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The Promise of Molecular Residual Disease (MRD) Testing for Patients With Colorectal Cancer

Discussants

Tanios S. Bekaii-Saab, MD
David F. and Margaret T. Grohne Professor of Novel Therapeutics for Cancer Research I
Professor, Mayo Clinic College of Medicine and Science
Chair, Hematology and Medical Oncology
Mayo Clinic Cancer Center
Phoenix, Arizona

Robert E. Schoen, MD, MPH
UPMC Endowed Chair in Gastroenterology, Hepatology and Nutrition
Professor of Medicine & Epidemiology
Department of Medicine
University of Pittsburgh
Pittsburgh, Pennsylvania

Abstract: Molecular residual disease (MRD) assays using circulating tumor DNA (ctDNA) have the potential to detect colorectal cancer recurrence earlier than current standard-of-care surveillance techniques, such as carcinoembryonic antigen measurement and follow-up with computed tomography. Residual cancer cells that increase the risk of disease recurrence and the ctDNA released from these cells into the bloodstream are not detectable through standard imaging but can be detected with MRD tests. Two types of MRD assays developed for use in oncology are tumor-informed, which detect mutations specific to a patient's tumor, and tumor-naive, which detect known ctDNA sequences that are not specific to a patient's tumor genomics. The tumor-informed MRD test has high sensitivity but requires tumor sequencing that takes longer to process, whereas the tumor-naive MRD test has a shorter turnaround time but a lower sensitivity. In prospective studies of these tests, patients with ctDNA-positive results were more likely to experience disease recurrence after surgery or definitive therapy than patients with ctDNA-negative results. Multiple platforms are already in clinical use or being developed as part of research studies. One such platform using the Oncodetect MRD test has confirmed the value of ctDNA testing and its association with recurrence-free survival at multiple timepoints (postsurgical, post-definitive therapy, and surveillance) in patients with colorectal cancer.

The Transformative Potential of Circulating Tumor DNA as a Biomarker for Detecting Molecular Residual Disease in Colorectal Cancer

Tanios S. Bekaii-Saab, MD

Assessing minimal residual disease through molecular assays, which is also known as molecular residual disease (MRD) assessment, is an evolving field in colorectal cancer (CRC). Although there are many clinical applications for this technology, more randomized clinical trials are needed to establish its predictive as well as prognostic value. Data on the horizon indicate that MRD assessment has utility in assisting clinical decision-making in patients who may be MRD-positive or MRD-negative.

Current Standard of Care for Monitoring Recurrence

For patients with stage III and high-risk stage II CRC, surgery followed by adjuvant chemotherapy based on clinical and pathologic risk stratification is recommended. At the same time, at least 50% of patients with stage III and stage II CRC can be cured by surgery alone and will not need chemotherapy. Still, despite completing appropriate treatment, more than 30% of patients with stage III CRC and approximately 15% of patients with stage II CRC experience disease recurrence.[1-3] Patients are treated with adjuvant chemotherapy because it enhances the likelihood of a cure. The decision to pursue chemotherapy is predicated on a discussion of prognosis and survival with patients, some of whom may refuse this treatment. Current monitoring tools have limited clinical utility to inform this discussion, which is crucial for educated decision-making.

The current standard of care for surveillance of stage II and III CRC includes clinical follow-up with measurement of carcinoembryonic antigen (CEA) levels every 3 to 6 months for 2 years, then every 6 months for the next 3 years.[4] Computed tomography (CT) imaging should be performed every 6 to 12 months from the time of surgery for a total of 5 years. Colonoscopy should be performed 1 year after surgery, then repeated either every year (for advanced adenoma) or at 3 years and 5 years (if there is no advanced adenoma).

Conventionally, for patients who had resection of either their CRC at an early stage or for select patients with oligometastatic disease that was resected, the monitoring tools that continue to be primarily used are standard imaging techniques, most commonly CT scan of the chest, abdomen, and pelvis. Occasionally, magnetic resonance imaging (MRI) of the liver and positron emission tomography CT scans are used as needed to complement CT and MRI in the presence of suspicious lesions.

Liver function tests may be important to detect early signs of recurrence in the liver. As mentioned, CEA level is also measured, although there is variation in the cut-off level that should be applied, and this test should not be used alone for assessment of recurrence risk. A meta-analysis found that at a threshold of 10 ng/mL, sensitivity for CEA level was 68% (95% CI, 53%-79%) and specificity was 97% (95% CI, 90%-99%).[5] When the threshold was decreased to 5 ng/mL, sensitivity was increased to 71% (95% CI, 64%-76%), whereas specificity was decreased to 88% (95% CI, 84%-92%). Further decreasing the threshold to 2.5 ng/mL improved sensitivity to 82% (95% CI, 78%-86%) but decreased specificity to 80% (95% CI, 59%-92%). Some patients will present to the clinic following their surgery, and their CEA level will be less than 10 ng/mL. A concern, of course, is for recurrence, so these patients go through a battery of testing that increases the anxiety of both the patient and their family, which in turn can make the provider anxious as well. Some patients will continue to maintain those same CEA levels for months or even years with no confirmed clinical recurrence. On the other hand, some patients will

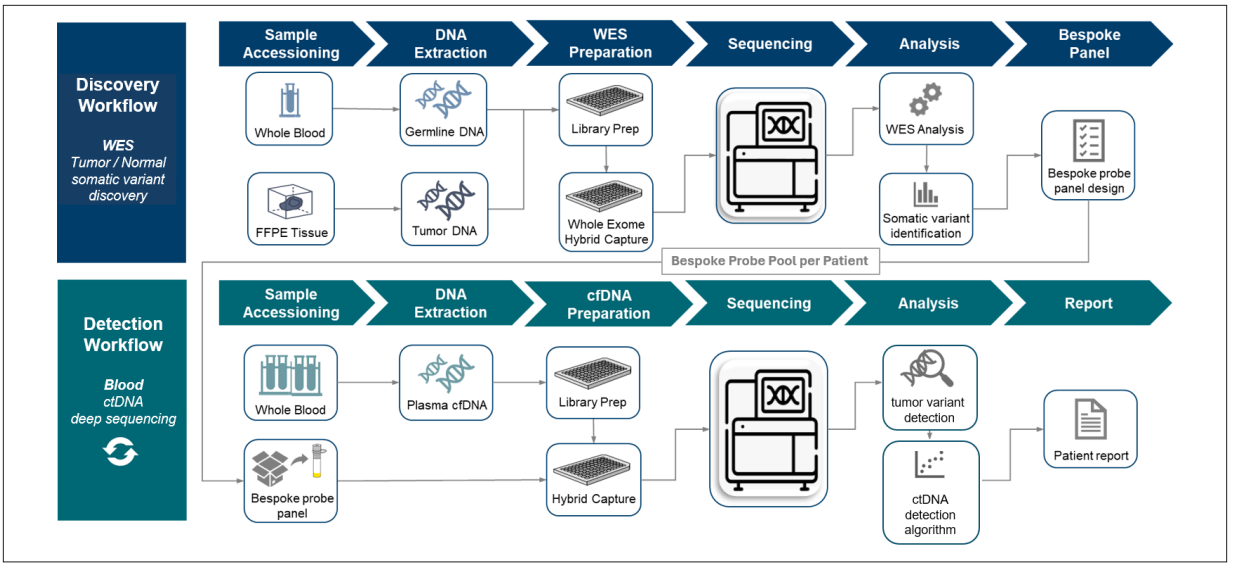

Figure 1. Workflow of the Oncodetect MRD tumor-informed test for recurrence risk in patients with stage III colorectal cancer. cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; FFPE, formalin-fixed paraffin-embedded; WES, whole-exome sequencing. Reprinted from Exact Sciences. Oncodetect MRD test. 2025. https://www.exactsciences.com/cancer-testing/oncodetect-mrd-providers/white-paper.[11]

not have elevated CEA levels even in the presence of cancer, further limiting the utility of the CEA test.

Circulating Tumor DNA as a Biomarker of Recurrence

In light of the lack of robust methods for assessing risk for recurrence, the use of MRD testing has been evaluated for this purpose. After surgery with curative intent, residual cancer cells can increase the risk of disease recurrence. These cells are not detectable through standard imaging. Instead, the detection of DNA released from these cancer cells into the bloodstream can be performed using tests for MRD. This circulating tumor DNA (ctDNA) is a subset of the cell-free DNA (cfDNA) found throughout the circulating blood and other bodily fluids.[6] The half-life of ctDNA in the circulation is extremely short—just 2 hours—making ctDNA a more dynamic blood-based biomarker compared with CEA.[7,8]

In CRC, ctDNA was first demonstrated as a method for detection of residual cancer in a study of patients with resected CRC.[7] Although the ctDNA levels varied widely prior to surgery, they had dropped precipitously in 17 patients with complete resections by the day of discharge (2 to 10 days after surgery), achieving a median decrease in ctDNA of 99.0% (range between 10th and 90th percentiles, 58.9%-99.8%). This decrease was observed as early as 24 hours after surgery. In contrast, 5 patients who had an incomplete resection showed either only a slight decrease at 24 hours in 2 patients (55% or 56%) or actually an increase in 3 patients (141%, 329%, and 794%). This increase was attributed to injury to the remnant tumor tissue during surgery. Among 16 patients with positive postoperative ctDNA status, 15 (94%) had a disease recurrence, whereas no recurrences were observed in the 2 patients who were ctDNA-negative after surgery.

More recently, the utility of ctDNA detection following surgery was evaluated in patients with CRC.[9] This meta-analysis using a random effects model found an association between ctDNA detection at the first timepoint after surgery and worse progression-free survival (hazard ratio [HR], 6.92; 95% CI, 4.49-10.64; $P<.00001$).

Tumor-Informed and Tumor-Naive MRD Assays

Two types of MRD assays have been developed and are used in oncology—tumor-informed and tumor-naive (also called tumor-agnostic).[10] Tumor-informed tests require tumor tissue, generating a patient-specific set of tumor variants (the tumor mutational profile). From this, a personalized test can be developed that targets that patient's tumor-specific variants from ctDNA in the blood. In contrast, tumor-naive assays apply a previously validated panel of cancer-driver genes and/or epigenomic signatures characteristic of a particular tumor type to detect ctDNA.

The Oncodetect MRD test is a tumor-informed test that, unlike the other available MRD assays, consists of 2 workflows: discovery and detection (Figure 1).[11] During the discovery process, tumor-specific variants are determined by whole-exome sequencing (WES) of DNA

Table 1. Comparison of Tumor-Informed and Tumor-Naive MRD Tests in CRC

Factor	Tumor-informed MRD test	Tumor-naive MRD test
Description	Detects mutations specific to an individual patient's tumor	Detects known circulating tumor DNA sequences that are not specific to the patient's tumor genomics
Genetic coverage	Customized panel of genes present in the patient's tumor	Panel of tumor mutations common to CRC tumors
Tissue sequencing	Required	Not needed
Turnaround time	Longer (3 to 4 weeks)	Shorter (1 to 2 weeks)

CRC, colorectal cancer; MRD, molecular residual disease.
Adapted from Abidoye O et al. *Cells*. 2025;14(3):161.[13]

extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue acquired through surgery or biopsy. WES identifies tumor-specific variants by subtracting those in a matched normal blood sample from those in the tumor sample. Variants in regions previously linked to clonal hematopoiesis of indeterminate potential (CHIP) are removed, and additional algorithms are applied to optimize detection sensitivity and specificity. Between 50 and 200 tumor-specific variants are identified through this discovery process for each patient. Subsequently, hybridization probes are designed to selectively bind to the DNA sequences containing these tumor-specific variants.

In the detection workflow, cfDNA is isolated from a patient's plasma sample and allowed to hybridize to these probes, capturing the DNA regions containing these tumor variants. The enriched sample is then sequenced. After algorithm processing, a ctDNA score is applied to each sample to determine if that sample is considered to be ctDNA-positive or ctDNA-negative. In addition, a quantitation of the amount of ctDNA, expressed as mean tumor molecules per milliliter (MTM/mL) of plasma, is reported.

Clinical Benefits and Drawbacks of MRD Tests

Tumor-naive MRD tests benefit from the lack of tumor tissue requirement, and also the ability to directly test a patient's plasma sample without the time required to build personalized assays to detect that patient's specific ctDNA.[12] However, these assays are limited by lower sensitivity. In contrast, tumor-informed MRD tests have improved sensitivity but require sequencing of the tumor tissue followed by design and validation of personalized polymerase chain reaction assays for each patient. This process takes weeks, which is a consideration when using these tests to guide a decision regarding adjuvant therapy. Table 1 compares some of the other benefits and drawbacks of each type of MRD test in CRC.[13]

The tumor-naive Guardant Reveal test was evaluated in a study of 103 patients with stage I to IV disease.[14] Of 70 patients with a single "landmark" plasma specimen from 1 month after completion of definitive therapy, 17 (24%) had detectable ctDNA after completion of definitive therapy and 15 of 17 (88%) of these patients experienced recurrence (the other 2 patients had clinical follow-up of <1 year). Of the 49 patients who had a ctDNA-negative landmark sample, 12 (24%) experienced recurrence (median time to recurrence was not reached). ctDNA-positive status was associated with recurrence prediction regardless of stage, neoadjuvant, or adjuvant therapy. The tissue-free test was further evaluated in patients with early CRC in the UK TRACC study part B.[15] Blood samples were taken from 143 patients after surgery. The 2-year recurrence-free survival (RFS) was 50.4% in patients who were ctDNA-positive and 91.1% in patients who were ctDNA-negative (HR, 6.5; 95% CI, 3.0-14.5; P<.0001). The median time from ctDNA detection to recurrence in the primary analysis population was 7.3 months (interquartile range, 3.3-12.5).

The tumor-informed test Signatera was evaluated in a prospective study of patients with stage I to III CRC (n=94 with samples from blood collection after surgery and n=75 with surveillance blood collection until month 36).[16] Patients who were ctDNA-positive 30 days following surgery were significantly more likely to experience disease relapse compared with ctDNA-negative patients (HR, 7.2; 95% CI, 2.7-19.0; P<.001). Additionally, ctDNA-positive status was also associated with a significant risk for relapse compared with ctDNA-negative status (HR, 17.5; 95% CI, 5.4-56.5; P<.001). In multivariate analysis, ctDNA-positive status remained independently associated with relapse. ctDNA surveillance suggested disease recurrence up to 16.5 months earlier than radiologic imaging. Serial ctDNA analyses revealed disease recurrence an average of 8.7 months (range, 0.8-16.5) ahead of standard-of-care radiologic imaging.

Utility of Incorporating MRD Testing

In an ideal world, physicians would have a tool that tells

them whether or not the patient in front of them with CRC requires adjuvant chemotherapy after surgery. For patients with stage III disease who have MRD-negative status, the available MRD tests may not yet be there but may be close for patients with stage II disease. In contrast, the decision is a bit more clear-cut in patients who are MRD-positive, as it is becoming clearer that these patients are likely to experience disease recurrence. However, more data are needed before this can be done universally with patients. The decision regarding chemotherapy requires a discussion, and the question, of course, that comes up during the discussion is what to do if the MRD test is negative, or what to do if the MRD test is positive.

An example of a scenario is a recent meeting I had with a patient who has stage IIIA CRC and is very reluctant to receive chemotherapy. After a discussion about the benefits and drawbacks of undergoing treatment, the decision was made to submit a sample for MRD testing. When the patient returned a few weeks later, she was MRD-negative. We then talked about what that means—that it moves the mark a bit towards a cure, but not with certainty. The patient continued to insist on not undergoing chemotherapy. At this point, it is an informed choice that makes sense. Therefore, the test is not yet being used as a decision tool but rather as one component of the physician and patient's consideration. In patients who have MRD-positive disease, I strongly advise working toward a pathway to consider adjuvant chemotherapy, as the data show they are more likely to benefit from chemotherapy than not.

Another important consideration is that MRD testing requires an extended surveillance period of up to 2 years. It is not enough to check the ctDNA level at a single point; the level must be assessed as a continuum. This is an important point to explain to the patient, as MRD testing is a commitment. One can argue that this will create even more anxieties for the patient, and that is actually disclosed to the patient from the beginning. In addition, we tighten up the follow-up schedule with the scanning schedule. Many times, we have found patients (whose MRD results turned positive before radiographic confirmation) with early metastasis to the liver or to the lungs that was addressed with local regional therapies, and this certainly has helped.

Disclosure

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Identification of Colorectal Cancer Recurrence Risk Using Circulating Tumor DNA

Robert E. Schoen, MD, MPH

The major issue for patients with stage III CRC—and to a lesser extent, stage II CRC—is the substantial rate of cancer recurrence. The standard-of-care monitoring tools, including CEA testing, colonoscopy, and CT scanning, are performed for early identification of a recurrence, which can trigger surgical or medication interventions that will improve outcomes. Asymptomatic rather than symptomatic recurrences have been shown to have better prognosis and survival rates. The currently available MRD assays using ctDNA in the setting of CRC are tumor-informed (Signatera and Oncodetect) as opposed to tumor-naive (Guardant Reveal). MRD studies of ctDNA reveal that in multivariable analyses including CEA, age, stage, and histology, ctDNA is overwhelmingly the most predictive factor for recurrence. Findings from the α-CORRECT study evaluating the new Oncodetect MRD test confirm the prognostic value of ctDNA in CRC.

α-CORRECT Study

The α-CORRECT (COloRectal cancer study to predict REcurrence using Circulating Tumor DNA) study was designed to evaluate the prognostic ability of an MRD test. The samples were used to evaluate Oncodetect, a patient-specific and tumor-informed assay, for determining recurrence risk in patients with stage III CRC.[1] A total of 137 adult patients who had a recent diagnosis of stage IIIA-C disease were enrolled between 2016 and 2020. A tumor tissue specimen, from either biopsy or surgical resection, was required from all patients; 3 patients did not meet this criterion and were not included in the study. Tumor DNA was obtained from FFPE tumor blocks from CRC tissue specimens, and germline DNA from the buffy coat of a blood draw.

Blood samples were obtained after surgery and prior to administration of adjuvant therapy, and then quarterly for the first 3 years and semiannually in the last 2 years for a 5-year follow-up. As depicted in Figure 2, these blood samples were grouped into 3 referent windows for analysis: postsurgical (PS; the single sample taken 3 to 12 weeks after surgery and before beginning adjuvant therapy); post-definitive therapy (PDT; the first sample collected after and within 6 months of completing adjuvant therapy, or at least 21 days after surgery for patients not treated with adjuvant therapy); and surveillance (the PDT sample and all subsequent samples). Plasma was extracted from each blood sample upon receipt and a separate aliquot was sent for a CEA level. Other data collected included tumor information, lymph node involvement, treatments received, and mismatch repair (MMR) status as determined by immunohistochemistry.

Each patient-specific tumor-informed MRD assay was developed with 2 phases: variant discovery and ctDNA detection. Variant discovery was accomplished with WES of paired tumor-normal samples from each patient, aiming to identify between 50 and 200 somatic variants for each patient's tumor (a mean of 172 [range, 50-200] variants per patient were identified). These patient-specific variants were filtered against CHIP variants, as reported in the literature. ctDNA detection was performed with hybrid capture methodology using DNA probes targeting the genomic regions containing each identified patient-specific variant. This allowed for target enrichment prior to DNA next-generation sequencing.

A bioinformatic algorithm was employed using the variant information obtained from sequencing, providing a ctDNA score to determine whether ctDNA was present (ctDNA-positive) or absent (ctDNA-negative) in the plasma samples. Samples were determined to be ctDNA-positive if either the ctDNA score was above an identified threshold, or if 2 or more individual variants showed a sufficiently high probability of cancer. Otherwise, the sample was classified as ctDNA-negative. Subsequently, adjusted criteria to improve specificity in the surveillance phase were applied, which included 2 changes: an increase in the ctDNA score threshold, and removal of the 2 high probability individual variants criterion.

Samples from a total of 124 patients were considered evaluable. Among these patients, 47% were female, 100% were not Hispanic or Latino (93.5% White, 1.6% Black, and 4.8% other), and most were aged 50 years or older (91%). The mean age at diagnosis was 64.5 years (standard deviation, 10.8). The vast majority of tumor sites were colon (95.2%), and the rest were rectum (4.8%). Tumor histology was adenocarcinoma (88.7%), mucinous adenocarcinoma (9.7%), medullary carcinoma or Signet-ring cell (0.8% each). All tumors were stage III (12.9%, 61.3%, and 25.8% were stage IIIA, IIIB, and IIIC, respectively). The histopathologic grade for tumors was low (21.0%), moderately differentiated (54.0%), or high (21.0%), and 79.0% were determined to be MMR-proficient (15.3% were

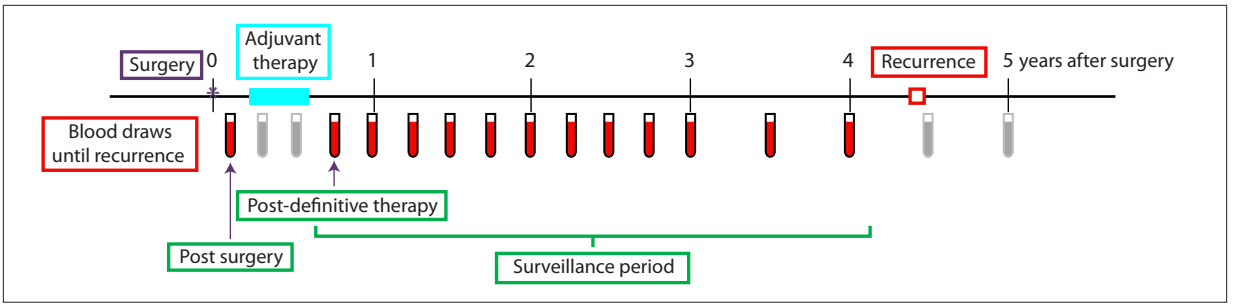

Figure 2. Schematic showing sampling timepoints and reference windows of the α-CORRECT study. Adapted from Diergaarde B et al. *J Surg Oncol.* 2025. doi:10.1002/jso.27989.[1]

MMR-deficient and 5.6% were MMR status unknown). Most patients (96.0%) received adjuvant therapy following surgery, and the majority of these (92.4%) were treated with 5-fluorouracil plus oxaliplatin.

The median follow-up time after surgery was 4.8 years (range, 0.2-5.8); during this surveillance period there were 27 recurrences among the 124 patients. Over this follow-up, plasma ctDNA status was found to be strongly associated with the risk of recurrence. A Cox proportional hazards regression model showed that during the surveillance period, the HR for recurrence for a patient with ctDNA-positive vs ctDNA-negative status was 49.6 (95% CI, 16.6-148.3; *P*<.0001). Figure 3A shows the association between ctDNA positivity and RFS in patients with 1 or more ctDNA-positive results vs patients with all ctDNA-negative results during surveillance.

Table 2 lists the HRs for recurrence, as well as the sensitivity and specificity for each time period. Recurrence HR for plasma ctDNA status at the PS timepoint was 9.6 (95% CI, 3.2-29.5) and for the PDT timepoint was 16.7 (95% CI, 6.9-40.3) (Figure 3B and Figure 3C). This allowed a calculation for the estimated 3-year RFS at the PS timepoint of 54.5% vs 96.1% for patients with ctDNA-positive vs ctDNA-negative status; at the PDT timepoint, the estimated 3-year RFS rates were 18.2% and 90.0%, respectively. The sensitivity for plasma ctDNA status was higher during the surveillance period (90.9%) than both the PS and PDT timepoints (77.8% and 47.6%, respectively), suggesting the importance of sampling during the surveillance period. Specificity during the surveillance period was 94.3%, indicating 82 of 87 patients who did not experience recurrence were ctDNA-negative.

Several clinicopathologic variables were assessed for their association with RFS. Univariate Cox model results found the following variables to be significantly associated with RFS: ctDNA status at all 3 time periods (surveillance, PS, and PDT timepoints); CEA status at the surveillance and PDT timepoints; pathologic tumor (pT) category; and Oncotype DX Colon Recurrence Score. However, in a multivariable analysis, during the surveillance period, ctDNA status was the only variable that remained significant for its association with RFS (HR, 39.9; 95% CI, 12.0-132.7; *P*<.0001). This was also true at the PS timepoint (HR, 8.7; 95% CI, 2.7-27.8; *P*=.0003). At the PDT timepoint, both ctDNA status (HR, 24.7; 95% CI, 8.6-70.5; *P*<.0001) and pT category (HR, 3.2; 95% CI, 1.2-8.4; *P*=.0207) remained significantly associated with RFS. In general, clinicopathologic variables did not add value to the association between ctDNA and RFS, and even when significant, were less informative than ctDNA status.

An exploratory analysis investigated the association between ctDNA status and location of recurrence. Most recurrence sites had 4 or fewer events, but the liver and lung had more occurrences (7 and 6, respectively, with 16 of 27 recurrences affecting both the liver and lung). The association between liver metastases and ctDNA-positive status has been reported in other studies as well.[2-5]

The α-CORRECT trial determined that ctDNA status was strongly prognostic for recurrence in patients with stage III CRC. Because the design of this study was limited to observation alone, no findings within this study altered treatment decisions. Outcomes from treatment trials based on ctDNA status are eagerly awaited.

Treatment Decisions Based on ctDNA Status

The DYNAMIC trial was designed to determine if a ctDNA-guided approach could reduce the need for adjuvant chemotherapy without compromising recurrence risk in patients with stage II CRC.[6] Patients (N=455) were randomized in a 2:1 ratio to have treatment decisions guided by either ctDNA results or standard clinicopathologic features. For patients randomized to the ctDNA-guided management strategy, a ctDNA-positive result at 4 weeks or 7 weeks after surgery triggered initiation of oxaliplatin-based or fluoropyrimidine chemotherapy. Patients in this arm who were ctDNA-negative did not receive chemotherapy. The primary endpoint was the 2-year rate of RFS. After a median follow-up of 37 months, the 2-year RFS with ctDNA-guided management was determined

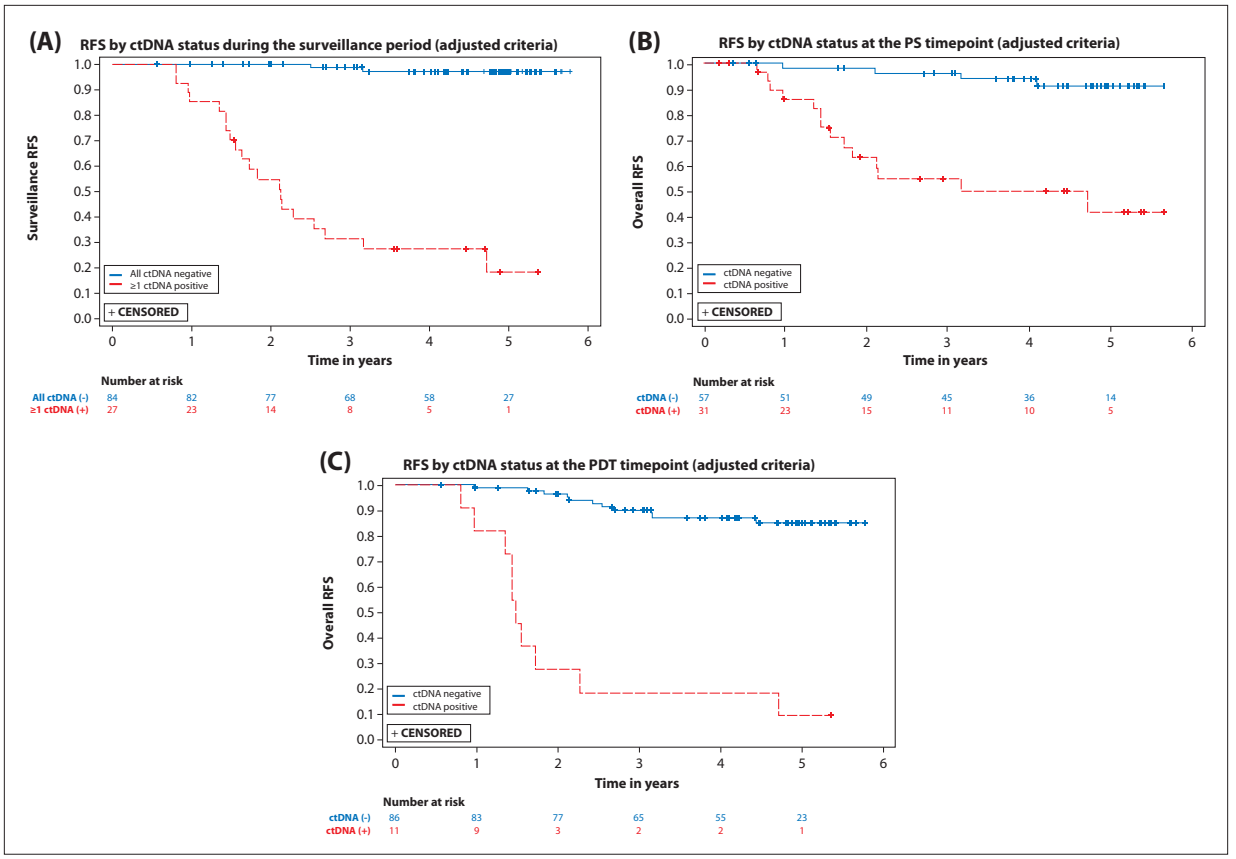

Figure 3. Association between 3-year RFS and ctDNA status in 124 patients in the α-CORRECT study. ctDNA, circulating tumor DNA; PDT, post-definitive therapy; PS, postsurgical; RFS, recurrence-free survival. Adapted from Diergaarde B et al. *J Surg Oncol.* 2025. doi:10.1002/jso.27989.[1]

to be noninferior to standard management (93.5% vs 92.4%; absolute difference, 1.1%; 95% CI, -4.1 to 6.2). The 3-year rate of RFS was 86.4% in patients who were ctDNA-positive and received adjuvant chemotherapy and was 92.5% in patients who were ctDNA-negative and did not receive adjuvant chemotherapy. Hence, ctDNA-guided management was noninferior to standard management in 2-year RFS but resulted in significantly less use of adjuvant therapy (28% in the standard management group vs 15% in the ctDNA-guided group (relative risk, 1.82; 95% CI, 1.25-2.65).

Several other clinical trials to evaluate the use of ctDNA status in guiding CRC treatment are ongoing. The results of these studies have the potential to change or inform current practice. Some of these studies come from CIRCULATE-Japan, a new type of adaptive platform clinical trial designed to further determine the association between clinical benefits and ctDNA analysis, with a goal of refining adjuvant therapy decisions. This design includes 3 studies—GALAXY, VEGA, and ALTAIR.[7]

An updated analysis of the GALAXY study, with an expanded cohort of patients with stage II/III resectable and metastatic stage IV CRC, has been reported.[8] At a median follow-up of 23 months (range, 2-49), ctDNA positivity was associated with significantly worse disease-free survival (HR, 11.99; 95% CI, 10.02-14.35; *P*<.0001) and overall survival (HR, 9.68; 95% CI, 6.33-14.82; *P*<.0001). Further, among those patients who experienced recurrence, ctDNA positivity was associated with a shortened overall survival (HR, 2.71; 95% CI, 1.64-4.47; *P*<.0001).

In another analysis of the GALAXY study (median follow-up, 16.74 months; range, 0.49-24.83), those patients with high-risk stage II/III disease and a ctDNA-positive status at 4 weeks following surgery had a significant benefit from adjuvant chemotherapy (adjusted HR, 6.59; 95% CI, 3.53-12.3; *P*<.001).[9] This association was seen across all pathologic stages, including stage II (adjusted HR, 5.84; 95% CI, 1.36-25.1; *P*=.018); stage III (adjusted HR, 7.02, 95% CI, 3.46-14.2; *P*<.0001); and stage IV (adjusted HR, 4.0; 95% CI, 1.85-8.8; *P*<.0001). A multivariate analysis showed that in patients with ctDNA-positive status, having no adjuvant chemotherapy was the most significantly negative prognostic factor (adjusted HR, 5.03; 95% CI, 3.17-8.0; *P*<.001).

Table 2. Hazard Ratios, Sensitivity, and Specificity, With CIs, for the Association Between RFS and ctDNA Status in the α-CORRECT Study

	PS (N=88)	PDT (N=97)	Surveillance (N=111)
Hazard ratio (95% CI)	9.6 (3.2-29.5)	16.7 (6.9-40.3)	49.6 (16.6-148.3)
Sensitivity n/N % (95% CI)	 14/18 77.8 (54.8-91.0)	 10/21 47.6 (28.3-67.6)	 20/22 90.9 (72.2-97.5)
Specificity n/N % (95% CI)	 53/66 80.3 (69.2-88.1)	 75/76 98.7 (92.9-99.8)	 82/87 94.3 (87.2-97.5)

ctDNA, circulating tumor DNA; PDT, post-definitive therapy; PS, postsurgical; RFS, recurrence-free survival.
Adapted from Diergaarde B et al. *J Surg Oncol*. 2025. doi:10.1002/jso.27989.[1]

VEGA is a randomized phase 3 study investigating whether no adjuvant therapy is noninferior to standard adjuvant therapy (capecitabine plus oxaliplatin for 3 months) in patients with high-risk stage II or low-risk stage III CRC if ctDNA status is negative at week 4 after curative surgery.

ALTAIR is a randomized double-blind phase 3 study planned to test the superiority of adjuvant chemotherapy (trifluridine/tipiracil) compared with placebo in patients with resected CRC and ctDNA-positive status after completion of primary adjuvant treatment.[10]

CIRCULATE-US is a prospective phase 2/3 randomized trial designed to investigate the role of ctDNA in risk stratification for treatment decisions to intensify and deintensify adjuvant chemotherapy in patients with stage III and high-risk stage II colon cancer.[11] Patients who are ctDNA-negative after surgery (cohort A) are randomized to receive either immediate adjuvant treatment with 5-fluorouracil and folinic acid or capecitabine plus oxaliplatin (FOLFOX6/CAPOX) or serial ctDNA surveillance and delayed adjuvant therapy. Patients in this latter arm who are found to develop subsequent positive ctDNA status are then enrolled in cohort B and re-randomized to 6 months of treatment with either FOLFOX6/CAPOX or 5-fluorouracil, folinic acid, oxaliplatin, and irinotecan (FOLFIRINOX). Patients who are ctDNA-positive after surgery are directly enrolled in cohort B and randomized to adjuvant treatment with either FOLFOX6/CAPOX or FOLFIRINOX. During phase 2, the primary endpoint for cohort A is time to positive ctDNA status; during phase 3, the primary endpoint is disease-free survival (inferiority). The primary endpoint for cohort B is disease-free survival (superiority) during both phases 2 and 3.

BESPOKE is a multicenter, prospective, observational cohort study expected to enroll 2000 patients with stage I-IV disease.[12] Patients will be followed with serial ctDNA testing for up to 2 years, with a primary endpoint of the impact of personalized ctDNA testing on adjuvant treatment decisions, and to measure asymptomatic CRC recurrence rates without imaging.

IMPROVE-IT2 is a multicenter, randomized trial evaluating the use of ctDNA-guided postsurgery surveillance vs standard-of-care surveillance with CT.[13,14] To be included, patients must have stage III or high-risk stage II CRC. The primary outcome of this study is the fraction of patients with relapse receiving intended curative resection or local treatment aiming at complete tumor destruction.

Disclosure

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Current Utilization of Molecular Residual Disease (MRD) Testing in Colorectal Cancer: Q&A

Tanios S. Bekaii-Saab, MD, and Robert E. Schoen, MD, MPH

H&O Does the radiology follow-up schedule remain the same in light of MRD testing?

Tanios S. Bekaii-Saab, MD: Currently, the guidelines are very loose regarding the frequency of radiologic imaging during follow-up of CRC, with recommendations of 3 to 6 months and 6 to 12 months as clinically indicated.[1] This is because there is currently no consensus about how frequently patients should be scanned. In fact, recent analyses have reported no survival benefit with CT vs no CT on follow-up, or with frequent vs less frequent CT scanning.[2-3]

In practice, if I see a patient with MRD-negative disease, I am more comfortable letting 6 months elapse before another CT scan because MRD assessments are every 3 months. If the MRD result turns positive, I reduce the interval between CT scans to every 3 months, because I want to see if the disease shows up earlier in an area where I can address it locally (oligometastatic disease).

H&O Are you currently utilizing MRD testing in tumor types beyond CRC, and if so, in what capacity?

Tanios S. Bekaii-Saab, MD: There are currently no other gastrointestinal tumors where we are applying MRD testing. The bulk of the data remain in CRC, where we have started considering its utility in the clinic. There are trials, which we are part of, that are assessing MRD in other tumor types such as liver and pancreas, but no significant data are available for these yet.

H&O Which MRD tests are you currently using in the clinic?

Tanios S. Bekaii-Saab, MD: Right now, we primarily use the tumor-informed test in the majority of our patients. The Signatera test had been the only version on the market for CRC, but the Oncodetect test was recently released.

H&O How do you think assays for the detection of MRD will evolve?

Robert E. Schoen, MD, MPH: One interesting evolution would be to have a quantitative assay. There still is a concern that some ctDNA-positive patients do not experience recurrence. If a ctDNA-positive result automatically triggers more aggressive chemotherapy but the patient does not need it, that is potentially a problem. All the MRD assays right now simply report a positive or a negative. Imagine if we had a quantitation where perhaps one timepoint was getting close to a threshold or even surpassing that threshold, but then the next one was reduced, it might generate a pause before beginning treatment. Likewise, if the result turned positive but just barely positive, one could consider waiting until the next timepoint to see whether the marker is rising. I think this is a potential area where MRD assays could become more informative.

Tanios S. Bekaii-Saab, MD: I agree. As MRD testing evolves, we are hoping to see more clinical trial data, with each test being more extensively validated. Another area that would benefit from improvement is the logistical side of testing, which is currently a key limitation for applying these tests in clinical decision-making. For patients with CRC who will receive adjuvant chemotherapy, that therapy must optimally be started within 8 weeks of surgery. The physician needs to have all the clinical decision-making tools on hand within 6 weeks.

H&O What is your view on how recent guideline changes will impact patient care?

Tanios S. Bekaii-Saab, MD: Including MRD testing via ctDNA as a prognostic biomarker for the management of patients with CRC in the National Comprehensive Cancer Network guidelines will make the testing more likely to be commercially reimbursed, creating a lesser reluctance to utilize them. However, this does not change the current challenge physicians face when ordering these tools, which is in the clarity of how exactly to apply them in clinical practice.

Robert E. Schoen, MD, MPH: Guideline changes regarding MRD testing can affect 2 areas: treatment and prognosis. Regarding treatment, the questions are whether I am going to treat and what to treat with. These are important questions, and we have to wait for the results

from randomized trials to see what they show and how they are going to affect practice. The more complicated area is regarding prognosis. The prognostic information gained from ctDNA status may be extremely valuable for some patients, providing accurate prediction of the risk of recurrence, although not everybody wants to know their prognosis. However, there are patients who would want as much information as possible about what their cancer status will be in the ensuing 5 years, and that information might guide and transform how they live their lives.

Tanios S. Bekaii-Saab, MD: The way we discuss prognostic tools with our patients is a big challenge. Inevitably, they will ask, "How does that matter to me? If you tell me that I have a disease that has a higher likelihood of coming back but you cannot do anything about it, how is that going to improve my outcome?"

Robert E. Schoen, MD, MPH: We do not yet know whether there is anything we can do about it.

Tanios S. Bekaii-Saab, MD: That is why today, every time I order an MRD test, I am with my patient, and we are having an extended discussion about the value of the findings, what they mean, how they are going to affect their outcome, and whether I can change that outcome or not. The patients, when we have this discussion, universally say, "I want to know today." But I do recognize that at Mayo we have a patient population that tends to be quite informed by the time we see them.

I have found that the best utility for MRD testing in the clinic is not necessarily limited to the earlier stages, although its use there is evolving, but in helping us stay ahead of early recurrences in the setting of treated oligometastatic disease. These patients tend to experience recurrences that are organ-limited, and as such are addressed locally instead of systemically. The MRD tests have helped to avoid chemotherapy for many of these patients, which I do not believe affects survival as much in the oligometastatic setting.

H&O What are the clinical implications of using an MRD test to allow for earlier recognition of recurrence as compared with conventional imaging?

Robert E. Schoen, MD, MPH: This raises the complicated issue of what to do for the patient with a positive ctDNA status but no recurrence identifiable on imaging. Of course, we do not really know the best intervention in this setting. We do know that the tests are harbingers of recurrent disease and that there is that median time of 7 to 10 months during which the test will turn positive before a finding is detected on the CT scan.

H&O Why are high sensitivity and high specificity across various clinical use cases important when considering the use of MRD testing?

Robert E. Schoen, MD, MPH: In terms of low specificity, meaning a high false-positive rate, the risk is of overtreatment, meaning that patients could be given a potentially toxic chemotherapy when they have not demonstrated evidence of recurrence and may not ever experience recurrence. With low sensitivity, or a high probability for a false-negative test, a negative test result does not well predict that the cancer is not going to recur, especially at a one-time sampling. For example, during surveillance, serial tests need to be performed over time to achieve adequate sensitivity. To have the greatest clinical utility, a test needs to have both excellent sensitivity and specificity.

H&O Typically, do you find much variance between clinical trial results and real-world experience when it comes to diagnostic testing? How do you reconcile the two?

Tanios S. Bekaii-Saab, MD: There is not a clinical trial on the planet that will 100% simulate what will happen in the clinic. That is the beauty of the art of medicine. I tell my fellows that science is easy, for us at least. The art is what has to be experienced in clinic, learning how to address the most challenging clinical issues. This is about 98% of what we do, and this is not represented fully in clinical trials. Most patients are going to be in the gray zone, where the art of medicine actually helps—experience, deep knowledge of the data, and deep understanding of the clinical course of the patient. Every individual sees the world a little bit differently. Their preferences are different, and their acceptance of treatment and of certain toxicities with treatment are different. Regardless, those tools are meant to assist us, not necessarily to replace us or replace decision-making.

H&O What other factors beyond test performance do you consider when selecting a specific diagnostic test?

Robert E. Schoen, MD, MPH: I would say the personality of the patient as well as the clinical situation. These are the factors that Dr Bekaii-Saab mentioned. At each visit, the physician must strive to understand the patient's needs, expectations, and concerns. Management must be tailored to each patient, especially in this very complex area of a cancer diagnosis, treatment, and prognosis. All of these factors are part of the art of medicine.

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