Int J Clin Oncol (2017) 22:421–430
DOI 10.1007/s10147-017-1105-2

INVITED REVIEW ARTICLE

Circulating tumor cells: clinical validity and utility

Luc Cabel[1,2] · Charlotte Proudhon[2] · Hugo Gortais[1] · Delphine Loirat[1,3] · Florence Coussy[1] · Jean-Yves Pierga[1,2,4] · François-Clément Bidard[1,2,5]

Received: 14 February 2017 / Accepted: 15 February 2017 / Published online: 25 February 2017

Abstract Circulating tumor cells (CTCs) are rare tumor cells and have been investigated as diagnostic, prognostic and predictive biomarkers in many types of cancer. Although CTCs are not currently used in clinical practice, CTC studies have accumulated a high level of clinical validity, especially in breast, lung, prostate and colorectal cancers. In this review, we present an overview of the current clinical validity of CTCs in metastatic and non-metastatic disease, and the main concepts and studies investigating the clinical utility of CTCs. In particular, this review will focus on breast, lung, colorectal and prostate cancer. Three major topics concerning the clinical utility of CTC are discussed—(1) treatment based on CTCs used as liquid biopsy, (2) treatment based on CTC count or CTC variations, and (3) treatment based on CTC biomarker expression. A summary of published or ongoing phase II and III trials is also presented.

Keywords Circulating tumor cells · Clinical validity · Clinical utility · Biomarkers · Clinical trials

✉ Luc Cabel
luc.cabel@curie.fr; luc_cabel@hotmail.fr

1 Department of Medical Oncology, Institut Curie, PSL Research University, 26 rue d'Ulm, 75005 Paris, France

2 Circulating Tumor Biomarkers Laboratory, SiRIC, Institut Curie, PSL Research University, Paris, France

3 Translational Immunotherapy Team, SiRIC, Institut Curie, PSL Research University, Paris, France

4 Paris Descartes University, Paris, France

5 Versailles-Saint-Quentin University, Paris-Saclay, Saint Cloud, France

Introduction

Circulating tumor cells (CTCs) were first described in 1869 [1]. Following the recent development of reproducible detection techniques, CTCs have been investigated as diagnostic, prognostic and/or predictive biomarkers in numerous types of cancer. In this review, after a brief reminder of detection techniques and the liquid biopsy concept, we present (1) an overview of the current clinical validity of CTCs in metastatic and non-metastatic disease, and (2) the main concepts and studies investigating the clinical utility of CTCs.

CTC detection techniques

CTCs are rare tumor cells; approximately 1 CTC per ml of blood released by primary tumors or metastases can be detected in peripheral blood. An ideal CTC detection platform must be able to isolate and detect all heterogeneous CTCs, while discarding the very large background of normal blood cells [2]. CTC selection is usually the first step of CTC detection, with positive or negative enrichment (Fig. 1). A detection step then further distinguishes (and possibly characterizes) CTCs from the remaining normal cells [2].

The selection step is based on the biochemical or biophysical properties of CTCs. Differences in cell-surface antigen expression between CTCs and leukocytes allow positive selection of CTCs and negative selection of leukocytes. In carcinoma, the most commonly used antigen for positive immunoselection of CTCs is the epithelial cell adhesion molecule (EpCAM), which is expressed by most carcinoma cells. The Cellsearch® system, which is the

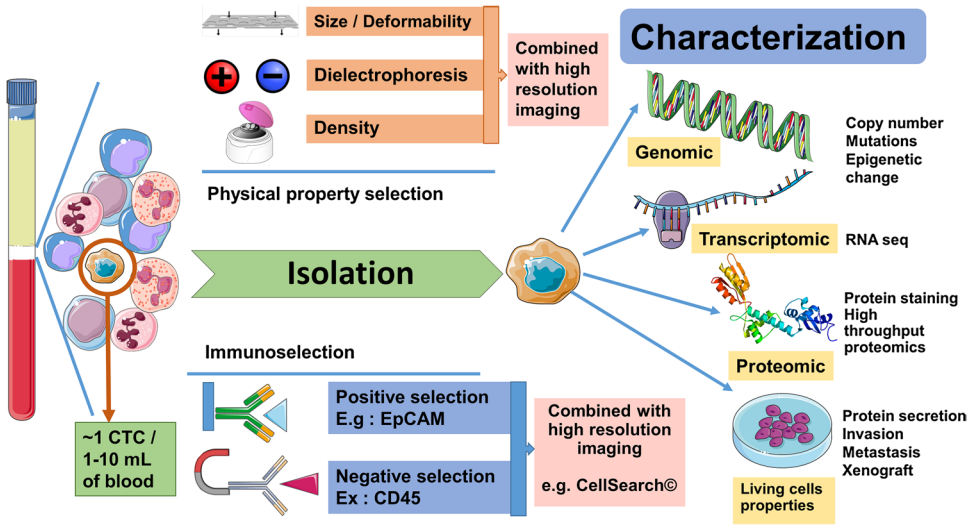

Fig. 1 Circulating tumor cells (CTC): detection techniques

only FDA-cleared detection technique, uses EpCAM-positive immunoselection as the CTC selection step. Negative immunoselection of leukocytes generally targets CD45, a pan-leukocyte antigen. Biophysics-based CTC selection is based on differences in size, deformability, bioelectric features or density between CTCs and leukocytes.

After CTC enrichment, verification of the identity of captured cells is generally based on high-resolution imaging combined with immunocytofluorescent staining. More sophisticated techniques include downstream high-throughput genomic, transcriptomic or proteomic characterization of isolated cells. A common definition of CTCs, used by CellSearch® for example, is an EpCAM+/cytokeratin+/DAPI+/CD45 cell detected in the blood [3]. Importantly, approaches based on detection of mRNA expression (by RT-PCR or probe-based assays) have been discontinued due to their lack of specificity and the results of these techniques will not be discussed in this review.

While different techniques allow the capture of CTCs with different phenotypes, their individual sensitivity and specificity are heterogeneous and vary according to the cancer type [4–6]. Because of its practical convenience (blood collection tubes allowing shipment of samples to remote laboratories) and FDA approval, most CTC clinical data have been obtained with the CellSearch® technique.

CTCs as liquid biopsy material: current status

Biopsies are invasive, costly, time-consuming, and potentially harmful and cannot be easily repeated during therapy. CTC detection is therefore very useful to avoid tissue biopsies, as CTCs can theoretically be characterized by all of the 'omic' assays that are usually performed on tissue biopsies. For instance, in breast cancer, point mutation [7], *ERBB2* amplification [8], copy number variation [9], DNA methylation [10], transcriptomic [11] and proteomic [12] analyses have been shown to be feasible on CTCs.

The CTC count depends on the technique used and the volume of blood screened, but the CTC count ultimately impacts the validity of any CTC-based biomarker analysis. In early and metastatic breast cancer, we have demonstrated that assessment of HER2 levels by immunocytofluorescence on CTC was unreliable in patients with low CTC count (<5 CTCs/7.5 ml) [13]. Numerous other 'omic' techniques require more material (DNA, RNA, proteins) than what is available in a single cell, and consequently require either pooling of CTCs (e.g., mixing DNA or proteins from different CTCs) or pre-analytical amplification steps that may generate artefacts. In this context, it is therefore difficult to interpret the heterogeneous results obtained by single-cell approaches, e.g., *PIK3CA* mutation in CTCs from breast cancer patients [7, 14] and to demonstrate any clinical validity of these results. In addition to the above-mentioned intrinsic limitations of CTC-based biomarker analyses, the recent development of circulating tumor DNA [15] has limited the relevance of CTCs for DNA mutation analysis, especially for the detection of single nucleotide variations. The detection of complex chromosomal rearrangements, such as translocations, remains difficult on circulating tumor DNA, but is feasible on CTCs [16].

The key advantage of CTCs, especially in relation to circulating tumor DNA, is to allow all other 'omic' analyses in addition to DNA sequence analysis, and especially to allow the study of mRNA and proteins expressed by tumor cells. Numerous proteins are the actual targets of antitumor drugs and can be used to tailor therapy. In prostate cancer, resistance to abiraterone and enzalutamide has recently been related to a splicing variant of the androgen receptor mRNA (AR-V7 [17, 18]). PD-L1 [19] and estrogen

receptor [20] are two examples of proteins whose expression levels by the tumor may influence response to immune checkpoint blockers and hormone therapy, respectively.

As of January 2017, there is currently no validated use of CTCs as a liquid biopsy technique in clinical practice, but some of these tests may become approved and reimbursed in the near future, pending further demonstration of their clinical validity and favorable economic evaluation profile [21].

Clinical validity of CTCs

Cancer screening

Studies using the Cellsearch® technique in early non-metastatic cancer have usually reported very low CTC detection rates (5–30% [22–30], depending on the cancer type). Moreover, the specificity of this technique is somewhat limited, as some circulating epithelial cells can be found in individuals with inflammatory disease or even in some healthy individuals [31]. The use of this system for cancer screening is therefore clearly impaired by its limited sensitivity and specificity. Although most of the other CTC detection techniques have not been shown to present better performances, striking results have been reported for early lung cancer detection using a filtration-based detection technique, ISET. In a preliminary report, Fiorelli et al. found that a CTC count >25 could help distinguish lung cancer from benign lesions in patients with abnormal lung imaging [32]. Using the same technology, Ilie et al. showed, in high-risk patients diagnosed with tobacco-induced chronic obstructive pulmonary disease, that only patients with detectable CTCs developed lung cancer during follow-up [33]. These results are now being investigated in a confirmatory study (NCT02500693), while our team has initiated a study on *BRCA1* carriers who are at high risk of breast and ovarian cancers (NCT02608346).

Prognostic value of CTCs in metastatic cancers

The highest level of clinical evidence for the prognostic value of elevated CTC count (CellSearch®) was obtained in a pooled analysis including data from 1944 individual metastatic breast cancer patients from 20 European studies [34]. In this study, coordinated by our group, 46.9% of patients had ≥5 CTCs/7.5 ml of blood at baseline, before the start of a new line of therapy. These patients had decreased progression-free survival (PFS) (hazard ratio [HR] 1.92, $p < 0.0001$] and overall survival (OS) (HR 2.78, $p < 0.0001$) compared to patients with a low CTC count (<5 CTCs/7.5 ml). Moreover, the addition of baseline CTC count to optimized clinicopathological models (including all available significant prognostic factors) significantly improved survival predictions.

Publication-based meta-analyses confirmed that an elevated baseline CTC count in metastatic colon cancer is an independent prognostic factor for PFS and OS with CellSearch® [35] or RT-PCR/immunostaining techniques [36]. The prognostic value of CTCa has also been demonstrated during chemotherapy [37–39]. Detection of CTCs undergoing epithelial−mesenchymal transition (EMT) also appeared to predict outcome in a study with plastin-3, an EMT marker [40]. In a phase II study, Krebs et al. found that patients with elevated baseline CTC counts (≥3 CTCs/7.5 ml) could benefit from an intensive chemotherapy regimen, unlike patients with low CTC counts [41]. This result suggests that CTC count may help to tailor treatment regimens in metastatic colorectal cancer patients; a confirmatory randomized clinical trial is currently underway (Table 1, NCT01640405).

In metastatic prostate cancer, CTCs have repeatedly been reported to be a strong independent prognostic factor, particularly in castration-resistant prostate cancer (CRPC) patients. Most of these studies have been reviewed elsewhere [42]. In a large prospective study on 711 CRPC patients previously treated by docetaxel, Scher et al. demonstrated that elevated CTC counts (≥5 CTCs/7.5 ml, CellSearch®) after 12 weeks of therapy was a strong prognostic factor [43]. Interestingly, the combination of CTC count and lactate dehydrogenase (LDH) level at 12 weeks was investigated as a surrogate marker of survival at the individual-patient level and fulfilled some of the surrogacy criteria; the 2-year OS was 46% in the low-risk group (low CTC and LDH) versus 2% in the high-risk group (≥5 CTCs/7.5 mL of blood and LDH >250 U/L) and 10% in the intermediate-risk group.

CTC count has also been shown to be an independent prognostic factor in non-small cell lung cancer [44, 45], and small cell lung cancer [46].

Prognostic value in non-metastatic cancers

CTCs have been extensively studied in breast cancer, in both neoadjuvant and adjuvant settings [22], mostly with the CellSearch® detection technique. In the neoadjuvant setting, a meta-analysis based on >2000 patients, reported by our group, showed that CTC detection was a strong independent prognostic factor [47], as each CTC detected added a further 'quantum' of poor prognosis in terms of distant disease-free survival (DFS) and OS as well as locoregional relapse-free survival. Inflammatory breast cancers have a higher CTC detection rate (almost 40%) [48], whereas CTC counts are generally lower in non-inflammatory breast cancers (approximately 20% before chemotherapy and 15% before surgery [22]). In the postoperative

Table 1 Clinical utility of CTCs: published or ongoing phase II or III trials

Trial name and organ	Primary objective	Results (for published trials)
Phase III		
SWOG S0500 [70] BREAST	Evaluate whether changing chemotherapy after one cycle of first-line chemotherapy in MBC patients with persistent CTC detection can improve the outcome	No significant increase of survival (PFS and OS) in patients randomized to the CTC-based management arm
CirCe01 NCT01349842 BREAST	Evaluate whether CTCs can guide chemotherapy from the third line of chemotherapy for MBC patients.	Ongoing
DETECT-III NCT01619111 BREAST	Evaluate the efficacy of lapatinib in HER2- negative MBC patients and HER2-positive CTC	Ongoing
STIC-CTC NCT01710605 BREAST	Assess the value of baseline CTCs to determine first-line treatment (hormone therapy vs chemotherapy) in hormone receptor-positive MBC patients. Randomization between treatment arms by the clinician or baseline CTC levels (hormone therapy if <5 CTCs chemotherapy if ≥5 CTCs)	Ongoing
VISNU-1 NCT01640405 COLON	Assess the value of first-line triplet chemotherapy (FOLFOXIRI-bevacizumab) vs doublet chemotherapy (FOLFOX-bevacizumab) in metastatic colorectal cancer patients with baseline elevated CTC counts (≥3 CTCs)	Ongoing
Phase II		
PESTRIN [72] BREAST	Evaluate lapatinib in HER2-negative MBC and HER2-positive CTCs	7/96 patients screened had HER2-positive CTCs, no objective response (0/7) was observed
Stebbing et al. [73] BREAST	Evaluate lapatinib in eliminating EGFR-positive CTCs in women with MBC	16/43 patients screened had EGFR-CTC(+). 6/14 evaluable patients had a decrease in CTC count, especially for EGFR-positive CTCs
Agelaki et al. [79] BREAST	Evaluate lapatinib in eliminating cytokeratin-positive CTCs in women with MBC	22 patients treated by lapatinib, 76.2% had a CTC decrease
Georgoulias et al. [74] BREAST	Evaluate trastuzumab vs placebo, added to chemotherapy, in HER2-negative primary tumor patients, with HER2(+)-CTCs before and after adjuvant chemotherapy	51 (89%) of the 57 analyzed patients had HER2-CTC(+) and were randomized. Median PFS was longer in the trastuzumab arm than in the placebo arm
Hainsworth et al. [80] BREAST	Evaluate trastuzumab + pertuzumab vs placebo in HER2-negative MBC and HER2-positive CTCs	14 patients treated: 12 cases of early progression <6 weeks, 1 partial response (12 weeks), 1 stable disease (12 weeks)
TREAT-CTC NCT01548677 BREAST	Evaluate trastuzumab in HER2-negative patients with primary breast cancer who, after completing (neo)adjuvant chemotherapy and surgery, have detectable CTCs in peripheral blood	Ongoing
CirCe T-DM1 NCT01975142 BREAST	Evaluate trastuzumab-emtansine (T-DM1) in HER2-negative MBC patients who have HER2-amplified CTCs	Ongoing
NCT01185509 BREAST	Evaluate the efficacy and safety of trastuzumab and vinorelbine in MBC with HER2-negative primary tumors and HER2(+)CTCs	Study was stopped: not enough confirmed responses to continue treatment

MBC metastatic breast cancer, *CTCs* circulating tumor cells

setting, Rack et al. showed that CTC detection before or after adjuvant chemotherapy in 21% of patients was also a prognostic factor [49]. In these studies, CTC detection appeared to be independent of other prognostic factors, such as lymph node involvement, breast cancer size, grade or subgroup.

In locally advanced pancreatic cancer, the prospective LAP07 study also showed CTC detection to be an independent prognostic factor for OS, although based on a low detection rate (9% with CellSearch®) [30]. Similar findings have been reported in non-metastatic colon cancer using CellSearch® [24, 25, 50] or RT-PCR [51] before surgery. In contrast, CTC detection by CellSearch® after surgery was not associated with poorer outcome in a large prospective study ($N = 472$ patients) [52]. In that study, Van Dalum et al. detected CTCs both before and after surgery. CTC detection rates were similar (24% before and 20% after surgery), but only preoperative CTC detection was a prognostic factor. Using Cellsearch®, only studies that analyzed CTCs before surgery found CTCs to be a prognostic factor [24, 25, 50], unlike postoperative CTC detection studies [24, 52]. However, there is no explanation for this clinical observation, which is apparently restricted to colorectal cancer. In patients with high-risk colorectal cancer, higher relapse rates were described for patients in whom CTCs were detected after completion of adjuvant chemotherapy [52, 53].

The prognostic value of CTCs has been less clearly demonstrated in other non-metastatic cancers, due to small-scale studies, as in lung cancer, for which few studies have found that patients with positive CTCs after surgery had a higher recurrence rate [54–56]. A review has discussed these studies in the non-metastatic setting [57].

In conclusion, CTC detection is an independent prognostic factor in the localized and metastatic settings in several cancer types (Fig. 2). However, CTCs are rare in the non-metastatic setting, at the limit of detection of current detection methods. For this reason, the cut-off in breast cancer is 5 CTCs per 7.5 ml in the metastatic setting, while it is generally 1 CTC per 7.5 ml in localized disease. Moreover, it is noteworthy that the CTC cut-off demonstrated to be a prognostic factor by one CTC detection technology cannot be extrapolated to another technique due to the sensitivity difference between techniques. Dichotomized CTC count (high or low) has generally been used. However, the absolute value of CTCs also has a prognostic value [3, 58]. Moreover, patients with very high CTC counts in blood generally have an extremely poor prognosis [59]. The timing of CTC detection is also crucial and positive CTCs before, during or after surgery may have different biological and clinical significance. The sensitivity and specificity of CTC detection are currently too low to allow the detection of minimal residual disease, unlike ctDNA [60].

Monitoring treatment response

Numerous studies in metastatic breast cancer have shown that women with high baseline CTC counts but low CTC counts after one cycle of chemotherapy have a significantly better prognosis than women with persistently elevated CTC counts [PFS median: 9.6 months, 95% CI 8.2–11.1 vs 4.8 (3.7–6.5), $p < 0.0001$ and OS median: 27 months 95% CI 21.7–31.5 vs 13.1, 95% CI 9.4–16.4, $p < 0.0001$)]. Moreover, this prognosis was almost the same as that of patients with low baseline CTC counts [61]. We showed that the combination of CTC count and variation of CTC count during treatment significantly increased the accuracy of multivariate prognostic models based on standard clinical and pathological characteristics, unlike serum tumor markers (CEA or CA15.3) [34]. Similar findings

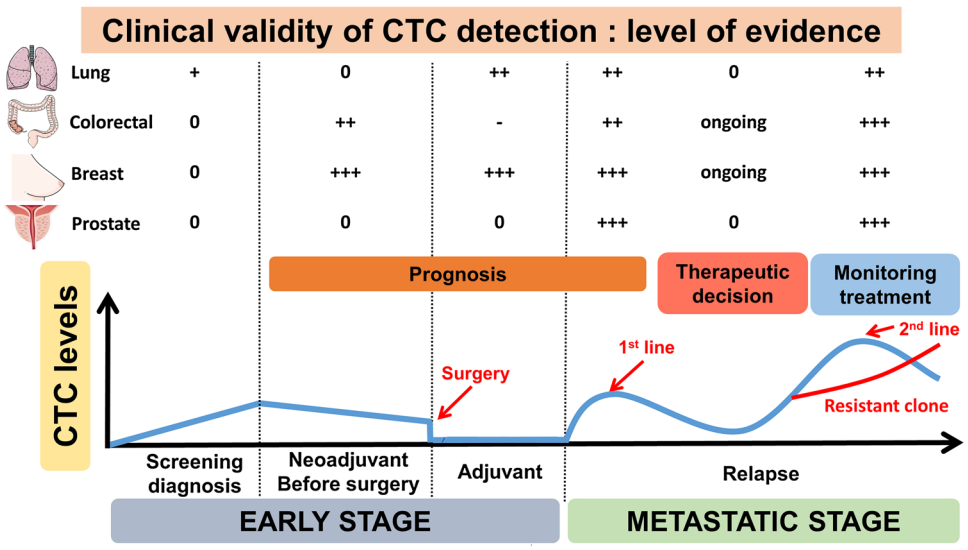

Fig. 2 Clinical validity of circulating tumor cells (CTC): level of evidence according to clinical settings

were reported in metastatic colon cancer, in which changes in CTC levels during chemotherapy were correlated with outcome [37–39]. In the largest study ($N = 319$ patients) [38], Cohen et al. showed that PFS and OS were significantly better in patients with high baseline CTC counts and low CTC counts at 3–5 weeks than in patients with high CTC counts at both time points (PFS 6.2 vs 1.6 months, $p = 0.02$; OS 11.0 vs 3.7 months, $p = 0.0002$). As observed in metastatic breast cancer, the outcome of patients with decreasing CTC counts was similar to that of patients with persistently low CTC counts. Variations in CTC levels have also been shown to be a prognostic factor in CRPC treated by docetaxel [62, 63] and in small cell lung cancer [46].

Due to the low rate of CTC detection in non-metastatic cancers, the clinical validity of changes in CTC counts during therapy is intrinsically limited; as reported by Poisson's law of rare events, a patient with 1 CTC detected before therapy and 0 CTC after therapy cannot be considered to present a true decrease in the CTC count. Keeping this statistical issue in mind, CTC 'decrease' was not significantly associated with pathological complete response of breast cancer treated by neoadjuvant chemotherapy [22]. During adjuvant therapy, persistently detectable CTCs after chemotherapy were associated with a negative impact on survival, while patients with 'increasing' CTC counts (i.e., 0 CTC before therapy and 1 CTC after therapy) did not have a poorer prognosis [49]. In ovarian cancer, CTC monitoring by means of a sensitive assay is more accurate than serum marker CA125 to predict response to chemotherapy and cancer relapse [64]. A phase 2 study conducted in 80 patients with non-metastatic gastric cancer showed that CTC decrease during neoadjuvant chemotherapy was an independent prognostic factor of survival [65], using a technique claiming an unusually high CTC detection rate (>90% of patients). In rectal cancer, a reduction in CTC counts (CellSearch®) was also observed in patients treated by neoadjuvant chemoradiotherapy with complete or partial remission, confirming the findings of two older studies using RT-PCR techniques [66, 67], but CTC detection was not a prognostic factor for post-treatment survival [27].

Altogether, these reports suggest that CTC monitoring is able to predict the efficacy of treatments, particularly in metastatic breast, colon and prostate cancers. However, this approach is limited by the low CTC detection rate before therapy, particularly in patients with non-metastatic cancer.

CTCs as liquid biopsy material

Several studies have correlated the phenotypic or genotypic characterization of CTCs with that of the matched tumor tissue, with a number of discordant results [7, 68]. A critical aspect of any CTC-based liquid biopsy approach is the number of CTCs examined. By comparing HER2 expression on CTCs with that of matched primary tumors in patients with early or advanced breast cancer, we demonstrated that CTC-based status may be unreliable in patients with low CTC counts. On the basis of these results, the validity of any predictive test based on CTC characterization can be expected to be limited to patients with high CTC counts, i.e., patients with a poorer prognosis. Building a clinical scenario taking into account both the intrinsic prognostic value of CTC counts and any predictive value of CTC characterization is feasible, but would require separately investigating patients with low and high CTC counts [69].

Clinical utility of CTCs

Although the clinical validity of CTC detection has been demonstrated, as described above, the clinical utility of CTC detection (i.e, does it improve patient outcome) has yet to be demonstrated before it can be implemented in routine clinical practice. Clinical trials assessing the clinical utility of CTCs have investigated three main concepts—(1) using CTCs as surrogate tumor material for liquid biopsy (e.g., AR-V7 detection predicting resistance to androgen-deprivation therapy), (2) CTC counts and CTC changes during therapy, and (3) targeting biological features that are specific for CTCs or related to metastatic spread. This last objective should be distinguished from the liquid biopsy concept, as the target is a molecular process or biomarker whose expression is specific to CTCs and not shared in common by all tumor cells. As CTCs reflect tumor heterogeneity, lead to metastasis and correlate with poorer prognosis, targeting molecular events responsible for CTC spread may help to improve patient outcome. Interventional phase 2 and 3 studies based on CTCs are summarized in Table 1 and several of them are described below.

Treatment based on CTCs used as liquid biopsy

The NCT02621190 phase II trial was proposed to study the splice variant of AR-V7, which has been shown to be a biomarker of resistance to anti-androgen therapy such as enzalutamide or abiraterone [17]. In the NCT02621190 trial, the investigators hypothesized that patients with AR-V7-positive CTCs would have a meaningful response to cabazitaxel, but this trial was withdrawn prior to enrollment.

Treatment based on CTC counts or CTC variations

The STIC CTC phase III randomized trial investigates the clinical utility of baseline CTC counts in first-line estrogen receptor-positive metastatic breast cancer patients (NCT01710605). In this trial, the type of first-line treatment

(hormone therapy or chemotherapy) is determined by clinicians (standard arm) or by baseline CTC level (CTC arm). In the CTC arm, patients with low baseline CTC counts are given hormone therapy, while patients with high CTC counts are treated by first-line chemotherapy. The rationale of this trial is that CTC count is a stronger prognostic factor than other clinical prognostic factors currently used by clinicians to choose between the two treatment modalities. In this trial, >700 patients have been randomized and the results are expected in 2017.

The utility of monitoring changes in CTC counts during therapy was investigated by the SWOG S0500 trial in first-line metastatic breast cancer [70]. Patients with CTC counts remaining >5 CTCs/7.5 ml (poorer prognosis) after one cycle of chemotherapy were randomized and were possibly switched earlier to second-line chemotherapy. After having included 595 patients and randomized 120 patients, this study was reported as negative; an early switch of chemotherapy did not improve OS in this subgroup of patients. Various explanations for this negative result have been proposed [71]; it is likely that this study selected patients displaying early chemoresistance and who are generally not responsive to further chemotherapy. Other clinical trials are currently ongoing, such as the CirCe01 trial, addressing the utility of a similar strategy in the third-line setting of metastatic breast cancer (NCT01349842).

Treatment based on CTC biomarker expression

Only limited proof of concept studies are available for treatment based on CTC biomarker expression. In the HER2-negative breast cancer setting, lapatinib, which binds both EGFR and HER2, was administered to patients with HER2-positive CTCs [72] or EGFR-positive CTCs [73]. No objective tumor responses were observed in these two studies, but these results could possibly be attributed to the limited efficacy of lapatinib administered as monotherapy in HER2-positive breast cancer. In the neoadjuvant setting, another study randomized 75 women with non-metastatic HER2-negative breast cancer and HER2-positive CTCs (by a RT-PCR based technique), to receive either trastuzumab or observation [74]. Twenty-seven of 36 (75%) HER2-negative women treated with trastuzumab became CK19 mRNA-negative compared to seven of 39 (17.9%) women in the observation arm ($p = 0.001$) and trastuzumab also decreased the risk of disease recurrence and prolonged DFS.

Conclusions and future perspectives

Although CTC count has been shown to be a strong prognostic factor and can be used to monitor the efficacy of treatment, CTC detection has not been approved in everyday clinical practice due to the absence of clinical utility.

Large-scale ongoing studies, especially in breast cancer, should determine whether CTC detection can change clinical practice. However, CellSearch® and other techniques using epithelial biomarkers may not capture all CTCs, as mesenchymal CTCs or stem cell-like CTCs present limited expression of epithelial markers, while they probably play a crucial role in cancer metastasis and drug resistance [75–77]. The clinical validity of new CTC detection techniques and the role of mesenchymal CTCs and stem cell-like CTCs should be investigated in larger studies, which may lead to the development of new anticancer strategy in the future. Improvement in the sensitivity of CTC detection could also improve monitoring of treatment response, cancer screening [32], and the use of CTCs as liquid biopsy. Lastly, another potential future application of CTCs would be to develop CTC-derived xenografts that could lead to drug screening [78].

Acknowledgements The Laboratory of Circulating Tumor Biomarkers is supported by grants from Institut Curie SiRIC (Grant INCa-DGOS-4654) and from the Innovative Medicines Initiative joint undertaking (under Grant agreement no. 115749; project Cancer-ID).

Compliance with ethical standards

Conflicts of Interest The authors declare no potential conflicts of interest.

References

1. Ashworth TR (1869) A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Aust Med J. 1869; 14: 146−147
2. Ferreira MM, Ramani VC, Jeffrey SS (2016) Circulating tumor cell technologies. Mol Oncol 10:374–394. doi:10.1016/j.molonc.2016.01.007
3. Coumans FAW, Ligthart ST, Uhr JW et al (2012) Challenges in the enumeration and phenotyping of CTC. Clin Cancer Res Off J Am Assoc Cancer Res 18:5711–5718. doi:10.1158/1078-0432.CCR-12-1585
4. Kirby BJ, Jodari M, Loftus MS et al (2012) Functional characterization of circulating tumor cells with a prostate-cancer-specific microfluidic device. PLoS One 7:e35976. doi:10.1371/journal.pone.0035976
5. Raimondi C, Nicolazzo C, Gradilone A et al (2014) Circulating tumor cells: exploring intratumor heterogeneity of colorectal cancer. Cancer Biol Ther 15:496–503. doi:10.4161/cbt.28020
6. Harb W, Fan A, Tran T et al (2013) Mutational analysis of circulating tumor cells using a novel microfluidic collection device and qPCR Assay. Transl Oncol 6:528–538
7. Pestrin M, Salvianti F, Galardi F et al (2015) Heterogeneity of PIK3CA mutational status at the single cell level in circulating tumor cells from metastatic breast cancer patients. Mol Oncol 9:749–757. doi:10.1016/j.molonc.2014.12.001
8. Mayer JA, Pham T, Wong KL et al (2011) FISH-based determination of HER2 status in circulating tumor cells isolated

with the microfluidic CEE™ platform. Cancer Genet 204:589–595. doi:10.1016/j.cancergen.2011.10.011
9. Kanwar N, Hu P, Bedard P et al (2015) Identification of genomic signatures in circulating tumor cells from breast cancer. Int J Cancer 137:332–344. doi:10.1002/ijc.29399
10. Chimonidou M, Kallergi G, Georgoulias V et al (2013) Breast cancer metastasis suppressor-1 promoter methylation in primary breast tumors and corresponding circulating tumor cells. Mol Cancer Res 11:1248–1257. doi:10.1158/1541-7786.MCR-13-0096
11. Mostert B, Sieuwerts AM, Kraan J et al (2015) Gene expression profiles in circulating tumor cells to predict prognosis in metastatic breast cancer patients. Ann Oncol 26:510–516. doi:10.1093/annonc/mdu557
12. Khoo BL, Warkiani ME, Tan DS-W et al (2014) Clinical validation of an ultra high-throughput spiral microfluidics for the detection and enrichment of viable circulating tumor cells. PLoS One 9:e99409. doi:10.1371/journal.pone.0099409
13. Ligthart ST, Bidard F-C, Decraene C et al (2013) Unbiased quantitative assessment of Her-2 expression of circulating tumor cells in patients with metastatic and non-metastatic breast cancer. Ann Oncol 24:1231–1238. doi:10.1093/annonc/mds625
14. Gasch C, Oldopp T, Mauermann O et al (2016) Frequent detection of PIK3CA mutations in single circulating tumor cells of patients suffering from HER2-negative metastatic breast cancer. Mol Oncol 10:1330–1343. doi:10.1016/j.molonc.2016.07.005
15. Bidard F-C, Weigelt B, Reis-Filho JS (2013) Going with the flow: from circulating tumor cells to DNA. Sci Transl Med 5:207ps14. doi:10.1126/scitranslmed.3006305
16. Pailler E, Adam J, Barthélémy A et al (2013) Detection of circulating tumor cells harboring a unique ALK rearrangement in ALK-positive non-small-cell lung cancer. J Clin Oncol 31:2273–2281. doi:10.1200/JCO.2012.44.5932
17. Antonarakis ES, Lu C, Wang H et al (2014) AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 371:1028–1038. doi:10.1056/NEJMoa1315815
18. Scher HI, Graf RP, Schreiber NA et al (2016) Nuclear-specific AR-V7 protein localization is necessary to guide treatment selection in metastatic castration-resistant prostate cancer. Eur Urol. doi:10.1016/j.eururo.2016.11.024
19. Mazel M, Jacot W, Pantel K et al (2015) Frequent expression of PD-L1 on circulating breast cancer cells. Mol Oncol 9:1773–1782. doi:10.1016/j.molonc.2015.05.009
20. Paoletti C, Larios JM, Muñiz MC et al (2016) Heterogeneous estrogen receptor expression in circulating tumor cells suggests diverse mechanisms of fulvestrant resistance. Mol Oncol 10:1078–1085. doi:10.1016/j.molonc.2016.04.006
21. Markowski MC, Frick KD, Eshleman JR et al (2016) Cost-savings analysis of AR-V7 testing in patients with metastatic castration-resistant prostate cancer eligible for treatment with abiraterone or enzalutamide. Prostate 76:1484–1490. doi:10.1002/pros.23232
22. Bidard F-C, Proudhon C, Pierga J-Y (2016) Circulating tumor cells in breast cancer. Mol Oncol 10:418–430. doi:10.1016/j.molonc.2016.01.001
23. Gazzaniga P, Gradilone A, de Berardinis E et al (2012) Prognostic value of circulating tumor cells in nonmuscle invasive bladder cancer: a cell search analysis. Ann Oncol 23:2352–2356. doi:10.1093/annonc/mdr619
24. van Dalum G, Stam G-J, Scholten LFA et al (2015) Importance of circulating tumor cells in newly diagnosed colorectal cancer. Int J Oncol 46:1361–1368. doi:10.3892/ijo.2015.2824
25. Bork U, Rahbari NN, Schölch S et al (2015) Circulating tumour cells and outcome in non-metastatic colorectal cancer: a prospective study. Br J Cancer 112:1306–1313. doi:10.1038/bjc.2015.88
26. Pierga J-Y, Bidard F-C, Mathiot C et al (2008) Circulating tumor cell detection predicts early metastatic relapse after neoadjuvant chemotherapy in large operable and locally advanced breast cancer in a phase II randomized trial. Clin Cancer Res 14:7004–7010. doi:10.1158/1078-0432.CCR-08-0030
27. Magni E, Botteri E, Ravenda PS et al (2014) Detection of circulating tumor cells in patients with locally advanced rectal cancer undergoing neoadjuvant therapy followed by curative surgery. Int J Colorectal Dis 29:1053–1059. doi:10.1007/s00384-014-1958-z
28. Loh J, Jovanovic L, Lehman M et al (2014) Circulating tumor cell detection in high-risk non-metastatic prostate cancer. J Cancer Res Clin Oncol 140:2157–2162. doi:10.1007/s00432-014-1775-3
29. Thalgott M, Rack B, Horn T et al (2015) Detection of circulating tumor cells in locally advanced high-risk prostate cancer during neoadjuvant chemotherapy and radical prostatectomy. Anticancer Res 35:5679–5685
30. Bidard FC, Huguet F, Louvet C et al (2013) Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. Ann Oncol 24:2057–2061. doi:10.1093/annonc/mdt176
31. Allard WJ, Matera J, Miller MC et al (2004) Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 10:6897–6904. doi:10.1158/1078-0432.CCR-04-0378
32. Fiorelli A, Accardo M, Carelli E et al (2015) Circulating tumor cells in diagnosing lung cancer: clinical and morphologic analysis. Ann Thorac Surg 99:1899–1905. doi:10.1016/j.athoracsur.2014.11.049
33. Ilie M, Hofman V, Long-Mira E et al (2014) "Sentinel" circulating tumor cells allow early diagnosis of lung cancer in patients with chronic obstructive pulmonary disease. PLoS One 9:e111597. doi:10.1371/journal.pone.0111597
34. Bidard F-C, Peeters DJ, Fehm T et al (2014) Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. Lancet Oncol 15:406–414. doi:10.1016/S1470-2045(14)70069-5
35. Huang X, Gao P, Song Y et al (2015) Meta-analysis of the prognostic value of circulating tumor cells detected with the cell search system in colorectal cancer. BMC Cancer 15:202. doi:10.1186/s12885-015-1218-9
36. Rahbari NN, Aigner M, Thorlund K et al (2010) Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. Gastroenterology 138:1714–1726. doi:10.1053/j.gastro.2010.01.008
37. Tol J, Koopman M, Miller MC et al (2010) Circulating tumour cells early predict progression-free and overall survival in advanced colorectal cancer patients treated with chemotherapy and targeted agents. Ann Oncol 21:1006–1012. doi:10.1093/annonc/mdp463
38. Cohen SJ, Punt CJA, Iannotti N et al (2008) Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 26:3213–3221. doi:10.1200/JCO.2007.15.8923
39. Sastre J, Maestro ML, Gómez-España A et al (2012) Circulating tumor cell count is a prognostic factor in metastatic colorectal cancer patients receiving first-line chemotherapy plus bevacizumab: a Spanish Cooperative Group for the Treatment of Digestive Tumors study. Oncologist 17:947–955. doi:10.1634/theoncologist.2012-0048
40. Yokobori T, Iinuma H, Shimamura T et al (2013) Plastin3 is a novel marker for circulating tumor cells undergoing the epithelial-mesenchymal transition and is associated with colorectal cancer prognosis. Cancer Res 73:2059–2069. doi:10.1158/0008-5472.CAN-12-0326

41. Krebs MG, Renehan AG, Backen A et al (2015) Circulating tumor cell enumeration in a phase II trial of a four-drug regimen in advanced colorectal cancer. Clin Colorectal Cancer 14:115–122. doi:10.1016/j.clcc.2014.12.006
42. Vlaeminck-Guillem V (2015) When prostate cancer circulates in the bloodstream. Diagn Basel Switz 5:428–474. doi:10.3390/diagnostics5040428
43. Scher HI, Heller G, Molina A et al (2015) Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. J Clin Oncol 33:1348–1355. doi:10.1200/JCO.2014.55.3487
44. Punnoose EA, Atwal S, Liu W et al (2012) Evaluation of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer: association with clinical endpoints in a phase II clinical trial of pertuzumab and erlotinib. Clin Cancer Res 18:2391–2401. doi:10.1158/1078-0432.CCR-11-3148
45. Krebs MG, Sloane R, Priest L et al (2011) Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. J Clin Oncol 29:1556–1563. doi:10.1200/JCO.2010.28.7045
46. Hou J-M, Krebs MG, Lancashire L et al (2012) Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. J Clin Oncol 30:525–532. doi:10.1200/JCO.2010.33.3716
47. Bidard F-C, Michiels S, et al (2016) IMENEO: International MEta-analysis of circulating tumor cell detection in early breast cancer patients treated by NEOadjuvant chemotherapy. In: Abstracts of the thirty-ninth annual CTRC-AACR San Antonio Breast Cancer Symposium, San Antonio, Texas, 6-10 December 2016. doi:10.1158/1538-7445.SABCS16-S3-01
48. Pierga J-Y, Bidard F-C, Autret A et al (2016) Circulating tumour cells and pathological complete response: independent prognostic factors in inflammatory breast cancer in a pooled analysis of two multicentre phase II trials (BEVERLY-1 and -2) of neoadjuvant chemotherapy combined with bevacizumab. Ann Oncol. doi:10.1093/annonc/mdw535
49. Rack B, Schindlbeck C, Jückstock J et al (2014) Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. J Natl Cancer Inst. doi:10.1093/jnci/dju066
50. Scholten L, Terstappen LWMM, van der Palen J et al (2012) Circulating tumor cells as a possible prognostic tool in newly diagnosed nonmetastatic colorectal cancer? ASCO Meet Abstr 30:395
51. Iinuma H, Watanabe T, Mimori K et al (2011) Clinical significance of circulating tumor cells, including cancer stem-like cells, in peripheral blood for recurrence and prognosis in patients with Dukes' stage B and C colorectal cancer. J Clin Oncol 29:1547–1555. doi:10.1200/JCO.2010.30.5151
52. Sotelo MJ, Sastre J, Maestro ML et al (2015) Role of circulating tumor cells as prognostic marker in resected stage III colorectal cancer. Ann Oncol 26:535–541. doi:10.1093/annonc/mdu568
53. Lu C-Y, Tsai H-L, Uen Y-H et al (2013) Circulating tumor cells as a surrogate marker for determining clinical outcome to mFOLFOX chemotherapy in patients with stage III colon cancer. Br J Cancer 108:791–797. doi:10.1038/bjc.2012.595
54. Yoon SO, Kim YT, Jung KC et al (2011) TTF-1 mRNA-positive circulating tumor cells in the peripheral blood predict poor prognosis in surgically resected non-small cell lung cancer patients. Lung Cancer 71:209–216. doi:10.1016/j.lungcan.2010.04.017
55. Bayarri-Lara C, Ortega FG, de Guevara ACL et al (2016) Circulating tumor cells identify early recurrence in patients with non-small cell lung cancer undergoing radical resection. PLoS One 11:e0148659. doi:10.1371/journal.pone.0148659
56. Zhu W-F, Li J, Yu L-C et al (2014) Prognostic value of EpCAM/MUC1 mRNA-positive cells in non-small cell lung cancer patients. Tumour Biol 35:1211–1219. doi:10.1007/s13277-013-1162-8
57. Cabel L, Proudhon C, Mariani P et al (2017) Circulating tumor cells and circulating tumor DNA: what surgical oncologists need to know? Eur J Surg Oncol. doi:10.1016/j.ejso.2017.01.010
58. Danila DC, Heller G, Gignac GA et al (2007) Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res 13:7053–7058. doi:10.1158/1078-0432.CCR-07-1506
59. Heidary M, Auer M, Ulz P et al (2014) The dynamic range of circulating tumor DNA in metastatic breast cancer. Breast Cancer Res 16:421. doi:10.1186/s13058-014-0421-y
60. Tie J, Wang Y, Tomasetti C et al (2016) Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 8:346ra92. doi:10.1126/scitranslmed.aaf6219
61. Cristofanilli M, Budd GT, Ellis MJ et al (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351:781–791. doi:10.1056/NEJMoa040766
62. de Bono JS, Scher HI, Montgomery RB et al (2008) Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 14:6302–6309. doi:10.1158/1078-0432.CCR-08-0872
63. Goldkorn A, Ely B, Quinn DI et al (2014) Circulating tumor cell counts are prognostic of overall survival in SWOG S0421: a phase III trial of docetaxel with or without atrasentan for metastatic castration-resistant prostate cancer. J Clin Oncol 32:1136–1142. doi:10.1200/JCO.2013.51.7417
64. Pearl ML, Dong H, Tulley S et al (2015) Treatment monitoring of patients with epithelial ovarian cancer using invasive circulating tumor cells (iCTCs). Gynecol Oncol 137:229–238. doi:10.1016/j.ygyno.2015.03.002
65. Ma J, Yao S, Li X-S et al (2015) Neoadjuvant therapy of dof regimen plus bevacizumab can increase surgical resection ratein locally advanced gastric cancer: a randomized controlled study. Medicine (Baltimore) 94:e1489. doi:10.1097/MD.0000000000001489
66. Zitt M, Zitt M, Müller HM et al (2006) Disseminated tumor cells in peripheral blood: a novel marker for therapy response in locally advanced rectal cancer patients undergoing preoperative chemoradiation. Dis Colon Rectum 49:1484–1491. doi:10.1007/s10350-006-0673-4
67. Kienle P, Koch M, Autschbach F et al (2003) Decreased detection rate of disseminated tumor cells of rectal cancer patients after preoperative chemoradiation: a first step towards a molecular surrogate marker for neoadjuvant treatment in colorectal cancer. Ann Surg 238:324–331. doi:10.1097/01.sla.0000086547.27615.e6
68. Gorges TM, Kuske A, Röck K et al (2016) Accession of tumor heterogeneity by multiplex transcriptome profiling of single circulating tumor cells. Clin Chem 62:1504–1515. doi:10.1373/clinchem.2016.260299
69. Bidard F-C, Pierga J-Y, Soria J-C et al (2013) Translating metastasis-related biomarkers to the clinic—progress and pitfalls. Nat Rev Clin Oncol 10:169–179. doi:10.1038/nrclinonc.2013.4
70. Smerage JB, Barlow WE, Hortobagyi GN et al (2014) Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. J Clin Oncol 32:3483–3489. doi:10.1200/JCO.2014.56.2561
71. Bidard F-C, Pierga J-Y (2015) Clinical utility of circulating tumor cells in metastatic breast cancer. J Clin Oncol 33:1622. doi:10.1200/JCO.2014.57.9714
72. Pestrin M, Bessi S, Puglisi F et al (2012) Final results of a multicenter phase II clinical trial evaluating the activity of single-agent lapatinib in patients with HER2-negative metastatic breast cancer and HER2-positive circulating tumor cells. A

proof-of-concept study. Breast Cancer Res Treat 134:283–289. doi:10.1007/s10549-012-2045-1
73. Stebbing J, Payne R, Reise J et al (2013) the efficacy of lapatinib in metastatic breast cancer with HER2 non-amplified primary tumors and egfr positive circulating tumor cells: a proof-of-concept study. PLoS One. doi:10.1371/journal.pone.0062543
74. Georgoulias V, Bozionelou V, Agelaki S et al (2012) Trastuzumab decreases the incidence of clinical relapses in patients with early breast cancer presenting chemotherapy-resistant CK-19mRNA-positive circulating tumor cells: results of a randomized phase II study. Ann Oncol 23:1744–1750. doi:10.1093/annonc/mds020
75. Yu M, Bardia A, Wittner BS et al (2013) Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 339:580–584. doi:10.1126/science.1228522
76. Aceto N, Bardia A, Miyamoto DT et al (2014) Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 158:1110–1122. doi:10.1016/j.cell.2014.07.013
77. Mitra A, Mishra L, Li S (2015) EMT, CTCs and CSCs in tumor relapse and drug-resistance. Oncotarget 6:10697–10711
78. Whittle JR, Lewis MT, Lindeman GJ et al (2015) Patient-derived xenograft models of breast cancer and their predictive power. Breast Cancer Res. doi:10.1186/s13058-015-0523-1
79. Agelaki S, Kalykaki A, Markomanolaki H et al (2015) Efficacy of lapatinib in therapy-resistant HER2-positive circulating tumor cells in metastatic breast cancer. PLoS One 10:e0123683. doi:10.1371/journal.pone.0123683
80. Hainsworth JD, Murphy PB, Alemar JR et al (2016) Use of a multiplexed immunoassay (PRO Onc assay) to detect HER2 abnormalities in circulating tumor cells of women with HER2-negative metastatic breast cancer: lack of response to HER2-targeted therapy. Breast Cancer Res Treat 160:41–49. doi:10.1007/s10549-016-3969-7