Minimal Residual Disease Detection using a Plasma-only Circulating Tumor DNA Assay in Patients with Colorectal Cancer

Aparna R. Parikh[1], Emily E. Van Seventer[1], Giulia Siravegna[1], Anna V. Hartwig[2], Ariel Jaimovich[2], Yupeng He[2], Katie Kanter[1], Madeleine G. Fish[1], Kathryn D. Fosbenner[1], Benchun Miao[3], Susannah Phillips[3], John H. Carmichael[3], Nihaarika Sharma[3], Joy Jarnagin[1], Islam Baiev[1], Yojan S. Shah[1], Isobel J. Fetter[1], Heather A. Shahzade[1], Jill N. Allen[1], Lawrence S. Blaszkowsky[1], Jeffrey W. Clark[1], Jon S. Dubois[1], Joseph W. Franses[1], Bruce J. Giantonio[1], Lipika Goyal[1], Samuel J. Klempner[1], Ryan D. Nipp[1], Eric J. Roeland[1], David P. Ryan[1], Colin D. Weekes[1], Jennifer Y. Wo[4], Theodore S. Hong[4], Liliana Bordeianou[5], Cristina R. Ferrone[5], Motaz Qadan[3], Hiroko Kunitake[5], David Berger[5], Rocco Ricciardi[5], James C. Cusack[3], Victoria M. Raymond[2], AmirAli Talasaz[2], Genevieve M. Boland[3], and Ryan B. Corcoran[1]

ABSTRACT

Purpose: Detection of persistent circulating tumor DNA (ctDNA) after curative-intent surgery can identify patients with minimal residual disease (MRD) who will ultimately recur. Most ctDNA MRD assays require tumor sequencing to identify tumor-derived mutations to facilitate ctDNA detection, requiring tumor and blood. We evaluated a plasma-only ctDNA assay integrating genomic and epigenomic cancer signatures to enable tumor-uninformed MRD detection.

Experimental Design: A total of 252 prospective serial plasma specimens from 103 patients with colorectal cancer undergoing curative-intent surgery were analyzed and correlated with recurrence.

Results: Of 103 patients, 84 [stage I (9.5%), II (23.8%), III (47.6%), IV (19%)] had evaluable plasma drawn after completion of definitive therapy, defined as surgery only ($n = 39$) or completion of adjuvant therapy ($n = 45$). In "landmark" plasma drawn 1-month (median, 31.5 days) after definitive therapy and >1 year follow-up, 15 patients had detectable ctDNA, and all 15 recurred [positive predictive value (PPV), 100%; HR, 11.28 ($P < 0.0001$)]. Of 49 patients without detectable ctDNA at the landmark timepoint, 12 (24.5%) recurred. Landmark recurrence sensitivity and specificity were 55.6% and 100%. Incorporating serial longitudinal and surveillance (drawn within 4 months of recurrence) samples, sensitivity improved to 69% and 91%. Integrating epigenomic signatures increased sensitivity by 25%–36% versus genomic alterations alone. Notably, standard serum carcinoembryonic antigen levels did not predict recurrence [HR, 1.84 ($P = 0.18$); PPV = 53.9%].

Conclusions: Plasma-only MRD detection demonstrated favorable sensitivity and specificity for recurrence, comparable with tumor-informed approaches. Integrating analysis of epigenomic and genomic alterations enhanced sensitivity. These findings support the potential clinical utility of plasma-only ctDNA MRD detection.

See related commentary by Bent and Kopetz, p. 5449

Introduction

Colorectal cancer is the third most commonly diagnosed and second leading cause of cancer death in the United States in men and women. While a majority of patients are diagnosed with early-stage disease, 5-year survival for patients with regional disease is only 71% (1). Surgery alone is often curative for stage I and II disease, and in higher risk disease, adjuvant chemotherapy can reduce the risk of recurrence (2). However, aside from risk stratification by tumor stage, clinical criteria, and carcinoembryonic antigen (CEA), there are no effective clinical tools to identify patients with postoperative minimal residual disease (MRD) who may be at highest risk for recurrence (2). An effective clinical tool to identify patients with MRD following completion of curative-intent therapy could identify patients who may benefit from additional systemic therapy or allow avoidance of unnecessary and potentially toxic therapy for lower risk patients with no evidence of MRD (2–5).

ctDNA is a promising noninvasive biomarker for MRD detection following curative-intent treatment in colorectal cancer and other cancer types. Detection of persistent ctDNA after surgery or adjuvant treatment effectively identifies patients with colorectal cancer with MRD who will ultimately recur without additional therapy (6–12). Accordingly, several prospective clinical trials of ctDNA-guided adjuvant therapy are currently underway to evaluate whether patients with evidence of MRD through ctDNA detection following surgery and/or adjuvant therapy may benefit from additional or more intensive systemic therapy to reduce recurrence risk (13–21).

[1]Department of Medicine, Division of Hematology and Oncology, Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, Massachusetts. [2]Guardant Health, Inc, Redwood City, California. [3]Department of Surgical Oncology, Massachusetts General Hospital, Boston, Massachusetts. [4]Department of Radiation Oncology, Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, Massachusetts. [5]Department of General and Gastrointestinal Surgery, Massachusetts General Hospital, Boston, Massachusetts.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Ryan B. Corcoran, Department of Medicine, Harvard Medical School, 149 13th St, Boston, MA 02129. Phone: 617-726-8599; Fax: 617-724-9648; E-mail: rbcorcoran@partners.org

Clin Cancer Res 2021;27:5586–94

doi: 10.1158/1078-0432.CCR-21-0410

Translational Relevance

Detection of persistent circulating tumor DNA (ctDNA) after curative-intent surgery to identify patients with minimal residual disease (MRD) who will ultimately recur has emerged as a potentially transformative approach in oncology. Early identification of patients with MRD through ctDNA detection could identify patients in whom additional therapy might salvage the chance of cure. To date, ctDNA MRD assays have employed a tumor-informed approach, requiring initial sequencing of tumor tissue to guide ctDNA detection, and thus cannot be used when a patient has insufficient tumor tissue for analysis. Here, we evaluate the first tumor-uninformed, plasma-only ctDNA assay integrating genomic and epigenomic signatures to detect MRD in patients with postoperative colorectal cancer, without requiring parallel tumor sequencing, which produced favorable sensitivity and specificity, comparable with tumor-informed approaches. These data highlight the feasibility and potential clinical utility of plasma-only ctDNA-guided MRD detection.

To date, most ctDNA assays designed for MRD detection rely on initial genomic profiling of tumor tissue to identify tumor-derived alterations specific for each individual patient, so that these precise alterations can be evaluated in ctDNA (6, 9, 22, 23). The rationale behind such "tumor-informed" approaches is that prior knowledge of the tumor-specific mutations may allow increased sensitivity for ctDNA detection and may improve specificity by enabling determination of which alterations are tumor-derived versus potential false positives arising from nontumor origins, such as clonal hematopoiesis of indeterminate potential (CHIP; refs. 24–26). However, a tumor-informed approach may pose several limitations. Importantly, in many cases, tumor cellularity may be limited, which would preclude the use of a tumor-informed approach. In one series, up to 9% of molecular analysis may be inadequate for tissue sequencing given low tumor cellularity, DNA yield, or quality (27). This issue is particularly relevant alongside the increasing use of neoadjuvant therapy in many tumor types, where surgical specimens may have insufficient tissue for molecular analysis due to treatment response. In contrast, a plasma-only ctDNA assay for MRD detection could offer several advantages, including more rapid turnaround time due to the need to analyze a single plasma sample, potential cost savings, and decreased logistical complexity. However, no studies have evaluated if a plasma-only MRD assay can detect ctDNA with clinically meaningful specificity and sensitivity.

We report results from a prospective, observational study in patients with stage I to IV colorectal cancer treated with curative-intent therapy to assess the ability of a plasma-only ctDNA assay to identify patients with MRD who would ultimately recur. In addition to standard detection of tumor-derived genomic alterations employed by most MRD assays, this tumor-uninformed assay (Guardant Reveal, Guardant Health) also integrates epigenomic signatures related to aberrant DNA methylation facilitating detection of ctDNA without requiring parallel assessment of tumor tissue.

Materials and Methods

Study population

This single-institution prospective study recruited patients with stages I–IV colorectal cancer treated with curative intent from August 2016 to May 2019 at the Massachusetts General Hospital Cancer Center (Boston, MA). The study was approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. All patients received treatment according to standard of care as per the treating medical oncology and surgical oncology teams. Data on neoadjuvant therapy, adjuvant therapy, and clinicopathologic information were collected from the electronic health record (EHR).

Sample collection

We collected 20 mL of peripheral blood in two 10 mL Streck tubes preoperatively (when available), approximately 4 weeks after surgery and approximately 4 weeks after completion of adjuvant therapy for patients who received additional treatment. "Landmark" timepoint was defined as the plasma specimen drawn approximately 1 month after completion of definitive therapy (surgery alone or completion of adjuvant therapy for patients who received adjuvant treatment). Longitudinal timepoints were defined by patients who had subsequent draws after their "landmark" timepoint, and, based on the methods employed by Reinert and colleagues, we also assessed performance in patients with evaluable "surveillance" draws, defined as a draw obtained within 4 months of clinical recurrence. Because of the variability of follow-up, a window of up to 6 weeks after recurrence scan was allowed for some serial draws to allow them to be collected at the patient's scheduled visit, provided that no intervening therapy was received. CEA at the landmark timepoint ($\pm$ 9 days) was obtained from the EHR. The timing of CEA draws was not mandated by the study and was performed according to the treating physician's discretion according to appropriate standard-of-care guidelines. Abnormal CEA was defined as >3.4 ng/mL (28). Serial blood collections from eleven patients who recurred but were negative at their landmark timepoint were analyzed. Plasma was separated within 1–4 days of collection through two different centrifugation steps (the first at room temperature for 10 minutes at 1,600 $\times$ g and the second at 3,000 $\times$ g for the same time and temperature). Plasma was isolated and stored at -80° until extraction. In a subset of samples (N = 72), cell-free DNA (cfDNA) was extracted from plasma using QIAamp Circulating Nucleic Acid Kit (QIAGEN) with 60 minutes of proteinase K incubation at 60°C.

Plasma genomic and epigenomic based analysis of cfDNA

Plasma (N = 180 samples; median, 4 mL; range, 1–4 mL) and extracted cfDNA (N = 72 samples; median, 60 ng; range, 4.4–100 ng) were transferred to a single site for analysis (Guardant Health). cfDNA was extracted from plasma as described previously (29). Extracted cfDNA was analyzed using the plasma-only Guardant Reveal test (formerly called LUNAR-1), which is a single sample next-generation sequencing test validated in early-stage colorectal cancer that integrates assessment of somatic alterations with an epigenomic cancer signature to identify the presence of methylation signatures associated with cancer versus normal DNA (**Fig. 1A**). The Guardant Reveal assay was designed to detect the presence of MRD without prior knowledge of the specific molecular alterations present in an individual patient's tumor.

For analysis, ctDNA fragments are partitioned and individual molecules within each partition are barcoded and then pooled and processed together through the rest of the library preparation. The libraries are enriched with an approximately 500 kb panel targeting both somatic and epigenomic regions using biotinylated bait oligonucleotides and sequenced on a NovaSeq 6000 System. Enriched

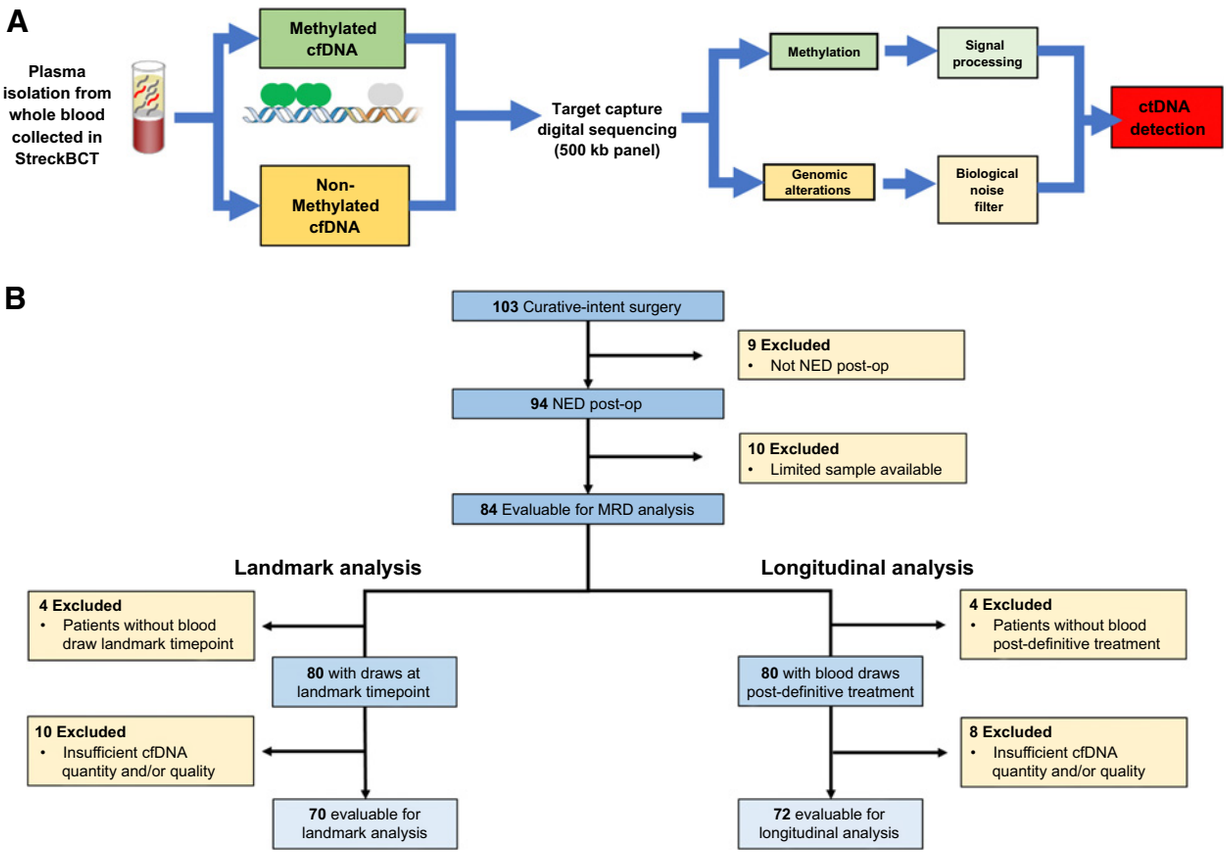

Figure 1.
Guardant Reveal plasma-only ctDNA assay schema (**A**) and patient enrollment and analysis groups (**B**).

samples are then amplified and sequenced. Sequencing data files containing raw data are analyzed using a proprietary bioinformatics pipeline software, trained to detect the presence of ctDNA based on multiple analytic features, including genomic variation (single-nucleotide variants and insertion-deletion alterations) and epigenomic signals, and to exclude common sources of interference such as CHIP. On the basis of this analysis, the plasma-only ctDNA test returns a result of either *ctDNA detected* or *ctDNA not detected*. For the current study, ctDNA analysis was performed blinded to the clinical data. Neither treating physicians nor patients were informed regarding the results of the ctDNA analyses.

Statistical analysis

The primary outcomes of the study were detection of ctDNA and recurrence-free survival (RFS) as assessed by standard radiographic imaging. RFS was measured from the day of completion of definitive treatment to first radiographic recurrence or death from colorectal cancer. For patients whose only treatment was surgery or surgery was the final intervention, definitive treatment was defined as day of surgery. For patients who received adjuvant chemotherapy, RFS was measured from the day of completion of adjuvant therapy. Patients were censored at the date of last follow-up or non-colorectal cancer–related death. Patients without clinical follow-up available were excluded from the study. Analysis was completed for patients with at least 1 year of follow-up and for the overall eligible cohort. The Kaplan–Meier method was used to describe survival outcomes. A log-rank test was used for HRs and all P values were based on two-sided testing with statistically significant differences at $P \leq 0.05$. Statistical analysis was performed using Graphpad PRISM version 8.0 for Windows, GraphPad Software.

Results

We evaluated the feasibility of tumor-uninformed MRD detection with a plasma-only MRD ctDNA assay in 103 patients with stage I–IV colorectal cancer undergoing curative-intent surgery. This assay (Guardant Reveal, Guardant Health) evaluates epigenomic signatures related to aberrant DNA methylation in addition to "standard" detection of tumor-derived genomic alterations employed by most MRD assays, without requiring parallel assessment of tumor tissue (**Fig. 1A**). Genomic alterations detected are filtered to remove variants of likely benign origin (e.g., CHIP). Overall, 84 patients were evaluable (**Fig. 1B**), and patient characteristics (9.5% stage I, 23.8% stage II, 47.6% stage III, and 19% stage IV) are shown (**Table 1**; Supplementary Tables S1 and S2). A total of 45% of patients received neoadjuvant therapy and 53.6% received adjuvant therapy. A total of 16 of 84 (19%) had surgery alone with no neoadjuvant or adjuvant therapy. Overall, 30 of 84 (35.7%) patients recurred with a median time to recurrence from surgery of 348.5 days (range, 35–887) and median time to recurrence from completion of definitive treatment of 211 days (range, 7–887). Blood was drawn a median of 30 days (range, 11–148) postoperatively and median of 32 days (range, 0–193) after completion

Table 1. Baseline patient and disease characteristics.

Characteristic	Overall cohort N = 84	%
Age (years) – median (range)	60 (35–84)	
Sex		
Female	33	39.3
Male	51	60.7
Stage at surgery		
I	8	9.5
II	20	23.8
III	40	47.6
IV	16	19.0
Sidedness		
Right	18	21.4
Transverse	5	6.0
Left	31	36.9
Rectal	30	35.7
Neoadjuvant treatment	38	45.2
Adjuvant treatment	46	54.8
Type of adjuvant treatment		
FOLFOX	31	67.4
CAPOX	7	15.2
FOLFOX + chemoxRT	3	6.5
5FU/LV	3	6.5
Other	2	4.3
Days on adjuvant treatment – median (range)	134.5 (28–463)	
Experienced disease recurrence	330	335.7
Days from surgery to recurrence – median (range)	348.5 (35–887)	
Days of clinical follow-up from surgery – median (range)	632.5 (33–1,246)	

of definitive therapy (**Table 1**). Four patients were excluded from the landmark analysis as they did not have an appropriate blood draw at after completion of definitive therapy. Of the 80 remaining patients, 10 patients were excluded from the primary analysis as after curative-intent specimens yielded extracted cfDNA that was of insufficient quantity or failed to pass sequencing quality control thresholds, leaving 70 (88% success rate) patients evaluable for the primary landmark analysis. Timing and receipt of neoadjuvant therapy, surgery, adjuvant therapy, and serial blood collections for each patient are shown (**Fig. 2**).

Primary "landmark" analysis

For the primary analysis, a single "landmark" plasma specimen drawn approximately 1 month after completion of definitive therapy (median, 31.5 days) was assessed, as early MRD detection is critical to enable therapeutic decisions during the standard window for adjuvant therapy initiation. Of 70 landmark evaluable patients, 17 of 70 (24%) patients had detectable ctDNA after completion of definitive therapy and 15 of 17 (88%) of these patients recurred (specificity 95.4%; Supplementary Fig. S1A; Supplementary Table S3). However, the 2 patients with detectable ctDNA but no recurrence both had clinical follow-up of less than 1 year. In patients with at least 1 year of clinical follow-up available at the data cutoff, 15 of 15 patients with detectable ctDNA (ctDNA-detected) recurred at a median of 162 days after definitive therapy [positive predictive value of recurrence of 100% (95% confidence interval, 78.2–100); HR, 11.28 ($P < 0.0001$)] (**Fig. 3A**). Of the 49 patients without detectable landmark ctDNA, 12 of 49 (24%) recurred (median time to recurrence, not reached). Sensitivity and specificity for recurrence was 55.6% (95% confidence interval, 35.3–74.5) and 100% (95% confidence interval, 90.5–100). Negative predictive value was 75.5% (95% confidence interval, 61.1–86.7). ctDNA detection predicted recurrence regardless of stage, neoadjuvant, or adjuvant therapy (Supplementary Table S4).

Longitudinal and surveillance ctDNA analyses

Prior studies have shown that sensitivity for recurrence prediction can be improved with longitudinal plasma monitoring. Overall, 9 of 14 (64%) of patients who recurred despite no detectable landmark ctDNA or who lacked landmark draws had at least one evaluable longitudinal specimen at a later timepoint (median, 3 per patient). Integrating longitudinal specimens increased sensitivity from 55.6% to 69.0% [HR, 12.26 ($P < 0.0001$)], with specificity remaining 100% (**Fig. 3B**; Supplementary Fig. S1B). Based on the methods employed by Reinert and colleagues (6), we assessed performance in patients with evaluable "surveillance" draws, defined as a draw obtained within 4 months of clinical recurrence, and observed that sensitivity improved to 91%.

Integration of genomic and epigenomic assessment of ctDNA

Because this is the first MRD assay to leverage ctDNA methylation analysis in addition to genomic alterations, compared with other ctDNA MRD assays that detect genomic alterations only, we assessed the relative contributions of integrating genomic and epigenomic signatures for ctDNA detection. ctDNA methylation and other epigenomic markers, such as ctDNA fragmentation, show promise in ctDNA-based early cancer detection approaches, which are tumor uninformed, and thus might also improve MRD detection (30–32). Across all ctDNA-positive specimens, 47% of samples were positive by both epigenomic and genomic calls, while 28% and 25%, respectively, were positive by either genomic or epigenomic calls alone (**Fig. 3C**). For the landmark analysis, using genomic calls alone, recurrence sensitivity would have been only 40.7%, and 48.2% using epigenomic calls alone. By integrating genomic and epigenomic calls, sensitivity improved to 55.6%. Similarly, in the longitudinal and surveillance analyses, respectively, sensitivity would have been only 55.2% and 72.7% with genomic calls alone or 48.3% and 63.6% with epigenomic calls alone, but sensitivity improved to 69.0% and 90.9% by integrating genomic and epigenomic calls. Thus, incorporating epigenomic calls with standard genomic calls increased relative MRD detection sensitivity by 25%–36% across analysis groups, suggesting ctDNA methylation may be an effective modality for MRD detection.

Analysis of CEA levels and recurrence

As a basis of comparison, we evaluated the ability of the standard-of-care colorectal cancer serum tumor marker CEA to predict recurrence in this same cohort. CEA values after definitive therapy were available for 56 patients at their landmark timepoint. Notably, CEA values failed to predict recurrence (**Fig. 4**; Supplementary Fig. S2).

Discussion

In this prospective cohort study, we report initial findings for one of the first plasma-only assays designed for tumor-uninformed ctDNA-based MRD detection, which demonstrated favorable sensitivity and specificity, comparable with previously reported tumor-informed approaches. This study also represents one of the first assessments of a ctDNA assay incorporating both genomic and epigenomic markers for MRD detection which also increased sensitivity.

One of the primary concerns with plasma-only assays is that specificity and sensitivity might be limited if the assay is not guided

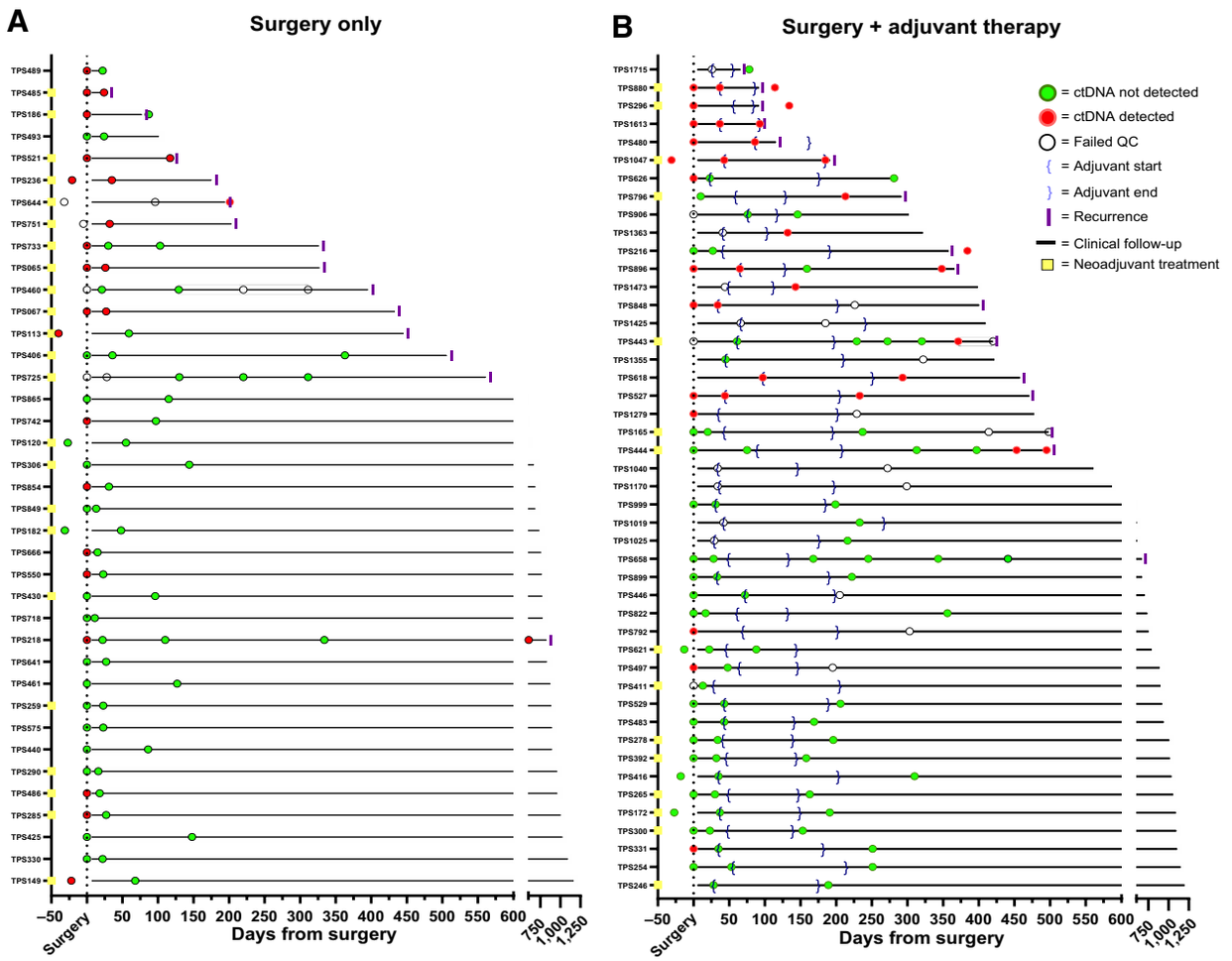

Figure 2.

ctDNA assay results and timing of treatment for each patient. Patients are ordered by total days of clinical follow-up from surgery for patients that received curative-intent surgery alone (**A**) and for patients that received curative-intent surgery followed by adjuvant therapy (**B**). Patients receiving neoadjuvant therapy are designated by a yellow square. Specimens obtained on the day of surgery were obtained preoperatively.

by specific alterations identified in the resected tumor. Notably, the loss of specificity is a critical concern, as noncancer-derived mutations are frequently present in the blood which could lead to false positives (24). In the current study, specificity was 100% in patients with at least 1-year minimum clinical follow-up, which aligns with specificity of other tumor-informed MRD approaches for colorectal cancer (6, 8). In the overall cohort, 2 patients with ctDNA detected had not yet recurred by the cut-off date. However, both patients had clinical follow-up of less than a year. Further analysis of larger cohorts is needed as high specificity of MRD detection will remain critical if MRD assays are to be used to select patients for additional or more intensive therapy. This would avoid situations in which patients who are cured are erroneously identified as MRD positive and subjected to potentially unnecessary therapy.

In addition, we found that sensitivity was comparable with the performance of many tumor-informed assays at the landmark timepoint. Landmark detection sensitivity is of central importance, as this assessment occurs within the time window in which treatment decisions are typically made. Previously reported "fixed panel" and "bespoke" tumor-informed MRD assays produced sensitivities of approximately 40%–50% when specifically assessing a single landmark timepoint obtained approximately 1-month after therapy (6, 9), which is comparable with the landmark sensitivity of 55.6% produced by this plasma-only tumor-uninformed assay. Furthermore, recent evidence has demonstrated that serial monitoring for recurrence can increase detection sensitivity. For example, Reinert and colleagues found that for patients with surveillance draws, sensitivity improved to 88% (6). Similarly, when evaluating patients with analogous surveillance draws in our cohort, defined as at least one draw within 4 months of clinical recurrence, we observed that sensitivity improved to 91%. Thus, these initial data suggest that this plasma-only assay can achieve performance characteristics comparable with currently approved tumor-informed approaches.

Importantly, we also evaluated whether assessment of epigenomic markers (specifically, changes in DNA methylation patterns) in ctDNA might increase the effectiveness for MRD detection. Indeed, methylation and other epigenomic markers, and DNA fragmentation patterns show promise in ctDNA-based early detection approaches, which are tumor uninformed, and thus may also help with MRD detection (30–34). Our data suggest that epigenomic methylation

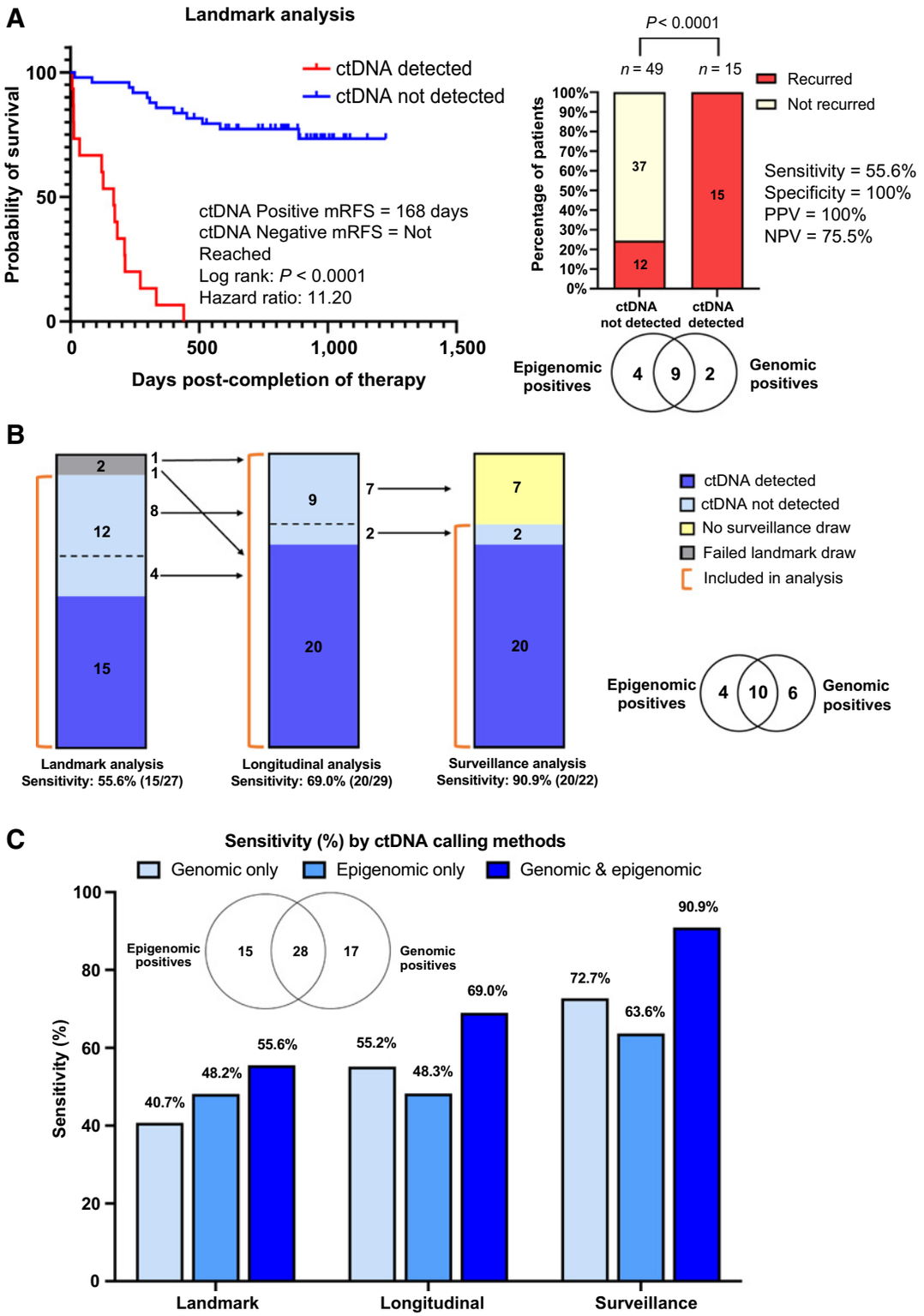

Figure 3.

A, RFS by landmark 1-month posttherapy ctDNA detection for patients with >1 year follow-up. Bar graph displays recurrence rates by ctDNA detection status with Venn diagram of ctDNA detection by calling methods (epigenomic only, genomic only, or both). **B,** Sensitivity analysis for landmark, longitudinal, and surveillance cohorts and Venn diagram of ctDNA detection for longitudinal and surveillance analyses by calling method. **C,** Recurrence sensitivity and Venn diagram of ctDNA detection by individual calling methods.

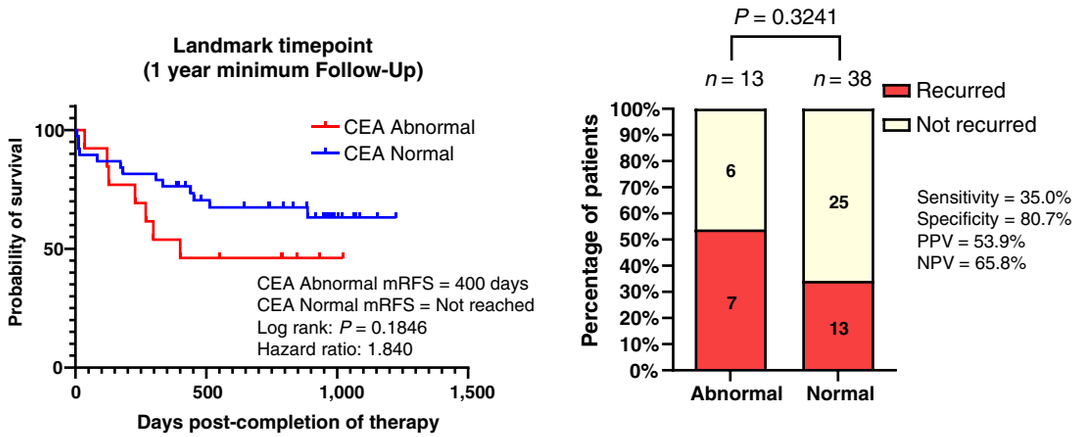

Figure 4.
RFS by landmark 1-month posttherapy CEA analysis for patients with >1 year follow-up. Bar graph displays recurrence rates by CEA status.

signatures in ctDNA can allow MRD detection with high specificity and may improve performance compared with detection of genomic calls alone, which is the method utilized by most MRD assays. Interestingly, we observed that while most cases showed ctDNA positivity by both genomic and epigenomic signatures, many cases were detected as positive by genomic or epigenomic calls alone (**Fig. 4A** and **B**), suggesting that integrating these two modalities may augment sensitivity for MRD detection. Specifically, integration of epigenomic signatures for landmark MRD detection increased sensitivity by a relative 36% compared with genomic alterations alone.

Overall, a plasma-only ctDNA assay for MRD detection may hold several advantages relative to tumor-informed approaches. These advantages include a more rapid turnaround time as only a single plasma draw is required, potential cost savings, and decreased logistical complexity. Moreover, a plasma-only assay would offer the potential for MRD detection even in situations where tumor tissue is not available or sufficient for use in tumor-informed ctDNA detection. Although sufficient tumor tissue is often available following a surgical procedure, some studies have suggested that in a subset of patients available tissue may be insufficient for molecular analysis, which would preclude the ability to utilize a tumor-informed approach for MRD detection (27). As neoadjuvant therapy becomes increasingly utilized as standard of care for many tumor types, a growing number of surgical specimens may yield insufficient tissue for molecular analysis after surgery, due to low tumor cellularity following a favorable treatment response (35). Thus, in some situations, a plasma-only ctDNA assay might offer the only means of assessing for MRD in certain situations.

While this study supports the potential of plasma-only ctDNA detection of MRD, this study also has several key limitations. First, the sample size is modest, and further evaluation in larger patient cohorts will be important to better define the assay performance characteristics. Second, while this study suggests the potential for effective MRD detection across multiple stages of colorectal cancer and across different treatment pathways (neoadjuvant and/or adjuvant therapy), a more focused and in-depth analysis will be important to understand the true performance in specific patient populations. Third, while this cohort provides a potentially valuable initial assessment the study design utilizing banked, isolated plasma samples, showed that in some cases the extracted ctDNA quantity or quality was below the recommended and optimal input levels for the assay. In particular, all of our samples had plasma input volumes of 4 mL or less, versus the recommended input of 8–10 mL, which may have affected overall performance characteristics. Finally, this study focused primarily on analysis of a single landmark timepoint and did not systematically incorporate serial longitudinal draws for all patients. While effective detection of MRD at a landmark timepoint early after completion of curative-intent therapy is clinically relevant, recent data suggest a potential value for serial monitoring during surveillance (6). Although incorporation of longitudinal and surveillance draws available for some patients did improve overall sensitivity from 55.6% to 69% and 91%, respectively, the lack of systematic longitudinal and surveillance draws across all patients precluded a comprehensive assessment.

In summary, we show that plasma-only, tumor-uninformed ctDNA-based detection of MRD is feasible and can produce comparable sensitivity and specificity to previously reported tumor-informed MRD approaches. These data also suggest that integration of epigenomic markers, such as DNA methylation analysis, may enhance detection sensitivity over standard genomic alteration detection methods alone and the integration of genomic and epigenomic assessment improves performance. The Guardant Reveal test is currently being utilized in several prospective clinical trials to assess the impact of ctDNA-guided adjuvant therapy (13, 17, 21). Ongoing prospective interventional studies will further evaluate the performance of this assay for MRD detection and to help guide treatment decisions.

Authors' Disclosures

A.R. Parikh reports personal fees from Foundation Medicine, Natera, Checkmate Pharmaceuticals, Eli Lilly, Pfizer, and Roche; and other from Puretech, PMV Pharma, BMS, Novartis, Plexxicon, Takeda, Macrogenics, C2I genomics outside the submitted work. E.E. van Seventer reports personal fees and other from Blueprint Medicines outside the submitted work. A.V. Hartwig reports being an employee and shareholder of Guardant Health. A. Jaimovich reports other from Guardant Health during the conduct of the study. Y. He reports being an employee and shareholder of Guardant Health. J.W. Franses reports personal fees from Foundation Medicine during the conduct of the study. L. Goyal reports receiving research funding (to institution) from Agios, Adaptimmune, Bayer, Eisai, Merck, Macrogenics, Genentech, Novartis, Incyte, Eli Lilly, Loxo Oncology, Relay Therapeutics, QED, Taiho Oncology, Leap Therapeutics, Bristol Meyers Squibb, and Nucana; scientific advisory committee (to self) from Agios Pharmaceuticals Inc, Alentis Therapeutics AG, H3Biomedicine, Incyte Corporation, QED Therapeutics, Sirtex Medical Ltd, and Taiho Oncology Inc.; consulting (to self) from Agios Pharmaceuticals Inc, Alentis Therapeutics, Genentech, Exelixis, Incyte Corporation, QED Therapeutics, Sirtex Medical Ltd, and Taiho Oncology Inc.; and DSMC (to self) from AstraZeneca. S.J. Klempner reports personal

fees from Merck, BMS, Eli Lilly, Astellas, Daiichi-Sankyo, Natera, Inc, and Pieris Oncology, and other from Turning Point Therapeutics outside the submitted work. E.J. Roeland has served as a consultant for Mitobridge Inc., Asahi Kasei Pharmaceuticals, DRG Consulting, Napo Pharmaceuticals, American Imaging Management, Immuneering Corporation, and Prime Oncology; additionally, E.J. Roeland has served on recent advisory boards for Heron Pharmaceuticals, Vector Oncology, and Helsinn Pharmaceuticals and has also served as a member on data safety monitoring boards for Oragenics, Inc, Galera Pharmaceuticals, and Enzychem Lifesciences Pharmaceutical Company. D.P. Ryan reports personal fees and other support from MPM; other support from Acworth Pharmaceuticals; personal fees from Iteos, Uptodate, McGraw Hill, and Boehringer Ingelheim; non-financial support from Exact Sciences; and grants and personal fees from SU2C during the conduct of the study; personal fees and other support from MPM; other support from Acworth Pharmaceuticals, Exact Sciences; personal fees from Iteos, Uptodate, McGraw Hill, and Boehringer Ingelheim, and grants and personal fees from SU2C outside the submitted work. T.S. Hong reports personal fees from Merck, Novocure, and Synthetic Biologics outside the submitted work. V.M. Raymond reports being an employee and shareholder of Guardant Health. A. Talasaz reports other support from Guardant Health, Inc. outside the submitted work. G.M. Boland reports grants from Takeda Oncology, Olink Proteomics, and Palleon Pharmaceuticals; grants and other from Novartis and Merck; and other from Nektar Therapeutics outside the submitted work. R.B. Corcoran reports personal fees from Abbvie, Pfizer, Astex Pharmaceuticals, Chugai, Elicio, Fog Pharma, Guardant Health, Ipsen, Mirati Therapeutics, Natera, Navire, Qiagen, Roivant, Shionogi, Tango Therapeutics, Taiho, and Zikani Therapeutics; grants and personal fees from Asana Biosciences and AstraZeneca; personal fees and other from Avidity Biosciences, C4 Therapeutics, Kinnate Biopharma, nRichDx, Remix Therapeutics, and Revolution Medicines; and other from Erasca outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

A.R. Parikh: Conceptualization, data curation, formal analysis, supervision, validation, investigation, methodology, writing–original draft, writing–review and editing. **E.E. Van Seventer:** Conceptualization, data curation, formal analysis, writing–original draft, writing–review and editing. **G. Siravegna:** Conceptualization, data curation, formal analysis, supervision, validation, investigation, writing–original draft, project administration, writing–review and editing. **A.V. Hartwig:** Data curation, formal analysis, writing–review and editing. **A. Jaimovich:** Data curation, formal analysis, writing–review and editing. **Y. He:** Data curation, formal analysis, writing–review and editing. **K. Kanter:** Data curation, formal analysis, writing–review and editing. **M.G. Fish:** Data curation, formal analysis, writing–review and editing. **K.D. Fosbenner:** Data curation, formal analysis, writing–review and editing. **B. Miao:** Data curation, formal analysis, writing–review and editing. **S. Phillips:** Data curation, formal analysis, writing–review and editing. **J.H. Carmichael:** Data curation, formal analysis, writing–review and editing. **N. Sharma:** Data curation, formal analysis, writing–review and editing. **J. Jarnagin:** Data curation, formal analysis, writing–review and editing. **I. Baiev:** Data curation, formal analysis, writing–review and editing. **Y.S. Shah:** Data curation, formal analysis, writing–review and editing. **I.J. Fetter:** Data curation, formal analysis, writing–review and editing. **H.A. Shahzade:** Data curation, formal analysis, writing–review and editing. **J.N. Allen:** Data curation, formal analysis, writing–review and editing. **L.S. Blaszkowsky:** Data curation, formal analysis, writing–review and editing. **J.W. Clark:** Data curation, formal analysis, writing–review and editing. **J.S. Dubois:** Data curation, formal analysis, writing–review and editing. **J.W. Franses:** Investigation, writing–review and editing. **B.J. Giantonio:** Data curation, formal analysis, writing–review and editing. **L. Goyal:** Data curation, formal analysis, writing–review and editing. **S.J. Klempner:** Data curation, formal analysis, writing–review and editing. **R.D. Nipp:** Data curation, formal analysis, writing–review and editing. **E.J. Roeland:** Data curation, formal analysis, writing–review and editing. **D.P. Ryan:** Data curation, formal analysis, writing–review and editing. **C.D. Weekes:** Data curation, formal analysis, writing–review and editing. **J.Y. Wo:** Data curation, formal analysis, writing–review and editing. **T.S. Hong:** Data curation, formal analysis, writing–review and editing. **L. Bordeianou:** Data curation, formal analysis, writing–review and editing. **C.R. Ferrone:** Data curation, formal analysis, writing–review and editing. **M. Qadan:** Data curation, formal analysis, writing–review and editing. **H. Kunitake:** Data curation, formal analysis, writing–review and editing. **D. Berger:** Data curation, formal analysis, writing–review and editing. **R. Ricciardi:** Data curation, formal analysis, writing–review and editing. **J.C. Cusack:** Data curation, formal analysis, writing–review and editing. **V.M. Raymond:** Conceptualization, data curation, formal analysis, supervision, validation, investigation, methodology, writing–original draft, project administration, writing–review and editing. **A. Talasaz:** Resources, data curation, formal analysis, supervision, project administration, writing–review and editing. **G.M. Boland:** Resources, data curation, formal analysis, supervision, investigation, methodology, writing–original draft, project administration, writing–review and editing. **R.B. Corcoran:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, writing–original draft, writing–review and editing.

Acknowledgments

This study was supported by a NIH/NCI Gastrointestinal Cancer SPORE (P50 CA127003, to R.B. Corcoran) and Stand Up To Cancer Colorectal Dream Team Translational Research Grant (SU2C-AACR-DT22-17, to R.B. Corcoran). Stand Up To Cancer is a division of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the Scientific Partner of SU2C. A.R. Parikh was supported by a Conquer Cancer Foundation of ASCO Career Development Award and an American Cancer Society Institutional Research Grant.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 1, 2021; revised March 23, 2021; accepted April 26, 2021; published first April 29, 2021.

References

1. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin 2020;70:145–64.
2. Chakrabarti S, Peterson CY, Sriram D, Mahipal A. Early stage colon cancer: Current treatment standards, evolving paradigms, and future directions. World J Gastrointest Oncol 2020;12:808–32.
3. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer 2017;17:223–38.
4. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
5. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol 2017;14:531–48.
6. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. JAMA Oncol 2019;5:1124–31.
7. Tarazona N, Gimeno-Valiente F, Gambardella V, Zuñiga S, Rentero-Garrido P, Huerta M, et al. Targeted next-generation sequencing of circulating-tumor DNA for tracking minimal residual disease in localized colon cancer. Ann Oncol 2019;30:1804–12.
8. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, et al. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. JAMA Oncol 2019;5:1710–7.
9. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, 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.
10. Tie J, Cohen JD, Lo SN, Wang Y, Li L, Christie M, et al. Prognostic significance of postsurgery circulating tumor DNA in nonmetastatic colorectal cancer: individual patient pooled analysis of three cohort studies. Int J Cancer 2021;148:1014–26.
11. Dasari A, Grothey A, Kopetz S. Circulating tumor DNA-defined minimal residual disease in solid tumors: opportunities to accelerate the development of adjuvant therapies. J Clin Oncol 2018;36:JCO2018789032.
12. Coakley M, Garcia-Murillas I, Turner NC. Molecular residual disease and adjuvant trial design in solid tumors. Clin Cancer Res 2019;25:6026–34.
13. U.S. National Library of Medicine. Circulating tumor DNA testing in predicting treatment for patients with stage IIA colon cancer after surgery. Available from: https://clinicaltrials.gov/ct2/show/NCT04068103.
14. Circulating tumor DNA based decision for adjuvant treatment in colon cancer stage II. Available from: https://clinicaltrials.gov/ct2/show/NCT04089631.

15. DYNAMIC-II: Circulating tumour DNA (ctDNA) analysis informing adjuvant chemotherapy in stage II colon cancer. Available from: https://trials.cancervic.org.au/details.aspx?ID=vctl_actrn12615000381583.
16. DYNAMIC-III: Circulating tumour DNA analysis informing adjuvant chemotherapy in stage III colon cancer: a multicentre phase II/III randomised controlled study. Available from: https://gicancer.org.au/clinical-trial/dynamic111/.
17. Lonardi S, Montagut C, Pietrantonio F, Elez E, Sartore-Bianchi A, Tarazona N, et al. The PEGASUS trial: Post-surgical liquid biopsy-guided treatment of stage III and high-risk stage. II colon cancer patients J Clin Oncol 38:15s, 2020 (suppl; abstr TPS4124).
18. BESPOKE study of ctDNA guided therapy in colorectal cancer. Available from: https://clinicaltrials.gov/ct2/show/NCT04264702.
19. Circulating tumor DNA analysis to optimize the operative and Postoperative Treatment for Patients With Colorectal Cancer - Intervention Trial 2. Available from: https://clinicaltrials.gov/ct2/show/NCT03637686.
20. Tracking mutations in cell free tumour DNA to predict relapse in early colorectal cancer. Available from: https://clinicaltrials.gov/ct2/show/NCT04050345.
21. Identification and treatment of micrometastatic disease in stage III colon cancer. Available from: https://clinicaltrials.gov/ct2/show/NCT03803553.
22. Schøler LV, Reinert T, Ørntoft M-BW, Kassentoft CG, Árnadóttir SS, Vang S, et al. Clinical implications of monitoring circulating tumor DNA in patients with colorectal cancer. Clin Cancer Res 2017;23:5437–5445.
23. Diehn M, Alizadeh AA, Adams H-P, Lee JJ, Klassen S, Palma JF, et al. Early prediction of clinical outcomes in resected stage II and III colorectal cancer (CRC) through deep sequencing of circulating tumor DNA (ctDNA). J Clin Oncol 35:15s, 2017 (suppl; abstr 3591).
24. Abbosh C, Swanton C, Birkbak NJ. Clonal haematopoiesis: a source of biological noise in cell-free DNA analyses. Ann Oncol 2019;30:358–9.
25. Gibson CJ, Steensma DP. New insights from studies of clonal hematopoiesis. Clin Cancer Res 2018;24:4633–42.
26. Hu Y, Ulrich BC, Supplee J, Kuang Y, Lizotte PH, Feeney NB, et al. False-positive plasma genotyping due to clonal hematopoiesis. Clin Cancer Res 2018;24:4437–43.
27. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017;23:703–13.
28. Nicholson BD, Shinkins B, Pathiraja I, Roberts NW, James TJ, Mallett S, et al. Blood CEA levels for detecting recurrent colorectal cancer. Cochrane Database Syst Rev 2015;2015:Cd011134.
29. Lanman RB, Mortimer SA, Zill OA, Sebisanovic D, Lopez R, Blau S, et al. Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS One 2015;10:e0140712.
30. Aravanis AM, Lee M, Klausner RD. Next-generation sequencing of circulating tumor DNA for early cancer detection. Cell 2017;168:571–4.
31. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature 2019;570:385–9.
32. Xie H, Mahoney DW, Foote PH, Burger K, Doering KA, Taylor WR, et al. Novel methylated DNA markers in plasma detect distant recurrence of colorectal cancer. J Clin Oncol 38:15s, 2020 (suppl; abstr 4088).
33. Taylor WC. Comment on 'Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA' by M. C. Liu et al. Ann Oncol 2020;31:1266–7.
34. Shen SY, Singhania R, Fehringer G, Chakravarthy A, Roehrl MHA, Chadwick D, et al. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. Nature 2018;563:579–83.
35. McDonald BR, Contente-Cuomo T, Sammut S-J, Odenheimer-Bergman A, Ernst B, Perdigones N, et al. Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. Sci Transl Med 2019;11:eaax7392.