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Circulating Tumor Cells and Colorectal Cancer

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Abstract

The significance of circulating tumor cells (CTCs) has been discussed for more than a century. The advent of modern technology has allowed for more reliable detection of CTCs, and recent studies have provided compelling evidence that CTCs predict clinical response in metastatic colorectal cancer (mCRC). Combination of CTC analysis with independent prognostic factors has demonstrated powerful synergy in some studies. The ability of CTCs to predict metastasis and therapy-specific response has high potential clinical utility, with early studies showing promising results in colorectal cancer (CRC). Reliable CTC detection has also allowed for examination of tumor cell dissemination during surgery, and there appears to be a heavy dependence on the approach chosen. This review discusses the evidence for CTC significance, with particular focus on detection methods, novel markers, and clinical outcomes in CRC. Numerous opportunities exist for preclinical, clinical, and translational studies to explore molecular determinants within CTCs, as well as the value of CTCs in directing targeted therapeutics.

Keywords

Circulating tumor cells; Colorectal cancer; CRC; Colon cancer; Rectal cancer; CTC detection; CTC enrichment; Stratification; Prognosis; Overall survival; Progression-free survival; Clinical response; Hepatic resection; Liver resection; Hepatic ablation; Hepatic metastasis; Liver metastasis; Cytokeratin; Tumor cell dissemination; Epithelial cell adhesion molecule; EpCAM; Survivin; Cetuximab; *KRAS*

Introduction

Colorectal cancer (CRC) remains the third most common cancer in the United States, with an overall 5-year survival rate of 64%, which has risen significantly in the past several decades [1]. The stage of diagnosis is the chief variable dictating this statistic. Nineteen percent of patients with CRC are diagnosed with an advanced stage, decreasing their 5-year survival rate to 11% [1]. This highlights the need for improved accessibility and reliability for diagnosis of CRC at earlier stages. Currently, reliable diagnostic techniques include colonoscopy, sigmoidoscopy, and CT, as well as the more costly CT virtual colonoscopy. Development of reliable diagnostic methods that are relatively inexpensive and less invasive may allow for earlier-stage diagnosis and significantly raise survival rates. Furthermore, identification of

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subgroups of patients who would benefit from adjuvant therapy is a high priority. This has been underscored within the past decade in CRC by patient response to cetuximab being determined by *KRAS* mutational status of the tumor [2]. In advanced-stage disease, the availability of circulating tumor cells (CTCs) may allow for better disease monitoring, especially in patients with metastatic CRC (mCRC) who do not have any measurable increase in carcinoembryonic antigen (CEA) or other markers.

The first clinical suggestion that metastasis might arise from primary tumor cells by intravasation has been traced to postmortem clinical observations by Ashworth [3] in 1869. This idea regained attention almost a century later when Engell [4] found evidence of CTCs in live cancer patients. However, follow-up studies by Engell [5] and others [6] found no correlation between survival and the number of tumor cells in the blood, likely because of poor cytologic criterion largely founded on cell morphology and size. Technological advances in subsequent years have increased the ability to accurately and reliably detect CTCs. Detection technology now includes reverse transcriptase polymerase chain reaction (RT-PCR), immunomagnetic separation, microchips, and several others that have been reviewed recently [7•].

The CellSearch System (Veridex LLC, Raritan, NJ) gained approval from the US Food and Drug Administration (FDA) in 2004 for metastatic breast cancer and is now also approved for metastatic prostate and colorectal cancer. This remains the only CTC detection method to have received FDA approval. Under this detection method, CTCs must possess the following properties: a round to oval shape by light scatter, an evident nucleus by 4′,6-diamidino-2-phenylindole (DAPI) staining, epithelial cell adhesion molecule positivity ($EpCAM^+$), and cytokeratin (CK)-8^+, -18^+, -19^+, and $CD45^-$ by immunofluorescence. This method is more efficient in sample size and processing time than other CTC enrichment methods, except for the CTC chip. However, it is limited by its requirement of EpCAM expression and therefore potentiates false-negative results. Nevertheless, this technology has allowed for reliable detection of CTCs (approximately 80–85% recovery of spiked samples) [8,9]. Studies using this technology have established CTCs as an independent prognostic indicator in metastatic breast cancer [9,10], castration-resistant prostate cancer [11], and mCRC [12••]. This review summarizes recent findings regarding CTCs in the clinic as a prognostic factor, novel efforts to improve CTC identification and enumeration, and the effect of resection on CTCs in the context of CRC. Figure 1 outlines CTC detection techniques and identification markers along with a putative CTC schematic for CRC. In the realm of preclinical and translational research, CTCs offer an exciting opportunity to explore new technologies for the recovery of live metastasis-initiating cells. The introduction of molecular characterization is expected to lead to important advances of relevance to prognostication and personalized therapy.

Prognostic Value in the Clinic

An early study by Sastre et al. [13] found the CellSearch system could identify CTCs in CRC patients and that CTC positivity correlated with disease stage (P=0.005). No significant correlation was found between tumor location, grade of differentiation, CEA levels, or lactate dehydrogenase (LDH) levels. A meta-analysis of nine studies conducted between 1998 and 2006 showed that CTC-positive patients, as detected by RT-PCR methods in blood samples collected from the tumor's draining vein, correlated with lymph node (LN)-positive patients (50%) versus LN-negative patients (21%) [14]. Furthermore, hepatic metastasis was found more often in CTC-positive patients (21%) than in CTC-negative patients (8%). These early reports demonstrated the feasibility and potential prognostic value of CTCs in CRC, allowing for larger-scale studies (Table 1).

Cohen et al. [15••] published one of the largest clinical studies of CTCs in mCRC involving 430 mCRC patients at 55 clinical centers in the United Kingdom, the Netherlands, and the United States. Patients were qualified for the study if initiating a new first-, second-, or third-line (with an epidermal growth factor receptor inhibitor) systemic therapy. Patients had peripheral blood collected before treatment initiation and at four time points after treatment initiation. For analysis, patients were grouped into favorable (<3 CTCs/7.5 mL blood) or unfavorable (≥3 CTCs/7.5 mL blood).

The study showed that median progression-free survival (PFS) and overall survival (OS) rates were approximately twice as high for patients in the favorable group based on low CTCs (PFS, 7.9 mo; OS, 18.5 mo) relative to the unfavorable group with elevated CTCs (PFS, 4.5 mo; OS, 9.4 mo) as determined at baseline. Importantly, this significance held to a similar extent when grouping was assigned by CTC count at any evaluated time point. Regardless of change to favorable or unfavorable, patients who switched their group classification 3 to 5 weeks after treatment initiation had a median OS between those of the baseline unfavorable and favorable groups. However, these patients maintained a PFS very close to the favorable group. A multivariate analysis of several significant factors (eg, CTC count, age, line of therapy) found that CTC number was a strong independent predictor of PFS and OS ($P \leq 0.001$) regardless of assessment time point. Interestingly, the study found a significant prognostic synergy between patient grouping by imaging response and CTCs. If patients continued to have elevated CTC counts for prolonged periods after therapy, this was associated with a worse prognosis.

Slightly more than a year later, Cohen et al. [15••] published a follow-up study with extended follow-up time points and expanded analyses using the same grouping described earlier [12••]. The difference in PFS and OS between favorable and unfavorable groups was more pronounced in patients receiving first-line therapy than in second- and third-line therapy patients. OS was prolonged in the favorable group relative to the unfavorable group regardless of whether they were receiving oxaliplatin, bevacizumab, or irinotecan, but PFS was increased only in the latter. The change in OS with baseline CTCs seems independent of age, and the change in PFS is not statistically different between CTC groups with patients older or younger than 65 years old. Eastern Cooperative Oncology Group performance status (ECOG PS) had no large effect on the significant difference of OS in the favorable and unfavorable CTC groups. However, the difference in PFS between favorable and unfavorable CTC groups was insignificant in patients with an ECOG PS of 1 or 2. An updated multivariate analysis reconfirmed baseline CTC number as an independent factor in PFS and even more so in OS.

Together, the two analyses of these mCRC patients by Cohen et al. [12••,15••] demonstrate that CTCs correlate significantly with PFS, and even more so OS, after treatment or at baseline, regardless of age, previous treatment, or disease stage. These data strongly argue for the use of CTCs as an independent prognostic factor in mCRC that may be combined with other factors to improve assessment, as demonstrated with radiographic imaging in the earlier publication.

The largest published mCRC CTC study to date involved 467 patients who received capecitabine, oxaliplatin, and bevacizumab as first-line therapy [16]. Half the patients also received cetuximab. Twenty-nine percent of patients had high CTCs (≥3 CTCs/75. mL blood) and were also more likely to have stage IV disease, to not have received adjuvant chemotherapy, and to have abnormal serum LDH levels relative to other enrolled patients. Patients with hepatic metastasis only or metastasis to additional organs had elevated CTC levels (33%) relative to other patients (12%). The study confirmed previous findings by Cohen et al. [12••,15••] that higher CTC count at baseline or 1 to 2 weeks after treatment correlated with prolonged PFS and OS in both treatment groups.

Patients who converted from the low- to high-CTC group between baseline and a follow-up time point had significantly different median PFS and OS rates, between those of consistently high- and low-CTC patients. Previously, Cohen et al. [12••,15••] published data demonstrating that patients meeting this criterion had a median PFS similar to that of consistently CTC-negative patients. This difference might be the result of slight differences in CTC group classification time point or, more likely, patient treatment history. CTC count after 1 to 2 weeks of treatment appeared to have stronger-than-expected correlations with response by CT compared with baseline CTC count. In support of findings by Cohen et al. [12••,15••], CTC level and Response Evaluation Criteria in Solid Tumors (RECIST) classification by imaging (eg, CT) synergistically predicted OS.

The possibility that CTCs might predict metastasis before detection by conventional methods has considerable impetus. Garrigós et al. [17•] conducted a small-scale study to measure CTCs in 16 patients with stage III and IV CRC. The authors used immunomagnetic beads for EpCAM and subsequent flow cytometry to identify $CD45^{-}$ and $CK7^{+}$ or $CK8^{+}$ cells, as it was more sensitive at CTC detection and enumeration than other available methods. The authors of this study did not examine CellSearch for their comparison. Two of the 16 patients had tumor relapse following resection and had elevated CTCs before relapse relative to the patients without tumor relapse. The use of CTCs as a predictor of metastasis would be a powerful clinical tool; therefore, a study with standardized CTC detection methods and a large sample size is warranted.

The Search for New Markers

Although effort is being made to validate current CTC identification technology, several novel markers have been identified (Table 2). Markers of therapy-specific response are very useful for adjuvant therapy stratification. For instance, Ronzoni et al. [18] evaluated response to bevacizumab in CRC patients and the utility of circulating endothelial cells (CECs) and endothelial progenitor cells as response predictors. Resting CECs, defined as $CD45^{-}$, $CD146^{+}$, $CD34^{+}$, and $CD106^{-}$, had the greatest enrichment in CRC patients relative to benign controls. Baseline CTCs had a strong correlation with response and PFS. No significant changes were found in the evaluated cell levels throughout treatment.

Substantial evidence is emerging for detection of upregulated mRNA in patient blood samples and its correlation with CTCs and prognosis. Findeisen et al. [19] screened 346 genes that are upregulated and found *SERPINB5* to be significantly upregulated in patients with elevated CTCs compared with benign controls. This elevation was detected in cell-spiking experiments and validated in patient blood samples. Future work with *SERPINB5* should determine whether it is differentially expressed in mCRC and if any prognostic value can be gained. Yie et al. [20] have shown that survivin mRNA detected by RT-PCR enzyme-linked immunosorbent assay was correlated with the disease stage of CRC patients. Approximately half the CRC patients tested positive for survivin expression, and half of these patients eventually suffered relapse. Survivin expression was also shown to be a better risk factor (P=0.048) for relapse than age, gender, disease stage, tumor penetration, nodal status, or plasma CEA.

Another study, by Wong et al. [21], found CK20 expression in LNs and blood of CRC patients. A follow-up study found that CK20-positive CTCs in CRC patients predicted metastasis (P<0.001) and had a highly significant impact on OS (P<0.0001) [22]. A randomized trial is being conducted to detect CTC levels by RT-PCR for CK20 and the impact of conventional versus anterior hepatic resection in mCRC patients [23]. The consequences of resection technique on tumor cell dissemination are discussed later in this review.

Koyanagi et al. [24] found that for LNs, CK immunohistochemistry (IHC; 30%) or measurement of CK blood levels by quantitative RT-PCR (qRT-PCR; 60%) was superior to

conventional pathologic LN examination by hematoxylin and eosin staining (17%) in detecting relapse in 12 relapsed CRC patients. CK IHC and qRT-PCR together identified 70% of relapsed patients. Combining mRNA markers for c-MET, melanoma-associated antigen 3 (MAGE-A3), β-1,4-*N*-acetylgalactosaminyltransferase (GalNAc-T), and CK20 showed a significant difference in PFS (P=0.014), but not OS, in the same patients.

Because of the overwhelming diversity within tumors, it is unlikely that any single marker will yield optimal identification fidelity. Gervasoni et al. [25] published a preliminary report on the use of molecular signatures by RT-PCR to identify patients using epithelial-specific genes. CK20, CK19, CEA, and guanylyl cyclase G (CGG) were shown to identify cancer patients versus healthy patients. Shen et al. [26] showed that CK20, survivin, and CEA levels were all independently higher by qRT-PCR in CRC patients versus normal controls. Moreover, all Dukes stages were also found to correlate with survivin (P<0.001), CK20 (P=0.011), or CEA (P<0.001) mRNA levels. Although these markers had increased sensitivity when combined, no data were shown for the markers in combination and their ability to predict any clinical outcomes. Future studies aiming to identify novel markers should corroborate the clinical significance using conventional detection methods (eg, CellSearch) and test in combination with conventional methods.

Improving CTC Detection Methods

With the availability of several methods for identification and enumeration of CTCs, investigators are faced with a difficult choice. Many methods that have higher sensitivity sacrifice accuracy and precision. Königsberg et al. [27] compared different nonautomated detection methods for CTC enrichment, including two density centrifugation methods, a density centrifugation and antibody-based method, and an immunomagnetic technique. Immunomagnetic enrichment using MACS HEA Microbeads (Miltenyi Biotec, Auburn, CA) that bind EpCAM had a superior recovery rate than the other methods in cell-spiking experiments and patient samples. CTC levels of ≥1 CTCs per 7.5 mL of blood were significantly correlated with PFS but not with OS. This disparity with other findings might be a result of shorter follow-up time points, a difference in CTC group threshold numbers, a difference in detection methods, or patient composition, as this study included mCRC patients without specifying other factors.

A high-throughput method for detection of *KRAS* mutations by a membrane array has been developed [28] and recently applied in the clinic to mCRC patients treated with cetuximab and FOLFOX4 (oxaliplatin + leucovorin + fluorouracil) or FOLFIRI (leucovorin + fluorouracil + irinotecan) [29•]. This technique is carried out by amplification of total RNA from peripheral blood, cDNA synthesis, hybridization to membrane arrays, and quantification of resultant spot intensities. A strong correlation existed between *KRAS* mutation status in the tumor and that of peripheral blood samples using the membrane array, with high sensitivity (84.8%) and high specificity (95.3%). As expected, mutant *KRAS* in primary tumor samples correlated strongly with lack of response to cetuximab. This strong response correlation was extended to mutant *KRAS* detection in peripheral blood by the membrane array (n=86; P< 0.0001). A multivariate analysis yielded a strong *KRAS* mutational status correlation with PFS and OS if detected in the tumor or in peripheral blood (n=86; P<0.0001).

This technique has been updated to utilize chemiluminescence to increase sensitivity [30]. Although the clinical study suggests a strong correlation with *KRAS* mutation status in the primary tumor and the peripheral blood, it cannot conclude that CTCs are the source. This is the case for all purely mRNA-based detection methods. Future experiments should determine whether the source of mutant *KRAS* in the peripheral blood is truly CTCs but may be complicated by limitations of current CTC enrichment methods. On the contrary, this may be

an advantage, as this technique does not rely on selection techniques based on markers commonly used for CTC enrichment (eg, EpCAM).

Antolovic et al. [31•] recently reported on the importance of the chosen EpCAM epitope in antibody-based selection and its impact on CTC count. The study showed that the use of two different antibodies resulted in disparate CTC detection by a CK20 RT-PCR assay. Although RT-PCR may be more sensitive to error than multiparametric detection systems such as CellSearch, EpCAM remains a widely used, exclusive selection factor in isolating CTCs. As such, evaluation of the significance of CTC detection dependency on EpCAM epitopes is warranted.

The Impact of Resection on CTCs

Disturbing tumor cells mechanically and causing shedding of tumor cells during resection have long been a concern. The question of whether these tumor cells remain viable and what they subsequently may do is still largely unanswered. CTC detection has allowed investigators to begin to reliably quantify this phenomenon. Uen et al. [32] published a large-scale study involving stage III and IV CRC patients undergoing curative resection. This study found that postoperative relapse was strongly correlated with LN metastases ($P<0.001$), as well as CTC level if elevated at pre- and postoperative time points. Pre- and postoperative CTC levels were not analyzed separately as a predictor of relapse, but depth of invasion ($P=0.032$), vascular invasion ($P=0.001$), and perineural invasion ($P=0.013$) were also found to predict relapse, although to a lesser extent. It should be noted that this study used a membrane array to detect human telomerase reverse transcriptase, CK19, CK20, and CEA mRNA levels for detecting CTCs.

Hepatic metastases in CRC were explored in a 20-patient study monitoring CRC patients before, during, and after resection or radiofrequency ablation (RFA) [33•]. This study found that preoperative and intraoperative CTC levels did not predict OS. Postoperative levels were predictive of OS and disease-free survival. It should be noted that the statistical analysis was performed using absolute CTC levels in contrast to the common categorical analysis by a threshold number of CTCs. An important observation is the sevenfold increase in intraoperative CTCs compared with preoperative levels. This enrichment was found to be in patients who underwent RFA (mean, 27 cells/7.5 mL blood) rather than resection (mean, 3 cells/7.5 mL blood). This finding is an important consideration in selecting a hepatic resection procedure in light of tumor dissemination. A lack of significant elevated CTC levels during resection also was reported by another study in patients with primary CRC or mCRC [34]. It should be noted that this study analyzed CTCs by flow cytometry and did not use EpCAM as a selection criterion. Future studies should examine the clinical impact of significant tumor cell dissemination by RFA. As a whole, the literature strongly suggests that the choice of resection method plays a key role in tumor cell dissemination, but the consequence of this is unclear.

Conclusions

In the 1950s, CTC levels were observed in equal number in cancer patients with or without relapse and deemed useless as a prognostic factor. Today, CTC detection by CellSearch is FDA approved for patient prognosis in metastatic breast, prostate, or colorectal cancer. Clearly, future advancements in accurate detection of CTCs will be essential in assessing the utility of CTC levels as a patient prognostic factor. Detailed analyses of isolated CTCs need to be conducted to elucidate what properties are unique to these cells. For instance, do CTCs express epithelial–mesenchymal transition markers (eg, vimentin, twist, fibronectin)? Are the CTCs detected viable? Isolation of viable CTCs should be pursued for ex vivo analysis and in vivo investigation in animals models. Identification of CTC-specific properties may provide

opportunities for therapeutic exploitation. For instance, insight could be gained from comparisons of disseminated tumor cells to intrinsic CTCs (eg, expression profiling). As for surgical method decisions in mCRC involving the liver, it is clear that RFA increases CTCs during the procedure. Data must be gathered on whether the disseminated tumor cells change clinical outcome.

The relationship of cancer stem cells (CSCs) with CTCs is entirely unclear at this point. In theory, CTCs must have tumor-initiating properties of CSCs but have additional intravasation and extravasation properties. Preliminary experiments should focus on the overlap with markers of CSCs and their levels in CTCs (ie, $CD133^+$). A recent finding indicates that a $CD26^+$ subpopulation of $CD133^+$ CRC cells have unique metastatic potential in CRCs [35]. More specifically, this subpopulation exclusively forms liver metastasis when injected into the cecal wall of mice. Furthermore, preliminary data from small groups of patients suggest that this marker may be useful in predicting metastasis.

Future efforts to improve CTC detection should explore the possibility of low EpCAM expression by CTCs, as this has been noted in epithelial–mesenchymal transitions, a process that CTCs may undergo during early stages of metastasis. The two large-scale patient studies by Cohen et al. [12••,15••] and Tol et al. [16] firmly place CTCs as an indicator of prognosis in mCRC. Examination of treatment regimen on CTC levels and the response of patients should be further explored to index therapeutic effects. Efforts to explore the role of CTCs in prediction of metastasis, as initiated by Garrigós et al. [17•], are highly warranted. Proof of CTCs as an indicator of future metastasis would be an extremely valuable tool in the clinic. With the available clinical data, CTC level should be incorporated with other traditional prognostic indicators to provide the best assessment possible for therapy stratification. The proven utility of CTCs needs to be integrated into clinics rather than viewed as a work in progress. In the spirit of Stephen Paget's metastasis model [36], which prevails more than a century later [37], with much effort we have found the "seeds"; now let us go to the field and stop them from being planted in the "soil."

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Jemal A, Siegel R, Ward E, et al. Cancer Statistics, 2009. CA Cancer J Clin 2009;59:225–249. [PubMed: 19474385]
2. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66:3992–3995. [PubMed: 16618717]
3. Ashworth T. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Aust Med J. 1869
4. Engell HC. Cancer cells in the circulating blood: a clinical study on the occurrence of cancer cells in the peripheral blood and in venous blood draining the tumour area at operation. Ugeskr Laeger 1955;117:822–823. [PubMed: 13247497]
5. Engell HC. Cancer cells in the blood: a five to nine year follow up study. Ann Surg 1959;149:457–461. [PubMed: 13637653]
6. Roberts S, Jonasson O, Long L, et al. Clinical significance of cancer cells in the circulating blood: two to five-year survival. Ann Surg 1961;154:362–370. [PubMed: 13742183]
7. Negin B, Cohen S. Circulating tumor cells in colorectal cancer: past, present, and future challenges. Curr Treat Options Oncol. 2010 Feb 9; (Epub ahead of print)]. This concise review describes current detection methods for CTCs.

8. Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 2004;10:6897–6904. [PubMed: 15501967]
9. Riethdorf S, Fritsche H, Müller V, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch System. Clin Cancer Res 2007;13:920–928. [PubMed: 17289886]
10. Hayes DF, Cristofanilli M, Budd G, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. Clin Cancer Res 2006;12:4218–4224. [PubMed: 16857794]
11. Danila DC, Heller G, Gignac GA, et al. Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res 2007;13:7053–7058. [PubMed: 18056182]
12. Cohen SJ, Punt CJA, Iannotti N, et al. Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. Ann Oncol 2009;20:1223–1229. [PubMed: 19282466] This was the first large-scale mCRC patient study examining CTCs. CTC level was shown to correlate with PFS and OS.
13. Sastre J, Maestro ML, Puente J, et al. Circulating tumor cells in colorectal cancer: correlation with clinical and pathological variables. Ann Oncol 2008;19:935–938. [PubMed: 18212090]
14. Katsuno H, Zacharakis E, Aziz O, et al. Does the presence of circulating tumor cells in the venous drainage of curative colorectal cancer resections determine prognosis? A meta-analysis. Ann Surg Oncol 2008;15:3083–3091. [PubMed: 18787906]
15. Cohen SJ, Punt CJA, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:3213–3221. [PubMed: 18591556] This extended analysis of a previous study provides updated follow-up time points. The impact of CTCs on prognosis is examined in several subgroups of patients.
16. Tol J, Koopman M, Miller MC, et al. Circulating tumour cells early predict progression-free and overall survival in advanced colorectal cancer patients treated with chemotherapy and targeted agents. Ann Oncol 2009;21:1006–1012. [PubMed: 19861577]
17. Garrigós N, Gallego J, Guillén-Ponce C, et al. Circulating tumour cell analysis as an early marker for relapse in stage II and III colorectal cancer patients: a pilot study. Clin Transl Oncol 2010;12:142–147. [PubMed: 20156783] This small study demonstrated the potential of CTCs to predict metastasis in CRC.
18. Ronzoni M, Manzoni M, Mariucci S, et al. Circulating endothelial cells and endothelial progenitors as predictive markers of clinical response to bevacizumab-based first-line treatment in advanced colorectal cancer patients. Ann Oncol. 2010 May 23; (Epub ahead of print).
19. Findeisen P, Matthias R, Matthias N, et al. Systematic identification and validation of candidate genes for detection of circulating tumor cells in peripheral blood specimens of colorectal cancer patients. Int J Oncol 2008;33:1001–1010. [PubMed: 18949363]
20. Yie SM, Lou B, Ye S, et al. Detection of survivin-expressing circulating cancer cells (CCCs) in peripheral blood of patients with gastric and colorectal cancer reveals high risks of relapse. Ann Surg Oncol 2008;15:3073–3082. [PubMed: 18670822]
21. Wong CSC, Cheung MT, Ma BBY, et al. Isolated tumor cells and circulating CK20 mRNA in pN0 colorectal cancer patients. Int J Surg Pathol 2008;16:119–126. [PubMed: 18387990]
22. Wong SCC, Chan CML, Ma BBY, et al. Clinical significance of cytokeratin 20-positive circulating tumor cells detected by a refined immunomagnetic enrichment assay in colorectal cancer patients. Clin Cancer Res 2009;15:1005–1012. [PubMed: 19188172]
23. Schmidt T, Koch M, Antolovic D. Influence of two different resection techniques (conventional liver resection versus anterior approach) of liver metastases from colorectal cancer on hematogenous tumor cell dissemination—prospective randomized multicenter trial. BMC Surg 2008;8:6. [PubMed: 18321372]
24. Koyanagi K, Bilchik AJ, Saha S, et al. Prognostic relevance of occult nodal micrometastases and circulating tumor cells in colorectal cancer in a prospective multicenter trial. Clin Cancer Res 2008;14:7391–7396. [PubMed: 19010855]

25. Gervasoni A, Monasterio Muñoz RM, Wengler GS, et al. Molecular signature detection of circulating tumor cells using a panel of selected genes. Cancer Lett 2008;263:267–279. [PubMed: 18262345]
26. Shen C, Hu L, Xia L, et al. Quantitative real-time RT-PCR detection for survivin, CK20 and CEA in peripheral blood of colorectal cancer patients. Jpn J Clin Oncol 2008;38:770–776. [PubMed: 18845519]
27. Königsberg R, Gneist M, Jahn-Kuch D, et al. Circulating tumor cells in metastatic colorectal cancer: efficacy and feasibility of different enrichment methods. Cancer Lett 2010;293:117–123. [PubMed: 20167419]
28. Chen YF, Wang JY, Wu CH, et al. Detection of circulating cancer cells with K-ras oncogene using membrane array. Cancer Lett 2005;229:115–122. [PubMed: 16157223]
29. Yen LC, Yeh YS, Chen CW, et al. Detection of KRAS oncogene in peripheral blood as a predictor of the response to cetuximab plus chemotherapy in patients with metastatic colorectal cancer. Clin Cancer Res 2009;15:4508–4513. [PubMed: 19549774] This study demonstrated the clinical feasibility of a novel membrane array for detecting CTCs and the importance of KRAS in CTCs for predicting cetuximab response.
30. Yang MJ, Chiu HH, Wang HW, et al. Enhancing detection of circulating tumor cells with activating KRAS oncogene in patients with colorectal cancer by weighted chemiluminescent membrane array method. Ann Surg Oncol 2010;17:624–633. [PubMed: 19937133]
31. Antolovic D, Galindo L, Carstens A, et al. Heterogeneous detection of circulating tumor cells in patients with colorectal cancer by immunomagnetic enrichment using different EpCAM-specific antibodies. BMC Biotechnol 2010;10:35. [PubMed: 20426872] This study demonstrated the significant impact of CTC identification method on CTC enumeration.
32. Uen YH, Lu CY, Tsai HL, et al. Persistent presence of postoperative circulating tumor cells is a poor prognostic factor for patients with stage I–III colorectal cancer after curative resection. Ann Surg Oncol 2008;15:2120–2128. [PubMed: 18481151]
33. Papavasiliou P, Fisher T, Kuhn J, et al. Circulating tumor cells in patients undergoing surgery for hepatic metastases from colorectal cancer. Proc (Bayl UnivMed Cent) 2010;23:11–14. This study found that RFA significantly increases the level of intraoperative CTCs.
34. Tralhão JG, Hoti E, Serôdio M, et al. Perioperative tumor cell dissemination in patients with primary or metastatic colorectal cancer. Eur J Surg Oncol (EJSO) 2010;36:125–129.
35. Pang R, Law WL, Chu ACY. A subpopulation of $CD26^{+}$ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell 2010;6:603–617. [PubMed: 20569697]
36. Paget S. The distribution of secondary growths in cancer of the breast. Lancet 1889;1:571–572.
37. Fidler IJ. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer 2003;3:453–458. [PubMed: 12778135]

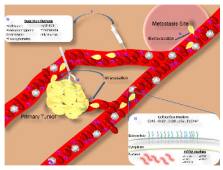

Fig. 1.

Circulating tumor cell (CTC) generation, identification, and detection. CTCs are shed from the primary tumor and intravasate by several possible mechanisms: direct shedding into existing blood vessels *(A)*, mechanical disruption (eg, resection; *[B]*), or shedding into angiogenic capillaries *(C)*. CTCs then travel through the bloodstream *(D)* and later extravasate at a potential site of metastasis *(E)*. CTCs can be detected in peripheral blood by collection *(F)* using a variety of methods *(G)*. Published markers for CTCs in colorectal cancer are listed *(H)*. CellSearch is a registered trademark of Veridex LLC, Raritan, NJ. qRT-PCR, quantitative reverse transcriptase polymerase chain reaction

Table 1

Clinical studies evaluating circulating tumor cells as a prognostic tool

Study	Inclusion criteria	Patients, *n*	CTC enumeration method	CTC positive threshold	Parameters examined	Main conclusion
Cohen et al. [12••]	mCRC initiating first-, second-, or third-line therapy with an EGFR inhibitor	430	CellSearch[a]	≥3/7.5 mL	Line of therapy, tumor type, ECOG PS, site of metastasis, CTC evaluation time point, PFS, OS	CTC number is an independent predictor of PFS and OS in mCRC
Cohen et al. [15••]	mCRC initiating first-, second-, or third-line therapy with an EGFR inhibitor	430	CellSearch	≥3/7.5 mL	Line of therapy, therapy choice, age, ECOG PS, PFS, OS	CTC prediction of PFS and OS is stronger in some treatment groups and subtypes
Garrigós et al. [17•]	Stage II or III CRC undergoing curative resection	16	Immunomagnetic beads or centrifugation enrichment followed by RT-PCR or FC	–	Detection method, relapse	Immunomagnetic enrichment and FC are the most efficient CTC detection methods; relapse in CRC may be correlated with CTC level
Katsuno et al. [14]	Curative surgery for CRC, blood collection from venous drainage of tumor at time of surgery	646	RT-PCR	–	LN, disease stage, hepatic metastasis	CTC levels correlate to LN positivity and disease stage
Papavasiliou et al. [33•]	CRC with hepatic metastases treatable with liver resection or tumor ablation, intact primary tumor	20	CellSearch	≥3/7.5 mL	Fong score, disease status, liver procedure, PFS, OS	Postoperative CTC may predict prognosis
Sastre et al. [13]	CRC, no preoperative chemo- or radiotherapy	127	CellSearch	≥3/7.5 mL	Disease stage, tumor location, differentiation, CEA and LDH levels	Reproducible CTC levels but correlated only with disease stage
Schmidt et al. [23]	>18 years old, eligible for hepatic resection by conventional and anterior approaches, no extrahepatic disease, liver cirrhosis, or positive LN	150	CK20 RT-PCR	–	Resection approach, OS, other parameters related to the surgery	Ongoing; primary aim is to conclude on tumor cell dissemination by conventional vs anterior hepatic resection
Tol et al. [16]	CRC with irresectable distant metastasis, ≥1 measurable disease parameter, WHO PS of 0 or 1, adequate organ functions	467	CellSearch	≥3/7.5 mL	CTC time point, CT imaging, PFS, OS	CTC number is an independent predictor of PFS and OS in mCRC; combination with CT imaging predicts OS more strongly
Tralhão et al. [34]	CRC	40	FC ($CD45^{-}$,CK^{+})	–	CTC levels influenced by surgery	No significant difference in CTC by surgical intervention

CEA carcinoembryonic antigen, *CK* cytokine, *CRC* colorectal cancer, *CTC* circulating tumor cell, *ECOG PS* Eastern Cooperative Oncology Group performance status, *EGFR* epidermal growth factor receptor, *FC* flow cytometry, *mCRC* metastatic CRC, *EGFR* epidermal growth factor receptor, *LDH* lactate dehydrogenase, *LN* lymph node, *OS* overall survival, *PFS* progression-free survival, *RT-PCR* reverse transcriptase polymerase chain reaction, *WHO PS* World Health Organization performance status

[a]Registered trademark of Veridex LLC, Raritan, NJ

Table 2

Novel methods for circulating tumor cell identification and main conclusion(s)

Study	CTC enumeration method	Main conclusion
Antolovic et al. [31•]	Immunomagnetic enrichment and CK20 RT-PCR	The clone used as an antibody for EpCAM-based enrichment alters results significantly
Chen et al. [28]	KRAS membrane array	KRAS membrane array is sensitive and specific when used on CRC blood samples.
Findeisen et al. [19]	346 candidate genes	*SERPINB5* expression is elevated in CRC blood
Gervasoni et al. [25]	CK20, CK19, CEA, and GCC RT-PCR	CTCs can be predicted by CK20, CK19, CEA, and GCC together
Königsberg et al. [27]	MACS HEA MicroBeads,[a] RosetteSep,[b] density centrifugation	EpCAM-coupled antibodies are a better detection method than cytometric methods
Konyanagi et al. [24]	CK20 IHC and c-MET, MAGE-A3, hTERT, and GalNAc-T qRT-PCR	CK20 IHC and qRT-PCR strongly predict DFS
Shen et al. [26]	Survivin, CK20, CEA qRT-PCR	CK20 and CEA mRNA correlates with disease stage and LN
Uen et al. [32]	hTERT, CK19, CK20, and CEA RT-PCR	Persistently elevated CTCs, LN, and vascular invasion are independent predictors of postoperative relapse
Wong et al. [22]	CK20 positive, cell morphology, and cell size	CK20 may be detected in CRC patients and is associated with disease status and LN
Yang et al. [30]	*KRAS* membrane array	Can detect down to three colon tumor cells/mL of blood with membrane array
Yen et al. [29•]	*KRAS* membrane array	*KRAS* mutation status in CTCs predicts response to cetuximab and affects PFS and OS
Yie et al. [20]	Survivin RT-PCR ELISA	Survivin is elevated in CRCs and correlated with metastasis

CEA carcinoembryonic antigen, *CK* cytokine, *CRC* colorectal cancer, *CTC* circulating tumor cell, *DFS* disease-free survival, *ELISA* enzyme-linked immunosorbent assay, *EpCAM* epithelial cell adhesion molecule, *GalNAc-T* β-1,4-*N*-acetylgalactosaminyltransferase, *GCC* guanylyl cyclase C, *hTERT* human telomerase reverse transcriptase, *IHC* immunohistochemistry, *LN* lymph node, *MAGE-A3* melanoma-associated antigen 3, *OS* overall survival, *PFS* progression-free survival, *qRT-PCR* quantitative RT-PCR, *RT-PCR* reverse transcriptase polymerase chain reaction

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