ctDNA/MRD Testing for Colon Cancer: A Work in Progress or Ready for Prime-Time Standard of Care?

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Abstract

In patients with surgically resectable colon cancer (CC), clinicopathologic characteristics translate into cancer staging and predict recurrence risk. Adjuvant chemotherapy reduces the risk of recurrence and is offered to high-risk patients. However, some patients are inevitably overtreated or undertreated; better risk stratification is necessary to improve outcomes after surgery. Circulating tumor DNA (ctDNA)–based minimum residual disease (MRD) assays sequence plasma cell-free DNA for tumor DNA to predict the presence of otherwise subclinical malignancy. Studies have demonstrated that detectable ctDNA after surgery for CC predicts a high rate of recurrence and improves prognostication. Recent clinical trials show promise for using ctDNA to guide therapy, in particular standard-risk stage II CC. Large, randomized studies evaluating ctDNA-guided adjuvant chemotherapy versus standard of care in stage III CC are ongoing. Current data are insufficient to recommend routine use of ctDNA to guide adjuvant chemotherapy in resectable stage III CC.

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Identifying the presence and extent of cancer is foundational to selection of anticancer therapy. Clinicopathologic findings are translated into tumor stage,[1] with colon cancer (CC) a standard example. CC staging incorporates depth of invasion of the primary tumor (T stage), lymph node involvement (N stage), and presence of distant metastases (M stage), which is translated into overall stage (I–IV) and is highly predictive of outcome. Circulating protein markers, such as CEA, and histopathologic features, such as histologic grade and the presence of lymphatic invasion, also inform recurrence risk.[2–5] Adjuvant chemotherapy is used to eradicate remaining imaging-occult micrometastatic disease after surgery and reduce recurrence. However, chemotherapy is associated with significant morbidity and cost to the health care system and patients. Thus, selection of patients for whom the benefits of chemotherapy outweigh the risks is critically important.

Large trials have examined the optimal duration and intensity of adjuvant chemotherapy for patients with resectable CC. Fluoropyrimidine-based therapy is typically recommended for high-risk stage II CC (primarily T4 tumors), with the addition of oxaliplatin for lymph node–positive (stage III) CC, for at least 3 and up to 6 months.[6–9] Residual neuropathy is a major concern for patients who receive oxaliplatin. Fluoropyrimidine monotherapy is appropriate for some patients with high-risk features other than T4 primary. Stage II cancers lacking high-risk risk features and all stage I tumors derive minimal overall survival (OS) benefit from adjuvant chemotherapy; these patients tend to undergo observation. Inevitably, some patients deemed high risk would not have experienced recurrence, and therefore receive chemotherapy without benefit. Conversely, a fraction of low-risk CC does recur and may have benefited from chemotherapy. Given that there were approximately 76,000 cases of stage I–III CC in the United States in 2023,[10] improved identification of patients who benefit from adjuvant chemotherapy is needed.

Minimum residual disease (MRD) testing uses sensitive molecular techniques to detect subclinical residual malignancy following definitive treatment. MRD approaches have a successful history of use in leukemia[11] and multiple myeloma,[12] in which sensitive flow cytometry of bone marrow aspirate may identify residual cancer. Patients who are MRD-positive are at higher risk of recurrence and may receive therapy intensification, whereas those who are MRD-negative may allow deintensification, with clear clinical benefits. Analogous testing has been more elusive in most solid tumors. CEA, for example, is insufficient to guide therapy, because a normal CEA level is common even with frank recurrence.[13,14]

Instead, techniques interrogating plasma cell-free DNA (cfDNA) for residual circulating tumor DNA (ctDNA) are being widely investigated and used clinically to guide decision-making for many cancers.[15] Potential applications for ctDNA detection include predicting recurrence or need for chemotherapy and as a biomarker for response to therapy, including immunotherapy. This review discusses ctDNA-based MRD assays in surgically resectable CC, focusing on clinical data supporting their use for predicting prognosis and benefit from adjuvant chemotherapy.

MRD Testing Based on ctDNA

cfDNA-based diagnostics are well established for a variety of purposes in cancer, such as comprehensive genomic profiling, in addition to nononcologic applications, such as noninvasive prenatal testing.[16,17] More recently, these techniques have been turned toward detection of residual cancer.[15] Single-gene assays can detect MRD; however, sensitivity is increased using large multigene panels.[18] These techniques primarily rely on sensitive massively parallel next-generation sequencing (NGS) of plasma DNA to detect tumor mutations. Assessment of ctDNA methylation[19,20] has also been used to accurately detect MRD. Complementary measures of MRD, such as novel protein biomarkers,[21] have also been proposed.

The current approach to clinically available ctDNA MRD assays broadly falls into 2 categories.[15] One method sequences cfDNA without relying on identifying mutations in tissue, referred to as *tumor-agnostic*. These assays have the advantage of speed

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and simplicity, but are vulnerable to confounding from mutations such as clonal hematopoiesis and may be less specific. Thus, carefully tuned bioinformatic processes are required to exclude confounders while maintaining sensitivity and specificity. DNA methylation assessment may also improve assay accuracy.[20] The second method relies on sequencing tumor DNA derived from tissue with either a broad panel of genes or whole-exome or whole-genome sequencing.[15] The result is a unique "tumor-informed" or "bespoke" mutational panel; these genes are then assayed in the cfDNA. Although highly specific to the tumor, tissue-informed assays require access to tumor tissue and add time and cost to the process. The clinical relevance of these differences is not yet known, especially when incorporating the most modern sequencing and bioinformatic processes. Given an observed transient increase in total cfDNA following surgery that may reduce test sensitivity,[22] a minimum waiting period (typically 2–4 weeks)[23–26] after surgical resection is required prior to the blood draw. Either assay type may be drawn at landmark timepoints, including postoperatively, during and after adjuvant chemotherapy, and during surveillance for recurrence.

Prognostic Utility of MRD Assays in CC

In an observational 2016 study, Tie et al[27] demonstrated that a ctDNA tumor-informed assay (based on the Safe-Sequencing System [Safe-SeqS]) drawn after surgical resection could predict recurrence in patients with stage II CC who did not receive chemotherapy (Table 1). Of the 14 patients with ctDNA-positive status, 100% experienced recurrence, compared with only 10% of those with ctDNA-negative status, with a hazard ratio (HR) of 18 (95% CI, 7.9–40). On serial testing during surveillance, ctDNA turned positive more frequently than CEA prior to recurrence on imaging, and median lead time of positivity for recurrence was significantly longer for ctDNA (167 days) than for CEA (61 days). In a follow-up study, among 96 patients with stage III CC, 92% of whom received adjuvant chemotherapy, those who were ctDNA-negative after resection had a 3-year disease-free survival (DFS) rate of 76%, compared with only 47% among those were ctDNA-positive after resection, with an HR of 3.6.[23] The difference between these studies is likely explained by higher baseline risk of recurrence for stage III versus stage II CC as well as a positive effect from adjuvant chemotherapy.

An observational study of 168 patients with stage III CC who underwent serial testing with the ctDNA MRD assay Signatera (Natera, Inc.) showed a recurrence rate of 80% among those who were ctDNA-positive after resection versus only 18% among those who were ctDNA-negative[22] (Table 1). Among those who remained ctDNA-positive after completion of adjuvant therapy, 100% experienced recurrence. This study also showed that total cfDNA levels were higher immediately after surgery and decreased steadily thereafter; sensitivity for residual ctDNA was greater as the total cfDNA levels decreased, supporting the need for a waiting period after surgery prior to MRD testing.

GALAXY is an observational cohort of CIRCULATE-Japan,[24,28] whereas BESPOKE is an observational study based in the United States[25,29] (Table 1). In both studies, patients underwent Signatera MRD testing as early as 2 weeks postoperatively and then received physician's choice of adjuvant therapy or surveillance. In updated results for GALAXY[28] including 2,058 patients with stage II–III colorectal cancer, 24-month DFS for patients who were ctDNA-negative at the postoperative timepoint was 89.3% versus 33.5% for patients who were ctDNA-positive (HR, 12). At the postoperative landmark timepoint, analytic sensitivity was 58.7% and specificity was 94%,[24] with sensitivity decreasing to 48% with data incorporating longer follow-up.[28] Serial surveillance testing improved sensitivity to 73% and specificity to 97%. Although MRD was highly predictive, higher T and N stage retained prognostic significance for recurrence. For 211 patients initially ctDNA-positive treated with adjuvant chemotherapy and followed with surveillance MRD timepoints, 61 with transient clearance (converted to ctDNA-negative then became ctDNA-positive) had similar 24-month DFS (2.3%) to the 66 patients who were persistently ctDNA-positive (2%), although recurrence was later for those with transient clearance. In contrast, 84 patients with sustained ctDNA clearance demonstrated 24-month DFS of 90.1%. Of note, this study was designed to recruit in concert for 2 interventional substudies of CIRCULATE-Japan—VEGA and ALTAIR—for patients with CC who were ctDNA-negative and ctDNA-positive, respectively, after resection.[30] In similar results, interim data for BESPOKE[25] included 689 patients with stage II–III CC and reported 91.6% 24-month DFS for patients who were ctDNA-negative postoperatively compared with 29.9% for those who were ctDNA-positive. However, longer follow-up is necessary in both studies to accurately assess sensitivity and specificity, because the potential for later relapses remains.

Finally, a study utilizing Guardant Reveal, a tumor-agnostic ctDNA assay from Guardant Health that relies on both somatic mutation and epigenomic methylation analyses, predicted recurrence in 84 patients with definitively treated stage I–IV CC.[20] Of 15 patients who were ctDNA-positive after definitive treatment and at least 1 year of follow-up, 100% experienced recurrence, compared with 12 of 49 (24.5%) who were initially ctDNA-negative. Addition of the epigenomic signature increased sensitivity by 25% to 36%. Taken together, these large observational studies demonstrate clear prognostic significance for ctDNA MRD assays. Importantly, traditional clinicopathologic risk criteria such N and T stage retained prognostic value, but were less effective than ctDNA alone at predicting recurrence.

Predictive Utility of MRD Assays in CC

The critical next question is whether ctDNA MRD may guide therapy intensification for patients previously predicted to be low-risk, and therapy de-escalation for those at risk for overtreatment. The landmark DYNAMIC II study was the first to demonstrate the viability of this approach in CC[31] (Table 1). In that study, 455 patients with stage II CC (including T4 primaries) were randomized to standard of care (SoC) or ctDNA-guided therapy with the tumor-informed Safe-SeqS assay. In the guided therapy arm, patients who were ctDNA-positive after resection received adjuvant chemotherapy and those who were ctDNA-negative underwent surveillance alone. Both arms demonstrated identical 3-year DFS, despite patients in the ctDNA-guided arm receiving chemotherapy less often (15%) than those in the SoC arm (28%). However, a higher proportion of patients in the ctDNA-guided arm (28/294; 9.5%) received oxaliplatin doublets than in the SoC arm (4/147; 2.7%). In the ctDNA-guided arm, 3-year DFS was not significantly different for patients who were ctDNA-negative (92.5%) versus ctDNA-positive (86.4%). These findings compare favorably to the investigators' prior observational study,[27] in which all similar patients with stage II CC who were ctDNA-positive and did not receive chemotherapy had experienced recurrence at 3 years.

Table 1. Trials Utilizing ctDNA MRD Testing in Resectable Colon Cancer

Assay	Trial	Population	Intervention/Design	Selected Results
Safe-SeqS, tumor-informed	ACTRN12612000326897[27]	Stage II CC (n=230)	Observational, ctDNA after resection, no ACT	3-y DFS: ctDNA− (n=164): 90% ctDNA+ (n=14): 0% HR, 18 (95% CI, 7.9–40)
	Tie et al, 2019[23]	Stage III CC (n=96)	Observational, ctDNA after resection, 91.6% received ACT	3-y DFS: ctDNA− (n=76): 76% ctDNA+ (n=20): 47% HR, 3.6
	DYNAMIC-II[31]	Stage II (T3 or T4, N0) (n=455)	ctDNA-guided therapy vs SoC	3-y DFS: ctDNA-guided: 91.7% SoC: 92.4% HR, 0.96 (95% CI, 0.51–1.82)
			ctDNA-guided therapy: ctDNA− → no ACT vs ctDNA+ → ACT	3-y DFS: ctDNA−: 92.5% ctDNA+: 86.4% HR, 1.83 (95% CI, 0.79–4.27)
	DYNAMIC-III[36]	Stage III CC (target n=1,000)	Randomized, SoC/physicians choice vs ctDNA-guided escalation/de-escalation	Pending
Personal Genome Diagnostics (PGDx), tumor-informed	MEDOCC-CrEATE[33]	Stage II CC (target n=1,320)	Randomized, SoC vs offer of ctDNA-guided therapy	Pending
Signatera, tumor-informed	Henriksen et al[22]	Stage III CC (n=168)	Observational, ctDNA after resection	Recurrence risk: ctDNA+: 80% ctDNA−: 18% HR, 7 (95% CI, 4–14)
			Observational, ctDNA after completion of ACT	Recurrence risk: ctDNA+: 100% ctDNA−: 10% HR, 51 (95% CI, 15–167)
	GALAXY: CIRCULATE-Japan observational cohort[24,28]	Stage II–III resectable CC (n=2,058)	ctDNA after resection	24-mo DFS: ctDNA−: 89.3% (95% CI, 87.2%–91.1%) ctDNA+: 33.5% (95% CI, 26.5%–40.7%)
		High-risk stage II–III CC (n=644)	ctDNA− after resection	ACT vs observation: HR, 1.71 (95% CI, 0.8–3.7)
			ctDNA+ after resection	ACT vs observation: HR, 6.59 (95% CI, 3.5–12.3)
	VEGA: CIRCULATE-Japan ctDNA− cohort[30]	ctDNA− high-risk stage II and low-risk stage III CC	Randomized to observation or ACT	Pending
	ALTAIR: CIRCULATE-Japan ctDNA+ cohort[30]	ctDNA+ stage II–III CC after SoC adjuvant therapy	Randomized to 6 months trifluridine/tipiracil vs placebo	Pending
	BESPOKE[25]	Stage II–III CC (n=689)	ctDNA after resection	24-mo DFS: ctDNA−: 91.6% (95% CI, 88.4%–93.9%) ctDNA+: 29.9% (95% CI, 13.3%–48.5%)
			ctDNA− after resection	ACT vs observation: HR, 1.5 (95% CI, 0.8–2.8)
			ctDNA+ after resection	ACT vs observation: HR, 3.1 (95% CI, 1.43–6.6)
	CIRCULATE-NA (NRG-GI008)[38]	Stage III and high-risk stage II CC (target n=1,912)	ctDNA−, randomized to ACT vs observation ctDNA+, randomized to SoC ACT vs FOLFOXIRI	Pending
	CIRCULATE (Europe)[37]	Stage II MSS CC (target n=2,310)	If ctDNA+, randomized to ACT vs observation	Pending

(*continued on next page*)

For patients receiving ctDNA-guided therapy, median time from surgery to start of chemotherapy was 83 days, 30 days longer than for SoC management, reflecting the additional time required to complete the assay.[31] Whether additional delay in adjuvant chemotherapy initiation impacts outcomes is controversial, given the lack of randomized studies and the potential for bias in observational studies.[32] Potential for delay has been raised as a concern for ctDNA-guided therapy; reassuringly, outcomes were not different in this study.

MEDOCC-CrEATE is a similar trial in the Netherlands that randomizes patients with ctDNA-positive standard-risk stage II CC to receive an offer of adjuvant chemotherapy versus surveillance.[33] A primary endpoint is to examine the willingness of patients to receive adjuvant chemotherapy if ctDNA-positive.

COBRA (NRG-GI005) was a large study using the LUNAR cfDNA platform, a tumor-agnostic assay from Guardant Health.[34,35] The study aimed to enroll 1,408 patients with stage IIA CC; if ctDNA-positive postoperatively, patients were randomized to observation or adjuvant chemotherapy. The primary endpoint was ctDNA clearance. A total of 635 patients were enrolled, of which 16 were ctDNA-positive (Table 1). Among these 16 patients, 3 of 7 (43%) who were randomized to surveillance achieved ctDNA

Table 1 (cont.). Trials Utilizing ctDNA MRD Testing in Resectable Colon Cancer

Assay	Trial	Population	Intervention/Design	Selected Results
Guardant Reveal, tissue-agnostic	PEGASUS[26]	Stage III and high-risk stage II (T4N0) CC (n=135)	ctDNA 4 weeks s/p surgery: ctDNA+ → 3 mo CAPEOX ctDNA− → 6 mo CAPE	24-mo DFS: ctDNA−: 90/100 (90%) ctDNA+: 23/35 (66%) HR, 3.91 (95% CI, 1.46–10.47)
			ctDNA+ cohort, s/p CAPEOX: ctDNA+ (27/35) → 24 received FOLFIRI ctDNA− (8/35) → surveillance	ctDNA after FOLFIRI: ctDNA+: 13/24 (54%) ctDNA−: 11/24 (46%) Relapses: 5 pre-FOLFIRI, 6 post-FOLFIRI
	NCT03803553[40]	Stage III CC, s/p SoC ACT (target n=500)	ctDNA− s/p ACT: surveillance ctDNA+ s/p ACT: MSS CC → FOLFIRI vs surveillance; MSI-H CC → anti–PD-1; MSS *BRAF* V600E CC → targeted therapy HER2+ CC → anti-HER2	Pending
	TRACC Part C[39]	Stage III and high-risk stage II (T4N0) CC (target n=1,621)	ctDNA after resection, randomized to ctDNA-guided vs SoC ACT	Pending
Guardant LUNAR, tissue-agnostic	NRG-GI005 (COBRA)[34,35]	Stage II CC without high-risk features (n=635)	ctDNA+ (n=16) randomized to surveillance or ACT	ctDNA clearance: Surveillance (n=7): 43% ACT (n=9): 11%

Abbreviations: ACT, adjuvant chemotherapy; CC, colon cancer; ctDNA, circulating tumor DNA; DFS, disease-free survival; HR, hazard ratio; MRD, minimum residual disease; MSI-H, microsatellite instability–high; MSS, microsatellite stable; SoC, standard of care.

clearance, compared with only 1 of 9 (11%) randomized to adjuvant chemotherapy. Given this result, the study was closed early for futility. This study in particular highlights the requirement for very high specificity (and therefore a high positive predictive value [PPV]) of MRD assays in low-risk populations such as this.

Several interventional trials with complex adaptive designs incorporating ctDNA-guided adjuvant therapy are underway for stage III and high-risk stage II CC (Table 1). Of these, data are available only for the PEGASUS trial.[26] This nonrandomized feasibility study enrolled 135 patients with stage III and high-risk (T4) stage II CC who were eligible for chemotherapy. CtDNA testing was obtained after resection and at regular intervals during therapy using Guardant Reveal. Patients who were ctDNA-positive received 3 months of CAPEOX, whereas those who were ctDNA-negative received 6 months of capecitabine. The patients who were ctDNA-negative and later became positive were intensified to CAPEOX. Patients who became ctDNA-negative, or who cleared their ctDNA, were then observed. Patients who were persistently positive after CAPEOX were transitioned to FOLFIRI to evaluate whether ctDNA clearance could be achieved. The 24-month DFS was better for those patients who were ctDNA-negative versus ctDNA-positive (90% vs 66%; HR, 3.91), as expected. Of 35 patients initially ctDNA-positive, only 8 of 35 (22.9%) converted to ctDNA-negative and entered surveillance. Of the remaining patients, 24 then received FOLFIRI, and of these, 11 of 24 (46%) converted to ctDNA-negative. PEGASUS was as a feasibility study, demonstrating that ctDNA testing could guide chemotherapy in the adjuvant setting. However, interpretation is limited by lack of randomization, low patient numbers, and a median reported follow-up of only 21 months.

The GALAXY and BESPOKE studies also reported an observational analysis of high-risk patients with stage II or III CC treated with chemotherapy versus surveillance[24,25,28] (Table 1). In GALAXY, for patients who were initially ctDNA-negative, no significant difference in DFS was observed between those who were and were not treated with adjuvant therapy.[24] In contrast, for patients who were initially ctDNA-positive, recurrence appeared significantly decreased in patients who received chemotherapy versus surveillance alone (HR, 6.59; 95% CI, 3.53–12.3). BESPOKE reported similar results, with no difference in recurrence for patients who were ctDNA-negative treated with or without adjuvant chemotherapy, whereas recurrence was decreased for patients who were ctDNA-positive receiving adjuvant therapy (HR, 3.1; 95% CI, 1.43–6.6).[25] These findings suggest a benefit from chemotherapy primarily for patients who are ctDNA-positive, with the caveat that these patients were not randomized, and the result may be subject to confounding.

Several large studies are ongoing in which patients with stage III or high-risk stage II CC are randomized to SoC or ctDNA-guided therapy, including DYNAMIC III for stage III CC guided by Safe-SeqS[36]; CIRCULATE-Japan interventional substudies ALTAIR and VEGA for patients who were ctDNA-positive and ctDNA-negative after resection, respectively, using Signatera[30]; CIRCULATE in Europe[37] for stage II CC and CIRCULATE-NA (North America)[38] for high-risk stage II and stage III CC using Signatera; TRACC Part C for stage III and high-risk stage II CC using Guardant Reveal[39]; and NCT03803553[40] (Table 1). This last study assigns patients with stage III CC who are ctDNA-positive after SoC treatment to FOLFIRI or targeted therapy if eligible, also using Guardant Reveal. In addition, a pooled analysis of various ctDNA-guided trials is planned. These studies will answer how best to use MRD assays to guide adjuvant therapy in various clinical scenarios, including surveillance versus deintensification alone for patients who are ctDNA-negative, intensification for those who are ctDNA-positive, or switch to alternative therapy for those who are persistently positive. These studies may also help address whether tumor-agnostic and tumor-informed assays are similar in performance for clinical use and determine the impact of delay in initiating therapy.

Discussion

In this review, we discussed the available clinical data evaluating the use of ctDNA MRD assays in resectable CC. These assays are

of clear prognostic significance that may exceed though not obviate clinicopathologic data. Prognostication has utility in providing reassurance to patients if ctDNA-negative or may prompt extra vigilance if ctDNA-positive. Indeed, patients in the BESPOKE study reported reduced anxiety around cancer recurrence and increased confidence they were receiving appropriate treatment.[41] However, use of these tests may add unnecessary anxiety for some patients. In addition, these tests add cost, though this may be balanced by avoiding chemotherapy. Patient reported outcomes and health economic analyses should be included as an important component of future ctDNA studies. Although not the primary focus of this review, MRD ctDNA assays are also commonly used for surveillance following surgical resection and may detect recurrence prior to becoming evident on imaging in definitively treated patients. Whether this information should prompt earlier therapy initiation is not yet known and sensitivity is known to be less for certain sites of recurrence such as lung and peritoneal metastases. Therefore, we cannot routinely recommend ctDNA MRD assays for prognostication or surveillance alone; use of the assay for this purpose should be the result of shared decision-making between the patient and the provider.

The true value of these assays will only be realized if they effectively improve outcomes by enabling intensification or de-escalation of therapy. Interventional trials DYNAMIC II[31] and PEGASUS[26] in addition to observational studies such as GALAXY[24,28] and BESPOKE[25] have demonstrated that detectable ctDNA may predict benefit from adjuvant chemotherapy. The DYNAMIC II trial identified the minority of patients with stage II CC who may benefit from chemotherapy while avoiding overtreatment, although notably DFS was not improved.[31] Therefore, for stage II, standard-risk CC, use of a tissue-informed ctDNA MRD assay is reasonable to guide therapy; patients who are positive should receive adjuvant chemotherapy while the remainder may be observed. This approach may be particularly helpful for older, more frail patients for whom the risks of chemotherapy are greater. However, given the potential differences in prescribing and practice patterns, the wider applicability of this study will need to be considered within the practice culture of each country. For high risk, particularly T4N0 stage II tumors, consideration of adjuvant therapy should not be based on ctDNA results alone but a shared decision-making process, taking into account competing risks and patient preference.

Clinicians must consider MRD assay performance together with the baseline risk of recurrence (ie, clinicopathologic characteristics) to apply PPV or negative predictive value (NPV) in a clinical context. High specificity is critical in lower-risk populations, because false-positives may be as frequent as true-positives in low prevalence situations. In the COBRA trial,[35] which screened 635 patients to identify 16 who were ctDNA-positive, the spontaneous clearance of ctDNA in the 3 of 7 patients undergoing observation raised several concerns. One is potential for false-positives in a low-risk population, which may have been mitigated by a more specific, improved assay. In this situation, the PPV was lower than ideal and could have subjected a significant fraction of patients who were ctDNA-positive to overtreatment, noting, however, that we currently overtreat many patients using clinical features only. Other considerations for COBRA include single time point clearance, stochastic sampling error, and the phenomenon of self-clearance. High sensitivity is important in higher-prevalence populations, as a negative test should safely select patients for de-escalation with a greater NPV. Further improvements in assay design and bioinformatic processing will hopefully drive improvements in assay accuracy.

Serial testing increases sensitivity for recurrence, although newly positive ctDNA ≥6 months from definitive treatment constitutes a different clinical scenario and is more appropriately considered surveillance, which is outside this review's specific focus. Indeed, MRD testing is being widely used clinically for surveillance to predict recurrence well before disease is detectable on imaging. The actionability of ctDNA MRD during surveillance is an important question that requires further study in parallel with investigation of ctDNA at landmark postoperative timepoints.

The PEGASUS trial demonstrated the feasibility of intensifying and adapting therapy for patients with stage III CC who are initially and persistently ctDNA-positive; however, applicability is limited by small numbers, lack of randomization, and relatively short follow-up.[26] The larger GALAXY and BESPOKE trials suggested benefit from chemotherapy in patients who were ctDNA-positive, whereas no benefit was observed in those who were ctDNA-negative; however, the studies were nonrandomized with still-limited follow-up, and should not be considered practice-changing.[24,25,28] Data from other studies in this population are not yet available. Therefore, given the lack of large, randomized trials with adequate follow-up in patients with high-risk stage II and stage III CC, we cannot currently recommend routine use of ctDNA to guide therapy in this population outside of a clinical trial.

Thus, ctDNA MRD testing is not ready for prime time in CC. Certainly, these data are promising, and results of ongoing clinical trials are urgently needed. As these trials and the technical specifications of these assays mature, sophisticated strategies for surveillance and adaptive therapies based on ctDNA will likely play a role in adjuvant therapy selection. Important questions remain. Both sensitivity and specificity remain concerns, because a nontrivial fraction of patients who are initially ctDNA-negative experience recurrence, and spontaneous clearance in patients who are ctDNA-positive occurs with substantial frequency. Assay type (tumor-informed vs tumor-agnostic) may or may not result in relevant clinical differences with the most modern assays. Duration and frequency of ctDNA-based surveillance and whether patients who are initially ctDNA-negative and convert to ctDNA-positive benefit from intensification will be important to address. Another question is choice of therapy in patients who are ctDNA-positive, such as immune checkpoint inhibitors as adjuvant therapy in microsatellite instability–high CC. Resulting delays due to assay processing may also have a negative effect and require ongoing process improvements. We expect answers in the near future, and that ctDNA MRD testing will soon take a more central role in resectable CC.

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References

1. Amin MB, Edge SB, Greene FL, et al, eds. AJCC Cancer Staging Manual, 8th edition. Springer; 2017.
2. Quah HM, Chou JF, Gonen M, et al. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. Dis Colon Rectum 2008; 51:503–507.
3. Thirunavukarasu P, Sukumar S, Sathaiah M, et al. C-stage in colon cancer: implications of carcinoembryonic antigen biomarker in staging, prognosis, and management. J Natl Cancer Inst 2011;103:689–697.
4. Kim CG, Ahn JB, Jung M, et al. Preoperative serum carcinoembryonic antigen level as a prognostic factor for recurrence and survival after curative resection followed by adjuvant chemotherapy in stage III colon cancer. Ann Surg Oncol 2017;24:227–235.
5. Niedzwiecki D, Bertagnolli MM, Warren RS, et al. Documenting the natural history of patients with resected stage II adenocarcinoma of the colon after random assignment to adjuvant treatment with edrecolomab or observation: results from CALGB 9581. J Clin Oncol 2011;29:3146–3152.
6. André T, Boni C, Navarro M, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol 2009;27:3109–3116.
7. André T, Vernerey D, Mineur L, et al. Three versus 6 months of oxaliplatin-based adjuvant chemotherapy for patients with stage III colon cancer: disease-free survival results from a randomized, open-label, international duration evaluation of adjuvant (IDEA) France, phase III trial. J Clin Oncol 2018;36:1469–1477.
8. Benson AB III, Venook AP, Adam M, et al. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer. Version 5.2024. To view the most recent version, visit https://www.nccn.org
9. Baxter NN, Kennedy EB, Bergsland E, et al. Adjuvant therapy for stage II colon cancer: ASCO guideline update. J Clin Oncol 2022;40:892–910.
10. Siegel RL, Wagle NS, Cercek A, et al. Colorectal cancer statistics, 2023. CA Cancer J Clin 2023;73:233–254.
11. Li W. Measurable residual disease testing in acute leukemia: technology and clinical significance. In: Li W, ed. Leukemia. Exon Publications; 2022: 79–100.
12. Medina-Herrera A, Sarasquete ME, Jiménez C, et al. Minimal residual disease in multiple myeloma: past, present, and future. Cancers (Basel) 2023;15:3687.
13. Primrose JN, Perera R, Gray A, et al. Effect of 3 to 5 years of scheduled CEA and CT follow-up to detect recurrence of colorectal cancer: the FACS randomized clinical trial. JAMA 2014;311:263–270.
14. Wille-Jørgensen P, Syk I, Smedh K, et al. Effect of more vs less frequent follow-up testing on overall and colorectal cancer-specific mortality in patients with stage II or III colorectal cancer: the COLOFOL randomized clinical trial. JAMA 2018;319:2095–2103.
15. Kasi PM, Fehringer G, Taniguchi H, et al. Impact of circulating tumor DNA-based detection of molecular residual disease on the conduct and design of clinical trials for solid tumors. JCO Precis Oncol 2022;6:e2100181.
16. Fiala C, Diamandis EP. Cell-free DNA analysis in cancer. N Engl J Med 2019;380:501–502.
17. Schobers G, Koeck R, Pellaers D, et al. Liquid biopsy: state of reproductive medicine and beyond. Hum Reprod 2021;36:2824–2839.
18. Chaudhuri AA, Chabon JJ, Lovejoy AF, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov 2017;7:1394–1403.
19. Murray DH, Symonds EL, Young GP, et al. Relationship between post-surgery detection of methylated circulating tumor DNA with risk of residual disease and recurrence-free survival. J Cancer Res Clin Oncol 2018;144: 1741–1750.
20. Parikh AR, Van Seventer EE, Siravegna G, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. Clin Cancer Res 2021;27:5586–5594.
21. Taylor MS, Wu C, Fridy PC, et al. Ultrasensitive detection of circulating LINE-1 ORF1p as a specific multi-cancer biomarker. Cancer Discov 2023; 13:2532–2547.
22. Henriksen TV, Tarazona N, Frydendahl A, et al. 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;28:507–517.
23. Tie J, Cohen JD, Wang Y, et al. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. JAMA Oncol 2019;5:1710–1717.
24. Kotani D, Oki E, Nakamura Y, et al. Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer. Nat Med 2023;29:127–134.
25. Kasi PM, Aushev VN, Ensor J, et al. Circulating tumor DNA (ctDNA) for informing adjuvant chemotherapy (ACT) in stage II/III colorectal cancer (CRC): interim analysis of BESPOKE CRC study. J Clin Oncol 2024;42(Suppl):Abstract 9.
26. Lonardi S, Pietrantonio F, Tarazona Llavero N, et al. LBA28 The PEGASUS trial: post-surgical liquid biopsy-guided treatment of stage III and high-risk stage II colon cancer patients. Ann Oncol 2023;34:S1268–1269.
27. Tie J, Wang Y, Tomasetti C, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 2016;8:346ra92.
28. Yukami H, Nakamura Y, Mishima S, et al. Circulating tumor DNA (ctDNA) dynamics in patients with colorectal cancer (CRC) with molecular residual disease: updated analysis from GALAXY study in the CIRCULATE-Japan. J Clin Oncol 2024;42(Suppl):Abstract 6.
29. Kasi PM, Sawyer S, Guilford J, et al. BESPOKE study protocol: a multicentre, prospective observational study to evaluate the impact of circulating tumour DNA guided therapy on patients with colorectal cancer. BMJ Open 2021;11:e047831.
30. Taniguchi H, Nakamura Y, Kotani D, et al. CIRCULATE-Japan: circulating tumor DNA-guided adaptive platform trials to refine adjuvant therapy for colorectal cancer. Cancer Sci 2021;112:2915–2920.
31. Tie J, Cohen JD, Lahouel K, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. N Engl J Med 2022;386: 2261–2272.
32. Sargent D, Grothey A, Gray R. Time to initiation of adjuvant chemotherapy and survival in colorectal cancer. JAMA 2011;306:1199; author reply 1200.
33. Schraa SJ, van Rooijen KL, van der Kruijssen DEW, et al. Circulating tumor DNA guided adjuvant chemotherapy in stage II colon cancer (MEDOCC-CrEATE): study protocol for a trial within a cohort study. BMC Cancer 2020;20:790.
34. Morris VK, Yothers G, Kopetz S, et al. NRG-GI005 (COBRA): phase II/III study of circulating tumor DNA as a predictive biomarker in adjuvant chemotherapy in patients with stage II colon cancer. J Clin Oncol 2020; 38(Suppl):Abstract TPS261.
35. Morris VK, Yothers G, Kopetz S, et al. Phase II results of circulating tumor DNA as a predictive biomarker in adjuvant chemotherapy in patients with stage II colon cancer: NRG-GI005 (COBRA) phase II/III study. J Clin Oncol 2024;42(Suppl):Abstract 5.
36. Australian New Zealand Clinical Trials Registry. Identifier ACTRN12617001566325. Circulating tumour DNA analysis informing adjuvant chemotherapy in stage III colon cancer: a multicentre phase II/III randomised controlled study (DYNAMIC-III). Accessed December 15, 2023. Available at: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12617001566325
37. Folprecht G, Reinacher-Schick A, Weitz J, et al. The CIRCULATE trial: circulating tumor DNA based decision for adjuvant treatment in colon cancer stage II evaluation (AIO-KRK-0217). Clin Colorectal Cancer 2022;21: 170–174.
38. Dasari A, Lin Y, Kopetz S, et al. NRG-GI008: colon adjuvant chemotherapy based on evaluation of residual disease (CIRCULATE-US). J Clin Oncol 2022;40(Suppl):Abstract TPS212.
39. Slater S, Bryant A, Chen HC, et al. ctDNA guided adjuvant chemotherapy versus standard of care adjuvant chemotherapy after curative surgery in patients with high risk stage II or stage III colorectal cancer: a multicentre, prospective, randomised control trial (TRACC Part C). BMC Cancer 2023;23:257.
40. ClinicalTrials.gov. Identifier NCT03803553. Identification and treatment of micrometastatic disease in stage III colon cancer. Accessed December 11, 2023. Available at: https://clinicaltrials.gov/study/NCT03803553
41. Kasi PM, Rivero S, Aushev VN, et al. Patient-reported outcomes from the BESPOKE CRC study. J Clin Oncol 2024;42(Suppl):Abstract 54.