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Evaluation of Circulating Tumor DNA and Carcinoembryonic Antigen Levels and Relationship with Clinicopathological Risk Factors and Prognostic Indices in Colorectal Cancer Patients

ctDNA and CEA Relationship in Colorectal Cancer

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Abstract: **Objective:** Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Circulating tumor DNA (ctDNA) has emerged as a promising biomarker for CRC management, offering real-time insights into tumor burden and genetic mutations. This study investigates the correlation between carcinoembryonic antigen (CEA) levels, ctDNA, clinicopathological factors, and treatment outcomes in early and advanced CRC patients.

Methods: The study retrospectively analyzed data from CRC patients, including those with early-stage disease who underwent curative treatment and those with metastatic disease. ctDNA levels, demographic data, and clinical parameters such as CEA, inflammatory indexes, and tumor characteristics were evaluated to determine correlations with treatment outcomes.

Results: The study included 20 patients, with 60% diagnosed at the metastatic stage. Among metastatic patients, the liver, bone, and lung were the most common metastasis sites. When the ctDNA levels of the patients were evaluated, the mean value was found to be 9.96±12 in patients with early stage (stage 2-3) colon cancer, while it was 9.75±13 in metastatic stage disease. No significant relationship was found between ctDNA levels in both early-stage disease and metastatic stage disease (p 0.903). Additionally, when the relationship between ctDNA levels and early-stage relapse was examined, no significant relationship was found between the ctDNA levels and early- stage relapse and patients who did not develop relapse (p 0.167). While CEA and ctDNA levels were measured, they did not demonstrate a significant relationship with treatment outcomes.

Conclusion: Despite its potential, the integration of ctDNA as a routine biomarker in CRC care faces challenges, including variability in measurement techniques and cost-effectiveness. However, ctDNA's ability to guide personalized treatment strategies and monitor disease recurrence holds promise. The study's findings align with previous research, suggesting ctDNA as a poor prognostic indicator, though further research is needed. ctDNA represents a significant advance in CRC management, offering non-invasive, real-time insights into tumor dynamics. Ongoing research is expected to solidify its role in personalized treatment planning, potentially leading to more effective and tailored therapies for CRC patients.

Key words: colorectal cancer, circulating DNA, tumor markers

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INTRODUCTION

Colorectal cancer (CRC) is a significant contributor to cancer-related illness and death globally. Studies have highlighted circulating tumor DNA (ctDNA) as a valuable biomarker for managing, diagnosing, and treating CRC. It has been demonstrated to be useful in assessing treatment response in advanced-stage disease and in determining the need for adjuvant treatment as well as treatment escalation or de-escalation in early-stage disease. CtDNA, which consists of freely circulating tumor-derived DNA fragments in the blood, offers several advantages over traditional laboratory-based biomarkers and radiological evaluations. One of its key benefits is the ability to provide real-time information on tumor burden and genetic changes, which is crucial in CRC for timely detection of disease recurrence or treatment response. The concept of 'minimal residual disease', commonly used in hematological malignancies, is also applicable to solid organ malignancies with ctDNA detection. In early-stage patients undergoing curative treatment like surgery, detecting measurable levels of ctDNA in the circulation is an important indicator for diagnosis and disease recurrence, impacting the need and timing of adjuvant treatment. CtDNA analysis can help identify driver mutations in the tumor, guiding personalized treatment strategies such as targeted therapies or immunotherapies. By evaluating ctDNA-based mutations from the circulation, clinicians can improve the effectiveness of treatment and patient survival rates by detecting the dominant colonic features in the tumor. However, the expanded use of ctDNA testing has presented some challenges, such as the lack of standardized measurements, variations in test sensitivity and specificity, and unresolved cost-effectiveness issues.

In this study, we aimed to assess the correlation of carcinoembryological antigen (CEA) levels, clinicopathological risk factors, and ctDNA levels with treatment outcomes in patients with early and advanced CRC.

METHODS

We began by reviewing the records of both early-stage and advanced-stage CRC (Colorectal Cancer) patients who underwent ctDNA testing at our hospital between 2015 and 2022. The inclusion criteria were as follows: patients over 18 years old who had received curative treatment, had undergone ctDNA testing, and tested positive for ctDNA; and patients diagnosed at the metastatic stage with measurable ctDNA levels before treatment were also included in the study. Among early-stage CRC patients, those who had ctDNA testing after curative treatment, tested positive, and were considered for adjuvant therapy were included. In metastatic CRC patients, treatment-naive individuals with measurable ctDNA levels were evaluated. In addition to pre-treatment ctDNA testing, demographic data such as patients' age, gender, comorbidities, diagnosis date, stage at diagnosis, and treatments received were examined. Simultaneously with the submission of ctDNA levels for both metastatic and early-stage diseases, full blood counts, inflammatory indices, tumor markers such as CEA, and biochemical parameters were included in the evaluation.

The correlation between ctDNA levels at the time of diagnosis and pathological features, prognostic indices at diagnosis, and tumor marker values were assessed. For early-stage patients who underwent surgery, the correlation between ctDNA levels measured before adjuvant therapy and the likelihood of disease recurrence was evaluated. In metastatic patients, ctDNA levels were correlated with the location and number of recurrences. Additionally, the correlation between ctDNA levels and molecular test results was evaluated in metastatic patients.

The data were transferred to the computer environment with SPSS v20.0 program and descriptive analysis was performed. Data were presented as mean (mean) ± standard deviation (SD), median, lower value (LV), upper value (UV), number (n) and percentage (%).

RESULTS

In this study, 20 patients diagnosed with early and advanced stage colorectal cancer who were followed up and treated at Çukurova University medical oncology clinic were included. 5 (%25) of our patients were female and 15 (%75) were male. Their average age was 53. While 12 (%60) of our patients were diagnosed at the metastatic stage, the remaining 8 (%40) patients were diagnosed as early stage disease. Only 8 of metastatic patients were recognized as denovo metastatic disease. When the patients were evaluated according to their metastasis areas, the most common were liver, bone and lung metastases, while lymph node and peritoneal metastases were less common. While 6 (%30) of the patients did not have an additional comorbid disease, 14 (%70) patients had an accompanying comorbid disease. The most common comorbidity was the combination of type-2 diabetes, hypertension and ischemic heart disease. When evaluated according to tumor location, 6(%30) patients were located in the right colon and 14 (%70) patients were located in the left colon. 14 patients (%70) were in the rectosigmoid region, 3 patients (%15) were in the transverse colon, 3 patients (%15) were in the cecum and hepatic flexura. While 8 (38.1%) of the patients did not undergo primary-directed surgery, 12 patients (57.1%) underwent primary-directed surgery. The average number of lymph nodes removed in the patients was over 15. R0 surgical margins were obtained in all patients. When both early stage and advanced stage patients were examined in terms of targeted mutations, 9 patients (%45) were found to be RAS mutant, 1 patients (%5) were found to be BRAF mutant, while 3 patients (%15) were found to have both RAS and BRAF was detected as wild. Only 1 of our patients had a familial syndrome, which was Lynch Syndrome. Since the patient was diagnosed at an early stage and did not develop recurrence, other advanced genetic molecular investigations and MSH status could not be evaluated. When microsatellite instability was evaluated in all patients, they were found to be stable (MSS). No patient was considered unstable (MSH). The demographic characteristics of the patients are detailed in Table 1.

All early-stage patients received adjuvant chemotherapy. The most commonly used chemotherapy regimens are; FOLFOX was preferred in 46.1% of cases, CAPOX, preferred in 38.4%, and single-agent capecitabine, preferred in 15.38%. When the treatment durations were examined, 3 patients (38%) received 3-month adjuvant treatment, while 5 patients (62%) received 6-month treatment (Figure 1). It was observed that 3 of the early-stage colon cancer patients included in the study developed recurrent disease with distant metastasis after the completion of adjuvant treatment.

When the metastatic patients included in the study were evaluated, 8 of the patients had de nova metastatic disease. The most common areas of metastasis in these patients were the liver, lungs and bones. There were no cases of single-organ metastasis. When first-line treatment options in metastatic stage disease were examined, FOLFOX was the most frequently applied regimen with 42.9%, while FOLFIRI was preferred as the second most common regimen with 14.3%. Monotherapy with capacitabine was used in 1 patient with 4.8%, FOLFIRINOX triplet regimen was preferred in 1 patient with 4.8%. Regarding the use of biological agents accompanying chemotherapy, biological agents could not be administered to 5 patients due to various complications. However, biological agents were added to the existing chemotherapy backbone in 7 patients. When examining the preferred biological agents, Bevacizumab was the most frequently used, at a rate of 42.9%. Less commonly, Cetuximab and Panitumumab were used, respectively.

Table 1. Baseline Characteristics of Patients With Colorectal Cancer

Characteristic	Patients (n:20)
Cancer Stage	
Stg-2	4 (%20)
Stg-3	4 (%20)
Stg-4	12 (%60)
Sex	
Men	15 (%75)
Women	5 (%25)
Age,median (range),y	53
Localization	
Left Colon and Rectum	14 (%70)
Right Colon and Transverse	6 (%30)
Mutations	
K/N/H RASm	9 (%45)
BRAFm	1 (%5)
MSS	13 (%65)
MSI	0
Unknown	7(%35)
Prior Adjuvant chemotheraphy	
Stg-2	4 (%20)
Stg-3	4 (%20)
Stg-4 (relapse recurrent)	4 (%20)
Metastatic Site	
Liver	8 (%40)
Lung	3 (%15)
Bone	2 (%10)
Lymph node	5 (%25)
Peritoneal	2 (%10)
Pathological Findings	
Histology Adenocarcinoma	19 (%95)
Mucinous carcinoma	1 (%5)
Differentiation	3(%15)
Well dif.	7 (%35)
Moderately dif.	2 (%10)
Poorly dif.	8 (%40)
Unknown	10 (%50)
LVI (positive)	10 (%50)
PNI (positive)	4 (%20)
Metastatic Lymph node	3 (%15)
N0	1(%5)
N1	
N2	
Surgery	
Elective Surgery	11(%55)
Emergency Surgery	1(%5)

*Abbreviations: MSI, microsatellite instability; MSS, microsatellite stability; LVI, lymphovascular invasion;PNI; perineural invasion

Only 3 patients diagnosed at the early stage were alive when overall survival was examined. The remaining 17 patients had died due to disease-related or other causes. CEA values at the time of diagnosis could not be evaluated in 6 patients due to inaccessibility, and were evaluated in 14 patients. The mean CEA mean values at the diagnosis were found to be 7.12 (>3). While C-reactive protein (CRP) levels at the time of diagnosis could not be evaluated in 6 patients due to lack of data, they were assessed in 14 patients. The mean CRP levels at diagnosis were 26.1 (>0.8). When the ctDNA levels of the patients were evaluated, the mean value was found to be 9.96±12 in patients with early stage (stage 2-3) colon cancer, while it was 9.75±13 in metastatic stage disease. No significant relationship was found between ctDNA levels in both early stage disease and metastatic stage disease (p 0.903). Additionally, when the relationship between ctDNA levels and early stage relapse was examined, no significant relationship was found between the ctDNA levels and early stage relapse and patients who did not develop relapse (p 0.167). When overall survival and ctDNA levels were evaluated, the mean ctDNA levels of the 3 patients who survived were 5.49, while the mean ctDNA levels of the 18 patients who had died were found to be 10.15. Although the ctDNA levels of surviving patients were numerically half of those in deceased patients, there was no statistical significance (p = 0.498). Similarly, when ctDNA levels were compared with CEA and CRP levels, no statistical significance was reached.

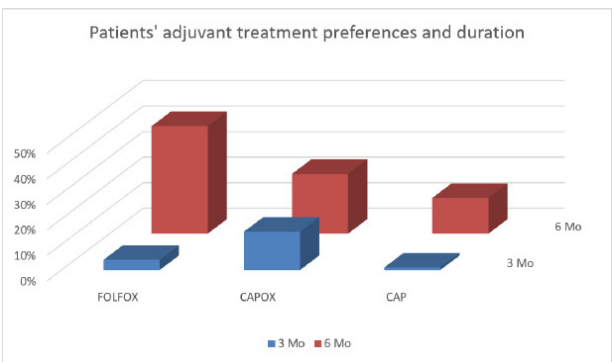

Figure 1. Patients Adjuvant Treatments and Durations

DISCUSSION

In this study, the aim was to investigate the relationship between circulating tumor DNA (ctDNA) levels, clinicopathological risk factors, and treatment outcomes in patients with early and advanced colorectal cancer (CRC), and to present these findings as survival data. Our cohort provides valuable insights into the potential role of ctDNA as a prognostic and predictive biomarker in the management of CRC.

Cell-free nucleic acids are fragments of extracellular DNA (cfDNA) or RNA (cfRNA) that can be detected in a variety of body fluids (1). These may be due to tumor apoptosis, necrosis or paraneoplastic releases. These tumor-associated nuclear fragmentations are called 'circulating tumor DNA' or 'circulating tumor RNA' when found in the blood or lymphatic circulation (2). Their half-life is approximately 114 hours. Depending on these half-lifes, ctDNAs constitute 0.1%-10% of cfDNAs (3,4). An increasing number of studies describe the potential uses of circulating tumor DNA (ctDNA) in the care of patients with colorectal cancer. However, unlike tissue biopsy, it has rapidly become widely used in the clinic because it is noninvasive, represents heterogeneous structures, and is easy and reproducible. Although the most common and well-known use of ctDNA is in blood, many other body fluids such as cerebrospinal fluid, saliva, pleural effusion, ascites, and urine samples can also be used(5-8). A number of different analytes are being investigated with different technologies, including not only these but also circulating tumor cells, tumor-educated platelets, exosomes, circulating nucleic acids, proteins and metabolites (9). Unfortunately, the detectable amount of ctDNA measured is closely related to tumor volume. For example, ctDNA has been shown to be detectable in 10–15% of patients with curatively treated stage II disease and 50–90% of patients with metastatic colorectal cancer(10-12). In our study, we examined ctDNA levels in blood, which is the most commonly analyzed source. Numerous studies have been heterogeneous, showing varied analyses and outcomes. However, more recent comprehensive studies indicate that ctDNA will soon be integrated into the routine care of both advanced and early-stage colorectal cancer patients, providing crucial guidance in patient management. There are many ongoing studies aiming to incorporate ctDNA usage in both early-stage and advanced-stage colorectal cancer treatment. When reviewing studies on why ctDNA is important in the early stages or how it can be integrated, the underlying hypothesis typically revolves around post-operative management (13). In patients receiving curative treatment, the objective is to provide prognostic and predictive insights into which patients should receive adjuvant therapy, how long it should last, and whether genomic analysis is warranted in early-stage disease (14-17). Additionally, ctDNA monitoring may enable the detection of early recurrences even before they become apparent on radiological scans in patients who have undergone curative treatment (18,19). In metastatic disease, ctDNA could be used to monitor treatment response, guide the selection of targeted therapies, identify resistance mutations that emerge post-treatment, and assess clonal evolution. As mentioned earlier, ctDNA levels tend to be higher in metastatic settings compared to early-stage disease. Although our study showed numerically higher ctDNA levels in metastatic cases, statistical significance was not reached, likely due to the heterogeneity of our patient group and the small sample size. Other studies have examined how ctDNA levels vary according to the site of metastasis (20).

The Gozila study, examined ctDNA levels at the metastasis site in colorectal cancers with single-organ metastasis. According to the study, the site with the lowest ctDNA level was peritoneal metastasis (21). In another study, higher ctDNA levels were observed in patients with liver metastases and tumor masses greater than 1 cm (22). Although this study did not include a sufficient number of patients with single-site metastases, ctDNA levels were found to be higher in patients with visceral metastases, aligning with findings from other studies.

CEA and other laboratory clinical risk factors evaluated in the early-stage disease group have been examined in many studies with ctDNA levels. Unfortunately, it has been shown that neither pathological risk factors nor laboratory markers such as CEA are sufficient to determine the risk of recurrence in patients. In fact, in the recent Galaxia and Dynamic studies, it has been proven that clinicopathological risk factors alone are not sufficient. In our study, when ctDNA measurements made before adjuvant treatment in earlystage patients were compared with clinicopathological risk factors of the patients, no statistically significant relationship was detected. Again, when both the amount of ctDNA measured in early-stage disease and metastatic-stage ctDNA elevations were examined, it was detected at lower rates compared to metastatic disease due to the lower disease burden in early-stage disease and the decrease in ctDNA secretions due to the disappearance of the primary tumor and the decrease in ctDNA levels secreted due to the primary mass. However, when the ctDNA levels of early-stage colon cancer patients and metastatic-stage colon cancer patients were examined in our study, although numerically higher levels were detected in metastatic-stage disease, this did not reach statistical significance. However, we think that this insignificance is due to the insufficient number of our patients. In a study examining the relationship between ctDNA and CEA after adjuvant treatment in early-stage disease and recurrence, 83% of patients with both ctDNA and CEA elevations after treatment subsequently experienced recurrence, while only 1 (17%) of patients with high CEA levels but negative ctDNA, experienced recurrence (23). In a different study evaluating imaging, CEA and ctDNA levels in terms of recurrence in resected early-stage colon cancer cases, it was observed that ctDNA testing did not provide a definite advantage over standard imaging and CEA measurement in the follow-up of resected colorectal cancer patients. In the study, the sensitivity of ctDNA in patients with recurrence was determined as 53.3%, imaging had a sensitivity of 60.0% and The sensitivity of CEA levels alone was 20.0%, while in the combined evaluation of ctDNA and imaging and CEA levels, the sensitivity of the combination was determined as % 73.3 (**24**).In this study, CEA levels could not be reached in 1 of 5 patients diagnosed with early-stage disease and who experienced recurrence, while 3 patients had CEA levels within the normal range, and only 1 patient had CEA elevation consistent with ctDNA. All 4 of the patients had high CEA levels at the time of diagnosis and before treatment.

Studies have confused the question of whether adjuvant treatment decisions should be made based on ctDNA or standard clinicopathological risk factors. In the latest ESMO 2024 study, it is suggested that if there is no correlation between ctDNA and standard clinicopathological risk factors, standard risk factors are still valid and treatment decisions should be made accordingly, and the obtained ctDNA results should be integrated into these risk factors (25). In our study, when the standard clinicopathological risk factors of patients who relapsed after early-stage curative treatment with ctDNA were evaluated, they constituted a high-risk patient group, similar to these finding.

Although many studies on ctDNA and colon cancer have been conducted to date, the first and only phase-3 randomized trial is the 'Dynamic' study. The results of this study are highly significant and are expected to bring about substantial changes in patient treatment management. The Dynamic study demonstrated that patients in the ctDNA-guided treatment arm received less chemotherapy compared to those in the standard treatment arm, which was based on clinicopathological risk factors. Despite this reduction in chemotherapy, the recurrence-free survival (RFS) outcomes at the 2-year follow-up were non-inferior. Additionally, when both ctDNA positive and negative arms receiving systemic treatment were compared, the ctDNA positive arm showed worse RFS. his suggests that the use of ctDNA can help prevent overtreatment, while also emphasizing the need for more intensive treatment in patients with high or positive ctDNA levels in the future (26). In our study, out of 9 early-stage patients

who received adjuvant treatment and had high ctDNA levels, only 3 did not experience recurrence, while the remaining 6 developed recurrence. Similar to other studies, our findings confirm that elevated ctDNA is a poor prognostic indicator.

This study, despite its contribution to the literature, also has serious deficiencies. First of all, the inclusion of both early and advanced stage disease groups, being a heterogeneous group, the low number of samples in both groups, the fact that ctDNA levels were only checked once and could not be evaluated during follow-up, and the lack of a control group in both arms are the deficiencies of our study.

CONCLUSIONS

In conclusion, ctDNA holds great promise as a valuable biomarker in the management of colorectal cancer, as it does in many other solid organ malignancies. Its ability to provide real-time and consistent information on tumor burden and associated driver genetic mutations, combined with the simplicity and non-invasiveness of its measurement through a blood test, represents a significant paradigm shift in the diagnosis, treatment, and monitoring of CRC patients. With ongoing research, we believe ctDNA will enhance the potential for personalized treatment planning, guiding decisions on when to intensify treatment or adopt a wait-and-see approach.

REFERENCES

1. Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer--a survey. Biochim Biophys Acta. 2007 Jan;1775(1):181-232. doi: 10.1016/j.bbcan.2006.10.001.
2. Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, Lindeman N, Lockwood CM, Rai AJ, Schilsky RL, Tsimberidou AM, Vasalos P, Billman BL, Oliver TK, Bruinooge SS, Hayes DF, Turner NC. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol. 2018 Jun 1;36(16):1631-1641. doi: 10.1200/JCO.2017.76.8671.
3. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. Cancer Discov. 2014 Jun;4(6):650-61. doi: 10.1158/2159-8290.CD-13-1014.
4. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, Thornton K, Agrawal N, Sokoll L, Szabo SA, Kinzler KW, Vogelstein B, Diaz LA Jr. Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008 Sep;14(9):985-90. doi: 10.1038/nm.1789.
5. De Mattos-Arruda L, Mayor R, Ng CKY, Weigelt B, Martínez-Ricarte F, Torrejon D, Oliveira M, Arias A, Raventos C, Tang J, Guerini-Rocco E, Martínez-Sáez E, Lois S, Marín O, de la Cruz X, Piscuoglio S, Towers R, Vivancos A, Peg V, Ramon y Cajal S, Carles J, Rodon J, González-Cao M, Tabernero J, Felip E, Sahuquillo J, Berger MF, Cortes J, Reis-Filho JS, Seoane J. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. Nat Commun. 2015 Nov 10;6:8839. doi: 10.1038/ncomms9839.
6. Kimura H, Fujiwara Y, Sone T, Kunitoh H, Tamura T, Kasahara K, Nishio K. EGFR mutation status in tumour-derived DNA from pleural effusion fluid is a practical basis for predicting the response to gefitinib. Br J Cancer. 2006 Nov 20;95(10):1390-5. doi: 10.1038/sj.bjc.6603428.
7. Wang Y, Springer S, Mulvey CL, Silliman N, Schaefer J, Sausen M, James N, Rettig EM, Guo T, Pickering CR, Bishop JA, Chung CH, Califano JA, Eisele DW, Fakhry C, Gourin CG, Ha PK, Kang H, Kiess A, Koch WM, Myers JN, Quon H, Richmon JD, Sidransky D, Tufano RP, Westra WH, Bettegowda C, Diaz LA Jr, Papadopoulos N, Kinzler KW, Vogelstein B, Agrawal N. Detection of somatic mutations and HPV in the saliva and plasma of patients with head and neck squamous cell carcinomas. Sci Transl Med. 2015 Jun 24;7(293):293ra104. doi: 10.1126/scitranslmed.aaa8507.
8. Reckamp KL, Melnikova VO, Karlovich C, Sequist LV, Camidge DR, Wakelee H, Perol M, Oxnard GR, Kosco K, Croucher P, Samuelsz E, Vibat CR, Guerrero S, Geis J, Berz D, Mann E, Matheny S, Rolfe L, Raponi M, Erlander MG, Gadgeel S. A Highly Sensitive and Quantitative Test Platform for Detection of NSCLC EGFR Mutations in Urine and Plasma. J Thorac Oncol. 2016 Oct;11(10):1690-700. doi: 10.1016/j.jtho.2016.05.035
9. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol. 2017 Sep;14(9):531-548. doi: 10.1038/nrclinonc.2017.14.
10. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SK,

Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih lM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Diaz LA Jr. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med. 2014 Feb 19;6(224):224ra24. doi: 10.1126/scitranslmed.3007094.

11. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, Silliman N, Tacey M, Wong HL, Christie M, Kosmider S, Skinner I, Wong R, Steel M, Tran B, Desai J, Jones I, Haydon A, Hayes T, Price TJ, Strausberg RL, Diaz LA Jr, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016 Jul 6;8(346):346ra92. doi: 10.1126/scitranslmed.aaf6219.
12. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, Wong R, Kosmider S, Ananda S, McKendrick J, Lee B, Cho JH, Faragher I, Jones IT, Ptak J, Schaeffer MJ, Silliman N, Dobbyn L, Li L, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating Tumor DNA Analyses as Markers of Recurrence Risk and Benefit of Adjuvant Therapy for Stage III Colon Cancer. JAMA Oncol. 2019 Dec 1;5(12):1710-1717. doi: 10.1001/jamaoncol.2019.3616. Erratum in: JAMA Oncol. 2019 Dec 1;5(12):1811. doi: 10.1001/jamaoncol.2019.5667.
13. Dasari A, Morris VK, Allegra CJ, Atreya C, Benson AB 3rd, Boland P, Chung K, Copur MS, Corcoran RB, Deming DA, Dwyer A, Diehn M, Eng C, George TJ, Gollub MJ, Goodwin RA, Hamilton SR, Hechtman JF, Hochster H, Hong TS, Innocenti F, Iqbal A, Jacobs SA, Kennecke HF, Lee JJ, Lieu CH, Lenz HJ, Lindwasser OW, Montagut C, Odisio B, Ou FS, Porter L, Raghav K, Schrag D, Scott AJ, Shi Q, Strickler JH, Venook A, Yaeger R, Yothers G, You YN, Zell JA, Kopetz S. ctDNA applications and integration in colorectal cancer: an NCI Colon and Rectal-Anal Task Forces whitepaper. Nat Rev Clin Oncol. 2020 Dec;17(12):757-770. doi: 10.1038/s41571-020-0392-0.
14. Henriksen TV, Tarazona N, Frydendahl A, Reinert T, Gimeno-Valiente F, Carbonell-Asins JA, Sharma S, Renner D, Hafez D, Roda D, Huerta M, Roselló S, Madsen AH, Løve US, Andersen PV, Thorlacius-Ussing O, Iversen LH, Gotschalck KA, Sethi H, Aleshin A, Cervantes A, Andersen CL. Circulating Tumor DNA in Stage III Colorectal Cancer, beyond Minimal Residual Disease Detection, toward Assessment of Adjuvant Therapy Efficacy and Clinical Behavior of Recurrences. Clin Cancer Res. 2022 Feb 1;28(3):507-517. doi: 10.1158/1078-0432.CCR-21-2404.
15. Parikh AR, Van Seventer EE, Siravegna G, Hartwig AV, Jaimovich A, He Y, Kanter K, Fish MG, Fosbenner KD, Miao B, Phillips S, Carmichael JH, Sharma N, Jarnagin J, Baiev I, Shah YS, Fetter IJ, Shahzade HA, Allen JN, Blaszkowsky LS, Clark JW, Dubois JS, Franses JW, Giantonio BJ, Goyal L, Klempner SJ, Nipp RD, Roeland EJ, Ryan DP, Weekes CD, Wo JY, Hong TS, Bordeianou L, Ferrone CR, Qadan M, Kunitake H, Berger D, Ricciardi R, Cusack JC, Raymond VM, Talasaz A, Boland GM, Corcoran RB. Minimal Residual Disease Detection using a Plasma-only Circulating Tumor DNA Assay in Patients with Colorectal Cancer. Clin Cancer Res. 2021 Oct 15;27(20):5586-5594. doi: 10.1158/1078-0432.CCR-21-0410.
16. Kotaka M, Shirasu H, Watanabe J, Yamazaki K, Hirata K, Akazawa N, Matsuhashi N, Yokota M, Ikeda M, Kato K, Aleshin A, Sharma S, Kotani D, Oki E, Takemasa I, Kato T, Nakamura Y, Taniguchi H, Mori M, Yoshino T. Association of circulating tumor DNA dynamics with clinical outcomes in the adjuvant setting for patients with colorectal cancer from an observational GALAXY study in CIRCULATE-Japan. *J Clin Oncol* 2022; 40: 9.
17. Tsukada Y, Matsuhashi N, Murano T, et al. Impact of postoperative integrated genomic and epigenomic signatures of circulating tumor DNA (ctDNA) on recurrence in resected colorectal cancer: initial report of a prospective ctDNA monitoring study COSMOS-CRC-01. *J Clin Oncol* 2022; 40: 168. DOI: 10.1200/JCO.2022.40.4_suppl.168
18. Wang Y, Li L, Cohen JD, Kinde I, Ptak J, Popoli M, Schaefer J, Silliman N, Dobbyn L, Tie J, Gibbs P, Tomasetti C, Kinzler KW, Papadopoulos N, Vogelstein B, Olsson L. Prognostic Potential of Circulating Tumor DNA Measurement in Postoperative Surveillance of Nonmetastatic Colorectal Cancer. JAMA Oncol. 2019 Aug 1;5(8):1118-1123. doi: 10.1001/jamaoncol.2019.0512.
19. Henriksen TV, Tarazona N, Reinert T, Carbonell-Asins JA, Renner D, Sharma S et al. Circulating tumor DNA analysis for assessment of recurrence risk, benefit of adjuvant therapy, and early relapse detection after treatment in colorectal cancer patients. Journal of Clinical Oncology. 2021;39(3 Suppl.):11. doi: 10.1200/JCO.2021.39.3_suppl.11
20. Bando H, Nakamura Y, Taniguchi H, Shiozawa M, Yasui H, Esaki T, Kagawa Y, Denda T, Satoh T, Yamazaki K, Sunakawa Y, Kato T, Goto M, Yuki S, Nishina T, Oki E, Shinozaki E, Matsuhashi N, Takahashi N, Tsuji A, Ohtsubo K, Wakabayashi M, Ikeno T, Hata M, Odegaard JI, Yoshino T. Effects of Metastatic Sites on Circulating Tumor DNA in Patients With Metastatic Colorectal Cancer. JCO Precis Oncol. 2022 Apr;6:e2100535. doi: 10.1200/PO.21.00535.

21. Xue J, Prabhakaran S, Prabhakaran S, Lim WM, Guerra G, Heriot A, Kong JC. The utility of ctDNA in colorectal cancer with peritoneal metastases. ANZ J Surg. 2023 Mar;93(3):506-509. doi: 10.1111/ans.18078.
22. Lee S, Park YS, Chang WJ, Choi JY, Lim A, Kim B, Lee SB, Lee JW, Kim SH, Kim J, Kwak JM, Yoon KC, Lee SH, Kim YH. Clinical Implication of Liquid Biopsy in Colorectal Cancer Patients Treated with Metastasectomy. Cancers (Basel). 2021 May 6;13(9):2231. doi: 10.3390/cancers13092231.
23. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, Wong R, Kosmider S, Ananda S, McKendrick J, Lee B, Cho JH, Faragher I, Jones IT, Ptak J, Schaeffer MJ, Silliman N, Dobbyn L, Li L, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating Tumor DNA Analyses as Markers of Recurrence Risk and Benefit of Adjuvant Therapy for Stage III Colon Cancer. JAMA Oncol. 2019 Dec 1;5(12):1710-1717. doi: 10.1001/jamaoncol.2019.3616. Erratum in: JAMA Oncol. 2019 Dec 1;5(12):1811. doi: 10.1001/jamaoncol.2019.5667.
24. Fakih M, Sandhu J, Wang C, Kim J, Chen YJ, Lai L, Melstrom K, Kaiser A. Evaluation of Comparative Surveillance Strategies of Circulating Tumor DNA, Imaging, and Carcinoembryonic Antigen Levels in Patients With Resected Colorectal Cancer. JAMA Netw Open. 2022 Mar 1;5(3):e221093. doi: 10.1001/jamanetworkopen.2022.1093
25. Soueidy C,Zaanan A,Gelli M, et al. Clinical impact of circulating tumor DNA to track minimal residual disease in colorectal cancer patients. Hopes and limitations. ESMO gastroint. Oncol. 2024; 4:100068. DOI:https://doi.org/10.1016/j.esmogo.2024.100068
26. Tie J, Cohen JD, Lahouel K, Lo SN, Wang Y, Kosmider S, Wong R, Shapiro J, Lee M, Harris S, Khattak A, Burge M, Harris M, Lynam J, Nott L, Day F, Hayes T, McLachlan SA, Lee B, Ptak J, Silliman N, Dobbyn L, Popoli M, Hruban R, Lennon AM, Papadopoulos N, Kinzler KW, Vogelstein B, Tomasetti C, Gibbs P; DYNAMIC Investigators. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. N Engl J Med. 2022 Jun 16;386(24):2261-2272. doi: 10.1056/NEJMoa2200075.