Tumor-proximal liquid biopsy to improve diagnostic and prognostic performances of circulating tumor cells

Etienne Buscail¹,²,³, Laurence Chiche¹,²,³, Christophe Laurent¹,²,³, Véronique Vendrely¹,²,³, Quentin Denost², Jérôme Denis⁴, Matthieu Thumerel², Jean-Marc Lacorte⁴, Aurélie Bedel¹,²,³, François Moreau-Gaudry¹,²,³, Sandrine Dabernat¹,²,³ and Catherine Alix-Panabières⁴,⁵

¹ INSERM U1035, Bordeaux, France
² CHU de Bordeaux, France
³ Université de Bordeaux, France
⁴ Laboratory of Rare Human Circulating Cells, University Medical Centre of Montpellier, France
⁵ Service de Biochimie Endocrinienne et Oncologie, Hôpital Pitié Salpêtrière Assistance Publique Hôpitaux de Paris, France

Keywords
cancer diagnostics; cancer prognosis; circulating tumor cells; liquid biopsy; vascular organ drainage

Correspondence
C. Alix-Panabières, Laboratory of Rare Human Circulating Cells, University Medical Centre of Montpellier, EA2415, Montpellier, France
E-mail: c-panabieres@chu-montpellier.fr

S Dabernat and C Alix-Panabières contributed equally to this article

(Received 31 March 2019, revised 4 June 2019, accepted 17 June 2019, available online 25 July 2019)

doi:10.1002/1878-0261.12534

Circulating tumor cell (CTC) detection and numeration are becoming part of the common clinical practice, especially for breast, colon, and prostate cancer. However, their paucity in peripheral blood samples is an obstacle for their identification. Several groups have tried to improve CTC recovery rate by developing highly sensitive cellular and molecular detection methods. However, CTCs are still difficult to detect in peripheral blood. Therefore, their recovery rate could be increased by obtaining blood samples from vessels close to the drainage territories of the invaded organ, when the anatomical situation is favorable. This approach has been tested mostly during tumor resection surgery, when the vessels nearest to the tumor are easily accessible. Moreover, radiological (including echo-guided based and endovascular techniques) and/or endoscopic routes could be utilized to obtain CTC samples close to the tumor in a less invasive way than conventional biopsies. The purpose of this article is to summarize the available knowledge on CTC recovery from blood samples collected close to the tumor (i.e., in vessels located in the drainage area of the primary tumor or metastases). The relevance of such an approach for diagnostic and prognostic evaluations will be discussed, particularly for pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, hepatocellular carcinoma, and non-small-cell lung cancer.

1. Introduction

Cancer diagnosis usually relies on information obtained using sequential procedures, including imaging data (CT, PET, MRI, ultrasonography, X-rays), changes in the levels of markers in bodily fluids (e.g., blood, urine), and mainly on the pathology examination of cancer cell or tissue samples, obtained by surgical biopsy or by fine-needle aspiration (fine-needle aspiration cytology, FNAC). Biopsy and FNAC are invasive procedures, especially in the case of deeply located tumors, and may present severe complications

Abbreviations
CRC, colorectal cancer; CTC, circulating tumor cell; CT, computed tomography; EMT, epithelial-to-mesenchymal transition; EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; FNAC, fine-needle aspiration cytology; HCC, hepatocellular carcinoma; MRI, magnetic emission imaging; NSCLC, non-small-cell lung cancer; OS, overall survival; PCR, polymerase chain reaction; PDA, pancreatic ductal adenocarcinoma; PET, positron emission tomography; PFS, progression-free survival; RT-qPCR, reverse transcription polymerase chain reaction.
such as infection, bleeding, or inflammation. More importantly, they also carry the risk of seeding tumor cells around the sampling area. Indeed, detached cells can be cleared by interstitial fluids to lymph nodes, or into the veins draining the tissue, thus entering the circulation. They might then extravasate at distant healthy tissues and contribute to metastasis formation. During fine-needle aspiration, cells can be dragged along the needle track, leading to the possibility of increasing the local dissemination (Shyamala et al., 2014). Moreover, if the fraction of tumor cells in the biopsy is too low for pathology/molecular analyses, particularly in tumors with strong desmoplastic reaction, repeated sampling is required, possibly delaying tumor management.

Besides diagnosis, cancer management would highly benefit from broadening the panel of the available prognostic/predictive markers to better stratify patients in view of precision medicine, and to follow the tumor response after treatment initiation. In particular, the outcome of pancreatic cancer is highly unpredictable, even in the case of resectable tumors, because predictive and prognosis markers are missing (Zhou et al., 2017). Consequently, effective and reliable biomarkers need to be identified for rapid diagnosis, especially when pathology-based diagnosis is not available or noncontributive.

Primary tumors and metastases release in the blood and other body fluids tumor-derived elements, such as circulating tumor cells (CTCs), nucleic acids, exosomes, and proteins. When identified as tumor-derived, these elements can be considered as an evidence of the presence of a tumor (Alix-Panabières and Pantel, 2014). The analysis of these circulating tumor-derived elements, called ‘liquid biopsy’ (Alix-Panabières and Pantel, 2013; Pantel and Alix-Panabières, 2010), might represent a noninvasive, safer, and faster alternative/complement to tissue biopsy. Tumor elements are released very early during cancer development. For example, in a mouse model of pancreatic cancer, CTCs with metastatic potential are already shed during the formation of the primary pancreatic adenocarcinoma, before it becomes detectable by histologic methods (Rhim et al., 2012). Liquid biopsies can also be used to detect disease progression or treatment resistance before the appearance of the first clinical signs (Riethdorf et al., 2018).

The first CTC proof was published in 1869 by Thomas Ashworth (1869). From the 1970s, the interest on CTCs has gradually increased thanks to the progress in the detection methods based on molecular biology techniques. In the last 20 years, new technologies for CTC enrichment, detection, and characterization with higher sensitivity have been developed, allowing CTC enumeration in different solid cancers (Lianidou et al., 2014). For instance, the US Food and Drug Administration (FDA) has approved the use of the CellSearch® test for CTC detection in patients in the clinical routine for metastatic breast cancer in January 2004 (Cristofanilli et al., 2004), and for the prognosis of advanced colorectal and prostate cancer in November 2007 (Cohen et al., 2006, 2009) and February 2008 (Resel et al., 2010), respectively (Millner et al., 2013; Riethdorf et al., 2018). Since then, increasing evidence indicates that CTC detection is a very promising tool, mostly of prognostic value in lung cancer, especially non-small-cell lung cancer (NSCLC), colorectal cancer (CRC), hepatocellular carcinoma (HCC), and pancreatic ductal adenocarcinoma (PDAC) (Hench et al., 2018; Pimienta et al., 2017).

Most studies have focused on CTC detection and counting in peripheral blood samples obtained by puncture of the median cubital vein. Fewer reports have tested the hypothesis that the chances of capturing and detecting CTCs might be higher in vessels closer to the tumor, especially in the main veins that drain blood from the organ invaded by the cancer. In this review, we will discuss studies that compared CTC yields in peripheral blood and in blood from vessels in the vicinity of the primary tumor (NSCLC, CRC, HCC, and PDAC). As blood sampling in the main vessels close to the tumors is feasible in the early management of cancer or during surgery, we focused on the diagnostic and prognostic values of this approach and considered the possible added value of the ‘close-to-the-tumor liquid biopsy’.

2. Basis for CTC analysis in the main veins close to the tumor site

The primary tumor releases a heterogeneous population of circulating cells, such as cells with metastatic potential, apoptotic or necrotic cells that are cleared by the organism, and live cells that can remain in a latent or dormant state in a distant organ (Massagué and Obenauf, 2016; Nguyen et al., 2009).

CTCs are disseminated mostly during metastasis formation. In fact, very few of the tumor cells released into the circulation will form metastases (Kessenbrock et al., 2010; Luzzi et al., 1998; Martin et al., 2017; Massagué and Obenauf, 2016). First, epithelial tumor cells in the primary tumor undergo a reversible phenotypic change, known as epithelial-to-mesenchymal transition (EMT). Consequently, cells detach from the tumor and spread out, using the surrounding fluids to move away, and enter the vessels by extravasation.
The first capillary bed that a metastatic cell encounters depends on the blood circulation pattern near the primary tumor. In most organs, the venous circulation leads to the right ventricle of the heart and into the lungs, whereas the gut venous circulation drains into the liver. This explains the high incidence of metastases in lungs and liver (Denève et al., 2013; Nguyen et al., 2009). For this reason, some authors have used blood samples from the vena cava upstream of the liver for CTC detection in patients with metastatic breast cancer (samples were obtained from an implanted vascular device) (Peeters et al., 2011).

3. Technologies for CTC enrichment and detection

Although the release of tumor cells from the primary tumor and/or metastases is deleterious for the patient, it also becomes an opportunity to obtain relevant information for precision medicine using a noninvasive procedure. Several technologies allow CTC enrichment and numeration (Alix-Panabières and Pantel, 2014). As CTCs are rare events, a first enrichment step is required to allow their detection. Specifically, CTCs’ physical properties (i.e., size, deformability, density, and electrical charges) can be used to differentially enrich them from the numerous surrounding cells present in blood (Alix-Panabières and Pantel, 2014; Harouaka et al., 2013; Pantel and Alix-Panabières, 2010). CTCs can also be enriched and detected on the basis of their biological properties. For instance, positive selection-based capture relies on the expression of tumor cell surface markers (most commonly EpCAM). This can be combined with the presence of epithelial-specific intracytoplasmic proteins (such as cytokeratin 19, CK19) and the absence of the blood-specific cell surface marker CD45. These features are the basis of the CellSearch® system. On the other hand, negative selection-based capture is an unbiased CTC enrichment step to eliminate the unwanted white blood cells. Antibodies against cell surface markers of the different blood cell types are used to pull down white blood cells, leaving the remaining supernatant enriched in CD45− endothelial cells and CTCs. After enrichment, the detection step is needed to confirm the presence of CTCs in the sample (Alvarez Cubero et al., 2017). This can be done using (a) immunocytological technologies (anti-epithelial antibodies), (b) molecular (RNA-based) technologies (e.g., RT-qPCR for epithelial mRNA), and (c) functional assays (e.g., EPISPOT assay that detects only viable CTCs) (Alix-Panabières and Pantel, 2014).

Despite improvements in the methods for CTC enrichment and detection, these cells remain rare in blood samples and difficult to identify. To maximize the chances of CTC recovery, it would seem logical to draw blood close to the site of the tumor. In the case of CRC, HCC, and PDAC, the primary tumor is connected to the vascular draining territory of the mesenteric and portal venous system, whereas lung cancer is linked to the pulmonary vein. These vessels are sufficiently large and resistant to allow direct vein puncture. Of note, the portal vein can be accessed by noninvasive ultrasonography puncture (Chapman and Waxman, 2016). The pulmonary vein is reachable only during surgery, but it is a good candidate to capture more CTCs, with a high prognostic value (Hashimoto et al., 2014). Conversely, in breast cancer and prostate cancer, tumor elements are released mostly in the lymphatic network and the internal iliac vasculature, respectively. As these draining systems cannot be punctured, CTC capture closer to the tumor has not been assessed in these cancer types. Therefore, this review will focus on CTC detection in the draining vessels of primary HCC, PDAC, CRC, and NSCLC.

4. Pancreatic ductal adenocarcinoma

Pancreatic ductal adenocarcinoma remains one of the deadliest cancers due to its late diagnosis and poorly efficient therapies (Buscail, 2017). Moreover, treatment is often delayed due to difficulties in proving the presence of malignant lesions (McGuigan et al., 2018). CTC numeration in the peripheral blood of patients with PDAC has been assessed as a diagnostic option, but rather unsuccessfully due to the low detection rates (Table S1). Most of these cohorts were quite small, and included only patients with metastatic or locally advanced tumors. Even in the cohorts that included patients with all tumor stages, metastatic cancers were the most frequent (>80%) (Lianidou et al., 2014). PCR-based or physical-based methods only slightly improved CTC detection rate (Table S1), whereas methods based on the expression of epithelial cell markers, such as CK19 or EpCAM, could have missed CTCs undergoing EMT. The most common site of PDAC spreading is the liver because the pancreas venous blood drains first into this organ (Figure 1) (Denève et al., 2013; Nieto et al., 2008). The liver filters pancreatic CTCs. If they do not stay in the liver, they will become highly diluted in the peripheral blood system (i.e., 1 tumor cell per 1 × 10⁷ blood cells, explaining the low detection rates) (Yu et al., 2011). To increase the chances of CTC detection, blood was
sampled directly from the portal vein prior to CTC sequestration in the liver (Chapman and Waxman, 2016). This approach was first tested in 20 patients with resectable PDAC in whom portal blood could be easily and safely sampled during surgery (Bissolati et al., 2015) (Table 1). CTCs were detected (CellSearch®) in nine portal blood samples (45%) and in four peripheral blood samples (20%) from these patients. In 25% of the 20 patients, CTCs were detected only in the portal blood sample, and would have been missed if only peripheral blood was used. The presence of CTCs in peripheral or portal blood did not correlate with long-term overall survival (OS) or progression-free survival (PFS). Conversely, CTC detection in the portal vein sample was associated with higher rate of liver metastases (Bissolati et al., 2015). Another study compared CTC identification in peripheral and portal vein blood samples in 41 patients undergoing upfront surgery for PDAC (Tien et al., 2016) (Table 1). CTCs were detected (CellSearch®) in 39% of peripheral and 58.5% of portal vein blood samples. The presence of CTCs in the portal blood was a predictive factor of liver metastasis. The short follow-up of this study (only 1 year after surgery) did not allow assessing the OS and progression-free survival (PFS). In 14 patients with borderline resectable (n = 7) or metastatic PDAC (n = 7), CTCs (CellSearch®) were identified in 21% of peripheral blood samples and in 100% of portal samples, drawn by pre-operative endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) performed for diagnostic and staging purposes (Catenacci et al., 2015). Moreover, the absolute CTC numbers were higher in the portal blood samples (83.2/7.5mL versus 0.4/7.5mL in the peripheral blood samples). No correlation with OS or PFS was reported. The authors also evaluated the suitability of portal vein CTCs for gene expression studies. They found that downregulation of tumor-suppressor genes had a strong prognostic value and could be used to stratify patients eligible for surgery, according to the relapse risk. Similarly, in 29 patients with locally advanced and metastatic tumors (Liu et al., 2018) (Table 1), CTCs were detected in 100% of the portal blood samples (obtained by ultrasonography-guided transhepatic puncture) and in 54% of the peripheral blood samples, with a higher CTC count in portal than in peripheral blood (282/7.5mL versus 21/7.5mL). Moreover, the CTC count was correlated with liver metastases, and patients with a portal vein CTC count higher than 150/7.5mL had shorter OS. Finally, in this study portal vein CTCs were cultured ex vivo to test the response to several common chemotherapies. CTCs were highly resistant to gemcitabine and other standard clinical regimens such as 5-FU and oxaliplatin. Moreover, CTC viability was reduced by deltarasin (Liu et al., 2018). This suggests that ex vivo CTC
culture might be a valuable tool for choosing the best therapy for each patient.

In these studies, CTC detection rate in peripheral blood was similar to what was previously reported (around 50%, as in Tables S1, 1), whereas in portal blood, it was on average 75%, for all tumor stages.

More studies with larger cohorts are needed to determine the value of this approach at early disease stages, particularly in patients with upfront resectable tumors. Interestingly, all studies found a correlation between liver metastases and portal CTCs, including in cohorts with resectable tumors. This suggests that CTC analysis in portal blood samples collected, for instance, during preoperative EUS-FNA could be used to better select patients for surgery, especially patients with undetectable micrometastases. Indeed, echoendoscopy with puncture is now the gold standard for histologic proof and formal diagnosis, but has some limitations. It carries variable negative predictive value, because the endoscopic ultrasound-guided fine-needle aspiration biopsy yield and the pathological analysis are largely operator-dependent. It is invasive and with high risk of morbidity, with possible induction of acute iatrogenic pancreatitis, sometimes compromising surgical management (Storm and Lee, 2016). CTC detection could contribute to decision-making, particularly for triggering neoadjuvant and adjuvant treatment (Table 6).

Taken together, the results of these pilot studies suggest that liquid biopsy in the portal vein may help improving pancreatic cancer prognosis evaluation, and could be associated with tumor sampling during EUS-FNA to improve PDAC management. For instance, CTC detection could be used as a companion diagnostic tool for the molecular/genetic analysis of cancer cells in patients with indication for neoadjuvant therapy. Indeed, preliminary data showed that CTCs could be useful to stratify patients and adjust the therapeutic options according to the cancer molecular characteristics (Soler et al., 2017).

Finally, functional testing of CTCs, such as detection/quantification of epithelial-specific secreted factors by isolated cells, is still in the early days, but patient management might benefit from such approaches in the future (Table 6).

5. Colorectal cancer

CRC is the third most common cancer in both sexes. The 5-year OS reaches almost 60%. About 50% of patients will develop metastatic disease that accounts for the majority of deaths (de Haas et al., 2011). After curative resection, approximately 30% of patients who

| Table 1. Comparison of CTC detection in peripheral and portal venous samples in patients with pancreatic ductal adenocarcinoma |
|---|
| **Number of patients** | **CTC count in peripheral blood** | **CTC count in portal blood** | **CTC detection rate in peripheral blood** | **CTC detection rate in portal blood** | **Prognostic value** |
| Liu et al. (2018) | 29 | Mean 281 | Mean 281 | 100% | n > 150 OS 9.2 months |
| Bissolati et al. (2019) | 20 | Mean 0.25 | Mean 0.25 | 20% | Positive for CTCs: OS 23.1 months |
| Tron et al. (2019) | 41 | Mean 71 | Mean 230 | 39% | Negative for CTCs: OS 9.2 months |
| Catraciuco et al. (2019) | 14 | Mean 0.7 | Mean 0.7 | 21% | Positive for CTCs: OS 23.1 months |

CTC, circulating tumor cell; EpCAM, epithelial cell adhesion molecule; PDAC, pancreatic ductal adenocarcinoma; ICC, immunocytochemistry; PCR, polymerase chain reaction; NA, not applicable; NS, not significant; OS, overall survival; PFS, progression-free survival; SD, standard deviation; Med, median.
develop metastases eventually die of metastatic disease. Although diagnosis of CRC by colonoscopy is routinely available, good prognostic markers to stratify patients according to the metastasis risk are still missing. It has been shown that CTC detection in peripheral blood is a good biomarker for poor prognosis such as PFS and OS in patients with metastatic CRC. Therefore, it could contribute to better tailor the patient general care. However, differently from breast and prostate tumors, CTC release in the peripheral blood by CRC is a rare event, and consequently, their detection is difficult in the clinical practice (less than 60% of positive patients for CTC detection rate) (Alix-Panabières and Pantel, 2014; Tan and Hao, 2018). PCR-based CTC detection methods did not improve sensitivity (Table S1). CTCs shed by CRC are disseminated via the mesenteric venous system that drains in the portal vein. The liver serves as a filter that retains many CTCs, including metastasis-initiating cells at the origin of liver metastases (Denève et al., 2013). Nevertheless, other CTC subpopulations can pass through this organ to reach the peripheral blood (Figure 1). Denève et al. showed a decreasing mesenterico-peripheral gradient of CTCs, with the liver as a frequent organ to accommodate distant metastases in CRC (Denève et al., 2013). Moreover, a study showed that immediately after tumor resection, CTC numbers decreased in the peripheral blood and in the local main vasculature of the tumor (Jiao et al., 2009) (Table 2). This study did not specify the percentage of patients with CTCs detected in the systemic circulation compared with the portal circulation, but the median CTC number before surgery, although very low in general, was higher in the portal circulation and hepatic vein. Moreover, CTC detection rate in the hepatic vein was lower than in the portal vein (17.5% versus 35%, respectively) (Rahbari et al., 2012), underlining the importance of the puncture site for CTC detection. In cohorts that included only patients with metastatic CRC, CTC detection rate was similar in peripheral blood and hepatic vein (46% and 54%, respectively), as well as the median CTC count (1 versus 2.5, respectively) (Connor et al., 2016) (Table 2). OS and PFS were worse in patients with CTC counts >3, suggesting that CTC detection and number could have a prognostic value in patients with metastatic CRC. When the tested population included only 20% of patients with metastatic diseases, CTC detection rate in mesenteric blood was almost twice higher than in peripheral blood (CellSearch®, 55.9% versus 29%, respectively). However, the small number of patients did not allow testing the correlation between CTC number and prognosis (Denève et al., 2013). This study also showed that using an EpCAM-independent CTC enrichment method followed by the functional EPISPOT enrichment assay significantly increased CTC detection rate to 55.4% in peripheral blood, and only slightly (to 65.9%) in mesenteric blood samples. CTC number was significantly higher in mesenteric blood than peripheral blood samples, and more CTCs were detected with the EPISPOT assay than with the CellSearch® system. CTC detection (both methods) inversely correlated with the presence of lymphatic emboli, and only the EPISPOT results correlated with the primary CRC grading. Finally, cancer-related survival was worse in patients without metastases but with more than 27 CTCs/15 mL of blood (only with the EPISPOT assay) (Table 2).

In conclusion, although CRC-related CTCs are rare, many groups performed studies to test their diagnostic and prognostic relevance. Blood sampling closer to the tumor did not increase significantly CTC detection and enumeration, including in the case of metastatic disease. It is possible that even close to the tumor, CRC-released CTCs are rare. However, the EPISPOT assay allowed increasing CTC detection in mesenteric blood (Denève et al., 2013). CTC detection correlated with bad prognosis. Studies on larger cohorts are needed to test the value of CTC detection for CRC management.

New personalized treatment strategies for metastatic colorectal cancer require a better understanding of tumor biology and informative biomarkers (Gbolaran and O’Neil, 2019). CTCs could assist the implementation of current emerging therapeutic sequences (Table 6).

6. Hepatocellular carcinoma

Hepatocellular carcinoma is the sixth most prevalent cancer responsible for one-third of all deaths by cancer (Ferlay et al., 2015). When diagnosed early enough, the 5-year OS can reach 50%. Conversely, less than 10% of patients with stage IV disease survive the first year after diagnosis. Thus, tools for early screening are urgently needed, especially in high-risk populations (patients with cirrhosis, hepatitis, and nonalcoholic steatosis hepatitis syndrome) who could greatly benefit from the available treatments in the case of early diagnosis. Currently, screening is based on the use HCC biomarkers, such as alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) (Tateishi et al., 2008). However, these markers show high false-positive rates. Like for other cancers, CTC detection in peripheral blood is not sensitive enough to allow HCC diagnosis (Table S1). Nevertheless, CTC detection correlates with poor PFS and OS (Fan et al., 2015).
| Number of patients | CTC enrichment/detection methods | CTC count in peripheral blood | CTC count in portal blood | CTC count in hepatic vein/central vein | CTC detection rate in peripheral blood | CTC detection rate in portal blood | CTC detection rate in hepatic vein (vena cava) | References |
|--------------------|---------------------------------|------------------------------|--------------------------|--------------------------------------|---------------------------------------|---------------------------------|------------------------------------------|------------|
| 80Δθ              | EpCAM⁺ CTC selection/CellSearch⁺ | NA                           | Mean 1.5 Med 0 Range 0–32 | Mean 0.3 Med 0 Range 0–5             | NA                                    | 35% ∑                                | 17.5% (via central line)              | Rahbari et al. (2012)            |
| 75Δ               | CD45⁻ leukocyte depletion EPISPOT⁺/EpCAM⁺ selection/CellSearch⁺ | EPISPOT⁺ Med 1.2 Range 0–92 CellSearch⁺ Med 0 Range 0–142 | EPISPOT⁺ Med 4 Range 0–247 CellSearch⁺ Med 2.7 Range 0–286 | NA                                   | EPISPOT 55.4% CellSearch⁺ 29%          | EPISPOT 65.9% ∑ CellSearch⁺ 55.9% ∑ | NA                                       | Denève et al. (2013)           |
| 29Δ               | EpCAM⁺ CTC selection/CellSearch⁺ | Open resection: Arterial Mean 1.82 Med 1 Range 0–6 Venous Mean 1.45 Med 1 Range 0–3 | Open resection: Mean 1.5 Med 0 Range 0–32 | Open resection: Mean 1.26 Med 87 Range 0–500 | Open resection: Mean 126 Med 87 Range 0–500 | NA                                    | NA                                       | Jiao et al. (2009)              |
| 31                 | EpCAM⁺ CTC selection/CellSearch⁺ | NA                           | NA                        | NA                                   | 17%                                    | 72% ∑                                | NA                                       | Wind et al. (2009)            |
| 63Δθ              | EpCAM⁺ CTC selection/CellSearch⁺ | Med 1 Interquartile range 0–4 | Med 2.5 Interquartile range 1–8 | Med 2.5 Interquartile range 1–8 | 46%                                    | NA                                    | 54% HV>3 △OS Multivariate analysis   | Connor et al. (2016)           |

Tumor stage of the studied population: Δ metastatic; θ neoadjuvant treatment before blood sampling.
Prognostic value not evaluated except for [46]
∑: statistically significant difference between portal and peripheral samples
Liver is connected with two major vascular systems: the hepatic veins that constitute the efferent pathway, and the hepatic artery and portal veins that compose the afferent pathway (Figure 2). CTCs from the primary liver tumor are first disseminated in microscopic portal vessels, and then in the centrolobular veins that drain into the main hepatic veins. CTCs can be detected also in the afferent system because HCC has a high propensity to colonize arterial vessels during neoangiogenesis (Figure 2) (Forner et al., 2012). We found only one study that compared CTC detection rates in function of the sampling site in patients with localized HCC (Table 3) (Sun et al., 2018). Detection rates in peripheral vein or artery blood samples (68% and 45%, respectively) were similar to previously published results (Tables 3, S1). As expected on the basis of the liver circulation, CTC recovery rate in portal blood and inferior vena cava did not increase compared with peripheral samples. Conversely, it was very high (80%) in the hepatic vein because this vessel drains all the microscopic lobular spaces that may receive CTCs. This study did not test the diagnostic/prognostic value of CTC detection in the different vessels. However, intrahepatic recurrence was strongly associated with CTC presence in peripheral blood samples (artery and vein) and with CTC micro-emboli (or clusters). In addition, high detection rate of CTCs or clusters in the hepatic vein was associated with the presence of lung metastases.

In conclusion, CTC detection in the hepatic vein shows a strong prognostic value, both for disease recurrence and for disease dissemination (Fang et al., 2014). It would be interesting to test whether CTC presence, particularly in the hepatic vein, could be used to stratify patients eligible for adjuvant therapy. Moreover, future studies should assess whether CTC analysis could facilitate the individualized therapeutic decision-making in HCC (Table 6).

7. Non-small-cell lung cancer

Lung and bronchus cancer remained the primary cause of death by cancer in 2017, representing around 25% of all deaths by cancer (Siegel et al., 2017). NSCLC accounts for 85% of all diagnosed lung cancers. This cancer is the most prevalent cancer in males and the third in females. Its 5-year OS depends on the disease stage, going from 92% for stage IA1 to less than 1% for metastatic stage IV. Overall, the 5-year survival rate is 18% (source: cancer.net). Even after surgery, tumor recurrence with distant metastases occurs in around 25% of patients, reaching approximately 29% in patients with stage I cancer (Goldstraw et al., 2016). Cytotoxic chemotherapy can slightly prolong survival in patients with tumor relapse. Indeed, it has been reported that the 5-year survival rate improved only by 4% to 5% for patients with stage I–III NSCLC, and by only few months for patients with stage IV.
tumors, possibly because tumor recurrence is detected too late (Johnson et al., 2014).

NSCLC-derived CTCs disseminate first in the pulmonary vein (Figure 3) (Popper, 2016). Cancer cells follow the main bloodstream through the heart and join the systemic circulation where metastasis-initiating cells can niche, mostly in the brain, bone marrow, adrenal gland, and liver. CTC detection in peripheral blood samples of patients with lung cancer has been evaluated in several studies (Table S1). Overall, the percentage of patients in whom CTC could be detected is quite low at all disease stages (around 53%) when
### Table 4. CTC detection in pulmonary vein and peripheral vein samples in patients with non-small-cell lung cancer

| Number of patients | CTC detection methods | CTC count in peripheral blood | CTC count in pulmonary vein blood | CTC detection rate in peripheral blood | CTC detection rate in pulmonary vein | Prognostic value | References |
|--------------------|-----------------------|-------------------------------|-----------------------------------|---------------------------------------|-------------------------------------|-----------------|------------|
| 36                 | OncoBEAM®             | Med 1.5 Range 0–15            | Med 7.5 Range 0–10               | 69.4%                                 | 83.3%                               | Shorter PFS associated with CTC clusters | Murlidhar et al. (2017) |
| 30 Δ               | Veridex®              | Mean 0.8 Med 0 Range 0–16     | Mean 1195 Med 81 Range 0–10034   | 16.7%                                 | 96.7%                               | NA              | Okumura et al. (2009) |
| 23                 | MACS+flow cytometry   | Med 5 Interquartile range 3–9 | Med 28 Interquartile range 3–9   | 91.3%                                 | 95.7%                               | High CTC count associated with lower PFS | Li et al. (2017) |
| 10                 | ScreenCell®+immunochemistry analysis of 549 human lung cells | Mean 22 Range 0–100           | Mean 65 Range 8–200              | 80%                                   | 100%                                | NS              | Chudasama et al. (2017) |
| 23 Δ               | ScreenCell®+hematoxylin-eosin method | Cluster (CTC >4) n = 6       | Cluster n = 15 Single CTC n = 4   | 30%                                   | 93%                                 | NS              | Sawabata et al. (2016) |
| 30                 | CellSearch®           | CTC ≥1/7.5mL n = 6 [1–4]     | CTC ≥187.5mL n = 23               | 22.2%                                 | 100%                                | High CTC count in peripheral blood associated with PFS and OS | Crosbie et al. (2016) |
| 32 Δ               | EpCAM-based microfluidic chip | Mean 3.1 CTC/7.5mL           | Mean 544 CTC/7.5mL NA             | NA                                    | NA                                  | Correlation with neoadjuvant therapy IT < SAPV CTC | Reddy et al. (2016) |
| 15 θ               | EpCAM-based microfluidic chip | NA                           | Mean 95.7 Range 0–855            | 6.6%                                  | 80%                                 | Correlation with neoadjuvant therapy IT < SAPV CTC | Tarumi et al. (2013) |

Tumor stage of the studied population: Δ metastatic; θ neoadjuvant treatment before blood sampling. ∑: statistically significant difference between pulmonary vein and peripheral samples. IT, induction chemotherapy; PV CTC, pulmonary vein CTC; SA, surgery alone.
using protein marker-based CTC enrichment methods. CTC detection in patients with metastatic disease is more efficient when using PCR-based methods (71%). Most studies reported a strong correlation between peripheral blood CTC detection and OS. Tumor recurrence also was associated with CTC detection (Gallo et al., 2017).

In 2005, CTCs were detected (RT-PCR) for the first time in the pulmonary vein of patients with NSCLC (Bernaudin et al., 2005). Since then, many studies have compared CTC detection rate in peripheral blood and close-to-the-tumor vessels. Like for PDAC and CRC, blood samples were collected close to the tumor drainage territory by puncturing the pulmonary vein. Most patients included in these studies had resectable tumors, and only a small percentage had metastatic disease. Overall, CTC detection rate in peripheral blood samples was similar to that of previously published works on peripheral blood only (Table 4; 45%, similar to the 53% in Table S1). Conversely, CTC detection rate in the pulmonary vein was about 91%. Of note, while the peripheral blood detection rates varied among reports (range 6.6–91.3%, mean 45.1% ± 34.1%), the pulmonary vein detection rate was quite reproducible (range 80–100%, mean 93.5% ± 7.3%). Similarly, the mean CTC count was higher in the pulmonary vein than in peripheral blood samples (544/7.5mL vs 3.1/7.5mL Table 4) (Reddy et al., 2016).

Results on the prognostic value of CTC presence in the pulmonary vein are heterogeneous. Some studies showed a correlation between CTCs and disease progression and OS (Crosbie et al., 2016; Li et al., 2017; Murlidhar et al., 2017; Tarumi et al., 2013). Other studies did not show a statistically significant association between CTC count or number of patients with CTCs in the pulmonary vein and OS or PFS (Chudasama et al., 2017; Hashimoto et al., 2014; Lv et al., 2018; Okumura et al., 2009; Reddy et al., 2016; Sawabata et al., 2016). However, a correlation between CTC levels and pathology, particularly tumor size, was described (Lv et al., 2018; Reddy et al., 2016). Sabawata’s data suggested a shorter PFS when CTC clusters were present in the pulmonary vein, which was further studied by Murlidhar et al. who showed that the presence of clusters in the pulmonary vein was a factor of poor prognosis (shorter PFS) (Murlidhar et al., 2017). Additional studies did not evaluate the prognostic value of CTCs (Chudasama et al., 2017; Okumura et al., 2009) or did not include long-term patient follow-up (Hashimoto et al., 2014). Finally, one study showed that PFS and OS were associated with high CTC rate in peripheral blood, but not in the pulmonary vein blood (Crosbie et al., 2016).

The link between CTC detection in lung cancer and the surgery technique was assessed by few authors. Particularly, it was shown that surgical manipulation significantly increased CTC number in the pulmonary vein and that this was associated with lymphatic invasion and a significant reduction of PFS and OS (Hashimoto et al., 2014, 2018). A recent work suggested that intraoperative manipulation contributes to the hematogenous dissemination of tumorigenic CTCs and circulating tumor micro-emboli (Table 5) (Lv et al., 2018). Similarly, it was reported that the CTC rate, including in the pulmonary vein, increases after endoscopic biopsy (Reddy et al., 2016). These data suggest that the pulmonary veins should be ligated before tumor mobilization to minimize tumor cell dissemination.

Table 5. CTC count after tumor mobilization in pulmonary vein and peripheral vein samples in patients with non-small lung cancer

| Number of patients | CTC detection methods | CTC count in pulmonary vein | CTC detection rate in peripheral blood | CTC detection rate in pulmonary vein | Prognostic value | References |
|--------------------|-----------------------|-----------------------------|----------------------------------------|--------------------------------------|-----------------|------------|
| 30 Δ               | CellSearch®           | Med 60                      | 6.7%                                   | 73.3%                                | NA              | Hashimoto et al. (2014) |
| 30 Δ               | CellSearch®           | Increase ΔCTC              | No sample                              | 80%                                  | PFS OS metastasis correlated with ΔCTC | Hashimoto et al. (2018) |
| 32                 | CellSearch®           | Mean 617                    | 25%                                    | 90.6% ∑                              | NS              | Lv et al. (2018) |

Tumor stage of the studied population: Δ metastatic; Δ neoadjuvant treatment before blood sampling

∑ statistically significant difference between pulmonary vein and peripheral samples

IT, induction chemotherapy; PV CTC, pulmonary vein CTC; SA, surgery alone.

Taken together, these results show that pulmonary vein puncture greatly increases the chances to detect CTCs originating from lung tumors, but the prognostic value for disease recurrence needs additional investigations with better categorization of the disease stages. Moreover, tumor cell dissemination during
| Cancer types | Diagnosis (samples during the diagnostic assessment) | Prognosis (samples during surgery) | Monitoring (postoperative recurrence—MRD) |
|--------------|-----------------------------------------------------|-----------------------------------|------------------------------------------|
| PDAC         | Echo-guided portal puncture (EUS-guided and external ultrasound-guided portal vein puncture) | Companion diagnostic test; Decision algorithm for neoadjuvant treatment | Direct portal vein puncture | Decision algorithm for adjuvant chemotherapy | External ultrasound-guided portal vein puncture | Confirmed disease relapse and monitoring metastatic disease |
| CRC          | External ultrasound-guided portal vein puncture | Companion test; Adapt therapeutic sequences | Direct portal vein puncture | Decision for adjuvant chemotherapy | External ultrasound-guided portal vein puncture | Confirmed disease relapse and adapt personalized treatment |
| HCC          | External ultrasound-guided portal vein puncture and hepatic vein sampling (transjugular) | Staging and treatment strategy (i.e., ablation, resection, chemoembolization) | Direct portal and hepatic vein puncture | Decision for adjuvant chemotherapy | External ultrasound-guided portal vein puncture and hepatic vein sampling (transjugular) | Staging and treatment strategy (i.e., ablation, resection, chemoembolization, liver transplantation) |
| NSCLC        | Endovascular procedure | Companion diagnostic test; Staging and treatment strategy | Direct pulmonary vein puncture (before and after tumor mobilization) | Decision for adjuvant therapy | Endovascular procedure | Confirmed disease relapse and monitoring metastatic disease and adapt treatment |

CRC, colorectal cancer; EUS, endoscopic ultrasound; HCC, Hepatocellular carcinoma; MRD, minimal residual disease; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma.
surgical procedure deserves to be better characterized. Nevertheless, CTC detection and characterization could help to obtain new insights into the tumor cell molecular features and design therapeutic strategies in NSCLC (Table 6).

8. Future directions

Analysis of the results obtained in different cancer types with similar approaches suggests that compared with peripheral CTC detection alone, combining CTC detection in the tumor-draining vein and peripheral blood at the time of surgery or by ultrasonography-guided puncture could improve the identification of patients at higher risk for cancer recurrence. Alternatively, diagnostic leukapheresis (DLA), a procedure recently introduced by Stoecklein’s group to screen liters of blood (Andree et al., 2018; Fischer et al., 2013), could improve the chance of CTC recovery. Indeed, processing DLA products using CellSearch® increased CTC yields up to 32-fold.

Importantly, feasibility of portal vein puncture by ultrasonography-guided puncture has already been successfully tested twice, allowing for the detection of CTCs for 100% of PDAC patients (Table 1 (Catenacci et al., 2015; Liu et al., 2018)). This approach is worth trying in HCC, since the only study testing CTC detection in the portal vein was promising for both CTC retrieval and prognostic performances (Table 3 (Sun et al., 2018)). For CRC, as CTC yields were not increased in mesenteric or portal vein, prognostic value was not tested in most of the studies. Thus, it is too early to recommend portal vein ultrasonography-guided puncture in CRC patients. By contrast, pulmonary vein puncture repeatedly increased chances of CTC detection and carried high prognostic value (Tables 4 and 5). However, besides surgery, it is feasible to sample the pulmonary vein using endovascular procedure (Haïssaguerre et al., 1998).

Moreover, additional studies on CTC detection in vessels close to the primary tumors are needed, particularly to obtain crucial information on the tumor biology and the metastatic cascade by genomic analysis of isolated single CTC. Indeed, we need to learn more on CTC heterogeneity during their journey in the bloodstream, and the selection of CTC subclones through specific filtering organs (e.g., the liver) (Joosse et al., 2018). This particular aspect could be evaluated by comparing peripheral CTCs versus tumor vicinity CTCs by single-cell analysis. Based on the hypothesis that CTCs represent cells at the origin of metastases, these cells could also predict the genetic landscape of the metastatic tumors. For example, in cancers that carry multiple genetic mutations, these alterations may not be homogeneously distributed and the tumor biopsy may not show all the mutations. Thus, CTC analysis can contribute to the genetic/molecular characterization of the tumor for prognostic/therapy stratification purposes (Table 6), and also to the discovery of new biomarkers.

Acknowledgements

We thank Dr. Elisabetta Andermarcher for assistance with her comments and proofreading of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

Alix-Panabières C and Pantel K (2013) Circulating tumor cells: liquid biopsy of cancer. Clin Chem 59, 110–118.
Alix-Panabières C and Pantel K (2014) Challenges in circulating tumor cell research. Nat Rev Cancer 14, 623–631.
Alvarez Cubero MJ, Lorente JA, Robles-Fernandez I, Rodriguez-Martinez A, Puche JL and Serrano MJ (2017) Circulating tumor cells: markers and methodologies for enrichment and detection. Methods in Mol Biol (Clifton, NJ) 1634, 283–303.
Andree KC, Mentink A, Zeune LL, Terstappen LW, Stoecklein NH, Neves RP, Driemel C, Lampignano R, Yang L, Neubauer H et al. (2018) Toward a real liquid biopsy in metastatic breast and prostate cancer: diagnostic leukapheresis increases CTC yields in a European Prospective Multicenter Study (CTCTrap). Int J Cancer 143, 2584–2591.
Ashworth TR (1869) A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Aust Med J 14, 146–149.
Bernaumin J, Coulon S, Saintigny P, Bazelly B, Ricci S, Le Pimpec Barthes E and Milleron B (2005) PD-085 detection of circulating cancer cells by real time RT-PCR in the pulmonary vein of patients with non-small cell lung cancer. Lung Cancer 49, S92. https://doi.org/10.1016/S0169-5002(05)80418-0.
Bissolati M, Sandri MT, Burtolo G, Zorzino L, Balzano G and Braga M (2015) Portal vein-circulating tumor cells predict liver metastases in patients with resectable pancreatic cancer. Tumor Biol 36, 991–996.
Buscail L (2017) Commentary: Pancreatic cancer: Is the worst to come? Int J Epidemiol 46, 1774–1775.
Catenacci DVT, Chapman CG, Xu P, Koons A, Konda VJ, Siddiqui UD and Waxman I (2015) Acquisition of
portal venous circulating tumor cells from patients with pancreaticobiliary cancers by endoscopic ultrasound. *Gastroenterology* 149, 1794–1803.e4.

Chapman CG and Waxman I (2016) Portal-vein blood samples as a new diagnostic entity for pancreatic cancer. *Expert Rev Gastroenterol Hepatol* 10, 665–667.

Chudasama D, Burnside N, Beeson J, Karteris E, Rice A and Anikin V (2017) Perioperative detection of circulating tumor cells in patients with lung cancer. *Oncol Lett* 14, 1281–1286.

Cohen SJ, Alpaugh RK, Gross S, O’Hara SM, Smirnov DA, Terstappen LW, Allard WJ, Bilbee M, Cheng JD, Hoffman JP, Lewis NL et al. (2006) Isolation and characterization of circulating tumor cells in patients with metastatic colorectal cancer. *Clin Colorectal Cancer* 6, 125–132.

Cohen SJ, Punji CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Pisac J, Morse MA, Mitchell E, Miller MC et al. (2009) Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol* 20, 1223–1229.

Connor AA, McNamara K, Al-Sukhni E, Diskin J, Chan D, Ash C, Lowes LE, Allan AL, Zogopoulos G, Moulton CA et al. (2016) Central, but not peripheral, circulating tumor cells are prognostic in patients undergoing resection of colorectal cancer liver metastases. *Ann Surg Oncol* 23, 2168–2175.

Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW et al. (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *New Engl J Med* 351, 781–791.

Crosbie PA, Shah R, Krysiak P, Zhou C, Morris K, Tugwood J, Booton R, Blackhall F and Dive C (2016) Circulating tumor cells detected in the tumor-draining pulmonary vein are associated with disease recurrence after surgical resection of NSCLC. *J Thoracic Oncol* 11, 1793–1797.

Denève E, Riethdorf S, Ramos J, Nocca D, Coffy A, Daurès J-P, Maudelonde T, Fabre J-M, Pantel K and Alix-Panabières C (2013) Capture of viable circulating tumor cells in the liver of colorectal cancer patients. *Clin Chem* 59, 1384–1392.

Fan J-L, Yang Y-F, Yuan C-H, Chen H and Wang F-B (2015) Circulating tumor cells for predicting the prognostic of patients with hepatocellular carcinoma: a meta analysis. *Cell Physiol Biochem* 37, 629–640.

Fang Z-T, Zhang W, Wang G-Z, Zhou B, Yang G-W, Qu X-D, Liu R, Qian S, Zhu L, Liu L-X et al. (2014) Circulating tumor cells in the central and peripheral venous compartment – assessing hematogenous dissemination after tranarterial chemoembolization of hepatocellular carcinoma. *Oncotargets Therapy* 7, 1311–1318.

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 136, E359–E386.

Fischer JC, Niederacher D, Topp SA, Honisch E, Schumacher S, Schmitz N, Föhrding LZ, Vay C, Hoffmann I, Kasprowicz NS et al. (2013) Diagnostic leukapheresis enables reliable detection of circulating tumor cells of nonmetastatic cancer patients. *Proc Natl Acad Sci USA* 110, 16580–16585.

Forner A, Llovet JM and Bruix J (2012) Hepatocellular carcinoma. *Lancet (London, England)* 379, 1245–1255.

Gallo M, De Luca A, Maiello MR, D’Alessio A, Esposito C, Chicchinelli N, Forgione L, Piccirillo MC, Rocco G, Morabito A et al. (2017) Clinical utility of circulating tumor cells in patients with Non-Small-Cell Lung Cancer. *Translat Lung Cancer Res* 6, 486–498.

Gbolahan O and O’Neil B (2019) Update on systemic therapy for colorectal cancer: biologics take sides. *Translat Gastroenterol Hepatol* 4, 9.

Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WEE, Nicholson AG, Groome P, Mitchell A, Bolejack V et al. (2016) The IASLC lung cancer staging project: proposals for revision of the TNM stage groupings in the forthcoming (Eighth) edition of the TNM classification for lung cancer. *J Thoracic Oncol* 11, 39–51.

de Haas RJ, Wicherts DA, Andreani P, Pascal G, Saliba F, Ichai P, Adam R, Castaing D, Azoulay D (2011) Impact of expanding criteria for resectability of colorectal metastases on short- and long-term outcomes after hepatic resection. *Ann Surg* 253, 1069–1079.

Haïssaguerre M, Jaïs P, Shah DC, Takahashi A, Hocini M, Quiniou G, Garrigue S, Le Mouroux A, Le Métayer P and Clémenty J (1998) Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *New Engl J Med* 339, 659–666.

Harouaka RA, Nisic M and Zheng S-Y (2013) Circulating tumor cell enrichment based on physical properties. *J Lab Automat* 18, 455–468.

Hashimoto M, Tanaka F, Yoneda K, Takuwa T, Matsumoto S, Okumura Y, Kondo N, Tsujimura T, Nakano T and Hasegawa S (2018) Positive correlation between postoperative tumor recurrence and changes in circulating tumor cell counts in pulmonary venous blood (PvCTC) during surgical manipulation in non-small cell lung cancer. *J Thorac Dis* 10, 298–306.

Hashimoto M, Tanaka F, Yoneda K, Takuwa T, Matsumoto S, Okumura Y, Kondo N, Tsubota N, Tsujimura T, Tabata C et al. (2014) Significant increase in circulating tumor cells in pulmonary venous blood during surgical manipulation in patients with
primary lung cancer. *Interact Cardiovasc Thorac Surg* **18**, 775–783.

Hench IB, Hench J and Tolnay M (2018) Liquid biopsy in clinical management of breast, lung, and colorectal cancer. *Front Med* **5**, 9.

Jiao LR, Apostolopoulos C, Jacob J, Szylro L, Johnson N, Tsim N, Habib NA, Charles Coombe R and Stebbing J (2009) Unique localization of circulating tumor cells in patients with hepatic metastases. *J Clin Oncol* **27**, 6160–6165.

Johnson DH, Schiller JH and Bunn PA (2014) Recent clinical advances in lung cancer management. *J Clin Oncol* **32**, 973–982.

Joossse SA, Souche F-R, Babayan A, Gasch C, Kerkhoven RM, Ramos J, Fabre J-M, Riethdorf S, König A, Wikman H et al. (2018) Chromosomal aberrations associated with sequential steps of the metastatic cascade in colorectal cancer patients. *Clin Chem*, **64**, 1505–1512. https://doi.org/10.1373/clinchem.2018.289819

Kessenbrock K, Plaks V and Werb Z (2010) Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* **141**, 52–67.

Li Y, Cheng X, Chen Z, Liu Y, Liu Z and Shaofa X (2017) Circulating tumor cells in peripheral and pulmonary venous blood predict poor long-term survival in resected non-small cell lung cancer patients. *Sci Rep* **7**, 4971.

Lianidou ES, Strati A and Markou A (2014) Circulating tumor cells as promising novel biomarkers in solid cancers*. Crit Rev Clin Lab Sci* **51**, 160–171.

Liu X, Li C, Li J, Yu T, Zhou G, Cheng J, Li G, Zhou Y, Lou W, Wang X et al. (2018) Detection of CTCs in portal vein was associated with intrahepatic metastases and prognosis in patients with advanced pancreatic cancer. *J Cancer* **9**, 2038–2045.

Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF and Groom AC (1998) Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *The American Journal of Pathology* **153**, 865–873.

Lv C, Zhao B, Wang L, Zhang P, Ma Y, Wang Y, Nan W, Ying W and Yang Y (2018) Detection of circulating tumor cells in pulmonary venous blood for resectable non-small cell lung cancer. *Oncol Lett* **15**, 1103–1112.

Martin OA, Anderson RL, Narayan K and MacManus MP (2017) Does the mobilization of circulating tumor cells during cancer therapy cause metastasis? *Nat Rev Clin Oncol* **14**, 32–44.

Massagué J and Obenauf AC (2016) Metastatic colonization by circulating tumor cells. *Nature* **529**, 298–306.

McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG and Stephen McCain R (2018) Pancreatic cancer: a review of clinical diagnosis, epidemiology, treatment and outcomes. *World J Gastroenterol* **24**, 4846–4861.

Millner LM, Linder MW and Valdes R (2013) Circulating tumor cells: a review of present methods and the need to identify heterogeneous phenotypes. *Ann Clin Lab Sci* **43**, 295–304.

Murlidhar V, Reddy RM, Fouladdel S, Zhao L, Ishikawa MK, Grabauskiene S, Zhang Z, Lin J, Chang AC, Carrott P et al. (2017) Poor prognosis indicated by venous circulating tumor cell clusters in early-stage lung cancers. *Can J Res* **77**, 5194–5206.

Nguyen DX, Bos PD and Massagué J (2009) Metastasis: From dissemination to organ-specific colonization. *Nat Rev Cancer* **9**, 274–284.

Nieto J, Grossbard ML and Kozuch P (2008) Metastatic pancreatic cancer 2008: Is the glass less empty? *Oncologist* **13**, 562–576.

Okumura Y, Tanaka F, Yoneda K, Hashimoto M, Takuwa T, Kondo N and Hasegawa S (2009) Circulating tumor cells in pulmonary venous blood of primary lung cancer patients. *Ann Thoracic Surg* **87**, 1669–1675.

Pantel K and Alix-Panabières C (2010) Circulating tumor cells in cancer patients: challenges and perspectives. *Trends Mol Med* **16**, 398–406.

Peeters DJE, Van den Eynden GG, van Dam P-J, Prové A, Benoy IH, van Dam PA, Vermeulen PB, Pauwels P, Peeters M, Van Laere SJ et al. (2011) Circulating tumor cells in the central and the peripheral venous compartment in patients with metastatic breast cancer. *Br J Cancer* **104**, 1472–1477.

Pimienta M, Edderkaoui M, Wang R, Pandol S (2017) The potential for circulating tumor cells in pancreatic cancer management. *Front Physiol*, **8**, 381. https://doi.org/10.3389/fphys.2017.00381.

Popper HH (2016) Progression and metastasis of lung cancer. *Cancer Metastasis Rev* **35**, 75–91.

Rahbabi NN, Bork U, Kircher A, Nimitz T, Schölch S, Kahlert C, Schmidt T, Steinert G, Ulrich AB, Reissfelder C et al. (2012) Compartamental differences of circulating tumor cells in colorectal cancer. *Ann Surg Oncol* **19**, 2195–2202.

Reddy RM, Murlidhar V, Zhao L, Grabauskiene S, Zhang Z, Ramnath N, Lin J, Chang AC, Carrott P, Lynch W et al. (2016) Pulmonary venous blood sampling significantly increases the yield of circulating tumor cells in early-stage lung cancer. *J Thoracic Cardiovasc Surg* **151**, 852–858.

Resel FL, Olivier GC, San JML, de Castro S Veganzones, Galante RI, Vidaurreta LM, de la OGV et al. (2010) Immunomagnetic quantification of circulating tumoral cells in patients with prostate cancer: clinical and pathological correlation. *Arch Exp Urol* **63**, 23–31.

Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK,
Vonderheide RH et al. (2012) EMT and dissemination precede pancreatic tumor formation. *Cell* 148, 349–361.

Riethdorf S, O’Flaherty L, Hille C and Pantel K (2018) Clinical applications of the cell search platform in cancer patients. *Adv Drug Deliv Rev* 125, 102–121.

Sawabata N, Funaki S, Hyakutake T, Shintani Y, Fujiwara A and Okumura M (2016) Perioperative circulating tumor cells in surgical patients with non-small cell lung cancer: Does surgical manipulation dislodge cancer cells thus allowing them to pass into the peripheral blood? *Surg Today* 46, 1402–1409.

Shyamala K, Girish HC and Murgod S (2014) Risk of tumor cell seeding through biopsy and aspiration cytology. *Journal of International Society of Preventive & Community Dentistry* 4, 5–11.

Siegel RL, Miller KD, Jemal A (2017) ‘Cancer statistics, 2017’ *CA Cancer J Clin* 67, 7–30.

Soler A, Cayrefourcq L, Mazel M and Alix-Panabières C (2017) ‘EpCAM-independent enrichment and detection of viable circulating tumor cells using the EPISPOT assay’. *Methods Mol Biol (Clifton, NJ)* 1634, 263–276.

Storm AC and Lee LS (2016) Endoscopic ultrasounds-guided techniques for diagnosing pancreatic mass lesions: can we do better? *World J Gastroenterol* 22, 8658–8669.

Sun Y-F, Guo W, Xu y, Shi Y-H, Gong Z-J, Ji Y, Du M, Zhang X, Hu B, Huang A et al. (2018) Circulating tumor cells from different vascular sites exhibit spatial heterogeneity in epithelial and mesenchymal composition and distinct clinical significance in hepatocellular carcinoma. *Clin Cancer Res* 24, 547–559.

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

Tan Y and Hao W (2018) The significant prognostic value of circulating tumor cells in colorectal cancer: A systematic review and meta-analysis. *Curr Probl Cancer* 42, 95–106.

Tarumi S, Gotoh M, Kasai Y, Matsuura N, Okuda M, Go T, Ishikawa S and Yokomise H (2013) Innovative method using circulating tumor cells for prediction of the effects of induction therapy on locally advanced non-small cell lung cancer. *J Cardiothoracic Surg* 8, 175.

Tateishi R, Yoshida H, Matsuayama Y, Mine N, Kondo Y and Omata M (2008) Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. *Hep Int* 2, 17–30.

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

Tien YW, Kuo H-C, Ho B-I, Chang M-C, Chang Y-T, Cheng M-F, Chen H-L, Liang TY, Wang CF, Huang CY et al. (2016) A high circulating tumor cell count in portal vein predicts liver metastasis from periampullary or pancreatic cancer: a high portal venous CTC count predicts liver metastases. *Medicine* 95, e3407.

Wind J, Tuynman JB, Tibbe AGJ, Swennenhuis JF, Richel DJ, van Berge Henegouwen MI and Bemelman WA (2009) Circulating tumor cells during laparoscopic and open surgery for primary colonic cancer in portal and peripheral blood. *Europ J Surg Oncol* 35, 942–950.

Yu M, Stott S, Toner M, Maheswaran S and Haber DA (2011) Circulating tumor cells: approaches to isolation and characterization. *J Cell Biol* 192, 373–382.

Zhou B, Jian-Wei X, Cheng Y-G, Gao J-Y, San-Yuan H, Wang L and Zhan H-X (2017) Early detection of pancreatic cancer: Where are we now and where are we going? *Int J Cancer* 141, 231–241.

**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1** Main studies for CTC detection in the peripheral blood of patients with PDAC, CRC, NSCLC, and HCC.