

Use of Molecular Assays and Circulating Tumor DNA in Early Stage Colorectal Cancer: A Roundtable Discussion of the Gastrointestinal Cancer Therapy Expert Group (GICTEG)

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Abstract. The use of genomic testing is rapidly emerging as an important clinical tool, both for cancer diagnosis, and for guiding treatment decisions in a wide range of malignancies, including gastrointestinal (GI) cancers such as colorectal cancer (CRC). Advances in technologies such as polymerase chain reaction (PCR) and next-generation sequencing (NGS) methods have made it possible to non-invasively screen for CRC, for example, through the use of blood- or stool-based testing, with high specificity. Tests are also available that can provide prognostic information beyond traditional clinicopathologic factors such as tumor size, grade, and nodal status, which can enable clinicians to more accurately risk stratify patients for recurrence. Lastly, in the setting of

resected CRC, tests are now available which can detect circulating tumor DNA (ctDNA) as a means for non-invasive minimal/molecular residual disease (MRD) monitoring, thereby potentially guiding the use of adjuvant chemotherapy and/or escalating or de-escalating therapy. The Gastrointestinal Cancer Therapy Expert Group (GICTEG) recently convened a virtual meeting to discuss current issues related to genomic testing in GI cancer, with the goal of providing guidance on the use of these tests for the practicing community oncologist, for whom GI cancer may be only one of many tumor types encountered. This article provides a summary of the discussion and highlights the key opinions of the GICTEG on this topic. *The Oncologist* 2021;9999:• •

Implications for Practice: The Gastrointestinal Cancer Therapy Expert Group (GICTEG) seeks to provide practical guidance and opinion on the treatment of GI malignancies including colorectal cancer (CRC) for the practicing community oncologist, in situations where guidelines from established bodies such as the National Comprehensive Cancer Network (NCCN) and/or the American Society for Clinical Oncology (ASCO) may be less clear. In the present report, clinical guidance on the use of molecular assays for a range of clinical indications in CRC is presented, including the use of circulating tumor DNA (ctDNA) to detect minimal/molecular residual disease (MRD) in patients with successfully resected early stage CRC.

INTRODUCTION

Despite improvements in prevention, screening, as well as advances in potentially curative surgical and adjuvant therapies, colorectal cancer (CRC) remains one of the most commonly diagnosed cancers, and a leading cause of cancer

death worldwide. Molecular assays are increasingly being used to prognosticate patients, guide treatment decisions, and monitor for disease recurrence in CRC. Several tests are now available and/or are under development, that offer the

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promise of simpler, more specific, less costly, and less invasive methods for screening and early detection, stratification of patients for recurrence risk, guidance regarding the utility of adjuvant treatment, and monitoring for disease recurrence. Treatment guidelines have not yet been established on the use of molecular testing and/or other emergent technologies in CRC.

ABOUT THE GICTEG AND ROLE OF FUNDING SOURCES

The Gastrointestinal Cancer Therapy Expert Group (GICTEG) comprises expert physicians and clinical researchers who have dedicated their careers to the treatment of patients with gastrointestinal (GI) malignancies. The purpose of the group is to meet periodically to discuss important developments related to management of GI cancers, with a particular emphasis on new findings and/or areas where guidance from established bodies, such as the National Comprehensive Cancer Network (NCCN) and the American Society for Clinical Oncology (ASCO), may be unresolved, and/or less well established. The goal is to elicit the group's collective opinions on a given topic as it relates to their own clinical practices, and more importantly, how this might impact oncologists in the community setting, who may not be as extensively versed in the treatment of GI cancers. Importantly, this article is not intended to replace any existing guidance or guidelines, nor to be an exhaustive review of the topic(s) in question. Rather, it is intended to present a concise synopsis of the most important data in the area, and summarize the opinion of the expert group, as gleaned from the meeting discussion.

In May of 2020, members of the GICTEG convened a virtual meeting to discuss current and emergent issues surrounding the use of molecular testing in colorectal and other GI cancers. The faculty members of the GICTEG were selected by Total Health Information Services, a medical information company, based on their expert experience on this topic. An unrestricted educational grant for this activity was provided by Natera Oncology. The faculty and Total Health jointly selected the main topics and general outline for the discussion. It is recognized that the panelists may, or may not have relationships with corporate entities, both related and/or unrelated to the topic in question. Content of the discussions, and any expert opinions presented herein, is intended to be based on the panelists' own expert clinical experience and insight, in alignment with current guidelines, and is understood not to be influenced by any corporate relationship or interest.

GENOMIC TESTING IN CRC SCREENING, AND ONGOING CLINICAL TRIALS

While not intended to be a comprehensive list of all CRC and/or pan-cancer screening tests that are currently under investigation, **Table 1** provides an overview of some the genomic tests and technologies that have been used to screen for CRC, including commercially available and investigational tests.¹⁻⁹ These non-invasive tests aim to assess for genomic, and epigenomic changes in bodily fluids (i.e., 'liquid biopsies'), which can detect the presence of cancer.

Exact Sciences' Cologuard®, one of the more widely known, non-invasive screening methods, is a stool-based assay that evaluates fecal hemoglobin, as well as several DNA markers including *KRAS* mutations, and epigenomic markers like aberrant methylation of the *NDRG4* and *BMP3* genes. The performance characteristics of the test were assessed in a prospective, multi-site, point-in-time study of adults at average risk for CRC, ages 50-84, who were scheduled to undergo colonoscopy. The test has a reported sensitivity of 92.3% for detecting CRC as compared with 73.8% for fecal immunohistochemical testing ($P=0.002$); sensitivity of the test was also better for detection of advanced precancerous lesions (42.4% vs. 23.8%; $P<0.001$), polyps with high grade dysplasia (69.2% vs. 46.2% $P=0.004$), and serrated sessile polyps of ≥ 1 cm (42.4% vs. 5.1%; $P<0.001$) however, the specificity of the test was lower (i.e., more false positives).^{7,8} Several investigational blood-based assays for CRC screening are also highlighted in **Table 1**.

Discussion

The group considered several questions related to the use of these tests as a means for screening, including where and how these tests are currently being used, the target population, and current guidance on their use. Overall, most medical oncologists generally become involved in the care of cancer patients once the diagnosis is made, and as such, these non-invasive screening tests are usually ordered by primary care physicians and/or gastroenterologists, as part of health maintenance visits. Because of this, most in the group had not used any of the available assays for early CRC detection (**Table 1**), outside of the setting of a clinical trial. The group recognizes that patients frequently have knowledge of these tests (e.g., through media, internet and/or social media sources) and often have questions about using them. With regards to the utility of blood-based genomic tests for CRC, the recent work of Lennon and colleagues was noted.⁹ In this prospective, interventional study of over 10,000 women not known to have cancer, those with a positive blood test were followed up with PET-CT scanning to confirm and localize the cancer. The group thought that studies of this type could be a useful model for other population screening methodologies, as a means to improve the level of evidence, and provide additional confidence before this type of testing may be routinely used in practice. Several important ongoing efforts including the GRAIL programs and ECLIPSE will help to refine the role for cell-free approaches in early CRC detection.^{2,4} It was agreed that the current gold standard for screening for early CRC remains colonoscopy.

GENOMIC TESTING IN EARLY STAGE CRC

Prognostic versus Predictive

In discussing genomic testing options that inform recurrence risk in early stage CRC, the group first noted the importance of highlighting the difference between tests that provide *prognostic* versus *predictive* information. Tests which provide *prognostic* information inform on the overall risk of disease recurrence in surgically resected patients

(i.e., disease biology), whereas tests which provide *predictive* information may inform decisions regarding the role of adjuvant, or postoperative therapies, to decrease recurrence risk (i.e., response to therapy).

Oncotype and Immunoscore

Molecular assays that may inform recurrence risk assessment in early stage CRC, including Oncotype Dx and Immunoscore, are highlighted in **Table 2**. Oncotype DX Colon (12-gene assay) has been used clinically as a means to assess risk of CRC recurrence in Stage II patients, based on its recurrence scoring system, however, it has not been validated as a means for predicting response to adjuvant chemotherapy. The prognostic performance of the test has also been validated across several major CRC trials including (QUASAR, CALGB 9581, NSABP C-07, and SUNRISE).¹⁰⁻¹³ Immunoscore is an image-based risk assessment tool that evaluates the host immune response (CD3+ and CD8+ cells) in the tumor microenvironment; it is intended to be used adjunctively with standard TNM classification, measuring the density of CD3+ and CD8+ T lymphocyte populations in the center and at the periphery of the tumor. (**Table 2**).¹⁴⁻²⁰ The test has been validated across several studies as a powerful prognostic tool in Stage II and III CRC patients (**Table 2**).

Discussion

While well-accepted clinical and pathologic factors, including lymphatic/vascular invasion (LVI), perineural invasion, T4 staging, and clinical obstruction or perforation remain essential in risk assessment and adjuvant chemotherapy decision-making for early stage CRC, the group weighed in on the prognostic-versus-predictive value of the growing number of molecular assays for risk assessment. The panelists agreed that tests such as Oncotype DX Colon and Immunoscore represent prognostic tests, and they have not been validated as predictive of chemotherapy benefit. Although these tests may inform whether patients are at particularly high risk of recurrence (i.e., prognosis), it is unclear whether, and to what degree, adjuvant chemotherapy may reduce that risk. The group therefore agreed that these tests cannot inform adjuvant chemotherapy decisions at the present time, given the lack of validated predictive information. This is in accordance with current NCCN guidelines, which note insufficient data to recommend the use of Oncotype or other available multi-gene assays (e.g., ColDx, ColoPrint) as a means to guide adjuvant chemotherapy treatment.²¹ Of note, the prognostic, but not predictive value of these tests in CRC stands in contrast to other cancer types, such as breast cancer, for which genomic tests (i.e., Oncotype Dx, MammaPrint, Breast Cancer Index) have been validated to inform the benefit of adjuvant therapies.

The group discussed recent data from the IDEA France collaborative group highlighting the potential role of Immunoscore in adjuvant chemotherapy decision making for Stage III colon cancer.²² In this study, which investigated the efficacy of 3 versus 6 months of oxaliplatin-based therapy in Stage III colon cancer, Immunoscore was a significant and independent predictor of DFS ($P=0.003$) after adjustment for sex, histological grade, stage, and MSI status in

multivariate analysis. Those with intermediate or high risk Immunoscore results had a benefit of 6 months of adjuvant mFOLFOX6 ($HR=0.53$; $P=0.0004$), whereas those with low risk Immunoscore did not have a significant benefit of the 6 month regimen relative to the 3 month regimen. Although these data are compelling, the group agreed that the results would need to be prospectively validated before Immunoscore testing would be used to more definitively inform treatment decisions.²² In addition, it is unclear mechanistically why a low Immunoscore signature would be associated with lack of benefit from chemotherapy. Most in the group agreed that, at the current time, recurrence risk in early CRC is best assessed on the basis of clinical and pathologic characteristics, pending further validation of additional assays in prospective clinical trials.

OTHER MOLECULAR MARKERS

The group agreed with current NCCN guidance recommending universal microsatellite instability (MSI) or mismatch repair (MMR) testing for all newly diagnosed patients with colon cancer, which may be performed using either immunohistochemistry (IHC) or polymerase chain reaction (PCR) platforms.²¹ In addition, the group noted that *BRAF* mutation testing should be reflexed in patients found to be MMR deficient (dMMR), as a means to identify patients who may benefit from genetic counseling. The presence of *BRAF* mutation would exclude conditions such as Lynch Syndrome (hereditary nonpolyposis colorectal cancer), and as such, germline testing would not be necessary in these individuals. The group also noted the prognostic impact of *BRAF* and *KRAS* mutations in CRC. In a study investigating patients with stage III CRC treated with adjuvant FOLFOX4 with or without cetuximab ($N=2559$), Taieb et. al. found that, among patients with microsatellite-stable (MSS) tumors, *KRAS* and *BRAF* V600E mutations were associated with shorter disease-free survival (DFS) and overall survival (OS).²³ These results suggest that *KRAS* and *BRAF* mutational testing may help to better stratify patients for adjuvant trials, though newer technologies may further refine stratification as discussed below.²³ Results from an earlier trial also suggested an adverse impact of *KRAS* ($HR=1.44$; $P<0.001$) and *BRAF* V600E mutation ($HR=1.37$; $P=0.009$) on DFS in Stage III CRC patients treated with FOLFOX.²⁴

NEXT GENERATION SEQUENCING (NGS) AND MOLECULAR RESIDUAL DISEASE (MRD) IN RESECTABLE CRC: WHAT TO TEST, AND WHEN?

The group considered the question of whether, in a patient with a clinically high risk for recurrence, they would do extended gene panel testing (e.g., NGS analysis for *BRAF*, *HER2*, etc.) on the primary tumor, or wait until a recurrence occurs and do genomic testing on a recurrence sample. Most in the group were in agreement that there is a high degree of concordance between the primary tumor and the metastatic site of recurrence for oncogenic driver alterations. Therefore, when selecting a tissue sample for genomic analysis, either primary tumor or metastatic site would

be appropriate for extended panel testing, depending on adequacy of tissue specimen available. The group did, however, recommend that, among patients treated with molecularly selected biologic agents such as BRAF-, HER2- and/or EGFR-directed therapies, a new tissue biopsy or blood based assay as described below should be strongly considered upon disease progression, as this may identify resistance mechanisms and inform subsequent treatment options, ideally in the setting of a clinical trial. For patients with metastatic disease, the group believes that essential testing includes MMR/MSI, *KRAS*, *NRAS*, *BRAF* (*HER2* for wild type *RAS* tumors) determinations, and if NGS has been performed, evidence of *NTRK* gene fusion and tumor mutational burden should be assessed.

CIRCULATING TUMOR DNA (ctDNA) TESTING FOR CRC RECURRENCE

Although a large portion of patients with CRC may be cured by surgery alone, a group of patients benefit from the addition of adjuvant chemotherapy as a means to eradicate micrometastatic disease.^{25,26} Chemotherapy, however, may lead to significant and in some cases potentially irreversible toxicities, such as oxaliplatin-associated peripheral neuropathy. A risk stratification strategy in these patients may therefore help avoid unnecessary chemotherapy treatment and its associated toxicities. Circulating tumor DNA (ctDNA) is released into the plasma in significant quantities as a result of tumor cell turnover and multiple other mechanisms, and as such, detection of minimal residual disease (MRD) via ctDNA assessment has emerged as a promising tool to guide risk stratification in adjuvant treatment. Morris and Kopetz, using pooled data from several cohorts, noted an increasing rate of postoperative ctDNA detection, as may be expected, with increasing pathologic stage, with 12%, 27% and 48% of Stage II, III and IV patients having detectable ctDNA after resection ($P < 1 \times 10^{-5}$).²⁷

In discussing genomic ctDNA testing for MRD, the group noted important differences between the tumor informed versus tumor uninformed (or agnostic) assays (**Figure 1**). Tumor agnostic assays evaluate large panels of genes, methylation patterns, and fragmentomic ctDNA signatures that are common drivers and/or are frequently mutated in CRC as a means to detect residual disease. Tumor informed assays, by comparison, create a tissue-based, specific signature of the patient's tumor which can be tested over time in the plasma. There are advantages and limitations to both approaches.²⁸ A tumor agnostic approach may detect a wide range of potentially druggable mutations in the tumor (if annotated and reported), and is therefore well suited to study heterogeneous tumors and/or the adaptive evolution of tumors over time in response to treatment. By comparison, the tumor informed approach utilizes a specific genomic fingerprint for the patient's tumor that is not expected to change over time and/or with treatment (i.e., generally more truncal genomic changes). While this does not enable, nor is it intended to identify potentially actionable targets over time, it has high sensitivity and specificity to detect recurrent disease that is present as ctDNA in the blood. An example of a tumor agnostic assay is LUNAR-1 (Guardant

Health, CA), and an example of a tumor informed assay is the Signatera™ test ctDNA developed by Natera (**Figure 1**).

The group noted findings from Parikh and colleagues (ASCO 2019) using a tumor uninformed approach that incorporates both genetic alterations and epigenetic markers to identify patients at high risk for recurrence (LUNAR-1 Assay). In this study, 72 patients with CRC underwent standard of care (SOC) that included surgery with or without neoadjuvant therapy (n=42) or surgery with adjuvant therapy with or without neoadjuvant therapy (n=30). Plasma samples were collected after a median of 31 and 37 days, respectively following surgery or completion of adjuvant therapy, respectively, and median follow up was 515 days. The study comprised approximately one-third of patients with Stage 0-II disease, one-third with Stage III disease, and one-third with stage IV disease at time of resection. Detection of ctDNA following SOC therapy had a recurrence PPV of 100% and an NPV of 76%, yielding a hazard ratio for recurrence of 9.22 ($P < 0.0001$). Similar findings were observed in the surgery (HR=8.7) and adjuvant therapy (HR=9.3) cohorts (for both, PPV, 100%; NPV, 76%, both $P < 0.0001$). These findings suggest that ctDNA detection post resection using a tumor uninformed approach may help identify patients at high risk for recurrence, and potentially who may benefit from adjuvant therapy as well as modified therapy approach.

The group reviewed additional evidence supporting the use of ctDNA to detect MRD. Tie and colleagues found that, in a population-based cohort study of 96 Stage III CRC patients, ctDNA was detectable in 21% of post-surgical samples and 17% of samples post chemotherapy, and ctDNA positivity was associated with a significantly increased risk of recurrence (**Table 3**). In multivariate analysis, post-surgical ctDNA status (HR=7.5; $P < 0.001$) demonstrated the strongest association with relapse free interval (RFI), followed by clinical risk factors (HR=2.5; $P = 0.008$).³⁰ Similar findings were also observed by the same group in a study examining ctDNA in Stage II CRC patients.³¹ Reinert and colleagues assessed ctDNA using a tumor-informed, multiplex, PCR-based NGS approach before and after surgery and adjuvant chemotherapy in a prospective multicenter cohort study of patients with Stage I – III CRC (N=130).³² ctDNA positivity at postoperative day 30 was also associated with a 7-fold increased risk for relapse in this study, as compared with ctDNA negative status (recurrence rate 70% vs. 11.9%; HR=7.2; $P < 0.001$). Following adjuvant chemotherapy, ctDNA positive patients had a 17 fold increased risk for relapse (relapse rate 100% vs. 13.7%; HR=17.5; $P < 0.001$), and monitoring of ctDNA during adjuvant treatment showed ctDNA clearance in 3 of 10 patients (30%) after adjuvant chemotherapy.³² In addition, with serial ctDNA monitoring following treatment, ctDNA positive status was associated with markedly reduced RFS (recurrence rate 93.3% vs. 3.3%; HR=43.5; $P < 0.001$); ctDNA was also the only factor significantly associated with RFS in multivariate analysis (HR=39.9; $P < 0.001$). Of note, when comparing longitudinal analysis of the same surveillance population (n=75) ctDNA showed higher sensitivity and specificity (88% and 98%, respectively) relative to carcinoembryonic antigen (CEA) monitoring (69% and 64%, respectively).³² Overall, the

group was in agreement that, although these studies are limited by relatively small sample size, they strongly demonstrate the potential of ctDNA to risk stratify CRC patients and to potentially inform adjuvant chemotherapy decisions, particularly in patients with Stage II and III disease.

Discussion

Given the significant and sometimes irreversible toxicity of chemotherapy, even when using the 3-month approach with CAPOX as suggested by the IDEA collaboration update, the group expressed agreement regarding the need to improve risk stratification of patients receiving adjuvant chemotherapy.³³ The group acknowledged that although compelling, there is insufficient data at present to support ctDNA-based MRD assessments as a means to guide the use of adjuvant chemotherapy. For example, data presented by Henriksen et al. at ASCO GI 2021 showed that 4 of 20 patients initially ctDNA positive postoperatively, and all of whom received adjuvant chemotherapy, did not have recurrence, implying that residual disease may have been cleared with the use of adjuvant treatment in these patients.³⁴ In view of the small sample sizes and low numbers of events in these retrospective studies, the group is in agreement with recent discussion by Overman and colleagues emphasizing the need for randomized prospective studies to answer this question.

Some in the group stated that they were currently offering ctDNA testing at their center to all patients as a means of complementing clinical factors and further informing recurrence risk estimates.³⁰ The group expressed that it would be ideal to have a validated test in which one could have the option to withhold treatment until serial monitoring demonstrates ctDNA test positivity (as indicative of MRD). The group also emphasized that testing should not be performed in the immediate post-surgical period, to avoid false positives which may create unnecessary anxiety for patients. In this regard, the group notes that timing of blood collection following resection is an especially important consideration. A recent study that included 453 patients with Stage I - III CRC undergoing elective surgery found an approximately 3-fold increase in the level of cell-free DNA following surgery ($P \leq 0.0001$) and this elevation was noted to persist for approximately 4 weeks.³⁵ Therefore, it is suggested that later sampling would reduce the potential for contamination with type (non-tumor) cfDNA. The group suggests 4 weeks post-surgery as a reasonable time frame for plasma sampling.

There was also agreement that serial ctDNA testing further improves the sensitivity. Reinert et. al., for example, found an improvement in sensitivity with 2 tests versus 1, and sensitivity was further improved with 3 tests.³² Therefore, if a patient is found to be ctDNA positive in the early post-surgery period, the group thought it would be reasonable to consider a 2nd test before treatment is given. Although the time between blood draw and initial ctDNA test result takes longer, a second blood draw (which takes less time than the initial) could be performed immediately following receipt of the first. Most in the group felt that MRD testing is more commonly ordered in Stage II cases, and less often in Stage III CRC, as it is not expected to

change management in the latter until more data in this setting is available. It was also agreed that, outside the setting of a clinical trial, ctDNA testing would generally not be ordered if it would not impact or otherwise change their treatment decision based on clinicopathologic factors. The group also notes a recent publication of the National Cancer Institute (NCI) Task Force on the application of ctDNA testing; this report noted multiple potential applications of ctDNA testing in CRC, including the detection of MRD, management of rectal cancer, and as a means to monitor clonal evolution of the tumor and response to targeted therapies and other systemic treatments. Notably, the NCI task force called for harmonization around timepoints for the collection of ctDNA, standardization of sample collection and clinical validation of testing, a need for patient and provider education on the applications of the technology, and the incorporation of ctDNA testing into treatment guidelines.³⁶

ctDNA TESTING: POTENTIAL CLINICAL SCENARIOS

Patient Case #1: What should we do when clinical risk is low, but ctDNA results are positive?

In a patient with Stage II colon cancer with low risk clinical characteristics, but with a positive ctDNA result, the group agreed that the best course of action would be to inform the patient of the high risk of recurrence, while noting that no data is yet available to ensure a benefit of treatment; in such a case some patients might decide to forgo treatment until the disease becomes radiologically detectable. Some experts, however, considered discussing adjuvant chemotherapy, managing the patient as a high-risk case, with monitoring of MRD to detect clearance, or persistence of ctDNA. Some in the group also thought a confirmatory repeat of the ctDNA testing might be indicated.

Patient Case #2: What should we do when clinical risk is high, but ctDNA results are negative?

In a patient with stage II colon cancer with high risk features who would normally be recommended adjuvant treatment, but who has a negative ctDNA result, the group agreed that based on current data and guidelines, treatment should not be withheld for such a patient. It was recommended to monitor, and consider discussion of CAPOX adjuvant therapy for 3 months versus 6 months. Some in the group also thought a repeat of the ctDNA test might be indicated here as well.

Patient Case #3: Relationship between molecular markers and ctDNA result?

The group then considered the case of a Stage II colon cancer patient with clinical low risk (i.e. well to moderately differentiated, $\leq T3$, adequate lymph node harvest, no lymphovascular or perineural invasion, and no evidence of obstruction or perforation), but who, on molecular testing was found to be *BRAF* wild type (WT) and MSI-high. Although adjuvant treatment would not be typically recommended in this scenario, the group considered what they would do if ctDNA (Signatera™) testing reported a positive ctDNA result, indicative of MRD. The group agreed, in such a scenario that this would require shared decision making with the patient, and that the patient should be informed of the high risk of recurrence while at the same

time noting the lack of data to clearly support a benefit from adjuvant treatment. Some in the group, however, considered discussing adjuvant chemotherapy, managing the patient as a high-risk case, and monitoring MRD for clearance or persistence of ctDNA. The clinician might also consider any preexistent conditions (e.g., neuropathy), comorbidities, or other patient factors (e.g., age, performance status) that might also preclude the use of chemotherapy.

Role of ctDNA in Surveillance

Many in the group felt that ctDNA testing, especially in the current time of COVID-19, may ultimately reduce the need for computed tomography (CT) testing for surveillance and its associated costs. For example, a strategy could be envisioned whereby one would only be CT scanned in the event of a positive ctDNA test; it was agreed such a strategy may completely change the established surveillance strategy. In this regard, the utility of routine CEA testing was also raised, given that CEA levels may be impacted by multiple factors unrelated to CRC. As such, CEA elevations may sometimes be harder to interpret as a definitive surrogate marker of disease recurrence, in the absence of radiological relapse. The group also highlighted potential limitations of ctDNA testing, including lack of data supporting the impact of earlier treatment (e.g., for recurrent metastatic disease) on modifying the overall disease trajectory in CRC, a lack of guidance regarding persistent ctDNA positivity *after* adjuvant treatment, uncertainty regarding the frequency of ctDNA testing, and a lack of consensus regarding the kinetics of ctDNA relative to clinically actionable (radiological) recurrence. Similar issues were raised in a recently convened National Cancer Institute task force on colon cancer, and remain important questions for the field.³⁶

FUTURE PERSPECTIVES

The group identified at least 2 key trials underway, which will help to further clarify the role of ctDNA in MRD testing, and evaluate the potential benefit of MRD status to inform use of adjuvant chemotherapy (**Table 4**). The group strongly encourages clinical trial enrollment for patients to further advance the genomic technologies described herein and support the validation of ctDNA MRD testing and other genomic assays in CRC. The group was also in agreement that routine incorporation of ctDNA testing might create a new paradigm for risk stratification as outlined by Yang and colleagues.³⁷ ctDNA genomic testing may also hold promise as a single blood draw screen for CRC, although it was agreed that a positive test would still be followed by a diagnostic colonoscopy. For CRC patients facing adjuvant treatment decisions, a sensitive and specific MRD test reflecting biologic tumor behavior may ultimately guide the use or avoidance of potentially toxic adjuvant therapies. Lastly, further refinements in the use of ctDNA to detect MRD in successfully resected CRC patients may dramatically reduce patient treatment intensity and cumulative side effects, as well as the associated costs of imaging for disease recurrence. The group is optimistic on the future of MRD testing in CRC and anticipates that this type of testing will emerge as a standard component of clinical decision making as data continues to rapidly emerge.

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39. Clinicaltrials.gov NCT04068103

TABLE 1. Selected Genomic Tests for CRC Screening Commercially Available and/or Under Investigation

Company	Test Description and Uses	Relevant Clinical Trials
Guardant ^{1,2}	ctDNA based blood test (LUNAR-2) currently under investigation for use in detecting CRC in an average risk population	ECLIPSE (NCT04136002): CRC screening study initiated in 2019; goal to enroll ~10,000 patients ages 45 – 84 planning to have a colonoscopy
Grail ^{3,4}	Pan-cancer early detection blood test utilizing cell-free (cf) nucleic acids (DNA and RNA)	PATHFINDER (NCT04241796): prospective, multicenter study enrolling ~6,200 participants; primary goal is to guide appropriate diagnostic workup for different cancer types
Freenome ^{5,6}	Multimomics platform utilizing cfDNA, cfRNA, proteomics, epigenetics, as well as computational biology and machine learning methods to generate early tumor and immune cancer signatures	PREEMPT CRC (NCT04369053): clinical study enrolling ~14,000 average risk participants undergoing colonoscopy; primary goal is to validate blood based test for CRC screening
Exact Sciences ^{7,8,9}	<p>ColoGuard® Stool based hemoglobin and DNA test; evaluates <i>KRAS</i> mutations, β-actin, and aberrant methylation of <i>NDRG4</i> and <i>BMP3</i></p> <p>Liquid biopsy blood test (CancerSEEK) currently under development for early detection of CRC and other cancers; not available for patients at present</p>	<p>NCT01397747 (DeeP-C)</p> <p>Initial evaluation of CancerSEEK test published as part of the DETECT-A study; study evaluated > 10,000 women aged 65 to 75 with no prior cancer history</p>

TABLE 2. Selected Genomic Tests for Risk Assessment in CRC

Test	Test Description and Uses	Relevant Clinical Trials
Oncotype DX Colon ¹⁰⁻¹³	12-gene genomic test (7 cancer-related, 5 reference); validated for use in Stage II MMR proficient (MMR-P) and Stage III A/B colon cancers; used to estimate recurrence risk (low, intermediate, high) and help guide treatment decisions; not predictive of adjuvant chemotherapy benefit	Validation studies confirm Recurrence Score (0-100) result predicts risk for local recurrence (QUASAR, CALGB 9581, NSABP C-07, SUNRISE)
Immunoscore Colon ¹⁴⁻²⁰	Image analysis based risk assessment tool targeting CD3+ and CD8+ cells and examining host immune response at the tumor site; intended for use adjunctively with TNM classification	In Stage II patients Immunoscore predicted a high risk subgroup (Immunoscore Low) with increased recurrence at 5 years (23% vs. 8%); suggested 7 of 10 high risk Stage II patients might be spared chemotherapy Stage III patients with Immunoscore High status had significantly better DFS (HR=0.59; P=0.0013), whereas 1 of 2 low risk (T1-3/N1) patients with Immunoscore Low status had significantly worse 3 year DFS (78% vs 92%)

TABLE 3. Impact of ctDNA Positive Status on Recurrence Risk³⁰

Post-Surgery	3 year RFI Kaplan-Meier	Hazard Ratio	P Value
ctDNA +	47%	3.8	< 0.001
ctDNA -	76%		
Post-Chemotherapy	3 year RFI Kaplan-Meier	Hazard Ratio	P Value
ctDNA +	30%	6.8	< 0.001
ctDNA -	77%		

RFI; relapse free interval

Table 4. Genomic Testing for MRD: Relevant Clinical Trials .

BESPOKE³⁸ NCT04264702	<ul style="list-style-type: none"> • Prospective observational study enrolling patients with successfully resected Stage II or Stage III CRC who have formalin-fixed, paraffin-embedded (FFPE) tissues and whole blood samples available for analysis. • Patients in this registry trial will receive tumor-informed ctDNA testing (Signatera™) and will be recommended for adjuvant treatment or observation by their treating physician. • A control arm will consist of cases with Stage II or III CRC with at least 2 years of follow up data. • The primary endpoint will be to assess the impact of tumor-informed ctDNA testing on adjuvant treatment decisions, and the rate of recurrence among patients diagnosed with CRC who remain asymptomatic • Additional secondary endpoints will assess clinical correlates of ctDNA testing, including clearance of MRD, percentage of patients undergoing surgery for oligometastatic recurrence, and quality of life (QoL) measures. • Target enrollment is 1000 patients.
COBRA³⁹ NRG GI005 NCT04068103	<ul style="list-style-type: none"> • Phase II/III trial that aims to evaluate the predictive benefit of tumor-uninformed ctDNA testing (LUNAR-1 assay) for patients with Stage IIA colon cancer following surgery, given that adjuvant therapy is generally not recommended for these patients. • Primary objective (Phase II portion) is the rate of ctDNA clearance for patients treated with or without adjuvant chemotherapy following surgical resection, and for the Phase III portion, the rate of recurrence free survival (RFS) for patients with ctDNA positive status treated with or without adjuvant chemotherapy following surgical resection of their Stage IIA disease. • Secondary objectives (e.g., prevalence of detectable ctDNA in Stage IIA CRC following resection), and exploratory objectives are also planned, including notably, association of quantitative ctDNA levels with time to event outcomes (e.g., RFS and OS) and cost effectiveness of ctDNA monitoring versus standard of care. • Patients will be randomly assigned to close observation or prospective ctDNA assessment; for those deemed high risk by ctDNA assessment (i.e., positive ctDNA or MRD +) patients will be assigned to 6 months of adjuvant chemotherapy, whereas those who are negative for ctDNA (MRD -) will remain on active surveillance. • Blood will be prospectively collected from the standard of care arm to be analyzed at a later time for ctDNA status, so as to enable comparison of ctDNA clearance with chemotherapy. • Approximately 1400 patients are expected to be enrolled.

Table 5. Use of Molecular Assays in CRC: Summary of GICTEG Practice Points

In CRC Screening	<ul style="list-style-type: none"> The group recognizes the availability of non-invasive molecular assays and technologies to detect early CRC, however, it was agreed that the current gold standard for early CRC detection remains colonoscopy
In Early Stage CRC	<ul style="list-style-type: none"> The group recognizes the availability of molecular assays (e.g., Oncotype DX colon, Immunoscore) as a means to inform overall recurrence risk in CRC (i.e., prognostic information) while noting that none of these tests have been shown to provide predictive information of chemotherapy benefit and the group cautions on the use of such assays to inform adjuvant therapy decisions.
In Surveillance for CRC Recurrence	<ul style="list-style-type: none"> The group recognizes the importance of emerging technologies, including, most notably, circulating tumor DNA (ctDNA) as a means to detect early disease recurrence and minimal residual disease (MRD) in initially resected patients, and to possibly further inform adjuvant treatment decisions and risk stratification in Stage II and III CRC patients Clinicians should understand the difference between tumor-informed and uninformed ctDNA approaches and the benefits and limitations for each While in agreement that ctDNA should not be used to inform treatment decisions at present, the group recognizes ongoing trials such as BESPOKE and COBRA which should help to further inform on the use of ctDNA in routine practice

Comparison of ctDNA Monitoring Approaches

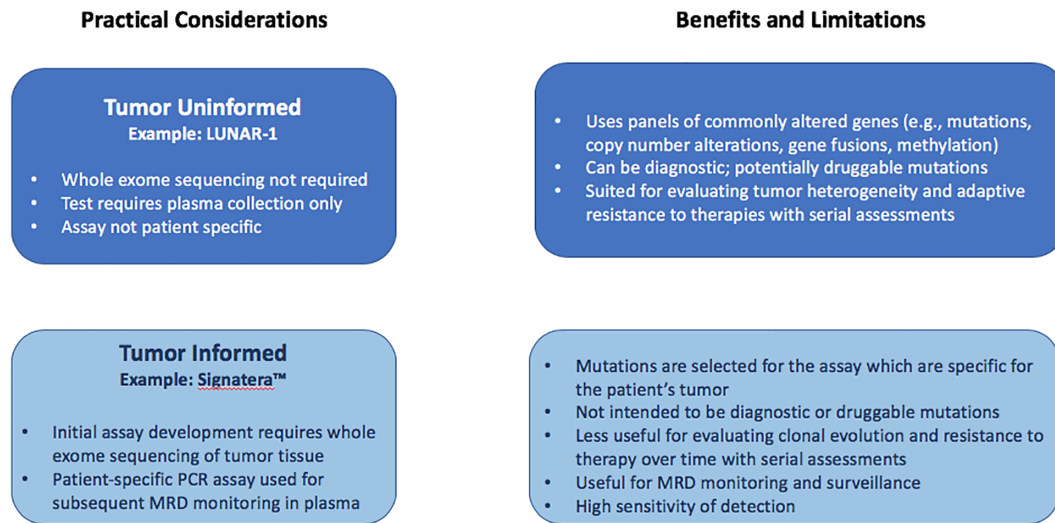


Figure 1. A Comparison of ctDNA Monitoring Approaches. ctDNA assessments in CRC can be either tumor informed (top) or tumor uninformed (bottom); practical considerations and the benefits and limitations of each approach are shown. Whereas the former method is more appropriately used to assess tumor heterogeneity, clonal evolution and/or resistance to therapies in CRC, the latter tumor-informed approach is more patient-specific and may be better suited for assessment of minimal residual disease (MRD) and ongoing surveillance for patients with initially resected CRC.