markedly increase CTC isolation (by up to 75-fold compared with the numbers harvested from 7.5 ml of blood) through the sampling of large volumes of blood[127,128]. However, this approach might be too complicated and invasive for use in a routine diagnostic test, particularly for cancer screening. A simpler device, named CellCollector, has also been developed to capture CTCs from a large volume of blood. CellCollector consists of an anti-EpCAM antibody-coated medical Seldinger guidewire that can capture CTCs in vivo following insertion into a cubital vein through a standard cannula[129]. This device enabled the detection of CTCs in 12 of 12 patients with breast cancer and 10 of 12 patients with NSCLC, including those with early stage, non-metastatic disease, whilst no CTCs were detected in volunteers without cancer[129]. Another study using CellCollector detected CTCs in 108 (58%) of 185 applications (before and after treatment) among 50 patients with lung cancer, compared with 23 (27%) of 84 applications using the CELLSEARCH platform[130]. However, these devices remain at an experimental stage of development and do not capture CTCs with only mesenchymal features, which might be more strongly associated with unfavourable clinical outcomes than other subtypes of CTCs[76].

The development of wearable devices that can monitor a condition over time is an important direction for future research in the field of clinical diagnostics. If CTCs passing through a vein could be detected using an imaging device worn on the hand or arm, an efficient and non-invasive approach to early cancer detection could become a reality. Such technology will not only enable the identification of CTCs in a much larger blood volume but also facilitate monitoring of the temporal differences in CTC release. Development of a wearable sensor device will require the optimization of injectable probes, such as those with the capacity to generate near-infrared light, to label CTCs

in vivo for detection, although in vivo CTC detection without labelling has also been reported in patients with melanoma (discussed further below)[131]. Clearly, the development of both CTC-specific markers and the sensor device is currently very challenging considering the potential background noise of such an approach to CTC detection in vivo. Advances of relevance for in vivo imaging of cancer include the optimization of surface-enhanced Raman scattering nanoparticles that, when coupled with spatially offset Ramen spectroscopy, could successfully image glioblastoma tumours in mice[132]. This study importantly demonstrates the feasibility of developing probes and coupled detection systems that can successfully penetrate tissue to the substantial depth required for diagnostic imaging[132]. Single-cell in vivo imaging of CTCs has already been demonstrated in mouse models of CRC using real-time confocal fluorescence microscopy[133], and the technological developments and potential applications of in vivo imaging of CTCs have been reviewed elsewhere[134]. In mouse models of melanoma, CTCs could also be detected using melanin as an intrinsic marker coupled with in vivo photoacoustic flow cytometry[135,136]. Further optimization and subsequent clinical analysis of this technology revealed a detection sensitivity of 1 CTC per litre of blood (~1,000 times better than that of pre-existing assays), with CTCs being detected in 27 (96.4%) of 28 patients with melanoma and 0 of 19 individuals without cancer[131]. Considering the current stage and speed of technological development and understanding of tumour cell biological and physical features, in vivo detection of CTCs for early cancer detection might soon be feasible. Multidisciplinary collaboration between cancer researchers, physicists, bioengineers and clinicians, both in the academic and industry settings, will be required to achieve this ambitious goal.

Molecular characterization of CTCs

In addition to increasing our ability to isolate and/or detect CTCs, further molecular characterization of the CTCs might enhance the possibility of using these cells to distinguish clinically significant and non-significant, indolent cancers. Identifying different subsets of CTCs, for example, distinguishing between dormant and proliferative CTCs and CTCs associated with different immune cells[137], might be clinically useful. In practice, this approach could involve the addition of immune-specific markers to CTC cluster analyses and/or RNA sequencing of CTC clusters versus single CTCs to identify which types of immune cells associate with CTCs[82,138] and what effects these interactions have on specific cellular pathways and, thus, on metastatic potential. Standard immunofluorescence analysis is limited by the number of markers that can be measured simultaneously; therefore, novel technologies, such as multiplex immunofluorescence analysis (for example, the MACSima imaging cyclic staining technology)[139] or mass cytometry, might be useful. Imaging mass cytometry exploits metal isotopes conjugated to antibodies for the simultaneous analysis of up to 42 markers[140]. Thus, this platform has the potential to provide additional phenotypic information on individual CTCs, CTC–CTC clusters and CTC–immune cell clusters to further refine their clinical utility. Alternative technologies including imaging cytometry, which combines principles of flow cytometry and fluorescent microscopy, also offer the potential to measure multiple molecular markers on CTCs and might, therefore, enable the identification of novel biomarkers for aggressive cancers in the early detection setting. Single-cell analysis technologies will need to be developed further to reduce their cost and complexity in order to facilitate their clinical application. In addition, the ex vivo culture of CTCs will widen the window of opportunity for molecular and phenotypic analyses and can provide information on the therapeutic vulnerabilities of cancer cells in individual patients[141,142]. Such molecular investigations might also help to identify markers to be used for in vivo detection of CTCs using a sensor device.

Combining CTCs with other biomarkers for early cancer detection

Blood is a rich source for cancer biomarker discovery. Taking advantage of blood sampling, the utility of CTCs as an early cancer detection tool might be further improved through combinations with other blood-based biomarkers, including circulating proteins, circulating cell-free tumour DNA (ctDNA), microRNAs (miRNAs), extracellular vesicles and immune cell subsets, each of which has advantages and disadvantages relative to CTCs (Table 2).

Plasma and serum proteins have long been explored and used for early cancer detection[143]. For example, PSA has a crucial role in prostate cancer detection although, owing to a lack of specificity, PSA testing can lead to overdiagnosis, particularly of indolent cancers[144]. In the aforementioned study exploring the potential for diagnosing aggressive prostate cancers based on CTC detection using the Parsortix system, CTCs and serum PSA had similar diagnostic accuracy[102]. Moreover, combining both CTC and PSA analyses substantially increased test accuracy[102].

Box 1

Future directions to facilitate the use of CTCs in early cancer detection

- Optimize epitope-independent circulating tumour cell (CTC) isolation strategies.
- Alter the anatomical site of blood sampling to increase CTC yield, for example, by using a tumour-draining vein for individuals with a very high risk of certain cancers.
- Increase the volume of blood sampled using approaches such as apheresis or antibody-coated insertable intravascular devices for CTC collection from blood passing through the vein.
- Optimize the blood sampling time (potentially through sampling during the night or resting phase) to ensure maximal CTC capture.
- Develop methods for in vivo imaging using injectable CTC probes and wearable detection devices.
- Further molecular characterization of CTCs to better identify clinically relevant, aggressive cancers and minimize overdiagnosis of inconsequential, indolent tumours.
- Investigate the application of machine learning and artificial intelligence to analyse large datasets and minimize interobserver and interlaboratory variability.
- Develop strategies for early multi-cancer detection using CTCs without cancer type-specific markers followed by further diagnostic work-up using organ-specific tumour markers and/or targeted imaging.

High-throughput targeted DNA methylation sequencing of plasma ctDNA has shown promise in cancer diagnosis[145,146], with the potential for early multi-cancer detection as well as prediction of the primary tumour site[147,148]. miRNAs have also shown potential as early cancer detection tools[149] based on the fact that their expression profile is often dysregulated in cancer[150]. A combination of these biomarkers alongside CTC detection might improve test sensitivity and specificity. For example, the combination of CTC detection and ctDNA quantification has shown increased sensitivity (compared with each biomarker alone) for predicting disease-free survival in patients with early stage triple-negative breast cancer[151]. This combination of CTC and ctDNA measurements might also be useful in the management of CRC[152] and in detection of primary lung cancers[153].

The antitumour immune response can be initiated early in cancer development[154], and changes in the proportions of peripheral blood leukocyte subsets have been reported as potential biomarkers for early cancer detection[155,156]. Whether cancer cell dissemination (that is, CTCs) affects the immune response and peripheral blood leukocyte composition remains to be determined, although combining CTC detection and phenotyping of circulating immune cells could potentially also improve the accuracy of early cancer detection tests. These examples highlight the promise of alternative circulating biomarkers and their combination with CTCs in early cancer diagnosis.

In addition to these circulating biomarkers, other non-invasive technologies, such as urinary biomarker assays and cancer imaging, are already in clinical use for early cancer detection. Urinary biomarkers are commonly used in the diagnostic work-up for bladder cancer detection[157], including nuclear matrix protein 22 (NMP22)[158] and bladder tumour antigen protein[159]. In addition, a *PCA3* (encoding urine prostate cancer antigen 3) mRNA test has been approved by the FDA for use in prostate cancer diagnosis[144]. However, the combined diagnostic value of CTCs and urinary markers is yet to be investigated. Radiological imaging is also commonly used in cancer diagnosis and population-based screening for breast[160] and lung[161] cancers. Studies have shown that CTCs have a similar diagnostic accuracy, with higher specificity but inferior sensitivity, to that of various imaging modalities (including mammography, ultrasonography and MRI) in patients with breast cancer[107] and a better prognostic accuracy in patients with lung cancer[162]; therefore, combining these two technologies is likely to improve the detection of clinically relevant cancers[163].

CTCs in cancer screening

CTCs are a feature of many types of cancers and, therefore, have great potential to be used as a biomarker for the detection of multiple cancer types. The benefit of a multi-cancer detection approach and the current efforts in this direction have been highlighted in a recent review[164]. On the basis of the evidence discussed herein, the detection of CTCs is likely to indicate the presence of an aggressive cancer somewhere in the body. Hence, the future development of CTC detection platforms

Table 2 | Advantages and disadvantages of different blood-based biomarkers for cancer detection

Biomarker	Advantages	Disadvantages
CTCs	Inherently highly specific for the presence of cancer;essential for cancer metastasis and, therefore, reflect cancer aggressiveness[102] as demonstrated by the prognostic capacity of CTCs[19,21]; contain all the genetic and other molecular materials of intact cells; detectable in patients with early stage cancer[54,116,117]; suitable for analyses of intrapatient tumour heterogeneity[98]; can be cultured for in vitro studies and drug sensitivity testing[176]	Rare in small-volume blood samples (typically <7.5 ml)[177]; current analysis technologies are time-consuming; current epithelial marker-based isolation systems do not enable detection of CTCs with mesenchymal phenotypes[176]; isolation technologies based on cell size might not capture small CTCs[176]
Circulating proteins	Well-established methods for clinical diagnostic use[178]; assessments are cost-effective[179]; requires only a small amount of blood (<1 ml) or even dried blood spots[180]	Limited accuracy, particularly cancer specificity[143]; multiple isoforms of proteins can exist and developing isoform-specific antibodies is difficult[179]; current detection methods are unsuitable for widespread clinical use in screening owing to low sensitivity (for example, analyte saturation leads to the hook effect, whereby high protein levels are falsely measured as low)[181]
ctDNA	Short half-life provides a near-real-time indication of tumour mutational burden[182]; isolated DNA is more stable than RNA or CTCs[183]; several ultrasensitive and relatively straightforward techniques are available for isolation and analysis[184]; epigenetic signature of ctDNA can potentially inform on the origin of the tumour[185]; ctDNA assessment can provide relevant information on cancer molecular subtype and, therefore, sensitivity to certain molecularly targeted treatments[186,187]	Discordance between mutations detected in tumour tissue and ctDNA[184]; technologies might still not be sensitive enough for early cancer detection[188]; some genetic variants are common across different tumour types[189]; low quantities of ctDNA compared to total cell-free DNA and therefore low-volume blood samples are not suitable for ctDNA detection[184,190]; typical blood draws of 10–18 ml are required[191]; background tumour-associated mutations often also detected in non-malignant cells (for example, *TP53* or other mutations in leukocytes, which are associated with clonal haematopoiesis of indeterminate potential in ageing individuals) can lead to false-positive findings[192]
miRNAs	Panels of miRNAs (~200) used in combination could potentially inform on tumour subtype[193]; relatively stable in circulation[194]	Role in tumour development unclear[195]; lack of tumour-specific miRNAs and most are detectable in non-cancer or benign conditions[196]; pre-analytical considerations and miRNA expression levels might be confounded by factors such as age, diet and medication use[197]
EVs	Contents protected by lipid bilayer; stable in circulation[198]; quantification can provide an indication of overall tumour burden[199]; highly heterogenous and potentially reflect the characteristics of the cell of origin[198]; reported to be involved in establishing pre-metastatic niches and, therefore, might be an early biomarker for aggressive cancer with metastatic potential[200]	Currently, no gold-standard procedures for EV isolation have been established (ultracentrifugation is most commonly used)[201]; current sensitivity and specificity depend on the analyte in EV collections[201]; procedures for EV isolation are expensive and time-consuming

CTC, circulating tumour cell; ctDNA, circulating cell-free tumour DNA; EV, extracellular vesicles, miRNA, microRNA.

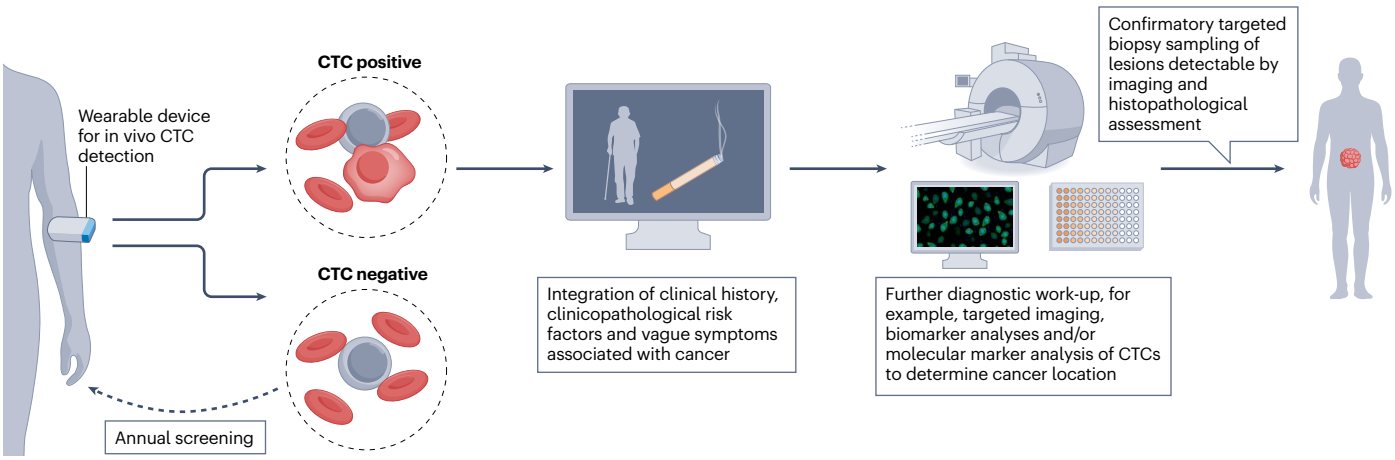

Fig. 4 | Our vision for the future role of CTCs in early cancer detection. Wearable devices might eventually be developed that enable non-invasive measurement of circulating tumour cells (CTCs) in a larger volume of blood than is currently feasible to sample routinely in a minimally invasive manner. Such devices could potentially directly image CTCs passing through a blood vessel based on labelling with injectable probes. Integration of clinical and family history with targeted imaging and biomarker analyses would inform localization of the tumour site in individuals with detectable CTCs, ultimately leading to a confirmed diagnosis. If the wearable device does not detect any CTCs, the individual might have to repeat the test at a regular interval (for example, annually) or sooner if they have a high risk of cancer or if prompted by any relevant symptoms.

with sufficient sensitivity as well as specificity might provide the first pan-cancer screening technology (Fig. 4).

Before being used for cancer screening in the general population, CTCs might first be applied for more targeted, risk-stratified screening of individuals deemed to be at high risk of developing particular malignancies based on genetic risk factors, lifestyle factors, and/or clinical and family history. Individuals with detectable CTCs could then be referred for further investigations, including organ-targeted imaging (such as mammography for those with an increased risk of breast cancer[165] or low-dose CT for those at high risk of lung cancer[166]), cancer-specific blood-based biomarker testing (serum PSA testing for suspected prostate cancer[144], CA19-9 testing for pancreatic cancer[167] and ctDNA and/or miRNA analyses for various cancer types[145,150,168]) or further molecular analysis of CTCs to locate the tumour such as tumour-specific antigen testing, DNA methylation analysis or copy-number aberration profiling[169]. After integration of the results of such tests with information on patient characteristics, risk factors and suspicious symptoms, a diagnosis might be made with or without biopsy sampling of the suspected tumour site, depending on the feasibility of biopsy sampling and the accuracy of non-invasive biomarkers. Individuals with a negative CTC test result could be considered as being cancer free or having a low risk of cancer and subsequently be re-screened for the presence of CTCs at regular intervals unless they experience any change in symptoms (Fig. 4).

Conclusions

In a rapidly evolving field, huge progress has already been made in understanding the processes involved in early cancer dissemination and metastasis. Micrometastases can be formed early in tumorigenesis and accumulating evidence indicates that CTCs can be detected at early stages in the development of aggressive cancers. Therefore, CTCs have great potential to be used for early cancer detection, enabling the identification of clinically relevant tumours while avoiding overdiagnosis of indolent disease. The current challenge lies in developing technologies to reliably harvest and analyse these scarce but implicative cells. With further technological developments, particularly those enabling highly sensitive detection of CTCs through non-invasive or minimally invasive sampling of a large amount of blood at a convenient time, we expect that CTC analysis will be successfully applied to change the paradigm of early cancer detection and thereby substantially improve outcomes for patients with cancer.

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Author contributions

R.L. and Y.-J.L. researched data for the article and wrote the manuscript. R.L., K.P. and Y.-J.L. made substantial contributions to the discussion of content. All authors reviewed and/or edited the manuscript before submission.

Competing interests

Y.-J.L. has received commercial research grants from ANGLE. K.P. has received personal fees from Agena, Illumina, and Menarini outside the submitted work and has a patent pending with the European Patent Office (application No. 18705153.7; PCT/EP2018/054052; Method of Detecting Cancer or Cancer Cells). The other authors declare no competing interests.

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ClinicalTrials.gov: https://clinicaltrials.gov/ct2/home
European Liquid Biopsy Society: www.elbs.eu
Europe's Beating Cancer Plan: https://ec.europa.eu/health/sites/health/files/non_communicable_diseases/docs/eu_cancer-plan_en.pdf
PANCAID: https://www.pancaid-project.eu/