original reports

Detection of Molecular Residual Disease Using Personalized Circulating Tumor DNA Assay in Patients With Colorectal Cancer Undergoing Resection of Metastases

Fotios Loupakis, MD, PhD[1]; Shruti Sharma, PhD[2]; Madiha Derouazi, PhD[3,4]; Sabina Murgioni, MD[1]; Paola Biason, PhD[1]; Mario Domenico Rizzato, MD[1]; Cosimo Rasola, MD[1,5]; Derrick Renner, BS[2]; Svetlana Shchegrova, PhD[2]; Allyson Koyen Malashevich, PhD[2]; Meenakshi Malhotra, PhD[2]; Himanshu Sethi, MPH[2]; Bernhard G. Zimmermann, PhD[2]; Alexey Aleshin, MD, MBA[2]; Solomon Moshkevich, MBA[2]; Paul R. Billings, MD, PhD[2]; Jonathon D. Sedgwick, PhD[3]; Marta Schirripa, MD[1]; Giada Munari, BD[1]; Umberto Cillo, MD[6]; Pierluigi Pilati, MD[7]; Angelo Paolo Dei Tos, MD[8]; Vittorina Zagonel, MD[1]; Sara Lonardi, MD[9,10]; and Matteo Fassan, MD, PhD[8,11]

abstract

PURPOSE More than 50% of patients with stage IV colorectal cancer (metastatic colorectal cancer [mCRC]) relapse postresection. The efficacy of postoperative systemic treatment is limited in this setting. Thus, these patients would greatly benefit from the use of a reliable prognostic biomarker, such as circulating tumor DNA (ctDNA) to identify minimal or molecular residual disease (MRD).

PATIENTS AND METHODS We analyzed a cohort of 112 patients with mCRC who had undergone metastatic resection with curative intent as part of the PREDATOR clinical trial. The study evaluated the prognostic value of ctDNA, correlating MRD status postsurgery with clinical outcomes by using a personalized and tumor-informed ctDNA assay (bespoke multiple PCR, next-generation sequencing assay). Postresection, systemic therapy was given to 39.2% of the patients at the discretion of the treating physician.

RESULTS Postsurgical, MRD positivity was observed in 54.4% (61 of 112) of patients, of which 96.7% (59 of 61) progressed at the time of data cutoff (hazard ratio [HR]: 5.8; 95% CI, 3.5 to 9.7; $P < .001$). MRD-positive status was also associated with an inferior overall survival: HR: 16.0; 95% CI, 3.9 to 68.0; $P < .001$. At the time of analyses, 96% (49 of 51) of patients were alive in the MRD-negative arm compared with 52.4% (32 of 61) in the MRD-positive arm. Patients who did not receive systemic therapy and were MRD-negative in the combined ctDNA analysis at two time points had an overall survival of 100%. In the multivariate analysis, ctDNA-based MRD status was the most significant prognostic factor associated with disease-free survival (HR: 5.78; 95% CI, 3.34 to 10.0; $P < .001$).

CONCLUSION This study confirms that in mCRC undergoing resection of metastases, postoperative MRD analysis is a strong prognostic biomarker. It holds promises for being implemented in clinical decision making, informing clinical trial design, and further translational research.

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cancer and the second-leading cause of cancer-related death in the United States.[1,2] Approximately 15%-25% of patients present with metastatic disease upon diagnosis,[3] and approximately 50% of patients with early-stage disease develop metastases.[4] Despite progressive improvements in therapeutic algorithms and molecular characterization over the past 15 years, the 5-year survival rate for metastatic CRC (mCRC) is below 20%.[2,5,6]

Although surgery with curative intent is a key option for selected cases, only a minority of patients achieve cure or long-term survival benefit. Reports have indicated that 17%-25% of patients with oligometastatic disease confined to a single organ (eg, liver) can attain cure, provided that the patient undergoes radical metastasectomy.[7] However, the value of adding postoperative adjuvant chemotherapy (ACT)[8] and/or targeted therapy[9] remains a matter of debate and active investigation.[10] Thus, surgery is the current recommended course of treatment, provided that it is technically feasible.[11] Notwithstanding, approximately 50% of patients relapse postresection.[4,12] When an R0 resection is achieved, relapse is due to the presence of postsurgical minimal or molecular residual disease (MRD).

CONTEXT

Key Objective

To examine the prognostic value of circulating tumor DNA (ctDNA) testing using a personalized multiplex PCR (mPCR) next-generation sequencing (NGS) assay (Signatera) for detecting minimal or molecular residual disease (MRD) and disease progression in the postsurgical setting for patients with metastatic colorectal cancer.

Knowledge Generated

Our data indicate that postsurgical, MRD-positive status as assessed by personalized mPCR NGS–based ctDNA assay predicts disease progression and reduced overall survival in patients with metastatic colorectal cancer and is the most significant prognostic factor when compared with other clinicopathologic factors. Our data also provide a compelling evidence on the superior performance of personalized mPCR NGS–based ctDNA assay over carcinoembryonic antigen and digital droplet PCR.

Relevance

Our study supports the potential of introducing ctDNA-based MRD testing into the design of clinical studies and in the standard of care.

During the postsurgical surveillance period, the current standard of care involves routine patient checkups, periodic computed tomography scans, and monitoring of carcinoembryonic antigen (CEA) levels.[11] However, each of these approaches have extensive limitations. Computed tomography imaging can only detect overt lesions and have shown limited sensitivity in detecting recurrent metastatic disease.[13] Similarly, reports have indicated limited clinical utility of CEA,[14] with limited sensitivity to detect recurrence (68%-82%).[15] Furthermore, CEA levels can paradoxically increase in response to chemotherapy.[16] Thus, better prognostic biomarkers are urgently needed for patients with oligometastatic CRC, to help improve patient outcomes, especially in the postresection setting.

Several studies have indicated the clinical utility of circulating tumor DNA (ctDNA) for MRD assessment, monitoring recurrence, and treatment response in patients with CRC.[17-19] Among a range of assays available,[20] digital droplet PCR (ddPCR) has been widely used to detect mutations in ctDNA.[21] However, in patients with mCRC, ddPCR is only applicable to patients who harbor the specific mutation in the tumor (eg, *KRAS* in most of the cases), making this approach less sensitive. Moreover, studies have shown *KRAS* mutation to occur in approximately 50% of patients with mCRC only.[22] Thus, studies that evaluate the clinical utility of ctDNA as a prognostic biomarker in patients with mCRC for MRD detection and predicting disease progression have been limited.

Here, we investigate the clinical validity of ctDNA testing by using a personalized and a tumor-informed multiplex PCR (mPCR), next-generation sequencing (NGS) assay (Signatera, bespoke mPCR-NGS) for MRD detection at the postoperative time point. This study evaluates the association of ctDNA-based MRD status with disease-free survival (DFS) in patients with mCRC. We also measured CEA and compared its performance with ctDNA-based MRD

detection, in predicting disease progression. Finally, we compared two orthogonal ctDNA detection methodologies (personalized mPCR-NGS assay *v KRAS* specific ddPCR) across samples, to identify the optimal method of ctDNA detection.

PATIENTS AND METHODS

A total of 136 patients were enrolled as part of the prospective PREDATOR study conducted at Istituto Oncologico Veneto, IRCCS, Padua, Italy, in collaboration with the Department of Medicine, University of Padua, Italy. All patients provided the informed consent. The study was granted Ethics Approval by Local Authorities and was conducted in accordance with the Declaration of Helsinki (CESC Istituto Oncologico Veneto ref no. 2018/66). The study involved collection of clinical and pathologic data and plasma samples at prespecified time points from patients with mCRC who underwent resection of metastases with curative intent, referred to the Istituto Oncologico Veneto. The primary objective was to measure the DFS from time of surgery to the first radiologic evidence of disease progression, and secondary end points included overall survival (OS) and translational analyses. All patients received treatment and follow-up in compliance with the standard clinical practice in this setting, according to investigator's choice. The ctDNA statistical analysis plan was developed before unblinding of the clinical data and followed for the analysis. The data assessors were blinded to patient outcome and sample order. Neither treating clinicians nor patients were informed about the ctDNA results.

Personalized mPCR-Based NGS Assay for ctDNA Detection

As previously described,[19] whole-exome sequencing was performed on formalin fixed and paraffin embedded tumor tissue along with matched normal blood samples. On analyzing the sequencing results, a set of 16 patient-

specific somatic clonal single nucleotide variants (SNVs) were selected for mPCR testing. The mPCR primers targeting the personalized SNVs were designed and synthesized to be subsequently used to identify and track ctDNA in a patient's plasma. For this, blood samples (20 mL) were collected from patients at predetermined time points and cell-free DNA (cfDNA) (median: 8.8 ng/mL; range: 2.3-397.9) per mL of plasma was extracted. Sequencing runs that were flagged for low coverage were resequenced after Clinical Laboratory Improvement Amendments process update (preparing or washing sequencers). Plasma samples with ≥ 2 SNVs detected above a predefined confidence threshold were deemed ctDNA-positive, and ctDNA concentration was reported as mean tumor molecules per mL of plasma.

Digital Droplet PCR

ddPCR analysis was performed at the Unit of Surgical Pathology, University of Padua, Italy. Plasma was isolated from whole blood with two subsequent centrifugations, first at $1,600 \times g$ for 10 minutes and the other on the supernatant at $3,000 \times g$ for 10 minutes, and was stored at –80°C until further analysis. DNA (median: 4.0 ng/μL; range: 0.8-7.2 ng/μL) was extracted from plasma using the Magcore Super automated nucleic acid extractor (Diatech, Jesi, Italy) according to the manufacturer's protocol.

Samples were prepared for ddPCR (QX200 ddPCR system; Bio-Rad, Berkeley, CA) using specific ddPCR Supermix with no dUTTP for probes (Bio-Rad) and probes supplied by the ddPCR KRAS Screening Multiplex Kit (Bio-Rad) that covered seven specific *KRAS* mutations: G12A (dHsaCP2500586), G12C (dHsaCP2500584), G12D (dHsaCP2500596), G12R (dHsaCP2500590), G12S (dHsaCP2500588), G12V (dHsaCP2500592), and G13D (dHsaCP2500598). QX200 droplet generator and C1000 Touch Thermo Cycler (Bio-Rad) were used for DNA amplification with the following protocol: 95°C for 10 minutes followed by 40 cycles of 94°C for 30 seconds and 55°C for 1 minute, and then 98°C for 10 minutes. Droplets were read in the QX200 droplet reader (Bio-Rad) and analyzed using the Quantasoft software version 1.0.596 (Bio-Rad). Among others, the software reports the value of fractional abundance calculated as the ratio of drops positive for the mutant allele to drops positive for both mutant allele and wild-type allele (percentage of mutant *KRAS* alleles). The sensitivity cutoff for the ctDNA detection assay was set at the lower limit of 0.02% mutant alleles.

Statistical Analysis

Statistical significance was assessed using Fisher's exact test for categorical variables. Survival analyses were performed using the Kaplan-Meier Estimator and the Cox method. These analyses were carried out in R-3.6.1 using packages survminer, survival, and coxphf.[23] The primary outcome measure was DFS assessed between the date of metastases resection and the date of the first evidence of progressive disease, as defined by RECIST criteria.[24] A multivariable Cox proportional hazards model was used to assess the most significant prognostic factor associated with DFS. The exploratory analysis evaluated the association of post-surgical ctDNA with OS and combined ctDNA analysis at two time points with DFS. All *P* values were based on two-sided testing, and differences were considered significant at $P \leq .05$.
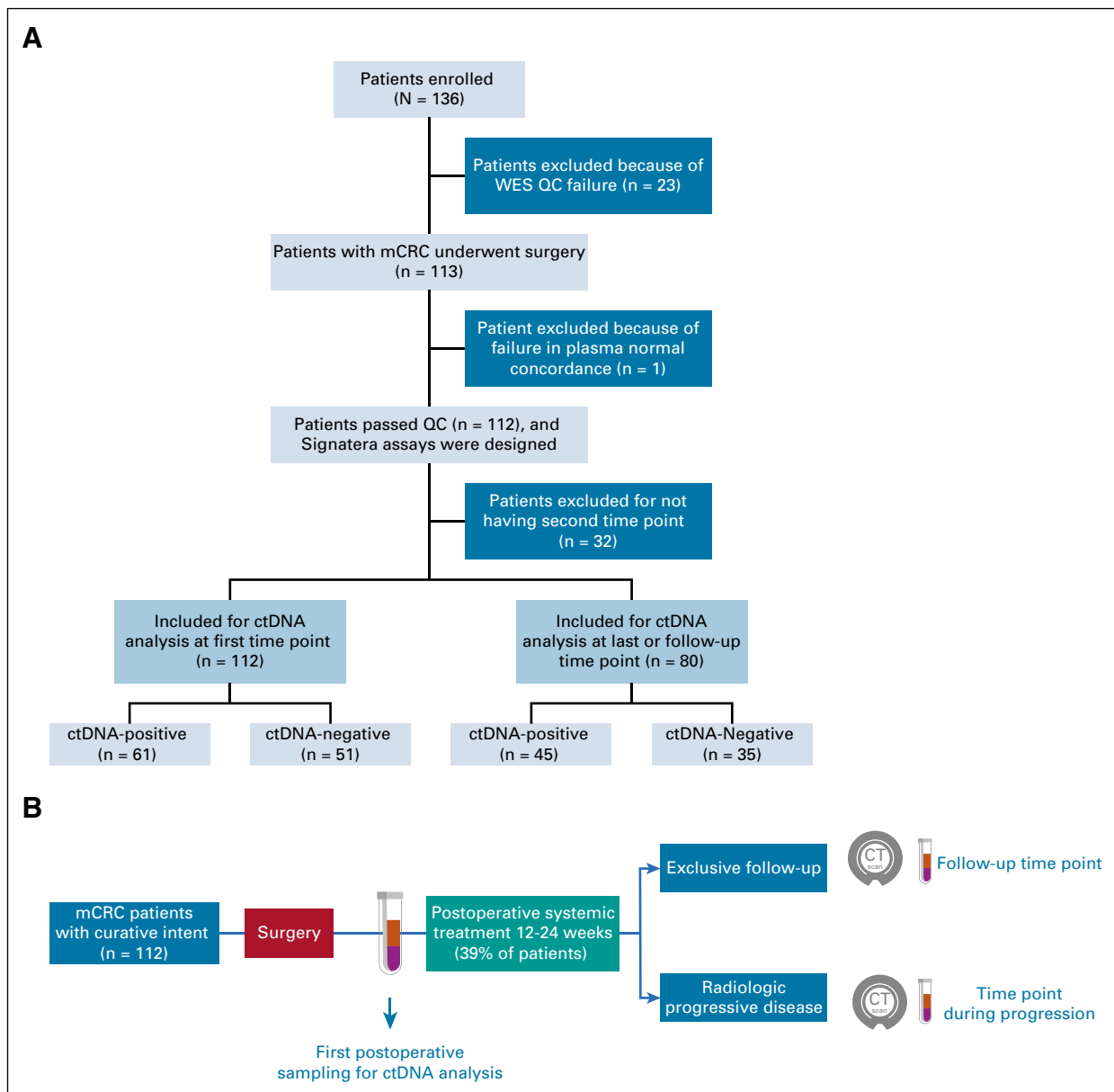
RESULTS

ctDNA analysis was performed on 192 plasma samples from 112 patients with a median follow-up of 10.7 months (range: 0.9-53.8 months), of which 73.2% (82 of 112) experienced disease progression. Plasma samples were collected at the first time point and at the time of radiologic evidence of progressive disease or last follow-up (Fig 1). Of 112 patients, 55 (49%) received preoperative treatment (doublet with or without biologic, FP monotherapy with or without biologic, and triplet with or without biologic) and 44 (39%) patients received postoperative treatment (doublet with or without biologic, FP monotherapy, and triplet with or without biologic), postresection. The median age of patients at diagnosis was 60.1 years. Of the 112 patients analyzed, 63 (56.2%) presented with synchronous tumors, with liver (n = 65/112; 58.0%) being the most common site of metastasis, followed by lung (n = 22/112; 19.6%), peritoneum (n = 16/112; 14.3%), and others (n = 9/112; 8.0%). Data on postsurgery clinical intervention and other clinicopathologic information were collected for all patients (Table 1).

Postoperative ctDNA Status Predicts Patient Outcomes

Plasma collected after surgery (median: 27 days; range: 8-99.5 days), before the start of ACT, was available for 112 patients. Of these, 61 (54.5%) were MRD-positive and 51 (45.5%) were MRD-negative. At first time point, the assay showed a sensitivity of 72% (59 of 82; Fig 2A), a specificity of 93.3% (28 of 30), and a positive predictive value of 96.7% (59 of 61). For MRD-positive patients that progressed, the median lead time was 3.16 months (range: 0.07-37.9 months). Of the two patients, that were ctDNA-positive and did not progress, one of them received ACT. MRD positivity was associated with a marked reduction in DFS, as compared with MRD-negative patients (HR: 5.8; 95% CI, 3.5 to 9.7; $P < .001$; Fig 2B). The MRD-positive status was also associated with an inferior OS (HR: 16.0; 95% CI, 3.9 to 68.0, $P < .001$; Fig 2C). At the time of data cutoff, 96% (49 of 51) of patients in the MRD-negative arm were alive compared with 52.4% (32 of 61) in the MRD-positive arm. In the multivariate analysis, ctDNA-based MRD status was the most significant prognostic factor associated with DFS when compared with other clinicopathologic factors (HR: 5.78, 95% CI, 3.34 to 10.0; $P < .001$; Fig 3).

Combined ctDNA Analysis at Two Time Points Is a Strong Predictor of Patient Outcomes

An exploratory combined ctDNA analysis at two time points (ie, baseline plus last follow-up time point–either the time of

FIG 1. CONSORT diagram and study design. (A) Flowchart showing patient enrollment, sample collection, and ctDNA analysis at the first postsurgical time point and at last or follow-up time point. ctDNA results (positive and negative) and number of patients who experienced disease progression are indicated. (B) Study schema showing timing of ctDNA sampling and CT imaging. Tumor tissue was available from 112 patients, who underwent colorectal cancer metastases resection with curative intent. CT, computed tomography; ctDNA, circulating tumor DNA; mCRC, metastatic colorectal cancer; QC, quality check; WES: whole exome sequencing.

radiologic progression or last evidence of radiologic disease's absence) was performed on 80 patients. Patients who were ctDNA-positive at both or turned positive at the second time point were categorized in the ctDNA-positive arm (n = 45), whereas patients who were ctDNA-negative at both or turned negative at the second time point were categorized in the ctDNA-negative arm (n = 35). All except one patient in the ctDNA-positive arm, 97.7% (44 of 45), experienced disease progression. Since in this cohort, a subset of patients received systemic therapy postsurgery (n = 30/80), which may affect

the performance of the assay for ctDNA assessment, we separately analyzed patients (n = 50) who did not receive systemic therapy. Of these 50 patients, a total of 35 patients progressed, of which 32 were ctDNA-positive, showing a sensitivity of 91.4% (Fig 2) and a specificity of 93.3% (14 of 15). This analysis showed that ctDNA-positive patients, not treated with systemic therapy, had a markedly reduced DFS (HR: 15.0; 95% CI, 4.3 to 49; $P <$ .001; Fig 4A). Furthermore, ctDNA-negative patients had an exceptional outcome with an OS of 100% with a 50-month follow-up (Fig 4B).

TABLE 1. Patient Demographics and Baseline Characteristics

Patient Characteristic	All Patients (N = 112), No. (%)
Sex	
Male	72 (64.2)
Female	40 (35.7)
Presentation of metastasis	
Synchronous	63 (56.2)
Metachronous	49 (43.7)
Event	
Progressive disease	82 (73.2)
Median age at diagnosis of metastatic disease, median (range), years	60.1 (22.1-83.3)
Site of metastasis	
Liver	65 (58.0)
Lung	22 (19.6)
Peritoneum	16 (14.3)
Others	9 (8.0)
Location of primary tumor	
Right	30 (26.8)
Left	82 (73.2)
Presurgical treatment	
Doublet with or without biologic, FP monotherapy with or without biologic, and triplet with or without biologic	55 (49.1)
Postsurgical systemic treatment	
Doublet with or without biologic, FP monotherapy, and triplet with or without biologic	44 (39.2)
CEA status	
Preoperative (n = 54)	
CEA-positive	41 (75.9)
CEA-negative	13 (24)
At the time of progression (n = 42)	
CEA-positive	25 (59.5)
CEA-negative	17 (40.4)

Abbreviations: CEA, carcinoembryonic antigen; FP, fluoropyrimidine.

ctDNA Assessment Using ddPCR Versus Personalized mPCR-NGS Methodology

Since ddPCR technology is used to detect a specific mutation in ctDNA, we selected a subset of patients (n = 27) with *KRAS* mutations for ddPCR analysis (Bio-rad commercial kits) and the results were compared with personalized mPCR-NGS technology at the postoperative time point. Our results indicate concordance in 55.5% (15 of 27) of patients and discordance in 44.5% (12 of 27) of patients (Fig 5A). Interestingly, all the discordant results were of the type where personalized mPCR-NGS was positive and ddPCR was negative, and 91.6% (11 of 12) of these patients developed disease progression. This suggests greater sensitivity of the personalized mPCR-NGS assay over ddPCR in accurately identifying patients with disease progression, even among patients whose tumors showed evidence of containing the *RAS* mutations of interest.

Comparison of CEA With ctDNA

In a subset of patients (n = 55) that had both ctDNA and CEA results available postoperatively, we compared CEA with ctDNA-based MRD status. As shown in Figure 5B, CEA was not predictive for DFS (HR: 1.5; 95% CI, 0.83 to 2.7; $P = .18$), whereas ctDNA testing using the personalized mPCR-NGS assay was significantly correlated with disease progression. Particularly, MRD-positive patients at the postsurgical time point had a markedly reduced DFS compared with MRD-negative patients (HR: 6.4; 95% CI, 3.0 to 13.0; $P < .001$; Fig 5C).

For patients who eventually progressed (n = 13), we compared the performance of the three assays: personalized mPCR-NGS, ddPCR, and CEA. We found that MRD assessment using the personalized mPCR-NGS was most sensitive in identifying recurrence with a sensitivity of 84.6% (11 of 13), whereas CEA and ddPCR showed the
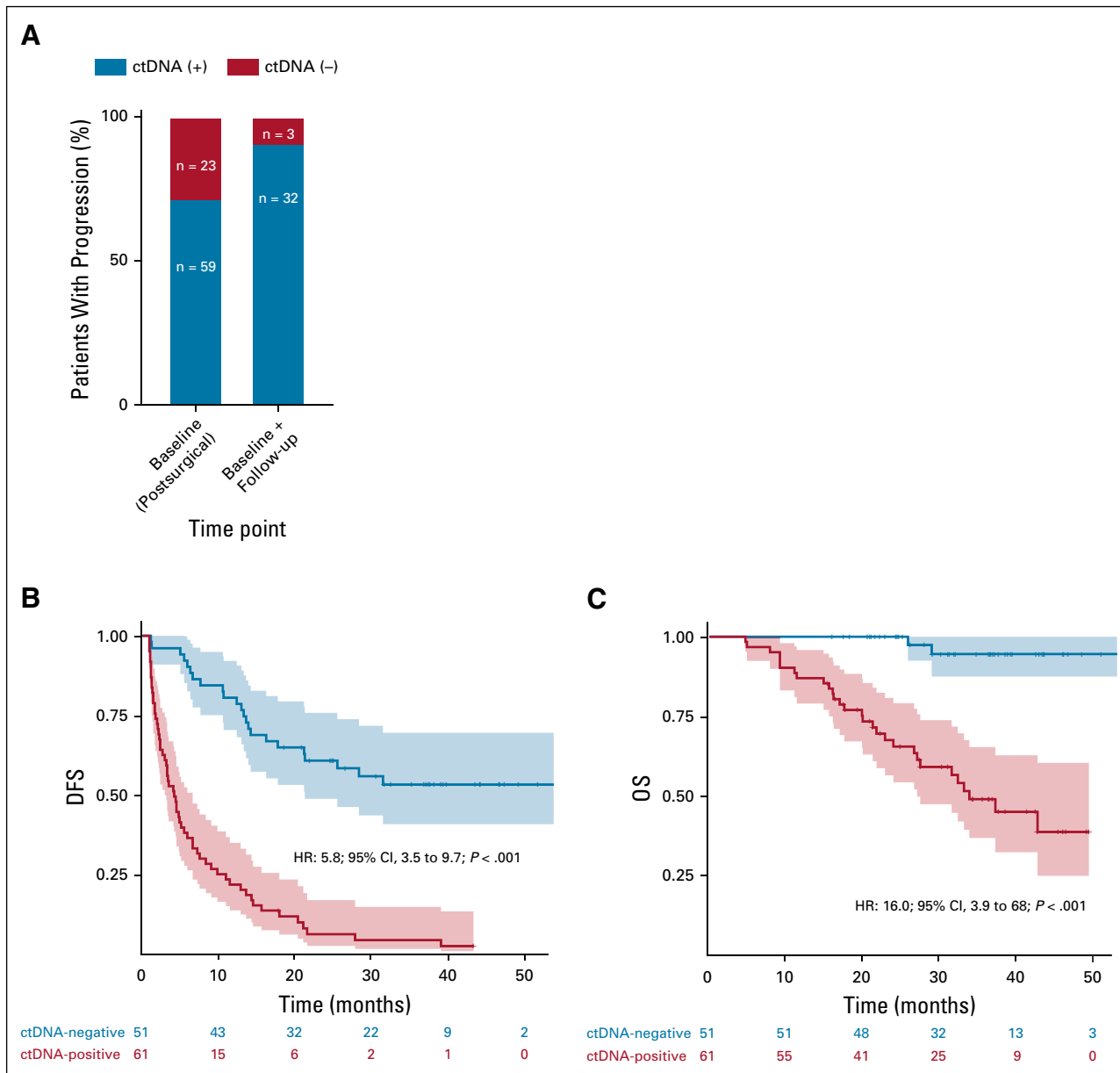
FIG 2. Association of postsurgical ctDNA status with disease progression and survival outcomes. (A) ctDNA status of percentage of patients with disease progression was assessed at a single postsurgical time point (baseline) and in combination of two time points (baseline + follow-up) in patients not treated with systemic therapy. (B and C) Kaplan-Meier estimates for 112 patients with metastatic colorectal cancer stratified by molecular residual disease status at postsurgical time point: DFS and OS. Shaded areas in the Kaplan-Meier plots indicate 95% CIs. ctDNA, circulating tumor DNA; DFS, disease-free survival; HR, hazard ratio; OS, overall survival.

sensitivity of 46% (6 of 13) and 38.4% (5 of 13), respectively (Fig 5D).

DISCUSSION

In the metastatic setting, ctDNA levels have shown to correlate with the resection radicality, suggesting ctDNA as a biomarker to detect MRD and facilitate with early detection of disease progression, thereby enabling a more informed or appropriate treatment decision.[25,26] Although studies centered on the postsurgical metastatic setting have been limited, a study by Overman et al[27] examined postliver hepatectomy mCRC cases and reported the sensitivity and specificity of 58% (95% CI, 41 to 74) and 100% (95% CI, 66 to 100), respectively. This study used a digital sequencing panel–based ctDNA assay for detecting residual disease in patients with a minimum follow-up of 1 year and showed correlation of ctDNA status with patient outcomes, as measured by RFS (HR: 3.1; 95% CI, 1.7 to 9.1; P = .002).[27] Similar to these findings, our study provides evidence on the prognostic value of ctDNA (mPCR-NGS assay) by identifying 96.7% (59 of 61) of MRD-positive

FIG 3. Disease-free survival by clinicopathologic variables and postsurgical ctDNA status: multivariate analysis of prognostic factors (ctDNA status, age, sex, site of metastasis, margins, postoperative treatment, and preoperative treatment) and their association with disease-free survival, as indicated by HR, analyzed across the cohort. ctDNA was the only significant prognostic factor. ***$P < .001$. AIC, akaike information criterion ctDNA, circulating tumor DNA; HR, hazard ratio.
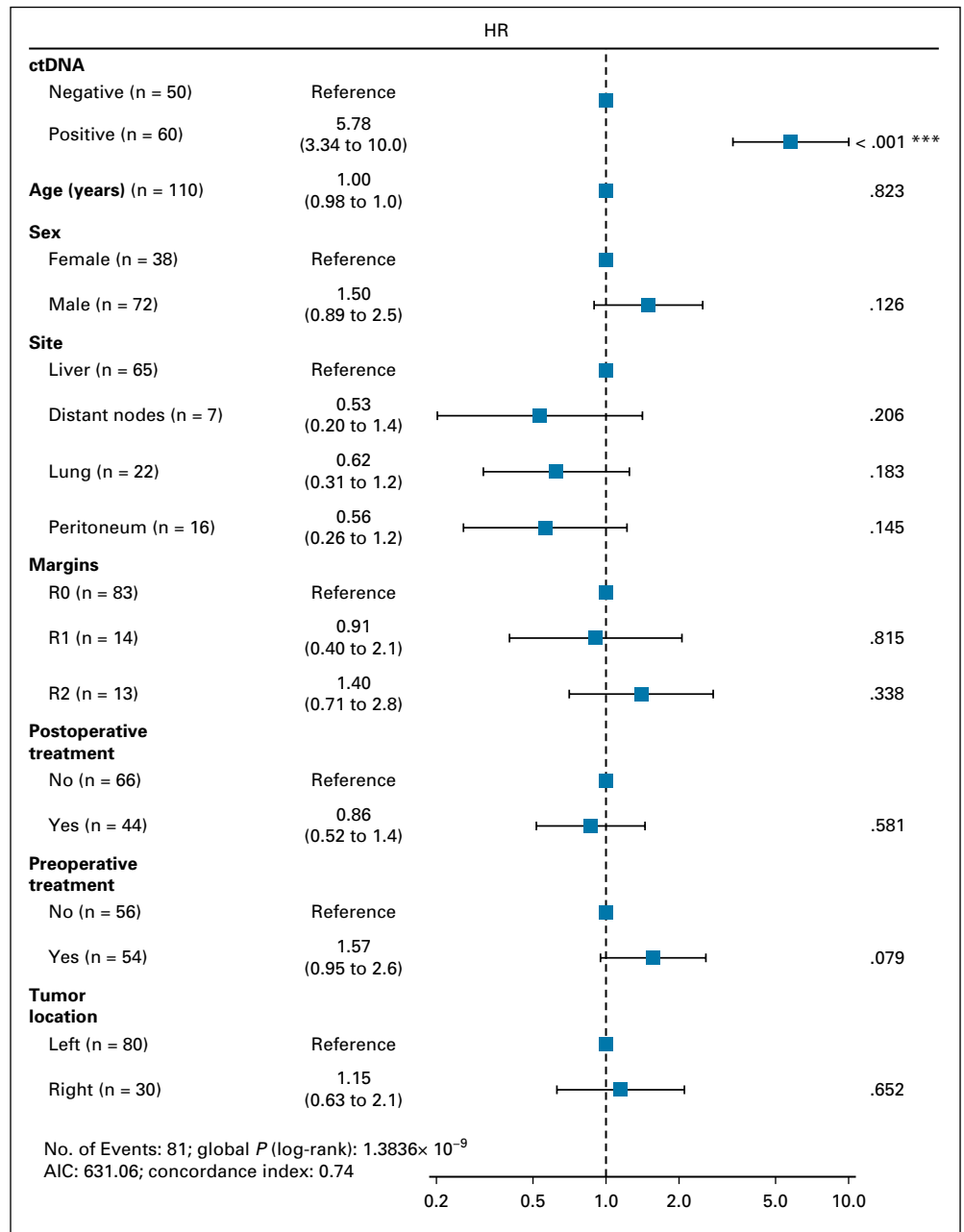
mCRC patients who experienced disease progression. Furthermore, postsurgical MRD-positive status was significantly correlated with reduced DFS (HR: 5.8; $P < .0001$) and OS (HR: 16.0; $P < .001$; Figs 2B and 2C). Taken together, these findings may provide support for clinical decision making for perioperative systemic treatment, including a rationale for a more aggressive follow-up.

In the present study, we also compared the use of personalized mPCR-NGS technology with ddPCR for ctDNA detection and to predict disease progression among KRAS-mutant patients (n = 14). Our results indicate higher sensitivity of ctDNA detection with the personalized mPCR-NGS assay (84.6%) versus ddPCR (38.4%; Fig 5D). Of

note, the design of Natera's mPCR-NGS assay considers clonal heterogeneity, which is a common characteristic of mCRC.[28,29] By contrast, orthogonal ctDNA detection tests such as ddPCR are designed to detect fewer tumor-specific SNVs at a time, providing less coverage for tumor heterogeneity.[29,30] Thus, these differences may explain lower sensitivity achieved with ddPCR. Although these results are encouraging and establish the superior performance of mPCR-NGS–based ctDNA assay over ddPCR and CEA, a validation of this comparison with a larger sample set is warranted.

To date, CEA has been the highly studied biomarker for CRC, although its prognostic value remains unclear. Here,
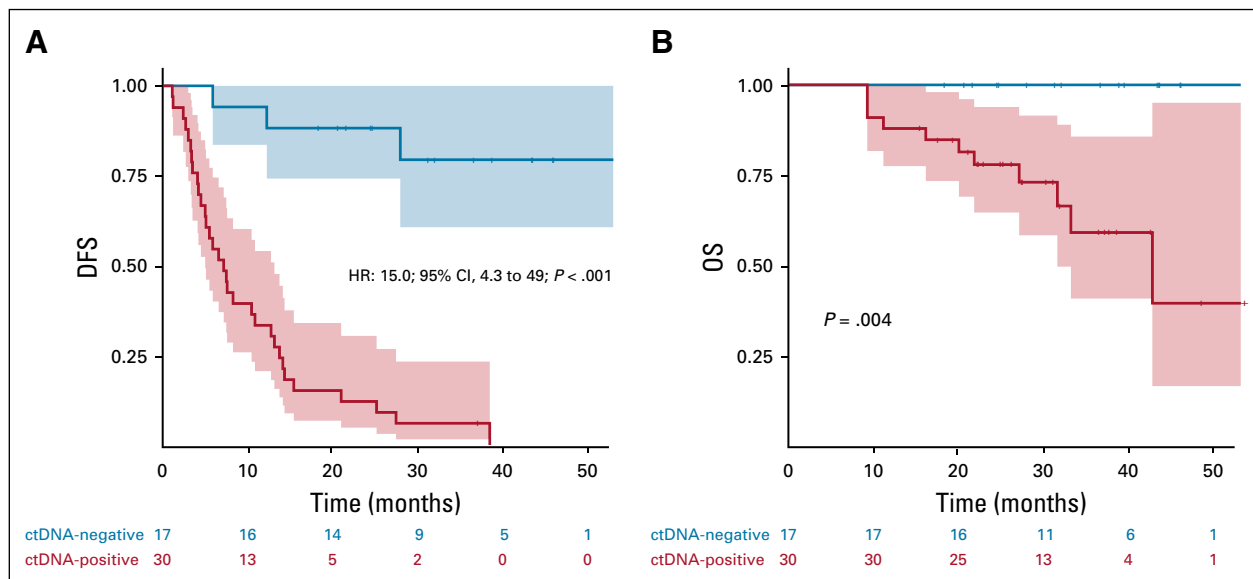
FIG 4. Survival outcomes on the basis of combined ctDNA analysis at two time points in patients not treated with systemic therapy. Kaplan-Meier estimates for 50 patients with metastatic colorectal cancer stratified by ctDNA status representing (A) DFS and (B) OS. Shaded areas in the Kaplan-Meier plots indicate 95% CIs. ctDNA, circulating tumor DNA; DFS, disease-free survival; HR, hazard ratio; OS, overall survival.

we show that within the same set of patients, the sensitivity of CEA in identifying patients with disease progression was only 46%, which was lower than personalized mPCR-NGS ctDNA assay but higher than ddPCR-ctDNA assay. Several studies have indicated the unreliability of CEA as a biomarker for CRC, with limited prognostic value.[14-16,31,32] By contrast, ctDNA has emerged as a promising cancer biomarker, which can detect MRD, therapeutic response to treatment, and recurrent disease with high sensitivity and specificity across cancers, including CRC.[19,33-37]

Limitations of the present study include a relatively small sample size and the retrospective design with the use of archived samples, because of which we observed a high degree of necrosis and low tumor cellularity in the tissue samples, resulting in a WES QC failure rate of 16.9%. This was because some patients received systemic treatment before liver or met resection, resulting in deteriorated sample quality. However, in real-world, prospective studies with analysis performed on primary or untreated samples, we have observed a WES QC failure rate of < 3%, which is compatible with clinical practice.[38] Another limitation of our study is that we only tested two time points (postsurgical) in this setting. Our future studies will include monitoring of ctDNA dynamics using serial testing at regular intervals within a subgroup of patients from the present analysis. Overall, we believe that the use of serial testing can allow for tailoring of treatment regimens, with treatment escalation in patients with progressive disease and the opportunity of early therapeutic interventions with more aggressive follow-up in patients who are ctDNA-positive but have not yet progressed. Additionally, previous studies have shown ctDNA clearance to be a proxy of treatment efficacy,

especially in the adjuvant setting where postoperative ctDNA negativity shows better outcomes compared with ctDNA-positive patients.[39] We acknowledge the limitation of our small data set to establish this evidence. However, we do see a clear pattern, wherein 100% of the patients who remain positive or turn positive at the second time point progress as compared with the ctDNA-negative patients.

Another interesting finding was the number of patients who progressed despite being MRD-negative at first time point (28%; 23 of 82) and later time point (8.6%; 3 of 35, ie, no systemic therapy; Fig 2A). Here, we speculate that the undetectable ctDNA levels, especially at the first time point, could be due to the surgery-induced increased cfDNA levels. Henriksen et al[40] showed that increased cfDNA levels observed within 4 weeks postsurgery could confound the detection of ctDNA in patients with CRC and bladder cancer. The study suggested a repeat ctDNA testing after 4 weeks in patients who were initially ctDNA-negative. Thus, our study supports the possibility of extending the period of MRD testing to 4-6 weeks postsurgery and implementing serial testing at regular intervals for monitoring disease progression. Other reasons for undetectable ctDNA could be low levels of ctDNA present in the patient after removal of the primary tumor (MRD time point) and indolent disease resulting in delayed time to progression or different anatomical site of progression. Our analysis showed that the median time to progression for ctDNA-negative patients on the basis of the first time point was significantly longer (P = .0002), that is, 12.8 months versus 4 months in ctDNA-positive patients.

Our work also suggests that clinical trials centered on patients with mCRC could benefit from the implementation
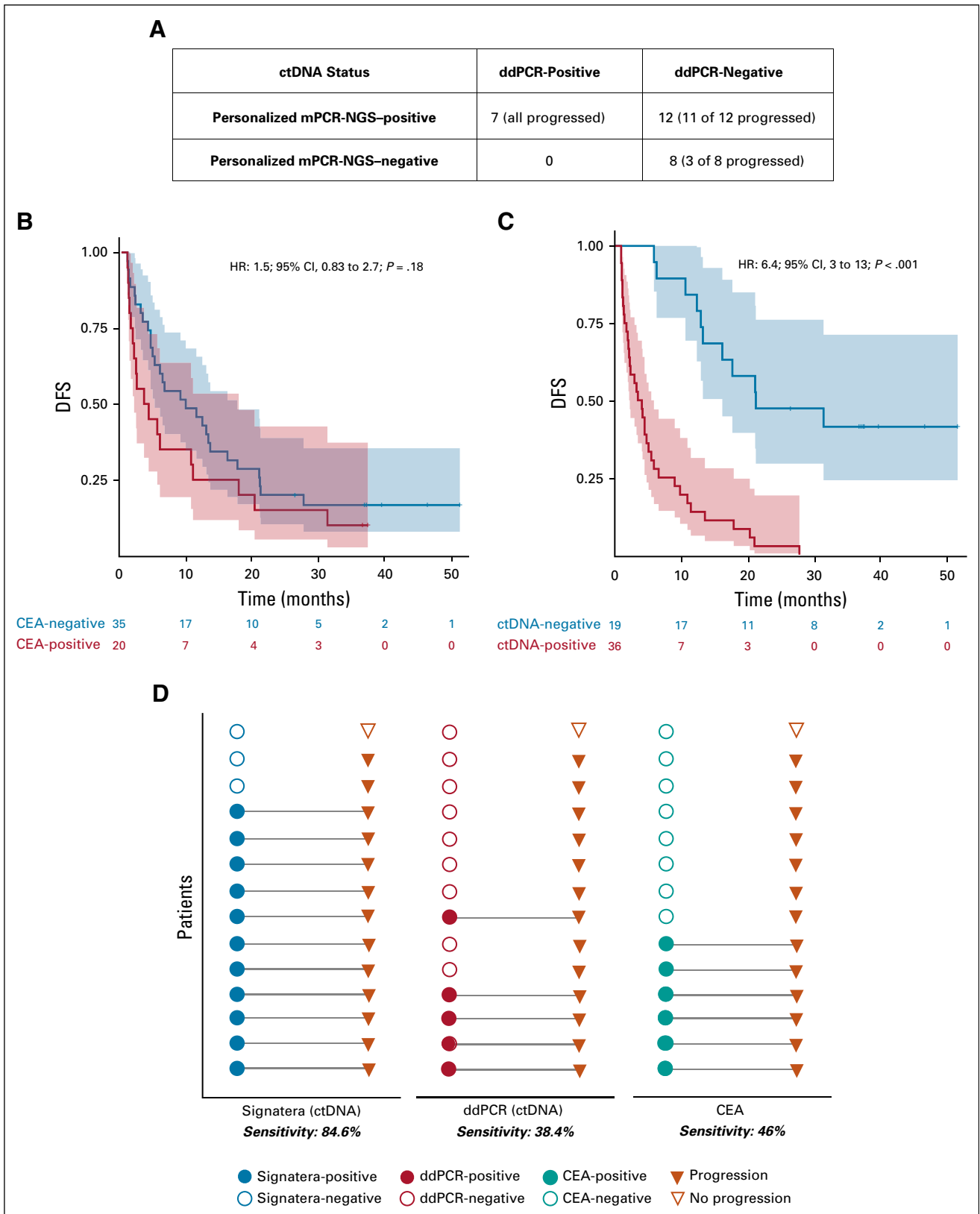
FIG 5. (A) ctDNA assessment using ddPCR versus personalized mPCR-NGS methodology. (B and C) DFS at postsurgical time point on the basis of CEA and ctDNA. Kaplan-Meier estimates for 55 patients with metastatic colorectal cancer representing DFS, in patients who had both CEA and ctDNA (assessed by personalized mPCR-NGS assay) results available postsurgically. Patients were stratified by CEA status or ctDNA status. Shaded areas in the Kaplan-Meier plots indicate 95% CIs. (D) Comparison of mPCR-NGS ctDNA technology with ddPCR and CEA in predicting disease progression in patients with *KRAS* mutation (n = 14). CEA, carcinoembryonic antigen; ctDNA, circulating tumor DNA; ddPCR, digital droplet PCR; DFS, disease-free survival; HR, hazard ratio; mPCR, multiplex PCR; NGS, next-generation sequencing.

of ctDNA testing in their design. For example, clinical trials could benefit from patient stratification on the basis of their postsurgical MRD status before randomly assigning patients into a treatment versus placebo arm. Additionally, MRD-guided trials could also benefit by enriching patients with high risk of relapse, leading to significant reductions in trial sample size and unnecessary treatment cost. Furthermore, the use of ctDNA as a surrogate end point for treatment response monitoring is being actively investigated, wherein an early indication of treatment efficacy (ctDNA clearance) relative to conventional strategies may lead to expedited approval of new therapies. Our present work supports the continuous expansion of the number of clinical studies in patients with mCRC using personalized ctDNA-based MRD analysis and provides direct evidence of the predictive and prognostic value of ctDNA, which could help clinicians and researchers with real numbers to design their clinical studies and support therapeutic decisions in the adjuvant setting.

AFFILIATIONS

[1]Oncology Unit 1, Department Oncology, Veneto Institute of Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Padua, Veneto, Italy

[2]Natera Inc, San Carlos, CA

[3]Cancer Immunology and Immune Modulation, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT

[4]AMAL Therapeutics, Genève, Switzerland

[5]Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy

[6]Hepatobiliary Surgery and Liver Transplant Unit, Department of Surgery, Oncology and Gastroenterology, University of Padua, Italy

[7]Unit of Surgical Oncology of the Digestive Tract, Veneto Institute of Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Padua, Italy

[8]Unit of Surgical Pathology, Department of Medicine (DIMED), University of Padua, Padua, Italy

[9]Oncology Unit 3, Department of Oncology, Veneto Institute of Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Castelfranco Veneto, Veneto, Italy

[10]Early Phase Clinical Trial Unit, Department of Oncology, Veneto Institute of Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Padua, Veneto, Italy

[11]Veneto Institute of Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Padua, Veneto, Italy

CORRESPONDING AUTHOR

Fotios Loupakis, MD, PhD, Unit of Oncology 1, Department of Oncology, Istituto Oncologico Veneto, IRCCS, Via Gattemelata, 64, 35128 Padua, Italy; e-mail: fotios.loupakis@iov.veneto.it.

EQUAL CONTRIBUTION

S.L. and M.F. contributed equally as senior authors.

AUTHOR CONTRIBUTIONS

Conception and design: Fotios Loupakis, Shruti Sharma, Himanshu Sethi, Bernhard G. Zimmermann, Alexey Aleshin, Marta Schirripa, Pierluigi Pilati, Angelo Paolo Dei Tos, Sara Lonardi, Matteo Fassan

Financial support: Madiha Derouazi, Alexey Aleshin, Angelo Paolo Dei Tos, Matteo Fassan

Administrative support: Fotios Loupakis, Alexey Aleshin, Matteo Fassan

Provision of study materials or patients: Fotios Loupakis, Sabina Murgioni, Himanshu Sethi, Giada Munari, Umberto Cillo, Pierluigi Pilati, Angelo Paolo Dei Tos, Matteo Fassan

Collection and assembly of data: Fotios Loupakis, Shruti Sharma, Sabina Murgioni, Paola Biason, Mario Domenico Rizzato, Cosimo Rasola, Derrick Renner, Svetlana Shchegrova, Himanshu Sethi, Bernhard G. Zimmermann, Alexey Aleshin, Marta Schirripa, Giada Munari, Angelo Paolo Dei Tos, Sara Lonardi, Matteo Fassan

Data analysis and interpretation: Fotios Loupakis, Shruti Sharma, Madiha Derouazi, Paola Biason, Derrick Renner, Svetlana Shchegrova, Allyson Koyen Malashevich, Meenakshi Malhotra, Himanshu Sethi, Bernhard G. Zimmermann, Alexey Aleshin, Solomon Moshkevich, Paul R. Billings, Jonathon D. Sedgwick, Marta Schirripa, Umberto Cillo, Angelo Paolo Dei Tos, Vittorina Zagonel, Sara Lonardi, Matteo Fassan

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by the authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Fotios Loupakis

Consulting or Advisory Role: Amgen, Sanofi, Bayer, Amal Therapeutics

Speakers' Bureau: Roche, Sanofi, Bayer, Amgen

Research Funding: Roche, Merck Serono, Amgen, Bayer

Travel, Accommodations, Expenses: Roche, Amgen, Merck Serono

Shruti Sharma

Employment: Natera

Stock and Other Ownership Interests: Natera

Madiha Derouazi

Employment: AMAL Therapeutics

Leadership: AMAL Therapeutics

Travel, Accommodations, Expenses: AMAL Therapeutics

Derrick Renner

Employment: Natera

Stock and Other Ownership Interests: Natera

Travel, Accommodations, Expenses: Natera

Svetlana Shchegrova
Employment: Natera
Stock and Other Ownership Interests: Natera

Allyson Koyen Malashevich
Employment: Natera
Stock and Other Ownership Interests: Natera

Meenakshi Malhotra
Employment: Natera
Stock and Other Ownership Interests: Natera

Himanshu Sethi
Employment: Natera
Stock and Other Ownership Interests: Natera
Research Funding: Natera
Patents, Royalties, Other Intellectual Property: Patents
Travel, Accommodations, Expenses: Natera

Bernhard G. Zimmermann
Employment: Natera
Stock and Other Ownership Interests: Natera
Honoraria: Natera
Patents, Royalties, Other Intellectual Property: Multiple patents and patent applications

Alexey Aleshin
Employment: Natera
Leadership: Natera
Stock and Other Ownership Interests: Natera
Consulting or Advisory Role: Mission Bio
Travel, Accommodations, Expenses: Natera

Solomon Moshkevich
Employment: Natera, Evidation Health
Stock and Other Ownership Interests: Natera
Patents, Royalties, Other Intellectual Property: Named as inventor on a patent filed by Natera Inc
Travel, Accommodations, Expenses: Natera

Paul R. Billings
Employment: Natera

Leadership: Biological Dynamics, OmniSeq, Alveo Technologies, Mission Bio
Stock and Other Ownership Interests: Natera, Trovagene, Biological Dynamics, OmniSeq, Alveo Technologies
Consulting or Advisory Role: Bethesda Group, Guidepoint Global

Jonathon D. Sedgwick
Employment: Boehringer Ingelheim
Travel, Accommodations, Expenses: Boehringer Ingelheim

Umberto Cillo
Consulting or Advisory Role: Springer Health Care, Novartis, Johnson & Johnson, Astellas, Sandoz
Speakers' Bureau: Eisai

Angelo Paolo Dei Tos
Consulting or Advisory Role: Bayer, Roche

Vittorina Zagonel
Consulting or Advisory Roles: Bristol Myers Squibb, MSD, Eisai, Italfarmaco
Speakers' Bureau: Roche, Bristol Myers Squibb, Astellas Pharma, Servier, AstraZeneca, MSD, Jansen, Ipsen
Research Funding: Bayer, Roche, Lilly, Astra Zeneca, BMS, Ipsen, Astellas Pharma
Travel, Accommodations, Expenses: Bayer, Roche, Servier

Sara Lonardi
Consulting or Advisory Role: Amgen, Merck Serono, Lilly, Servier, AstraZeneca, Incyte, Daiichi Sankyo, Bristol Myers Squibb
Speakers' Bureau: Roche, Lilly, Bristol Myers Squibb, Servier, Merck Serono, Pierre Fabre, GlaxoSmithKline, Amgen
Research Funding: Amgen, Merck Serono, Bayer, Roche, Lilly, AstraZeneca, Bristol Myers Squibb

Matteo Fassan
Consulting or Advisory Role: Astellas Pharma, Tesaro, GlaxoSmithKline, Diaceutics, Diaceutics, Roche
Research Funding: Astellas Pharma, QED Therapeutics, Macrophage Pharma

No other potential conflicts of interest were reported.

REFERENCES

1. American Cancer Society: Cancer Facts and Figures 2020. Atlanta, GA, American Cancer Society, 2020

2. Siegel RL, Miller KD, Goding Sauer A, et al: Colorectal cancer statistics, 2020. CA Cancer J Clin 70:145-164, 2020

3. van der Geest LG, Lam-Boer J, Koopman M, et al: Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. Clin Exp Metastasis 32:457-465, 2015

4. Lin J, Peng J, Zhao Y, et al: Early recurrence in patients undergoing curative resection of colorectal liver oligometastases: Identification of its clinical characteristics, risk factors, and prognosis. J Cancer Res Clin Oncol 144:359-369, 2018

5. Costi R, Leonardi F, Zanoni D, et al: Palliative care and end-stage colorectal cancer management: The surgeon meets the oncologist. World J Gastroenterol 20:7602-7621, 2014

6. Ottaiano A, Circelli L, Lombardi A, et al: Genetic trajectory and immune microenvironment of lung-specific oligometastatic colorectal cancer. Cell Death Dis 11:275, 2020

7. Tomlinson JS, Jarnagin WR, DeMatteo RP, et al: Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol 25:4575-4580, 2007

8. Kopetz S, Chang GJ, Overman MJ, et al: Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. J Clin Oncol 27:3677-3683, 2009

9. Hurwitz H, Fehrenbacher L, Novotny W, et al: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335-2342, 2004

10. Kawaguchi Y, Vauthey JN: The landmark series: Randomized control trials examining perioperative chemotherapy and postoperative adjuvant chemotherapy for resectable colorectal liver metastasis. Ann Surg Oncol 27:4263-4270, 2020

11. Benson AIB, Venook AP, Al-Hawary MM, et al: NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 2.2021. JNCCN 19:329-359, 2021

12. Pan Z, Peng J, Lin J, et al: Is there a survival benefit from adjuvant chemotherapy for patients with liver oligometastases from colorectal cancer after curative resection? Cancer Commun (Lond) 38:29, 2018

13. Van Cutsem E, Verheul HM, Flamen P, et al: Imaging in colorectal cancer: Progress and challenges for the clinicians. Cancers (Basel) 8:81, 2016

14. Goldstein MJ, Mitchell EP: Carcinoembryonic antigen in the staging and follow-up of patients with colorectal cancer. Cancer Invest 23:338-351, 2005

15. Nicholson BD, Shinkins B, Mant D: Blood measurement of carcinoembryonic antigen level for detecting recurrence of colorectal cancer. JAMA 316:1310-1311, 2016

16. Sorbye H, Dahl O: Carcinoembryonic antigen surge in metastatic colorectal cancer patients responding to oxaliplatin combination chemotherapy: Implications for tumor marker monitoring and guidelines. J Clin Oncol 21:4466-4467, 2003

17. Chakrabarti S, Xie H, Urrutia R, et al: The promise of circulating tumor DNA (ctDNA) in the management of early-stage colon cancer: A critical review. Cancers (Basel) 12:2808, 2020

18. Osumi H, Shinozaki E, Yamaguchi K, et al: Clinical utility of circulating tumor DNA for colorectal cancer. Cancer Sci 110:1148-1155, 2019

19. Reinert T, Henriksen TV, Christensen E, et al: Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. JAMA Oncol 5:1124-1131, 2019

20. Elazezy M, Joosse SA: Techniques of using circulating tumor DNA as a liquid biopsy component in cancer management. Comput Struct Biotechnol J 16:370-378, 2018

21. Postel M, Roosen A, Laurent-Puig P, et al: Droplet-based digital PCR and next generation sequencing for monitoring circulating tumor DNA: A cancer diagnostic perspective. Expert Rev Mol Diagn 18:7-17, 2018

22. Ottaiano A, Normanno N, Facchini S, et al: Study of Ras mutations' prognostic value in metastatic colorectal cancer: STORIA analysis. Cancers (Basel) 12:1919, 2020

23. R Core Team. R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing. https://cran.r-project.org/

24. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45:228-247, 2009

25. Benešová L, Hálková T, Ptáčková R, et al: Significance of postoperative follow-up of patients with metastatic colorectal cancer using circulating tumor DNA. World J Gastroenterol 25:6939-6948, 2019

26. Norcic G, Jelenc F, Cerkovnik P, et al: Role of specific DNA mutations in the peripheral blood of colorectal cancer patients for the assessment of tumor stage and residual disease following tumor resection. Oncol Lett 12:3356-3362, 2016

27. Overman MJ, Vauthey J-N, Aloia TA, et al: Circulating tumor DNA (ctDNA) utilizing a high-sensitivity panel to detect minimal residual disease post liver hepatectomy and predict disease recurrence. J Clin Oncol 35:3522, 2017 (15 suppl)

28. Molinari C, Marisi G, Passardi A, et al: Heterogeneity in colorectal cancer: A challenge for personalized medicine? Int J Mol Sci 19:3733, 2018

29. Zhang H, Liu R, Yan C, et al: Advantage of next-generation sequencing in dynamic monitoring of circulating tumor DNA over droplet digital PCR in cetuximab treated colorectal cancer patients. Transl Oncol 12:426-431, 2019

30. Vanova B, Kalman M, Jasek K, et al: Droplet digital PCR revealed high concordance between primary tumors and lymph node metastases in multiplex screening of KRAS mutations in colorectal cancer. Clin Exp Med 19:219-224, 2019

31. Tong G, Xu W, Zhang G, et al: The role of tissue and serum carcinoembryonic antigen in stages I to III of colorectal cancer—A retrospective cohort study. Cancer Med 7:5327-5338, 2018

32. Litvak A, Cercek A, Segal N, et al: False-positive elevations of carcinoembryonic antigen in patients with a history of resected colorectal cancer. J Natl Compr Cancer Netw 12:907-913, 2014

33. Christensen E, Birkenkamp-Demtröder K, Sethi H, et al: Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. J Clin Oncol 37:1547-1557, 2019

34. Coombes RC, Page K, Salari R, et al: Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. Clin Cancer Res 25:4255-4263, 2019

35. Abbosh C, Birkbak NJ, Wilson GA, et al: Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 545:446-451, 2017

36. Magbanua MJM, Swigart LB, Wu HT, et al: Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann Oncol 32:229-239, 2021

37. Bratman SV, Yang SYC, Iafolla MAJ, et al: Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nat Cancer 1:873-881, 2020

38. Yukami H, Nakamura Y, Watanebe J, et al: Minimal residual disease by circulating tumor DNA analysis for colorectal cancer patients receiving radical surgery: An initial report from CIRCULATE-Japan. Presented at ASCO, Virtual Meeting, 2021

39. Naidoo M, Gibbs P, Tie J: ctDNA and adjuvant therapy for colorectal cancer: Time to re-invent our treatment paradigm. Cancers (Basel) 13:346, 2021

40. Henriksen TV, Reinert T, Christensen E, et al: The effect of surgical trauma on circulating free DNA levels in cancer patients-implications for studies of circulating tumor DNA. Mol Oncol 14:1670-1679, 2020

- - -