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ORIGINAL CONTRIBUTION

Clinical Validity of Tumor-Informed Circulating Tumor DNA Analysis in Patients Undergoing Surgery of Colorectal Metastases

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BACKGROUND: Accurate biomarkers to monitor tumor load and response in metastatic colorectal cancer patients undergoing surgery could optimize treatment regimens.

OBJECTIVE: This study aimed to explore the clinical validity of tumor-informed quantification of circulating tumor DNA in blood using ultradeep sequencing.

DESIGN: Resection specimens from 53 colorectal cancer patients were analyzed for tumor-specific mutations in 15 genes. These mutations were used to measure the presence of circulating tumor DNA in preoperatively collected plasma samples using hybrid capture-based sequencing. Additional postoperative measurements were performed 1 week after surgery in 16 patients.

SETTINGS: The study was conducted at the Radboud University Medical Center.

PATIENTS: A total of 53 colorectal cancer patients undergoing surgery of metastases were included.

MAIN OUTCOME MEASURES: The detection of circulating tumor DNA.

RESULTS: At least 1 tumor-specific mutation was detected in all tumor samples. In preoperative plasma samples, circulating tumor DNA was detected in 88% (37/42) of systemic treatment-naïve patients and in 55% (6/11) of patients who received preoperative chemotherapy. More specifically, circulating tumor DNA was detected in 0% (0/3) of cases with a subtotal or partial pathologic response and in 75% (6/8) of cases without a pathologic response in the resection specimen ($p = 0.06$). In postoperative plasma samples, circulating tumor DNA was detected in 80% (4/5) of patients with an incomplete resection and in 0% (0/11) of those with a complete resection ($p = 0.003$).

LIMITATIONS: The study was limited by the heterogeneity of the cohort and the small number of postoperative plasma samples.

CONCLUSIONS: These data indicate that tumor-informed circulating tumor DNA detection in the plasma of patients undergoing surgery for metastatic colorectal cancer is feasible and may have clinical value in response monitoring and predicting residual disease. Prospective studies are needed to establish the clinical utility of circulating tumor DNA analysis to guide treatment decisions in these patients. See **Video Abstract** at http://links.lww.com/DCR/B990.

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VALIDEZ CLÍNICA DEL ANÁLISIS DE ADN DEL TUMOR CIRCULANTE INFORMADO POR EL TUMOR EN PACIENTES SOMETIDOS A CIRUGÍA DE METÁSTASIS COLORRECTALES

ANTECEDENTES: Los biomarcadores precisos para monitorear la carga tumoral y la respuesta en pacientes con cáncer colorrectal metastásico que se someten a cirugía podrían optimizar los regímenes de tratamiento.

OBJETIVO: Este estudio explora la validez clínica de la cuantificación informada por el tumor del ADN tumoral circulante en sangre mediante secuenciación ultraprofunda.

DISEÑO: Se analizaron muestras de resección de 53 pacientes con cáncer colorrectal en busca de mutaciones específicas del tumor en quince genes. Estas mutaciones se usaron para medir la presencia de ADN tumoral circulante en muestras de plasma recolectadas antes de la operación usando secuenciación basada en captura híbrida. Se realizaron mediciones postoperatorias adicionales una semana después de la cirugía en dieciséis pacientes.

AJUSTES: El estudio se realizó en el centro médico de la universidad de Radboud.

PACIENTES: Se incluyeron un total de 53 pacientes con cáncer colorrectal sometidos a cirugía de metástasis.

PRINCIPALES MEDIDAS DE RESULTADO: La detección de ADN tumoral circulante.

RESULTADOS: Se detectó al menos una mutación específica de tumor en todas las muestras de tumor. En muestras de plasma preoperatorias, se detectó ADN tumoral circulante en el 88% (37/42) de los pacientes sin tratamiento sistémico previo y en el 55% (6/11) de los pacientes que recibieron quimioterapia preoperatoria. Más concretamente, en el 0% (0/3) de los casos con respuesta patológica subtotal o parcial y en el 75% (6/8) de los casos sin respuesta patológica en la pieza de resección (p = 0,06). En muestras de plasma postoperatorio se detectó ADN tumoral circulante en el 80% (4/5) de los pacientes con una resección incompleta y en el 0% (0/11) de los que tenían resección completa (p = 0,003).

LIMITACIONES: El estudio estuvo limitado por la heterogeneidad de la cohorte y el pequeño número de muestras de plasma postoperatorias.

CONCLUSIONES: Estos datos indican que la detección de ADN tumoral circulante informado por el tumor en el plasma de pacientes sometidos a cirugía por cáncer colorrectal metastásico es factible y puede tener valor clínico en el control de la respuesta y la predicción de la enfermedad residual. Se necesitan estudios prospectivos para establecer la utilidad clínica del análisis de ADN tumoral circulante para guiar las decisiones de tratamiento en estos pacientes. Consulte **Video Resumen** en http://links.lww.com/DCR/B990. *(Traducción—Dr. Mauricio Santamaria)*

KEY WORDS: Circulating tumor deoxyribonucleic acid; Colorectal cancer; Liquid biopsies; Next-generation sequencing.

Colorectal cancer (CRC) is the third most commonly occurring cancer worldwide.[1] Nearly 50% of CRC patients either present with or will develop metastases, mostly to the liver.[2,3] Approximately half of these metastatic patients qualifies for surgery, often in combination with systemic treatment.[2,4] Monitoring of tumor load and response could contribute to more individualized treatment strategies, such as timely resection in patients with minimal response to neoadjuvant treatment or administration of adjuvant treatment after resection in the case of residual disease.[5] Currently, accurate biomarkers to monitor treatment response or predict residual disease are not available.

Numerous studies focus on the use of circulating tumor DNA (ctDNA), which allows for noninvasive and serial molecular assessment of the tumor.[6,7] This ctDNA reflects the presence of tumor cells and may be used to monitor disease progression and treatment response.[8,9] As ctDNA comprises only a small fraction of the total circulating cell-free DNA (cfDNA), highly sensitive next-generation sequencing (NGS) techniques and specialized data analyses are needed to measure the presence and level of ctDNA. Only recently, techniques became available to assess ctDNA with acceptable sensitivity for application in treatment monitoring.[7,10] Still, few studies have focused on ctDNA analysis in CRC patients receiving surgery of metastases, although a biomarker enabling personalized treatment in this patient group is needed. This study evaluated the clinical validity of tumor-informed detection of ctDNA in plasma using ultradeep sequencing in patients with metastatic CRC undergoing surgery.

MATERIALS AND METHODS

Patients and Sample Collection

In this study, 53 colorectal adenocarcinoma patients were enrolled who underwent surgery for their metastases at the GI surgery department of the Radboud University Medical Center between July 2017 and November 2019. Patients were included consecutively when they met the inclusion criteria and provided written informed consent. Preoperative blood samples were collected on the day of surgical treatment, and postoperative blood samples were collected 1 week after surgery. Clinical data of all patients were retrieved. The study was ethically approved

by the Internal Review Board of the Radboud University Medical Center (Committee on Research Involving Human Subjects [CMO] 2016-2805), meets the criteria of the Dutch code of proper use of human samples, and was conducted in accordance with the Declaration of Helsinki.

Tumor Tissue Analysis

Clinical analysis of tumor size was performed by reviewing CT scans, and the clinical response was evaluated according to Response Evaluation Criteria in Solid Tumors.[11] Surgical specimens of metastatic tissue were histologically evaluated by 2 gastroenterology-dedicated pathologists for tumor diameter, radicality of surgery, and pathologic response in the case of preoperative chemotherapy. Pathologic response was estimated by scoring tumor regression based on the presence of residual tumor cells and the extent of fibrosis. This tumor regression score was translated in the following classification: complete response indicated absence of tumor cells replaced by abundant fibrosis; subtotal response included presence of <10% residual viable tumor cells scattered throughout fibrosis; partial response corresponded to the presence of >10% viable tumor cells; and no response indicated the presence of exclusively tumor cells without fibrosis. Tumor load from metastases and primary tumor before surgery were estimated based on the sum of longest diameters from pathologic revision combined with clinical size estimations from CT scans in centimeters. For molecular analysis, DNA from formalin-fixed paraffin-embedded metastatic resection specimens was isolated using the Chelex-100 (Bio-Rad, Hercules, CA) method, as previously described.[12] DNA concentrations were measured using the Qubit Broad Range kit (Thermo Fisher, Waltham, MA). A total of 70 ng of this tumor tissue-derived DNA was used for library preparation with a customized single-molecule molecular inversion probe–based NGS panel and paired-end sequenced with 300 cycles on a NextSeq 500 instrument (Illumina, San Diego, CA).[13,14] This panel was designed to cover regions with a high frequency of somatic mutations in GI tumors, with a focus on CRC and esophageal cancer, based on available literature and relevant databases (Catalogue of Somatic Mutations in Cancer and The Cancer Genome Atlas available via cBioPortal, accessed 20-04-2018).[15,16] The panel consists of 15 genes (*APC*, *ARID1A*, *BRAF*, *CDKN2A*, *CTNNB1*, *ERBB2*, *FBXW7*, *GNAS*, *KRAS*, *NRAS*, *PIK3CA*, *RNF43*, *SMAD4*, *TGFBR2*, and *TP53*) and 56 mononucleotide repeat markers to measure microsatellite instability. Data are bioinformatically analyzed using standard procedures.[12–14]

Plasma Analysis

Blood samples were collected in special cfDNA collection tubes (Roche, Basel, Switzerland) and processed within 4 days using 2 centrifugation steps: first at 1600 g for 10 minutes to isolate plasma and subsequently at 16,000 g for 10 minutes to remove cellular debris. Plasma was stored at −80°C until further processing. Isolation of cfDNA from 4- to 10-mL plasma was performed with the QIAamp Circulating Nucleic Acid kit (Qiagen, Hilden, Germany). DNA concentrations were measured using the Qubit High Sensitivity kit (Thermo Fisher). Library preparation was performed with the Accell NGS Hyb kit (Swift, Ann Arbor, MI). cfDNA input ranged from 10 to 120 ng with a mean of 67.4 ng. Adapters contained unique molecular identifiers (UMIs) consisting of 8 random bases and a patient specific barcode. These UMIs enable grouping of reads derived from the same target DNA molecule, hereby facilitating error correction and calculation of number of mutated target DNA molecules. To extract the regions of interest, a hybridization capture was performed with a customized probe set (Twist, San Francisco, CA), targeting the same 15 genes as the single-molecule molecular inversion probe–based panel used for tissue sequencing. Paired-end sequencing was performed on a NextSeq 500 instrument (Illumina) using 300 cycles. For the analysis, BCL files were demultiplexed using the Illumina BCL2FASTQ Conversion Software (version 2.20). Resulting FASTQ files were subsequently aligned to the hg19 reference genome using Burrow-Wheller Aligner (BWA; version 0.7.8). Aligned reads were grouped and deduplicated using the read-specific UMI information (FGBIO, version 0.8.1). Unique reads that were based on only 1 deduplicated read (ie, singletons) were discarded. To detect small somatic variants, variant calling was performed on the filtered reads using Genomic Analysis ToolKit (GATK) Mutect2 (version 4.1.5.0, Broad Institute, Cambridge, MA). All tumor-specific variants were also manually checked inIntegrative Genomics Viewer (IGV) (version 2.4, University of California, San Diego, CA and Broad Institute, Cambridge, MA). For variants only detected in IGV, variant specific mean and SD of the variant allele frequency (VAF) in negative samples were calculated to determine the reliability of the variant. Only variants with a VAF higher than the mean plus 3 times the SD of the negative samples were designated as true variants. Technical validation demonstrated that all mutations present with a VAF of 0.5% to 5% in artificial human control template DNA standards (SeraCare, Milford, MA, and Horizon Discovery, Cambridge, United Kingdom) that were covered by the panel could be detected using 25-ng input DNA (Supplemental Table 1 at http://links.lww.com/DCR/B991). The number of mutant molecules per mL plasma was calculated with the mutant VAF, the volume of plasma used for isolation, and the total number of DNA molecules.[9]

Droplet Digital PCR

For droplet digital PCR (ddPCR) analysis, a QX200 system (Bio-Rad) was used together with a *KRAS* multiplex

primer-probe assay (Bio-Rad, assay ID 1863506) to target 7 hotspot mutations in codon 12 and 13 according to the manufacturer's instructions. cfDNA isolated from 2- to 4-mL plasma was used as input with a mean of 31.5 ng.

Data Evaluation

Differences in tumor characteristics, clinical and pathologic response measurements, and radicality of surgery were compared among patients using the Fisher exact probability test for categorical variables and Mann-Whitney (rank sum) test for nonparametric continuous variables. Correlation was assessed using Spearman rank correlation coefficient. Statistical tests were performed in IBM Statistical Package for the Social Sciences (SPSS) (version 25), and figures were generated using the R software (version 3.6.2) and GraphPad Prism (version 5.03).

TABLE 1. Clinicopathological characteristics of patients undergoing surgery of metastases of colorectal cancer (n = 53)

Clinicopathological characteristics	*Total, n (%)*	*ctDNA detection, n (%)*
Age, median (range)	67 (37-86)	
Gender		
Male	37 (69.8%)	29 (78.4%)
Female	16 (30.2%)	14 (87.5%)
Clinical TNM stage		
IV A	32 (60.4%)	27 (84.4%)
IV B	7 (13.2%)	7 (100%)
IV C	14 (26.4%)	9 (64.3%)
Primary tumor		
Location		
Colon	43 (81.1%)	35 (81.4%)
Rectum	10 (18.9%)	8 (80%)
pT stage		
pT2	8 (15.1%)	7 (87.5%)
pT3	34 (64.2%)	27 (79.4%)
pT4	11 (20.8%)	9 (81.8%)
pN stage		
pN0	18 (34%)	16 (88.9%)
pN1	15 (28.3%)	10 (66.7%)
pN2	20 (37.7%)	17 (85%)
Metastatic site		
Liver only	32 (60.4%)	27 (84.4%)
Peritoneum only	6 (11.3%)	2 (33.3%)
Multiple organs	15 (28.3%)	14 (93.3%)
Treatment		
No prior systemic treatment	42 (79.3%)	37 (88.1%)
Chemotherapy	11 (20.8%)	6 (54.6%)
Clinical response		
Stable disease	4 (36.4%)	3 (75%)
Partial response	7 (63.6%)	3 (42.9%)
Pathologic response		
No	8 (72.7%)	6 (75%)
Partial	2 (18.2%)	0 (0%)
Subtotal	1 (9.1%)	0 (0%)

The number of patients where ctDNA was detected in preoperative plasma and the percentage is shown.
ctDNA = circulating tumor DNA.

All p values were based on 2-sided testing, and p values <0.05 were considered significant.

RESULTS

Patient Characteristics

A total of 69 plasma samples were analyzed from 53 patients enrolled in this study (Table 1). All patients underwent surgery of their metastases, mainly consisting of liver resection and hyperthermic intraperitoneal chemotherapy surgery. A total of 32 patients presented with metachronous metastases. The other 21 patients presented with synchronous metastases, of whom 7 underwent surgery of the primary tumor several months earlier. Fourteen patients underwent simultaneous resection of both primary tumor and metastases. In total, 32 patients presented with liver metastases only, 6 patients presented with peritoneal metastases only (one solitary lesion invading the urinary bladder), and 15 patients had metastases in multiple organs, including the liver, peritoneum, lung, ovary, and pancreas. Preoperative induction chemotherapy was given to 11 patients with locally advanced disease to make the tumor surgically resectable.

Mutation Analysis in Tissue and Plasma

With a panel of 15 genes, at least 1 tumor-specific mutation was identified in 100% of the analyzed metastatic tissues. A total of 171 somatic mutations were found, mostly in *APC*, *TP53*, and *KRAS* (Fig. 1A). Only 1 patient presented with a microsatellite instable tumor (2%).

The average yield of cfDNA in the preoperative samples was 23.1-ng/mL plasma. cfDNA samples were sequenced to a total mean depth of 24,033x, and after deduplication and filtering, the mean depth was 4551x. At least one tumor-specific mutation identified in the paired tissue analysis could be detected in plasma in 81% (43/53) of patients (Fig. 1A). Of the tumor-specific mutations found in tissue, 70% (119/171) could be identically detected in the paired preoperative cfDNA samples (Supplemental Table 2 at http://links.lww.com/DCR/B991). The number of mutant molecules ranged from 3.5 to 3837/mL plasma in preoperative plasma samples and correlated with estimated tumor load (Spearman $\rho = 0.3$, $p = 0.03$) (Fig. 1B). Carcinoembryonic antigen levels showed no correlation with the number of mutant molecules per mL plasma and estimated tumor load ($p = 0.07$ and $p = 0.9$, respectively). Patients with liver metastases had significantly higher levels of ctDNA compared with patients with peritoneal metastases (mean mutant molecules/mL plasma 125.3 vs 3.3; $p = 0.01$) (Fig. 1B). To explore the sensitivity of the analyses, ddPCR analysis for *KRAS* codon 12 and 13 mutations was performed on cfDNA of 10 patients. NGS and ddPCR analyses were 100% concordant (Supplemental Table 3 at http://links.lww.com/DCR/B991). The *KRAS* VAF measured with NGS correlated with the fractional

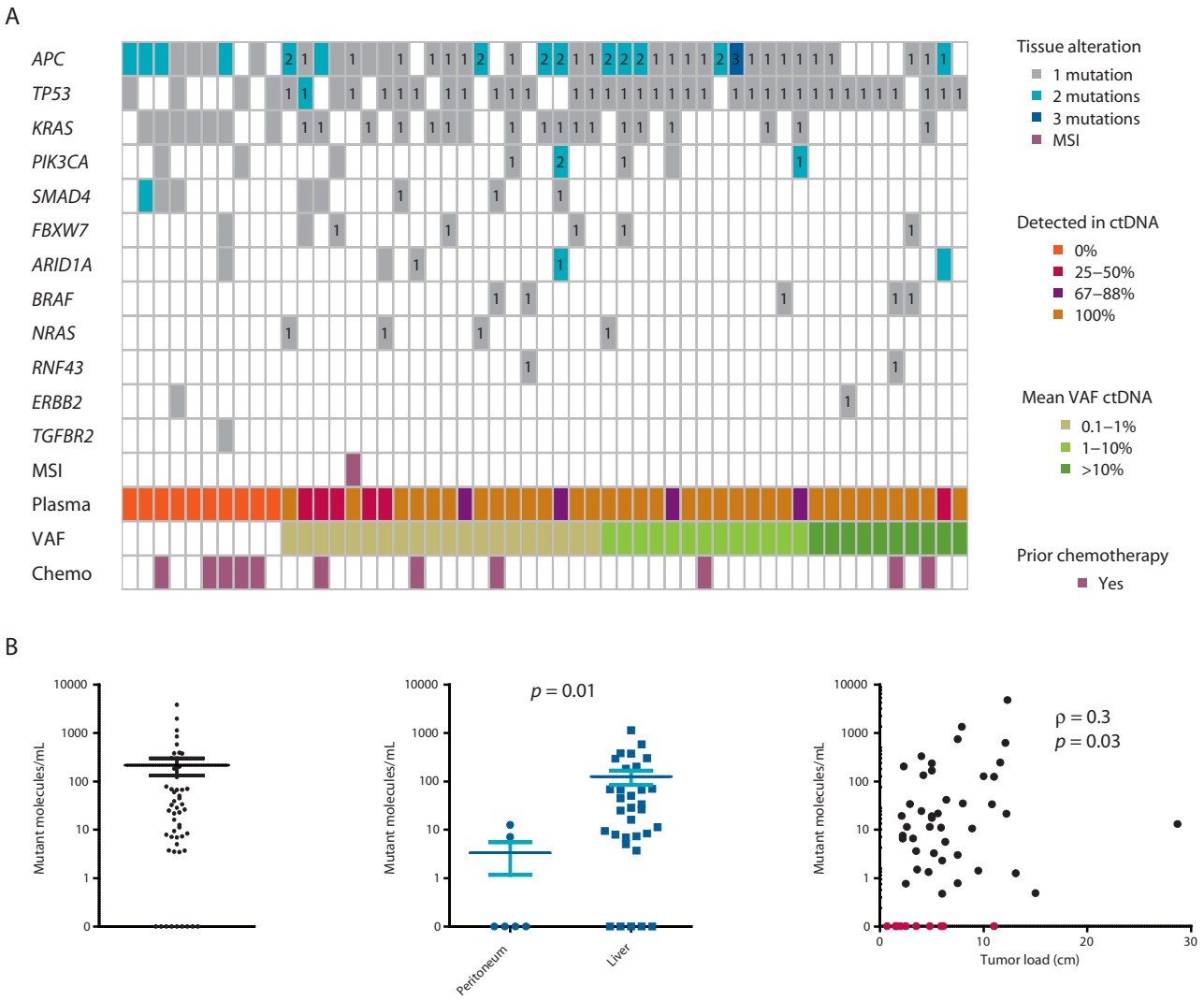

FIGURE 1. Preoperative ctDNA analysis. A, Overview of all mutations found in tumor tissue and their paired preoperative plasma samples for all 53 patients. Different patients are shown horizontally. The colors show the number of alterations found in tissue, and the numbers show the number of tumor-informed mutations detected in ctDNA. The row marked as plasma presents the percentage of tumor-informed mutations detected in ctDNA. The row VAF indicates the mean VAF for that patient in the ctDNA. B, The number of mutant molecules per mL plasma plotted for every patient, comparison of the number of mutant molecules per mL plasma between patients with liver metastases and peritoneal metastases, and correlation of number of mutant molecules per mL plasma with estimated tumor load before surgery in cm. Median and interquartile range are shown. Tumor load of ctDNA negative samples is displayed in red. chemo = chemotherapy; ctDNA = circulating tumor DNA; MSI = microsatellite instability; VAF = variant allele frequency.

abundance measured with ddPCR (Spearman ρ = 0.9, $p = 0.03$).

In the preoperative plasma samples of patients who had no systemic treatment before surgery, ctDNA was detected in 88% (37/42) of patients (Supplemental Figure 1 at http://links.lww.com/DCR/B991). The 5 patients without detectable ctDNA had a significantly smaller estimated tumor load (mean diameter 2.84 vs 6.93 cm; $p = 0.01$), and all had metastases confined to a single organ (5/5 vs 25/37; $p = 0.006$).

ctDNA Detection After Chemotherapy Associated with Lack of Pathologic Response and Incomplete Resection After Surgery

In 55% (6/11) of plasma samples of patients who received chemotherapy, ctDNA was detected preoperatively (Fig. 2A). More specifically, ctDNA was detected in 0% (0/3) of patients with a subtotal or partial pathologic response and in 75% (6/8) of patients without signs of a pathologic response ($p = 0.06$). The clinical response measurements based on CT scans only correctly predicted the

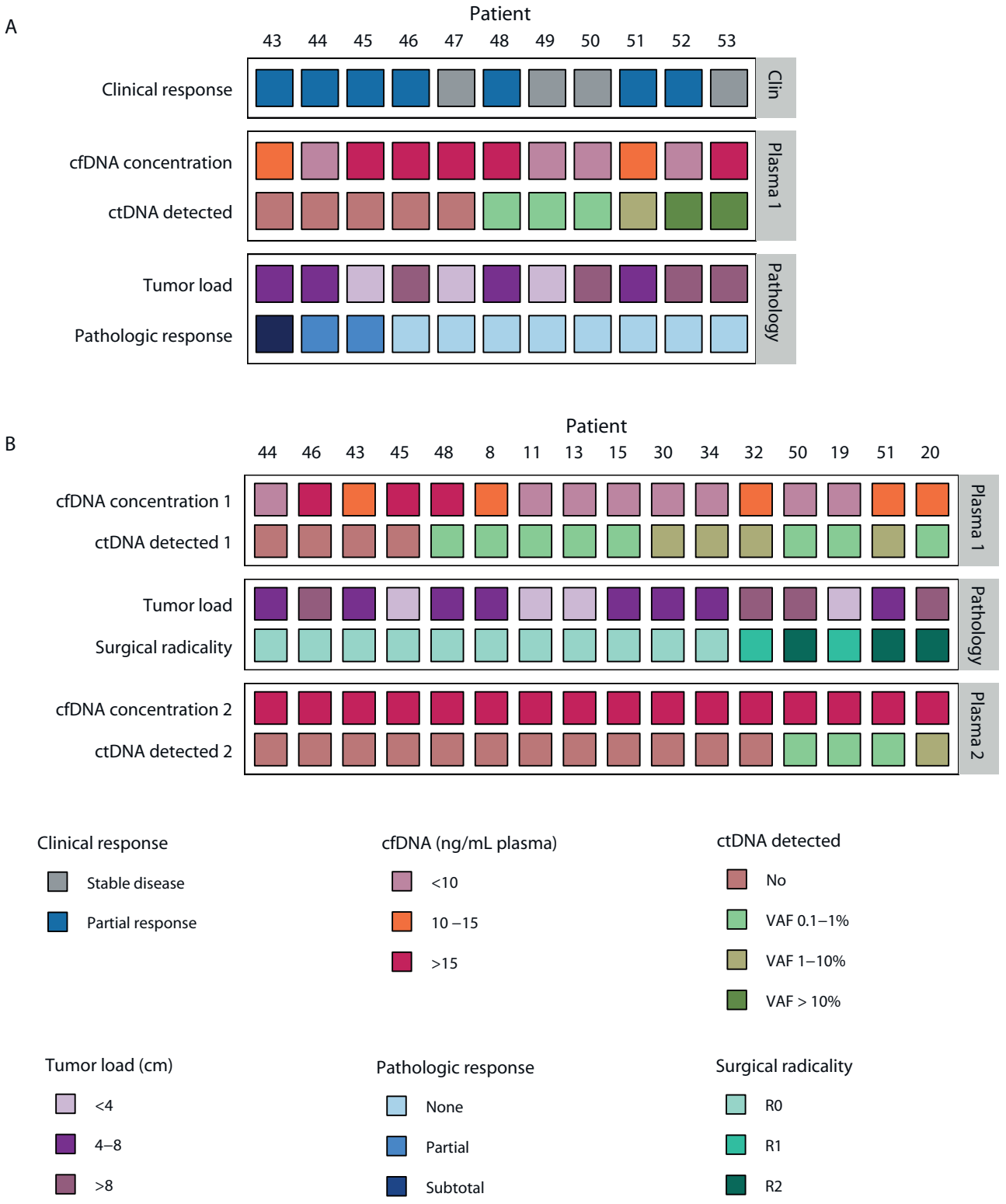

FIGURE 2. Overview of clinical, plasma, and pathologic tumor characteristics. A, Patients with preoperative chemotherapy (n = 11). B, Patients with both preoperative (plasma 1) and postoperative (plasma 2) analysis (n = 16). Patients are ordered based on the mean VAF detected in ctDNA. Clin = clinical; cfDNA = cell-free DNA; ctDNA = circulating tumor DNA; VAF = variant allele frequency.

absence of pathologic response for 50% (4/8) of patients ($p = 0.66$).

An additional postoperative plasma sample was available for 16 patients. Five of these patients had an incomplete resection, consisting of 3 hyperthermic intraperitoneal chemotherapy resections with incomplete cytoreduction. The average yield of cfDNA in these postoperative samples was 180.02 ng/mL plasma. cfDNA samples were sequenced to a total mean depth of 31,527x, and for unique sequencing reads, this was 5810x. In none of the 4 patients without preoperative ctDNA detection, ctDNA was detected after surgery. ctDNA was detected postoperatively in 33% (4/12) of the remaining patients (Fig. 2B). More specifically, ctDNA was detected in 80% (4/5) of patients with an incomplete resection (R1/R2) and in 0% (0/7) of patients with a complete resection (R0) ($p = 0.003$).

DISCUSSION

In this study, we evaluated the clinical validity of tumor-informed ctDNA detection using ultradeep sequencing in metastatic CRC patients who were eligible for surgery. With this approach, ctDNA was detected in 88% of preoperative baseline samples. Detection of ctDNA in patients who underwent preoperative chemotherapy was associated with lack of pathologic response. Moreover, postoperative detection of ctDNA was associated with the incomplete removal of metastases during surgery.

Several studies have been performed on ctDNA analysis in advanced metastatic CRC patients receiving systemic treatment. However, studies specifically focusing on CRC patients receiving surgery of metastases are less prominent. In addition, within the available studies, the cohort size remains limited,[4,17,18] and material is heterogeneous as different treatment combinations were offered.[19,20] Sensitive ddPCR analysis has been widely used to detect ctDNA in metastatic CRC patients[20,21] but is limited to specific hotspots and does not fully cover tumor heterogeneity. NGS-based technologies that allow for calculation of the number of target DNA molecules analyzed are able to detect a large variety of mutations in multiple genes.[18,22] Therefore, the assay can be applied for a broad spectrum of tumors each having unique mutations. Moreover, when multiple tumor-specific mutations are analyzed simultaneously, the sensitivity is higher, and the chances of detecting the very low levels of ctDNA during treatment will increase.[10] In this study, the clinical validity of a custom 15 gene-based NGS panel was evaluated in 53 metastatic CRC patients that were eligible for surgical treatment. This demonstrated that the sensitivity of the assay is in a clinically relevant range. Moreover, we confirmed that the detection of *KRAS* mutations using our NGS approach was similar to that of ddPCR analysis in 10 patients, proving the methodological sensitivity of the test.

With our NGS approach, we could detect ctDNA in 88% of patients without systemic treatment shortly before surgery. This detection rate was similar to that described in other surgically resectable metastatic CRC cohorts, ranging from 80% to 87%.[19,22,23] Only in patients with a relatively low tumor load did ctDNA remain undetected. This was also reflected in the significantly lower levels of ctDNA in patients with peritoneal disease compared with patients with liver metastases, which can be explained by the larger volume of liver metastases and peritoneal metastases spreading via the peritoneal cavity and not the bloodstream. These results are consistent with earlier observations that ctDNA levels are variable and depend on tumor burden and type of metastasis.[24,25]

In patients who received preoperative chemotherapy, we found that ctDNA detection was an indicator for absence of pathologic response in the tumor. Other studies also found this decreased detection of ctDNA in patients who received chemotherapy.[19,22] Moreover, Pellini et al[18] also showed that ctDNA can sensitively detect a residual tumor after preoperative chemotherapy. Interestingly, we found that ctDNA detection seems to be a better predictor for pathologic response than the clinical response measurements evaluated with response evaluation criteria in solid tumors. This suggests an additive value of ctDNA to monitor response before surgical intervention and an increased sensitivity to predict response when ctDNA is combined with current modalities.

In patients without detectable ctDNA preoperatively, ctDNA was also not found after surgery. We detected ctDNA postoperatively in 80% of patients who underwent an incomplete resection of their metastases, indicating that ctDNA detection reflects the radicality of surgery and the presence of a residual tumor. This finding was previously observed by Reinert et al[4] in 1 CRC patient where the ctDNA analysis indicated that surgery was not radical. Furthermore, Benešová et al[26] specifically compared ctDNA analysis and radicality of surgery of metastatic CRC, and their numbers were highly comparable with our results. Assessment of the surgical specimen can be performed with histologic examination, whereas ctDNA could also be helpful in detecting the potential presence of occult metastases.[26] In studies with long-term follow-up, ctDNA detection after surgery was described to be a prognostic marker for impaired outcome.[23,27] Whether this might be a useful biomarker to identify patients with residual disease that may benefit from adjuvant treatment needs to be evaluated in future large prospective studies.

Although our study underlines that ctDNA can be detected in patients with residual metastatic disease, a limitation of our study is the small number of postoperative samples, as this time point was added after inclusion

of the majority of patients. This small number combined with lack of long-term follow-up data led to deliberate omission of survival analysis as the right conditions could not be guaranteed. Moreover, the postoperative plasma samples were obtained 1 week after surgery. At that time point, we found an increase in the total cfDNA concentration compared with the plasma sample obtained before surgery, probably caused by damage and inflammation induced by the surgical intervention. This increase in background cfDNA levels may have masked the ctDNA present. Indeed, Henriksen et al[28] found that trauma-induced cfDNA persists for up to 4 weeks and thus recommend sample collection 4 weeks after surgery. Furthermore, for monitoring of residual disease, multiple time points for follow-up are desirable.[29] Another limitation of our study is the requirement of tissue for tumor-informed analysis. Here, we analyzed the available metastatic resection specimens, and we used mutations in driver genes that are normally present in all neoplastic cells. However, tissue biopsies are collected as standard of care and mostly resection specimens of the primary tumor are available for mutation profiling. Moreover, ctDNA analysis may be performed without prior knowledge of the tumor genotype, provided that white blood cells are analyzed to exclude clonal hematopoiesis variants that could otherwise be misclassified as tumor derived.[18]

Our data indicate that ultradeep sequencing of ctDNA may have a role as a biomarker in clinical practice for personalized treatment decisions in metastatic CRC patients eligible for surgery. ctDNA-based prediction of response to chemotherapy when combined with current measurements can potentially support the decision to advance, delay, or even omit a surgical procedure. Furthermore, the presence of ctDNA after surgery may identify patients with residual disease. Currently, adjuvant treatment is not given as standard of care in this patient group. The results of our study open possibilities to assess whether ctDNA detection after surgery may aid in selecting patients who might benefit from adjuvant treatment.[30]

CONCLUSION

A tumor-informed NGS approach for ctDNA analysis was evaluated for its clinical validity in patients undergoing surgery of colorectal metastases. This sensitive ctDNA analysis showed potential to monitor response to chemotherapy and to predict completeness of surgery. Further analyses of the clinical utility of this approach in larger prospective metastatic CRC cohorts are needed before adaptation of treatment can be based on ctDNA levels. In the future, ctDNA may be part of a set of response predictors to guide personalized treatment.

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