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ORIGINAL ARTICLE – COLORECTAL CANCER

Circulating Tumor Cells in Patients Undergoing Resection of Colorectal Cancer Liver Metastases. Clinical Utility for Long-Term Outcome: A Prospective Trial

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ABSTRACT

Background. Patients with resected colorectal cancer liver metastases display heterogeneous clinical behavior. The identification of new prognostic factors would help in making more accurate decisions.

Objective. The aim of this study was to evaluate the survival impact of circulating tumor cells (CTCs) in this setting.

Methods. We conducted a prospective study of patients with resected liver metastases of colorectal cancer. Patients were included in the study from February 2009 to January 2013. The CellSearch System™ was employed for the detection of pre- and postsurgery CTCs. A positive test was defined as two or more CTCs/7.5 mL of blood. Recurrence rate, disease-free survival, and overall survival were calculated, and univariate and multivariate analyses were performed.

Results. Forty-four patients were included in our study. After a median follow-up of 60 months (range 28–74), 32 patients experienced recurrence (72.7%). The CTCs number was determined and the test was positive in 8 patients (18.6%) before surgery and 13 patients (29.5%) after surgery. The postoperative detection of CTCs was not related to any clinical outcome; however, the preoperative detection of CTCs was significantly related to behavior. All patients in the preoperative CTC-positive group relapsed, versus 65% in the CTC-negative group ($p = 0.051$). Disease-free survival was 19 months in the preoperative CTC-negative group versus 7 months in the CTC-positive group ($p = 0.01$). Additionally, overall survival was 69 months in the preoperative CTC-negative group versus 17 months in the CTC-positive group ($p = 0.004$). Preoperative CTC count remained significant in multivariate analysis.

Conclusions. In this cohort of colorectal cancer liver metastases patients, the presence of two or more preoperative CTCs was associated with disease progression and poor survival despite complete resection.

Liver resection offers a chance of cure for patients with colorectal liver metastases. Surgery combined with chemotherapy (ChT) is the standard approach and achieves 5-year survival rates of up to 50–60%.[1] However, clinical behavior and the probability of relapse are heterogeneous among these patients, with up to 30% of patients dying due to relapse in the first 2 years.[2] Hence, identifying prognostic factors is desirable for accurate decision making. Several clinical and pathological factors have been studied as prognostic determinants, and some clinical risk scores have been proposed.[3,4] Nonetheless, treatment has improved, principally due to major advances in surgical techniques and the availability of more effective systemic therapies. Therefore, the classic prognostic factors have been called into question, and there is a need for novel biomarkers.

Circulating tumor cells (CTCs) are malignant cells shed from the primary tumor or its metastases that gain access to the vasculature through intravasation. CTCs are evaluated for the identification of patients at high risk for relapse, stratification of patients to specific adjuvant therapies, and monitoring response to treatment in breast, lung, or

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prostate cancer. In colorectal cancer, the presence of CTCs has been associated with worse prognosis in both local and metastatic settings.[5,6] Studies conducted in patients with colorectal liver metastases are scant and heterogeneous, and have used different detection methods.[7] The CellSearch System™ is the only method approved by the US FDA for use in clinical practice.

The aim of this study was to evaluate the prognostic value of CTCs measured using the CellSearch System™ for patients undergoing hepatic resection of colorectal liver metastases.

METHODS

Patients

Patients were prospectively recruited at our institution between February 2009 and January 2013. This study was approved by the Hospital de Navarra Ethics Board, and all patients signed an informed consent form.

The inclusion criteria were: patients aged > 18 years, colorectal cancer with synchronous or metachronous liver metastases, metastatic disease confined to the liver, and radical surgery for liver metastases (as well as primary tumors if synchronic) performed at our institution. Neoadjuvant and adjuvant ChT were allowed. The exclusion criteria included: patients with any previous neoplasia (except carcinoma in situ), macroscopic residual disease, and life expectancy < 3 months. If a patient was included in the study and radical surgery was not subsequently achieved, he or she was censored.

The treatment schedule was planned for each patient following clinical practice, and the investigators were blinded to the CTC account.

Patient data, including sex, age, location of the primary tumor, pN stage, presence of synchronous or metachronous metastases, time to appearance of metachronous metastases, number of metastases, carcinoembryonic antigen (CEA) level at diagnosis, RAS status, neoadjuvant ChT administration, response to neoadjuvant ChT, adjuvant ChT, type of liver surgery, liver resection margin involvement, size of the largest metastases, and pathological regression, were collected. The CEA level was dichotomized according to its normal or elevated value. Patients were stratified by age (older or younger than 70 years) and number of metastases (≤ 3 or > 3). Response to neoadjuvant ChT was evaluated according to Response Evaluation Criteria In Solid Tumors (RECIST) criteria, and pathological regression was stratified as a complete response if any cancer cell was evidenced: $\leq 50\%$ and $> 50\%$ residual cancer cells. If tumor growth was found within 1 mm of the resection margin, it was reported as involved. The Fong clinical risk score was calculated and categorized as high risk (3–5 points) or low risk (0–2 points).

Circulating Tumor Cell Detection

Peripheral blood samples were collected and enumerated at two time points: before surgery and 2 weeks postoperatively. On each occasion, 7.5 mL of blood was drawn in CellSave™ tubes for optimal preservation of CTCs over a 96 h maximum period. Sample preparation and detection of CTCs was undertaken using the CellSearch™ assay, as previously described and validated. Briefly, the samples were processed in a semiautomatic system (AutoPrep System™) that selects CTCs using a surface marker (EpCAM). CTCs were immunomagnetically separated and fluorescently labeled using the CellSearch System™ (Veridex/Immunicon Corp.). CTC counts were verified by manual review by two trained hematologists independently, and discordant data were reviewed and checked by an external consultant.

The test was considered positive if the CTC count was ≥ 2/7.5 mL of blood, and was considered negative if the CTC count was 0–1.

Follow-Up

Follow-up was performed according to clinical practice. Symptoms and CEA levels were recorded every 3 months for 2 years, every 6 months for 3 years, then annually for a period of 10 years. Computerized Tomography (CT) scan was performed every 6 months for 2 years, then annually for a period of 5 years.

Statistical Methods

Patient characteristics are summarized as frequencies and percentages, except for age, which is summarized as median and range. The analysis of association and prediction of recurrence was tested using the Chi-square method for contingency tables. Disease-free survival (DFS) was measured from the date of resection to the date of diagnosis of disease recurrence, date of death for any reason, or the date of last known follow-up, while overall survival (OS) was measured from the date of resection to the date of death or the date of last known follow-up. Kaplan–Meier curves and the log-rank test were used to compare survival distributions according to CTC status. The association between variables and survival was evaluated using univariate and multivariate Cox regression models. Significant or near-significant variables ($p < 0.1$) in the univariate analysis were included in the multivariate analysis.

Data were prospectively entered into an anonymized database, updated to 15 January 2018. IBM SPSS Statistics 20 for Windows was used for statistical calculations.

RESULTS

Patient Characteristics and Recurrence

Forty-four patients were included in the study between February 2009 and January 2013. The main characteristics of the entire series are shown in Table 1. The median age of patients was 62 years, and 23% of patients were older than 70 years. The primary tumor was located in the right colon, left colon, or rectum in 16%, 45%, and 38% of patients, respectively. Local lymph nodes were affected in 63% of patients. Liver metastases were synchronous in 50% of patients, and 27% of patients had more than three metastases. The Fong clinical risk score was calculated, and 66% of patients had a score of ≤ 2 points, and no patients had a score of 4 or 5 points. Sixty percent of patients received neoadjuvant ChT, and up to 80% of patients received adjuvant ChT.

A blood test was obtained in 43 patients before surgery and 38 patients after surgery. The test was positive (two or more CTCs) in 8 patients (18.6%) before surgery. Fifty percent of patients maintained a positive CTC count (≥ 2) after surgery, while the remaining 50% of patients negativized this parameter (< 2). Thirteen patients (29.5%) had a positive postsurgery test, only four of whom (30%) had a positive presurgical CTC count.

After a median follow-up of 60 months (range 28–74 months), 32 patients experienced recurrence (72.7%), and, at the time of analysis, 19 patients (43.2%) were alive.

The recurrence was liver-only in 13 patients (41%), extrahepatic in 11 patients (34%), and hepatic + extrahepatic in 8 patients (25%). All patients who were CTC-positive preoperatively relapsed, while only 65% of patients who were CTC-negative developed recurrence ($p = 0.051$). With regard to postoperative CTCs, 68% of the CTC-negative patients relapsed, versus 84% in the CTC-positive patient group ($p = 0.27$).

Disease-Free Survival

The median DFS was 18 months. Kaplan–Meier curves showed a significant difference in DFS between patients with preoperative negative CTCs and patients with preoperative positive CTCs (19 vs. 7 months; $p = 0.01$) (Fig. 1); however, the difference was not significant between CTC-negative and CTC-positive patients in the postoperative setting (21 vs. 13 months). In the univariate analysis, preoperative positive CTCs were associated with worse prognosis (hazard ratio [HR] 2.7, 95% confidence interval [CI] 1.2–6.2; $p = 0.016$). The other factor associated with significantly worse prognosis was the number of liver metastases (HR 2.9, 95% CI 1.4–6.1; $p = 0.004$). The remaining factors did not show any differences (Table 2). In the multivariate analysis (Table 3), the preoperative CTCs and number of liver metastases remained significant.

Overall Survival

Patients with preoperative negative CTCs obtained a median OS of 69 months compared with those who were CTC-positive preoperatively, who obtained a median OS of 17 months ($p = 0.004$) (Fig. 1). The difference was not significant in the postoperative setting. In the univariate analysis, preoperative CTC-positive status was again associated with worse prognosis (HR 3.6, 95% CI 1.4–8.1, $p = 0.007$). The number of liver metastases, as well as neoadjuvant ChT, showed a value close to statistical significance. In this analysis, women had a better prognosis than men (HR 4.5, 95% CI 1.3–15.2, $p = 0.015$). The univariate analysis is shown in Table 2. In the multivariate analysis (Table 3), sex, preoperative CTCs and neoadjuvant ChT remained significant. In patients with preoperative positive CTCs, the risk of death was increased seven times after adjusting for other variables.

DISCUSSION

This study explores the prognostic value of preoperative CTCs in resected colorectal liver metastases. This test was a strong and independent prognostic factor for OS and DFS in our series. Patients with 0–1 CTCs had a median DFS of 19 months, compared with 7 months in patients with two or more CTCs. Similarly, the median OS was 17 months in CTC-positive patients compared with 69 months in CTC-negative patients. In addition, all patients with preoperative positive CTCs relapsed.

There are many retrospective series and one meta-analysis on CTC detection in metastatic colorectal cancer;[8] however, the methodologies and results are mixed.

Some studies evaluated peripheral blood CTCs as well as tumor cells in the bone marrow. It should be noted that the meaning of the two situations may be different. Second, the method for detecting CTCs is variable throughout studies, and the different techniques used were not always validated. Finally, most studies included resectable and unresectable liver metastases, as well as widespread metastatic disease. Our study focused on resected colorectal metastases, and the detection of CTCs was performed in peripheral blood using the CellSearch

TABLE 1 Patient characteristics of the entire series

Group	*n*	%
Sex		
Male	31	70.5
Female	13	29.5
Age, years		
Median (range)	62.5 (45–79)	
Location of primary tumor		
Right colon	7	16
Left colon	20	45.5
Rectum	17	38.5
Lymph node status		
N0	16	36.5
N+	28	63.5
Synchronous metastases		
Yes	23	52.3
No	21	47.7
Number of liver metastases		
1–3	32	72.7
> 3	12	27.3
Fong clinical risk score		
1	12	27.3
2	17	38.6
3	15	34.1
CEA ≥ 5		
Yes	10	65.5
No	19	34.5
RAS status		
Wild-type	19	55.9
Mutated	15	44.1
Neoadjuvant chemotherapy		
Yes	27	61.4
Partial response	17	65.4
Estabilization	9	34.6
No	17	38.6
Synchronous surgery		
Yes	9	20.5
No	35	79.5
Type of surgery		
Limited resection	37	84.1
Right or left hepatectomy	7	15.9
Pathological regression in patients with neoadjuvant chemotherapy (n = 27)		
Complete response	1	3.7
< 50% residual tumor	11	40.7
> 50% residual tumor	15	55.6
Adjuvant chemotherapy		
Yes	37	84.1
No	7	15.9

CEA carcinoembryonic antigen

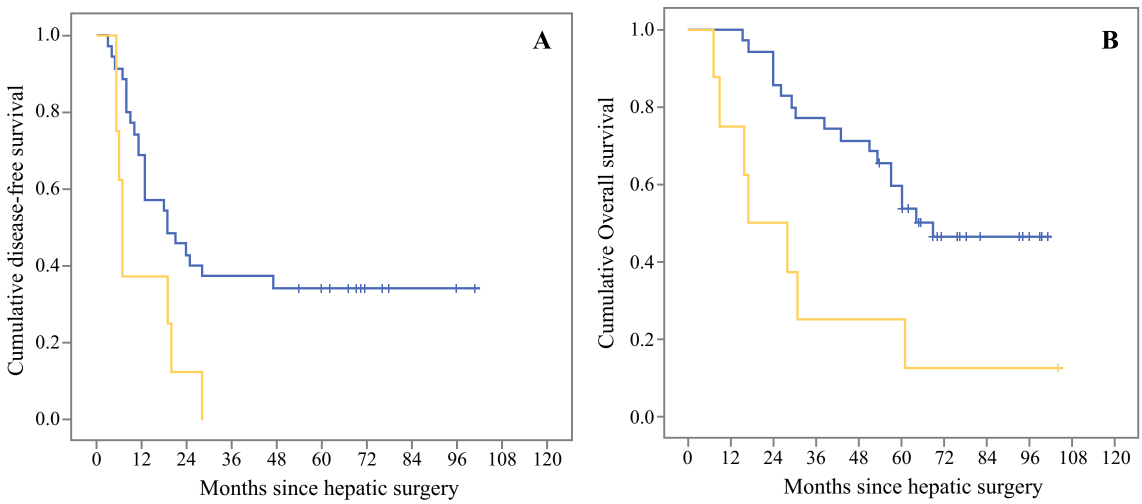

FIG. 1 **a** Disease-free survival according to preoperative CTC status (0–1 shown in blue; ≥ 2 shown in yellow) in patients with resected colorectal liver metastases (median 19 vs. 7 months; $p = 0.01$). **b** Overall survival according to preoperative CTC status (0–1 shown in blue; ≥ 2 shown in yellow) in patients with resected colorectal liver metastases (median 69 vs. 17 months; $p = 0.004$). *CTC* circulating tumor cell

TABLE 2 Univariate analysis for disease-free survival and overall survival

Variable	Level	DFS		OS	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value
Sex	Women/men	2.0 (0.8–4.5)	0.117	4.5 (1.3–15.2)	**0.015**
Age		1.0 (0.9–1.0)	0.451	1.0 (1.0–1.1)	0.462
Preoperative CTCs	0–1/≥ 2	2.7 (1.2–6.2)	**0.016**	3.6 (1.4–8.1)	**0.007**
K-ras	Wild-type/mutated	1.4 (0.6–3.0)	0.396	0.8 (0.4–1.9)	0.604
Fong clinical risk score	0–2/3–5	1.6 (0.8–3.3)	0.194	1.5 (0.7–3.3)	0.323
Location	Left/right	1.0 (0.4–2.6)	0.981	1.5 (0.6–3.9)	0.434
Lymph node status	N0/N+	1.2 (0.6–2.5)	0.593	1.8 (0.7–4.6)	0.193
Neoadjuvant chemotherapy	No/yes	1.8 (0.9–3.8)	0.112	2.4 (1.0–5.7)	0.054
CEA	≤ 5/> 5	1.1 (0.4–2.6)	0.908	1.0 (0.4–2.7)	0.990
Postoperative CTCs	0–1/≥ 2	1.8 (0.8–3.8)	0.141	1.3 (0.5–3.2)	0.545
Synchronous surgery	No/yes	1.8 (0.7–4.6)	0.238	2.3 (0.7–7.7)	0.175
Affected margin	No/yes	1.5 (0.7–3.3)	0.352	1.5 (0.6–3.6)	0.375
Number of liver metastases	1–3/≥ 4	2.9 (1.4–6,1)	**0.004**	2.1 (0.9–4.8)	**0.084**
Pathological regression	≥ 50%/< 50%	1.1 (0.5–2.4)	0.755	1.7 (0.8–3.8)	0.197

Bold values indicate statistical significance ($p < 0.005$)

DFS disease-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval, *CTCs* circulating tumor cells, *CEA* carcinoembryonic antigen

TABLE 3 Multivariate analysis for disease-free survival and overall survival

Variable	Level	DFS		OS	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value
Sex	Women/men	–	–	6 (1.7–22)	**0.006**
Neoadjuvant chemotherapy	No/yes	–	–	3.6 (1.3 –9.6)	**0.012**
Preoperative CTCs	0–1/≥ 2	3.2 (1.1–8.7)	**0.026**	5.3 (1.8–15.5)	**0.002**
Number of liver metastases	1–3/≥ 4	2.7 (1.2–6.1)	**0.018**	1.3 (0.5–3.2)	0.540

Bold values indicate statistical significance ($p < 0.005$)

DFS disease-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval, *CTCs* circulating tumor cells

System™, a validated tool approved by the FDA. Our results are in line with most papers reporting a significant role of preoperative CTCs. Similarly, the detection of other circulating biological materials, such as circulating tumor DNA (ctDNA),[9–11] are also being developed as prognostic tools.

The optimal cut-off value defining CTC positivity in patients with colorectal cancer is unresolved. Different studies used various cut-off values to assess the clinical significance of CTCs. The highest cut-off value used is three or more CTCs/7.5 mL of blood;[6] however, cut-off values of one or more CTCs/7.5 mL, or two or more CTCs/7.5 mL have also been associated with poor prognosis in patients with colorectal cancer.[12,13] It should be noted that the cut-off value may be different between metastatic disease and disease-free settings. This difference is well-established in breast cancer.[14,15] In our study, we selected two or more CTCs/7.5 mL. For a cut-off value of three or more CTCs/7.5 mL, the difference in OS was also significant (69 vs. 16 months; $p = 0.00$), but the number of patients with three or more CTCs was small (six patients). For one or more CTCs/7.5 mL, no differences were shown, which may be because the detection of one CTC has low sensitivity and can be found in healthy subjects or patients with nonmalignant diseases.[16] In this sense, the detection of two or more CTCs/7.5 mL can be assumed to be a pathologic value. In a meta-analysis of CTCs in colorectal cancer, the impact on prognosis was independent of the cut-off point selected.[8]

Some clinical scores have been proposed as a prognostic approach for predicting recurrence after liver resection; the Fong Clinical Risk Score is probably the most extended predictor. This score evaluates node-positive primary, disease-free interval < 12 months, number of metastases > 1, diameter of liver disease > 5 cm, and CEA > 200 ng/mL; however, the improvement in surgical techniques, as well as the more intensive and effective ChT used, may have modified the impact of these factors. In our series, the Fong Score was not a prognostic factor measured as a discrete variable or as a dichotomous variable (< 3 points vs. ≥ 3 points; data not shown). Of the five factors included in this score, the only significant variable in our study was the number of metastases > 3 for DFS. However, for OS, not even the number of liver metastases remained significant.

In our analysis, sex remained significant in the multivariate analysis. We can suggest some hypotheses as to why this is so, such as the proportion of rectal cancer being higher than usual in our study (40%); a worse prognosis in men with rectal cancer has been described;[17] and the average age of the men in our study was slightly higher than that of women (64 vs. 59 years; $p = 0.10$).

The need for neoadjuvant ChT is the last significant factor in the multivariate analysis. In our series, patients who required treatment with neoadjuvant ChT had a worse prognosis. One possible explanation is that the need for neoadjuvant ChT is probably a surrogate factor of a complex clinical situation with more advanced disease.

There are several limitations to our study. First, the number of patients recruited was relatively small, and, second, the study was conducted in a single institution. Testing a greater number of patients and performing an external validation would be desirable for establishing the role of CTCs as prognostic factors in the preoperative setting of patients with liver metastases from colorectal cancer.

CONCLUSIONS

Our series suggests preoperative CTCs as a useful tool for predicting the clinical behavior of colorectal cancer after liver surgery. These data need to be evaluated individually according to the characteristics of the patient, the extent of the surgery planned, and the eventual subsequent treatments. In this respect, the evaluation of tumor biology, beyond anatomical data, seems to be a promising approach.

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REFERENCES

1. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, et al. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomised, controlled, phase 3 trial. *Lancet Oncol.* 2013;14(12):1208–15.
2. de Jong MC, Pulitano C, Ribero D, Strub J, Mentha G, Schulick RD, et al. Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. *Ann Surg.* 2009;250(3):440–8.
3. Nordlinger B, Guiguet M, Vaillant JC, Balladur P, Boudjema K, Bachellier P, et al. Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Française de Chirurgie. Cancer. 1996;77(7):1254–62.
4. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. *Ann Surg.* 1999;230(3):309–18; discussion 318–21.
5. Cohen SJ, Punt CJA, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol.* 2009;20(7):1223–9.

6. Sastre J, Maestro ML, Gomez-Espana A, Rivera F, Valladares M, Massuti B, et al. 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.* 2012;17(7):947–55.
7. Ma B, Wang L, Gao P, Song Y, Wang Z. Letter to the editor: a meta-analysis of preoperative circulating and disseminated tumor cells are negative predictors of survival in patients undergoing hepatic resection of colorectal liver metastases. *Int J Colorectal Dis.* 2016;31(8):1523–4.
8. Groot Koerkamp B, Rahbari NN, Büchler MW, Koch M, Weitz J. Circulating tumor cells and prognosis of patients with resectable colorectal liver metastases or widespread metastatic colorectal cancer: a meta-analysis. *Ann Surg Oncol.* 2013;20(7):2156–65.
9. Tie J, Kinde I, Wang Y, Wong HL, Roebert J, Christie M, et al. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. *Ann Oncol.* 2015;26(8):1715–22.
10. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. *Nat Med.* 2008;14(9):985–90.
11. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature.* 2012;486(7404):532–6.
12. Seeberg LT, Waage A, Brunborg C, Hugenschmidt H, Renolen A, Stav I, et al. Circulating tumor cells in patients with colorectal liver metastasis predict impaired survival. *Ann Surg.* 2015;261(1):164–71.
13. Gazzaniga P, Raimondi C, Gradilone A, Biondi Zoccai G, Nicolazzo C, Gandini O, et al. Circulating tumor cells in metastatic colorectal cancer: do we need an alternative cutoff? *J Cancer Res Clin Oncol.* 2013;139(8):1411–6.
14. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med.* 2004;351(8):781–91.
15. Lucci A, Hall CS, Lodhi AK, Bhattacharyya A, Anderson AE, Xiao L, et al. Circulating tumour cells in non-metastatic breast cancer: a prospective study. *Lancet Oncol.* 2012;13(7):688–95.
16. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, 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(20):6897–904.
17. Berger MD, Yang D, Sunakawa Y, Zhang W, Ning Y, Matsusaka S, et al. Impact of sex, age, and ethnicity/race on the survival of patients with rectal cancer in the United States from 1988 to 2012. *Oncotarget.* 2016;7(33):53668–78.