ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN SCIENCE FOR OPTIMAL CANCER CARE

ORIGINAL ARTICLE

Clinical validation of liquid biopsy-RECIST (LB-RECIST) in metastatic colorectal cancer (mCRC) patients: findings from the PLATFORM-B study

V. Martelli[1,2,3,4], J. Vidal[1,3], J. Gibert[5], M. C. Fernández-Rodríguez[5], J. Linares[1,3], P. García-Alfonso[6], D. Páez[7], V. Alonso[8], M. A. Gomez-España[3,9], M. Guix[1,3], C. Santos[3,10], G. Duran[11], E. Élez[3,12], R. Garcia-Carbonero[13], R. Ferreiro[14], E. Pineda[15], J. Sastre[16], M. T. Cano[3,9], J. L. Manzano[17], F. Losa[18], E. Aranda[3,9], F. Rivera[19], A. Sibilio[1,3], R. Toledo[20], J. Tabernero[3,12], R. Salazar[3,10], B. Bellosillo[5] & C. Montagut[1,2,3*], on behalf of the Spanish Cooperative Group for the Treatment of Digestive Tumours (TTD)

[1]Hospital del Mar Research Institute Barcelona, Medical Oncology Department, Hospital del Mar, Barcelona; [2]Universitat Pompeu Fabra, Barcelona; [3]Centro de Investigación Biomédica en Red de Oncología (CIBERONC-ISCIII), Madrid, Spain; [4]Department of Internal Medicine and Medical Specialties (DiMI), School of Medicine, Università degli Studi di Genova, Genoa, Italy; [5]Hospital del Mar Research Institute Barcelona, Pathology Department, Hospital del Mar, Barcelona; [6]Medical Oncology Department, Hospital Gregorio Marañón, Madrid; [7]Medical Oncology Department, H. Santa Creu i Sant Pau, U705. CIBERER, Barcelona; [8]Medical Oncology Department, Hospital Universitario Miguel Servet, Instituto de Investigación Sanitaria de Aragón (IISA), Zaragoza; [9]Medical Oncology Department, IMIBIC, Reina Sofía Hospital, University of Cordoba, Córdoba; [10]Medical Oncology Department, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL)-CIBERONC, Campus Bellvitge, University of Barcelona, Barcelona; [11]Unidad de Gestión Clínica Intercentros de Oncología Médica, Hospitales Universitarios Regional y Virgen de la Victoria, IBIMA, Málaga; [12]Medical Oncology Department, Vall d'Hebron Barcelona Hospital Campus, Vall d'Hebron Institute of Oncology (VHIO), Barcelona; [13]Medical Oncology Department, Hospital Universitario 12 de Octubre, Instituto de Investigación Sanitaria Hospital 12 de Octubre (Imas12), UCM, Madrid; [14]Medical Oncology Department, Hospital Ramón y Cajal, Madrid; [15]Medical Oncology Department, Hospital Clínic, Barcelona; [16]Medical Oncology Department, Hospital Universitario Clínico San Carlos, IdISSC, Madrid; [17]Medical Oncology Department, ICO, H. Germans Trias i Pujol, Barcelona; [18]Medical Oncology Department, Hospital Sant Joan Despí - Moisés Broggi, ICO-Hospitalet, Barcelona; [19]Medical Oncology Department, Hospital Marques de Valdecilla, IDIVAL, Santander; [20]Biomarker and Clonal Dynamic Laboratory, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Available online 3 September 2025

Background: Circulating tumor DNA (ctDNA) variations predict tumor response to systemic treatment (so-called molecular response) earlier than radiological assessment. However, a standardized categorization of molecular response is an unmet clinical need. Liquid biopsy-RECIST (LB-RECIST), based on aggregate variant allele frequency (aggVAF; sum of all detected variant allele frequencies in a sample) variations, has been proposed to stratify molecular response. Metastatic colorectal cancer (mCRC) may be an attractive clinical scenario for LB-RECIST clinical implementation; however, specific data on clinical validity is still lacking.

Patients and methods: The prospective PLATFORM-B study enrolled 130 mCRC patients who received standard frontline treatment and underwent serial ctDNA analysis at baseline and week 8 of treatment. ctDNA was analyzed by next-generation sequencing (Oncomine Colon cfDNA Assay; Ion Torrent S5). LB-RECIST, both qualitative (changes in ctDNA detection) and quantitative (percentage variations of aggVAF), were used to categorize molecular response, and were correlated with clinical outcomes, including progression-free survival (PFS) and overall survival (OS).

Results: ctDNA results were available for 106 patients at baseline and 90 patients at week 8 of treatment. Single timepoint $aggVAF_{WEEK8}$ >0% showed significantly worse survival outcomes compared to $aggVAF_{WEEK8} = 0\%$ (PFS $P < 0.0001$; OS $P = 0.0069$). Complete clearance of ctDNA at week 8 (ctDNA complete response, CCR) demonstrated the best prognostic and predictive values [median (m) OS 41.8 months; mPFS not reached (NR)], similar to persistent undetectable ctDNA (ctDNA non-measurable disease, CND; mOS 41.1 months; mPFS NR). Conversely, patients with ctDNA partial response (CPR) and ctDNA progressive disease (CPD) had the worst clinical outcomes (mOS 16.4 and 25.5 months, and mPFS 12.7 and 11.9 months, respectively).

Conclusions: LB-RECIST is prognostic and predictive of clinical outcomes in frontline mCRC. The clinical utility of LB-RECIST to guide early treatment decisions is warranted through interventional trials.

Key words: liquid biopsy, LB-RECIST, ctDNA, metastatic colorectal cancer, response assessment, aggregate VAF

*Correspondence to: Prof. Clara Montagut, Medical Oncology Department, Hospital del Mar Research Institute Barcelona, Hospital del Mar, Passeig Marítim 25-29, 08003 Barcelona, Spain. Tel: +34-93-248-3137; Fax: +34-93-248-3366
E-mail: cmontagut@psmar.cat (C. Montagut).

INTRODUCTION

The analysis of circulating tumor DNA (ctDNA) in peripheral blood samples—commonly referred to as liquid biopsy—is a rapid, minimally invasive, and highly reproducible technique for real-time assessment of tumor molecular biology.[1,2] This innovative approach can be applied throughout all phases of cancer management, from early detection to the advanced setting, and is already transforming clinical practice.[3,4] Metastatic colorectal cancer (mCRC) typically sheds high amounts of ctDNA and harbors universal, easily detectable trunk mutations (e.g. *APC*, *TP53*, *KRAS, NRAS*). Recent technological advancements have led to unprecedented advances in the clinical use of liquid biopsy in mCRC. Currently, three major clinical applications of ctDNA are entering routine clinical practice in mCRC[5]: (i) biomarkers detection to guide treatment selection,[6-8] (ii) monitoring of treatment response, and (iii) detection of emerging resistant clones to inform rechallenge strategies.[9-13] International guidelines recommend ctDNA analysis in mCRC to guide first-line therapy when tissue testing is not feasible or when urgent therapeutic decisions are needed and suggest its use for anti-epidermal growth factor receptor (EGFR) inhibitor rechallenge in later lines.[14] However, the use of liquid biopsy for real-time treatment response monitoring remains an unmet clinical need.[15]

The Radiological Response Evaluation Criteria in Solid Tumors (RECIST) serve as the current standard for assessing treatment response.[16,17] RECIST relies on measuring dynamic changes in target lesion diameters via imaging technologies such as computed tomography scanning and magnetic resonance imaging. Although it is widely used in both clinical research and practice, RECIST has several limitations. First, the timing of evaluation occurs every 8-12 weeks, which fails to capture disease progression or treatment response in real-time. Second, RECIST only detects macroscopic disease, potentially overlooking the emergence of resistant subclones. Finally, certain ambiguous scenarios, such as pseudo-progression, which can lead to unnecessary treatment modifications, or stable disease, which may mask underlying disease progression, further complicate its interpretation.

Liquid biopsy offers a potential solution by enabling real-time response assessment, as ctDNA levels in the bloodstream correlate with tumor burden. Indeed, changes in ctDNA levels during treatment (molecular response) often predict tumor response earlier than radiological assessment in solid tumors.[18] However, a standardized framework for classifying molecular response is still lacking. Although several criteria have been proposed, most studies were conducted on small cohorts[18-20] or evaluated responses to specific treatment regimens.[21,22] Among these, the liquid biopsy-RECIST (LB-RECIST) criteria proposed by Gouda et al. have emerged as a promising tool for early molecular response assessment. Using a digital droplet PCR (ddPCR)-based approach,[23,24] LB-RECIST was used to analyze samples collected at baseline, mid-treatment, and disease progression from a large population of patients treated across different tumor types, regimens, and treatment lines.[25] This framework introduced the concept of aggregate variant allele frequency (aggVAF)—the sum of VAFs for all detected somatic mutations within a single sample—demonstrating its strong correlation with survival outcomes and radiological responses. Two main response categories have been established: qualitative LB-RECIST, which assesses changes in ctDNA detectability during treatment, and quantitative LB-RECIST, which is based on percentage variations in aggVAF.[26] Despite the promising potential of LB-RECIST, ctDNA levels can vary across tumor types, treatment regimens, and lines of therapy. Although mCRC represents a compelling candidate for LB-RECIST implementation, specific data on the clinical validity and reproducibility in this setting is still lacking.

In the present study, we aim to evaluate the clinical validity of LB-RECIST in patients diagnosed with mCRC undergoing frontline standard therapy, utilizing data from the multicenter, prospective, non-interventional PLATFORM-B clinical study.[27]

MATERIALS AND METHODS

Patients

This retrospective analysis included all patients enrolled in the prospective PLATFORM-B study, conducted across 15 tertiary referral Spanish Centers from January 2018 to December 2020. The eligibility criteria included patients aged $\geq$18 years with histologically confirmed mCRC and planned first-line treatment with chemotherapy plus cetuximab or bevacizumab according to *RAS* mutational status.[27] The specific chemotherapy backbone (FOLFOX or FOLFIRI) administered was left to the discretion of the treating physician and was not prospectively collected in the study database.

ctDNA analysis

Whole blood samples were collected before the initiation of first-line treatment (baseline) and at week 8 of treatment (week 8) using cell-free DNA collection tubes (Roche Diagnostics, Indianapolis, IN). Tubes were sent within 72 hours of blood collection to the central laboratory, where plasma isolation was obtained via centrifugation. Aliquots were stored at −80°C until ctDNA extraction.

Cell-free DNA (cfDNA) was extracted following the manufacturer's protocols using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany), QIAsymphony DSP Circulating DNA Kit (Qiagen, Hilden, Germany), or MagMAX Cell-Free DNA Kit (Thermo Fisher Scientific, Walthman, MA). A minimum of 4 ml of plasma was required for the ctDNA assay, to obtain at least 20 ng of extracted cfDNA. ctDNA at baseline and week 8 was analyzed through next-generation sequencing (NGS) assay (Oncomine Colon cfDNA Assay; Ion S5 system).

The Ion Torrent Oncomine Colon cfDNA Assay covers 14 hotspot regions in genes (*AKT1, BRAF, CTNNB1, EGFR, ERBB2, FBXW7, GNAS, KRAS, MAP2K1, NRAS, PIK3CA,*

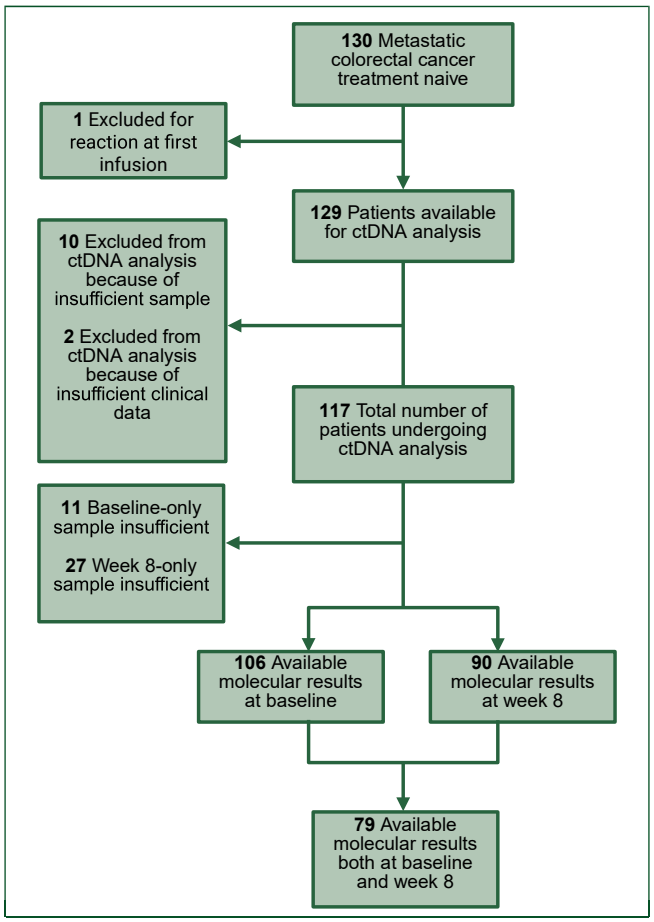

Figure 1. Study consort diagram.
ctDNA, circulating tumor DNA.

SMAD4, TP53, and *APC*) with a sensitivity threshold of 0.1%. Cases with <20 ng of cfDNA were considered insufficient for NGS analysis.

Variant allele frequencies (VAFs) were calculated as the number of variant reads divided by the total number of reads for each variant position. Aggregate VAF (aggVAF) was calculated as the sum of all VAFs detected in the sample. In case of undetectable mutations within a sample, aggVAF was 0%.

Variations in detectability of ctDNA between baseline and week 8 were used to determine molecular response according to qualitative LB-RECIST criteria[25] Dynamic changes in aggVAF between the two timepoints were calculated to assess molecular response as per quantitative LB-RECIST criteria.

Molecular response assessment

To evaluate the prognostic and predictive role of aggVAF, we included patients with plasma samples available from least at one timepoint (baseline and/or week 8). For assessment of LB-RECIST criteria, we only analyzed paired plasma samples from baseline and week 8 (Figure 1).

Qualitative and quantitative and LB-RECIST criteria were used as previously described.[25,26] In brief, qualitative LB-RECIST was based on changes in ctDNA detectability between baseline and week 8, identifying four distinct groups: group 1 (G1, detectable ctDNA at baseline, persisting as detectable at week 8 of treatment); group 2 (G2, detectable ctDNA at baseline, converting to undetectable at week 8); group 3 (G3, undetectable ctDNA at baseline, becoming detectable at week 8); group 4 (G4, persistently undetectable ctDNA at both baseline and week 8). Quantitative LB-RECIST classified patients into five categories based on percentage changes in aggVAFs between the two timepoints: ctDNA complete response (CCR, complete aggVAF clearance after initial detectability), ctDNA partial response (CPR, decrease in aggVAF levels >10%), ctDNA stable disease (CSD, no variations in aggVAF levels or changes within ±10%), ctDNA progressive disease (CPD, increase in aggVAF >10% or *de novo* ctDNA detection), and ctDNA non-measurable disease (CND, undetectable both at baseline and week 8). Figure 2 summarizes the LB-RECIST criteria and defines each molecular response category.

Study design

The PLATFORM-B study was a prospective, observational, multicentric study conducted in 15 Spanish hospitals. Tumor response was assessed locally following RECIST v1.1.[16] The final protocol was approved by an independent ethics committee, and all patients gave their written informed consent before enrollment. The study was conducted in accordance with the Declaration of Helsinki.[27]

Statistical analysis

Categorical variables were presented as frequencies and percentages. Survival curves were estimated using the Kaplan–Meier method and compared with the log-rank test. Cox proportional hazards models were employed to evaluate the prognostic significance of aggVAF levels and molecular response categories, adjusting for potential confounders. *Chi*-square or Fisher's exact tests were used to compare categorical clinicopathologic characteristics, and Mann–Whitney *U*-test was used for continuous variables. All statistical tests were two-sided, and a *P* value of < 0.05 was considered statistically significant. PFS was defined as the time from initiation of first-line treatment (standard chemotherapy plus cetuximab or bevacizumab) until tumor progression or death, whichever occurred first, and OS was defined as the time from initiation of first-line treatment to death from any cause. Statistical analyses were conducted using R version 4.1.2 (November 2021).

RESULTS

A total of 130 mCRC patients were included in the study from February 2017 to April 2018. One hundred patients had *RAS* wild-type (*RAS*-wt) mCRC and received first-line doublet chemotherapy plus cetuximab, whereas 30 patients had *RAS* mutant (*RAS*-mut) mCRC and were treated with first-line doublet chemotherapy in combination with bevacizumab. The median follow-up was 25 months (range: 1-49 months). The study flowchart is summarized in Figure 1. Patient clinicopathological characteristics are provided in Table 1.

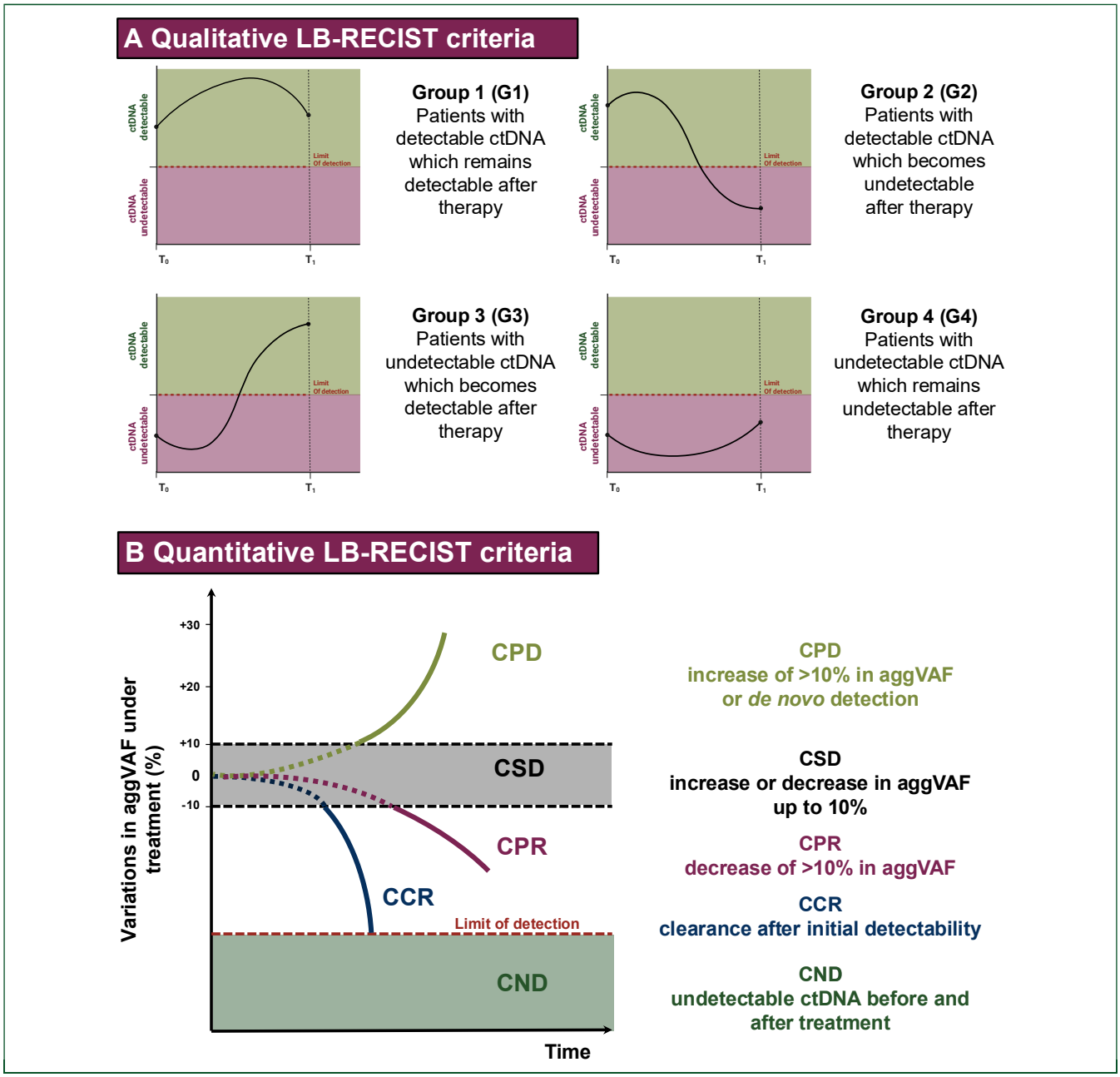

Figure 2. Liquid biopsy RECIST criteria for molecular response in solid tumor. *Qualitative criteria* (A) classify patients into four categories based on ctDNA detectability at baseline and the second timepoint. *Quantitative criteria* (B) define molecular response dynamics based on relative changes in aggVAF at the second timepoint compared with baseline.
aggVAF, aggregate variant allele frequency; CCR, ctDNA complete response; CND, ctDNA not detectable; CPD, ctDNA progressive disease; CPR, ctDNA partial response; CSD, ctDNA stable disease; ctDNA, circulating tumor DNA; LB-RECIST, liquid biopsy RECIST.

Prognostic and predictive value of aggVAF at baseline and week 8 in mCRC patients

ctDNA genomic results were available for 106 patients at baseline and 90 patients at week 8. The median aggVAF at baseline ($mAggVAF_{BASELINE}$) was 8.7%, which was adopted as a cutoff to classify patients into two prognostic groups. Patients with higher aggVAF demonstrated significantly worse median OS (mOS) compared with patients with lower $mAggVAF_{BASELINE}$ (mOS 20.3 months versus 39 months; $P = 0.021$), but no statistically significant difference in median PFS (mPFS) was observed (mPFS 13.8 months versus 19.6 months; $P = 0.17$) (Figure 3A and B). No correlation was observed between $aggVAF_{BASELINE}$ and clinicopathological features.

At week 8, the mAggVAF ($mAggVAF_{WEEK8}$) was 0%. Patients with $aggVAF_{WEEK8}$ values >0% had significantly worse outcomes in terms of both PFS (mPFS 11.9 versus 19 months; $P < 0.0001$) and OS (mOS 17 versus 41.8 months; $P = 0.0069$), compared with patients with $aggVAF_{WEEK8}$ value of 0% (Figure 3C and D). Regardless of baseline levels and dynamic variations in ctDNA, single timepoint assessment of $aggVAF_{WEEK8}$ was statistically significant in discriminating PFS and OS, suggesting that the absence of ctDNA as early as after only 8 weeks of systemic treatment

Table 1. Baseline clinicopathological characteristics of patients

Patient characteristics	*Overall* *N = 117, n (%)*	*Baseline* *ctDNA+ n = 73, n (%)*	*Baseline* *ctDNA− n = 33, n (%)*	*P value*
Age in years, median (range)	66 (27-85)	67 (39-85)	67 (27-83)	—
Sex				0.52
Female	38 (40)	33 (45)	12 (26)	—
Male	69 (60)	40 (55)	21 (64)	—
Primary tumor location				0.67
Right	25 (21)	16 (22)	5 (15)	—
Left	58 (50)	34 (47)	18 (55)	—
Rectum	34 (29)	23 (32)	10 (30)	—
Primary tumor present, yes	79 (68)	54 (74)	16 (48)	**0.01**
***RAS* mutational status**				1.0
Wild type	89 (76)	56 (77)	25 (75)	—
Mutant	28 (24)	17 (23)	8 (25)	—
Number of metastatic sites				1.0
<3	101 (85)	61 (84)	28 (85)	—
≥3	18 (15)	12 (16)	5 (15)	—
Metastases timing				**0.008**
Synchronous	88 (75)	59 (81)	18 (55)	
Metachronous	29 (25)	14 (19)	15 (45)	
Metastatic sites				0.48
Nodes	32 (27)	22 (30)	7 (21)	0.82
Lung	42 (35)	24 (33)	12 (36)	0.9
Liver	84 (71)	57 (78)	19 (58)	**0.037**
Peritoneum	30 (25)	15 (21)	11 (33)	0.22
Other	15 (13)	8 (11)	6 (18)	0.35
First-line treatment				0.13
Doublet CT w/o moAb	8 (7)	6 (8)	1 (3)	—
Doublet CT + cetuximab	89 (76)	56 (77)	25 (75)	—
Doublet CT + bevacizumab	20 (17)	11 (15)	7 (21)	—
Mutations with highest incidence at baseline				—
TP53	39 (33)	40 (55)	NA	—
APC	34 (29)	37 (51)	NA	—
Mutations with highest incidence at week 8				—
TP53	15 (13)	9 (12)	6 (18)	0.55
APC	12 (10)	11 (15)	1 (3)	0.10

CT, chemotherapy; ctDNA, circulating tumor DNA; moAbs, monoclonal antibodies; NA, not applicable; w/o, without.

is, by itself, a powerful predictor of response to treatment and long-term survival in first-line mCRC.

Furthermore, to address the potential bias introduced by patients with *RAS*-wt tumors in tissue but mutant ctDNA at baseline,[28,29] a subgroup analysis was carried out excluding these patients ($n = 7$) from the cohort receiving anti-EGFR therapy. The prognostic impact of ctDNA dynamics was reassessed in this restricted population. No significant association was observed between aggVAF at baseline and either PFS or OS. However, the predictive value of aggVAF at week 8 was maintained, showing a statistically significant correlation with both PFS and OS (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2025.105760). These findings support the robustness of molecular response at week 8 as a prognostic biomarker, even when patients with potential misclassification based on ctDNA *RAS* status are excluded.

Clinicopathologic correlates of baseline ctDNA detectability

To better characterize the clinical and biological correlates of ctDNA shedding,[30] we compared baseline characteristics between patients with baseline detectable (ctDNA+) and undetectable (ctDNA−) ctDNA. Notably, the presence of the primary tumor was significantly more frequent among baseline ctDNA+ patients ($P = 0.01$). Similarly, synchronous presentation of metastases was associated with ctDNA positivity ($P = 0.008$), whereas metachronous disease was more prevalent in baseline ctDNA− cases. In terms of metastatic spread, liver involvement was more common in ctDNA+ patients ($P = 0.037$). No significant differences were observed in sex, sidedness, *RAS* mutational status, or the number of metastatic sites ($P > 0.05$) (Table 1).

Molecular response to first-line treatment in mCRC patients according to LB-RECIST criteria

Paired baseline and week 8 ctDNA results were available for 79 patients. Through the quantitative LB-RECIST criteria, molecular response to treatment was classified into CCR ($n = 32$), CND ($n = 18$), CPR ($n = 20$), CPD ($n = 8$), and CSD ($n = 1$).

According to qualitative LB-RECIST criteria, patients were categorized into G1 ($n = 22$), G2 ($n = 32$), G3 ($n = 7$), and G4 ($n = 18$).

Patients in the CCR group had the most favorable clinical outcomes, with mPFS not reached (NR) and a mOS of 41.8 months. The CND group showed the second-best survival rates, closely resembling those observed in the CCR group (mPFS NR; mOS 41.1 months). In both these response

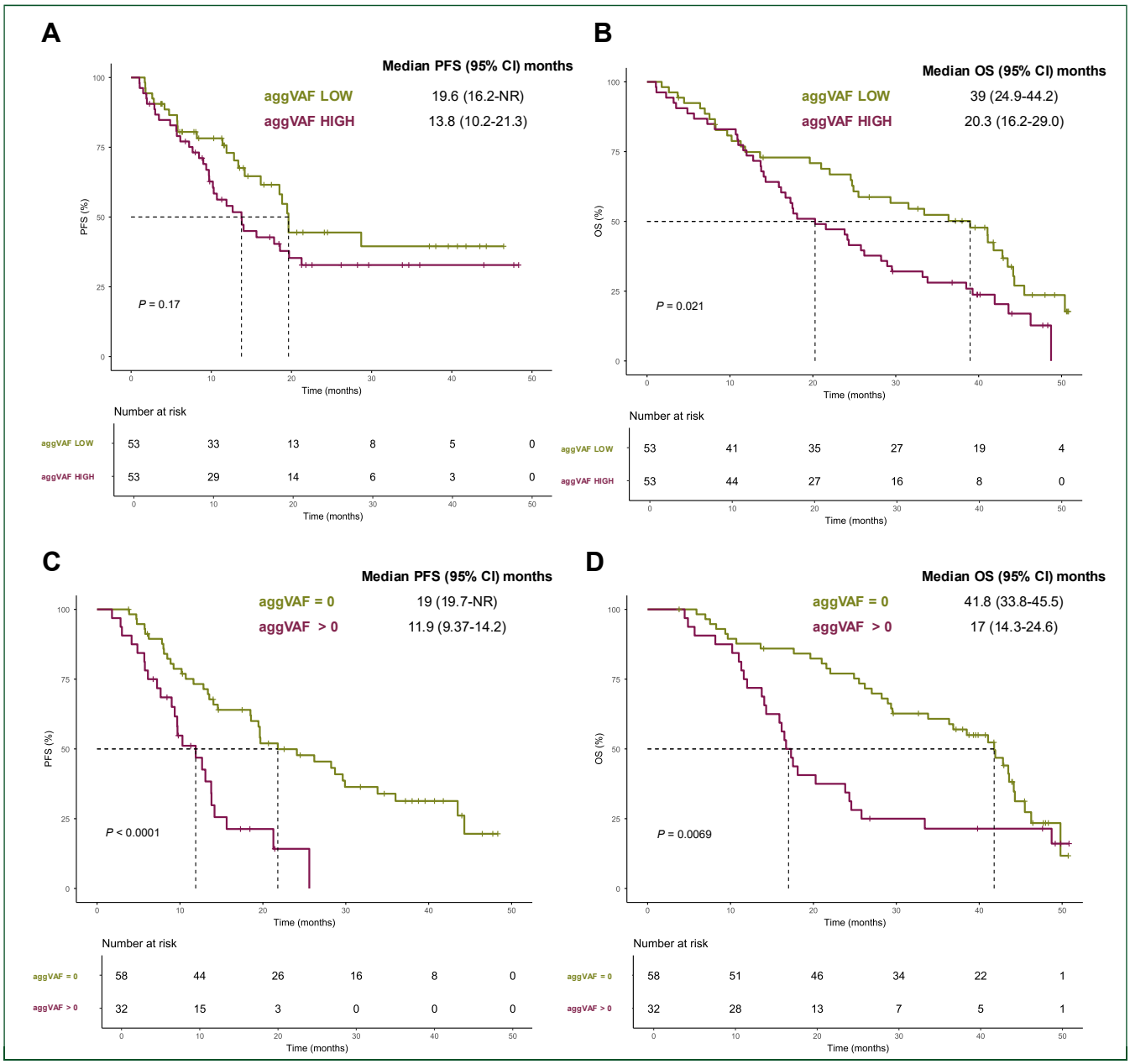

Figure 3. Survival outcomes according to aggVAF at baseline and week 8. Kaplan—Meier curves for PFS and OS are represented based on aggVAF values at baseline (A, B) and week 8 (C, D). The mAggVAF was calculated at both timepoints ($mAggVAF_{BASELINE}$ = 8.7%; $mAggVAF_{WEEK8}$ = 0%). Statistically significant differences in outcomes were observed in all comparisons, except for PFS at baseline (A).
aggVAF, aggregate variant allele frequency; CI, confidence interval; High, higher than the median; Low, lower than the median; mAggVAF, median aggVAF; NR, not reached; OS, overall survival; PFS, progression-free survival.

categories, median PFS was not reached, probably due to the fact that patients underwent radical surgery (CCR = 8 patients; CND = 4 patients) after first-line treatment based on imaging results indicating a partial or complete response to therapy. To further clarify the prognostic value of qualitative LB-RECIST categories, we repeated the PFS analysis after excluding patients who underwent surgery following first-line treatment (CCR: $n = 8$; CND: $n = 4$). Importantly, the prognostic relevance of the CCR and CND groups remained statistically significant even after this exclusion, as shown in Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2025.105760.

In contrast, the CPR and CPD groups showed the poorest outcomes, with mPFS values of 12.7 and 11.9 months and mOS values of 16.4 and 25.5 months, respectively (Figure 4).

A similar pattern of survival outcomes was observed within the qualitative LB-RECIST groups: mPFS for G1, G2, G3, and G4 were 11.9 months, NR, 14.2 months, and NR, respectively. Corresponding mOS values were 16.4 months, 41.8 months, 33.4 months, and 41.1 months. Altogether, persistent detectable ctDNA (G1) or conversion to detectable ctDNA (G3) were predictive of poor survival outcomes, whereas persistent undetectable (G4) or conversion to undetectable (G2)

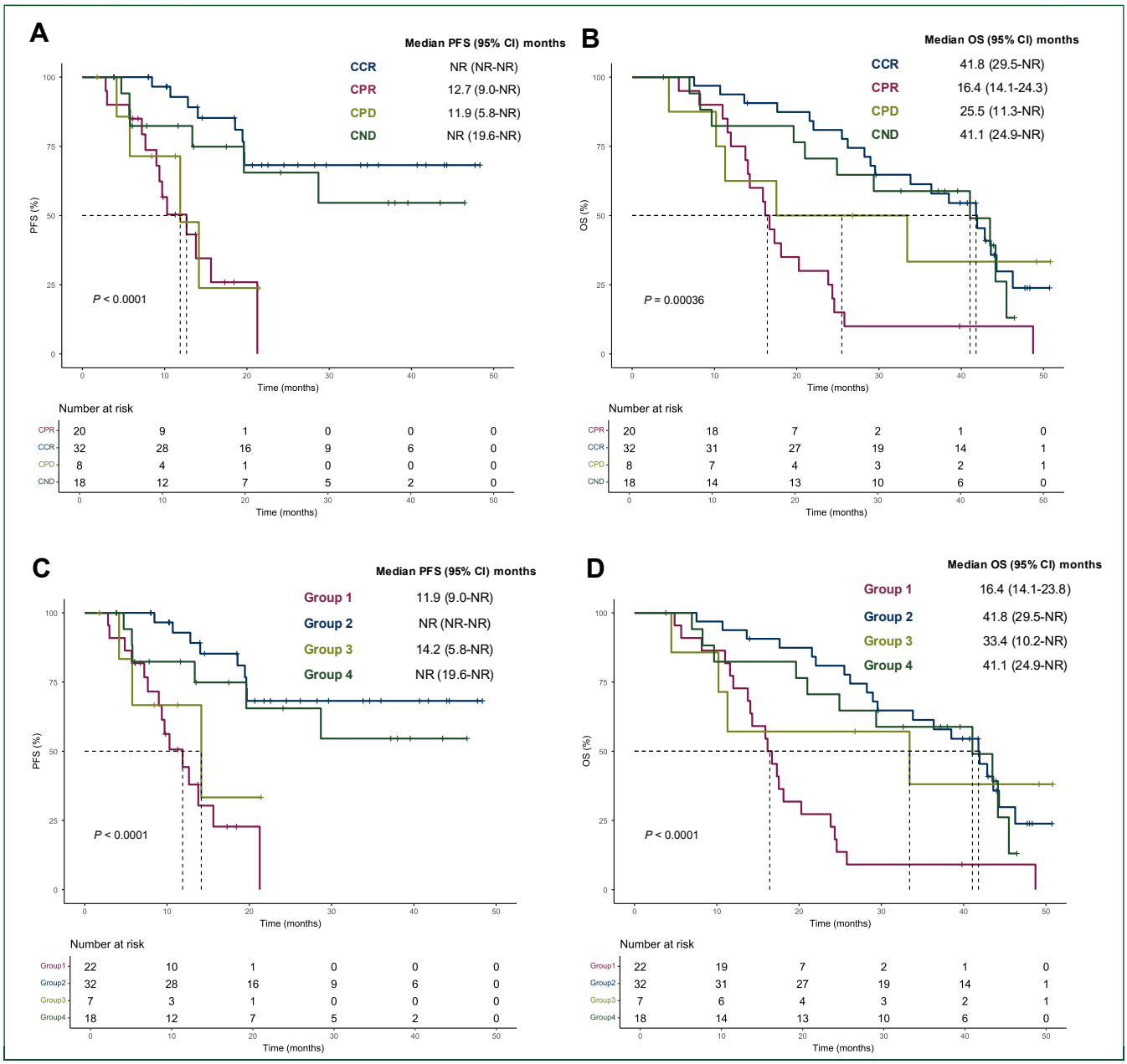

Figure 4. Survival outcomes according to liquid biopsy RECIST (LB-RECIST) criteria. Dynamic changes in aggVAF between baseline and week 8 were evaluated in $N = 79$ patients to assess molecular response according to quantitative LB-RECIST criteria (A, B). Variations in the detectability of ctDNA between baseline and week 8 were considered to define response as per qualitative LB-RECIST criteria (C, D).
aggVAF, aggregate variant allele frequency; CCR, ctDNA complete response; CI, confidence interval; CND, ctDNA not detectable; CPD, ctDNA progressive disease; CPR, ctDNA partial response; CSD, ctDNA stable disease; NR, not reached; OS, overall survival; PFS, progression-free survival.

were predictive of long-lasting clinical benefit to treatment and good long-term survival (Figure 4).

As an exploratory analysis, we considered only patients with concordant *RAS* status between tissue and ctDNA. Survival analyses restricted to this concordant cohort confirmed the prognostic value of molecular response categories, both for qualitative and quantitative LB-RECIST criteria (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2025.105760).

To contextualize these findings, survival outcomes were also evaluated according to best response assessed by radiological RECIST: mOS was 41.1 months in the CCR subgroup ($n = 12$), 29.5 months in patients with CPR ($n = 63$), 21.5 months for stable disease CSD ($n = 27$), and 5.3 months for CPD ($n = 12$). Median PFS ranged from 19.6 months (CCR) to 2.9 months (CPD) (Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2025.105760).

Cox regression analyses revealed statistically significant differences in PFS and OS across quantitative LB-RECIST categories ($P < 0.0001$). The same level of significance was observed in the qualitative LB-RECIST groups.

The hazard ratio (HR) values closely aligned with the Kaplan–Meier curves, reinforcing the observed survival

differences. Comparisons were made using CPR as the reference category for the quantitative LB-RECIST classification and G1 for the qualitative classification. No significant difference was observed between CPR and CPD (HR = 0.76, $P = 0.627$), as their survival curves were nearly overlapping. In contrast, both CCR and CND showed significantly lower HRs compared with CPR, indicating a protective effect. Since all HR values were <1, they suggest a reduced risk of progression or death relative to CPR. These findings highlight the consistency between HR estimates and Kaplan—Meier survival plots, providing complementary insights into the prognostic value of LB-RECIST categories. A complete overview of HRs is provided in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2025.105760.

Multivariate Cox regression analyses showed that LB-RECIST (both qualitative and quantitative) was the strongest prognostic and predictive marker of clinical benefit (for both OS and PFS), independently of clinicopathological features such as primary tumor location, *RAS* status, presence of the primary tumor, or liver metastases (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2025.105760).

DISCUSSION

There is an unmet clinical need for a standardized, reliable, and reproducible method to assess molecular response to systemic treatment in solid tumors. LB-RECIST has recently been proposed as a potential standardized criterion, based on a cohort of patients with diverse tumor types and treatment regimens.[26] However, ctDNA dynamics and detectability may vary depending on tumor type, treatment scheme, and line of treatment. Given that mCRC harbors truncal mutations (*TP53, KRAS, APC*) that are universally present in mCRC tumors and are abundant and easily detected in ctDNA, LB-RECIST represents a promising tool for application in this clinical setting. Therefore, we used mCRC as a proof-of-concept model to validate LB-RECIST, with the potential for its future adaptation to a pan-cancer framework.

Patients who achieved complete ctDNA clearance after initial detection (CCR; G2) experienced the best response to treatment in terms of PFS, followed by those patients with undetectable ctDNA at both baseline and week 8 (CND; G4). Notably, undetectable aggVAF at week 8 emerged as a strong predictor of treatment response, irrespective of baseline ctDNA levels. In contrast, increasing levels of aggVAF at week 8, the emergence of new mutations (CPD; G3), or the persistence of detectable mutations throughout the treatment (CPR, CSD; G1) correlated with significantly worse treatment outcomes. Evaluation of dynamic ctDNA changes from baseline to week 8 using qualitative and quantitative LB-RECIST criteria was a valid molecular response classifier, significantly predicting PFS. Additionally, a single timepoint analysis at week 8 ($aggVAF_{WEEK8}$) also demonstrated strong predictive value, highlighting its potential as a clinically relevant biomarker. Undetectable aggVAF at week 8 of therapy (including CCR/CND in the quantitative LB-RECIST; or G2/G4 in the qualitative ones) may support treatment de-escalation or even discontinuation, reducing patient burden and minimizing unnecessary toxicity. Conversely, persistent ctDNA or *de novo* detection (including CSD/CPD/CPR quantitative LB-RECIST; or G1/G3 qualitative LB-RECIST) may indicate the need for treatment modification, either by switching regimens or intensifying ongoing therapy to counteract the emergence of resistant clones. Interventional prospective randomized clinical trials are required to establish the clinical utility of LB-RECIST in guiding treatment decisions and optimizing patient outcomes.

Interestingly, our findings demonstrate that the LB-RECIST criteria are applicable and reproducible using a different analytical method—specifically, the NGS platform, Oncomine Colon cfDNA Assay on the Ion S5 system—compared with the ddPCR approach used in the original report.[25] This platform differs in genome coverage and sensitivity, yet it yielded consistent results, reinforcing the robustness of LB-RECIST in assessing molecular response.[27] Notably, most detected mutations were classical truncal mutations (*TP53; APC*) with high allele frequency in the blood of patients, suggesting that genome coverage and assay sensitivity may not be critical limitations in the context of mCRC. However, an important consideration is that in our study, ctDNA was assessed at week 8 (after three treatment cycles), and it remains unclear whether earlier timepoints for ctDNA analysis would provide similarly reliable predictive value.

Regarding the prognostic value of ctDNA, baseline aggVAF showed a statistically significant correlation with OS, confirming its role as a prognostic marker, consistent with previous results from other groups.[31] Moreover, aggVAF at week 8 was also predictive of OS, further reinforcing its clinical relevance. Undetectable ctDNA is often associated with 'non-shedder' tumors, which can sometimes exhibit aggressive clinical features, such as peritoneal carcinomatosis. However, in our study, patients with undetectable ctDNA at both baseline and week 8 (CND; G4) exhibited a very good prognosis. These findings highlight the prognostic relevance of ctDNA undetectability at any timepoint during treatment and underscore its potential utility in guiding clinical decision-making. Moreover, our analysis showed that patients with undetectable ctDNA at baseline more frequently had metachronous disease and a trend toward reduced liver involvement, supporting the hypothesis that ctDNA negativity in this context may reflect a less aggressive tumor biology.[32]

Unlike partial response in radiological RECIST criteria, the LB-RECIST CPR group exhibited poor survival outcomes, with an mOS even worse than that of CPD patients. Although definitive conclusions cannot yet be drawn, we hypothesize that applying a uniform percentage threshold (e.g. 10%) for both increases and decreases in aggVAF may lack the accuracy needed to distinguish CPR from CPD. A more stringent threshold for decreases in aggVAF might be required to accurately identify true CPR, akin to the

adjustments made in radiological RECIST criteria for evaluating treatment responses.[16] It is important to emphasize that the ±10% threshold adopted in LB-RECIST was originally proposed by Gouda et al. as part of a retrospective analysis across multiple tumor types and treatment settings.[25] Our study does not aim to formally validate this specific cutoff, but rather provides an independent disease-specific confirmation of its prognostic relevance in mCRC. Further refinement and prospective validation of molecular response thresholds remain warranted.

Overall, our data support the LB-RECIST criteria as an accurate and reproducible method for assessing molecular response in mCRC patients receiving first-line therapy, aligning with findings from the initial results reported by Gouda et al.[25] Nevertheless, some limitations should be acknowledged. First, the relatively small sample size may limit the generalizability of our findings, particularly for less frequent molecular response categories, such as CSD, which was represented by only one patient in our cohort. Second, the analytical method used for ctDNA assessment, based on a 14-gene targeted panel, presents intrinsic limitations. While cost-effective and clinically feasible, such a restricted panel may not fully capture the genomic heterogeneity of colorectal cancer.[33] Consequently, aggVAF should be interpreted as a surrogate rather than a direct measure of tumor burden. Broader genomic platforms or tumor fraction estimation algorithms could improve the precision of molecular response assessment.[34]

Furthermore, a subset of patients classified as CCR or CND underwent surgical resection after week 8 and discontinued systemic treatment. Although we were able to re-analyze survival outcomes excluding these cases—confirming the prognostic value of LB-RECIST categories—detailed information on the nature of surgical procedures (e.g. timing, intent, and resected sites) was not systematically collected in our dataset. This lack of granularity prevents deeper exploration of the role of locoregional treatments. Future studies should aim to prospectively integrate standardized data on surgical and ablative interventions, which may help refine the clinical application of LB-RECIST in the management of mCRC.

Another limitation of our study is the lack of comparative analysis with the standard serum biomarker carcinoembryonic antigen (CEA), which remains a conventional tool for monitoring mCRC. Although CEA data were available for some patients, the absence of centralized measurement protocols posed a high risk of inter-laboratory variability. For this reason, we opted not to include CEA comparisons to avoid potential bias, but we acknowledge that direct comparisons with ctDNA dynamics would be valuable and should be pursued in future centrally coordinated studies.

Finally, our study focused on assessments at only two timepoints. Although this proved to be a strong predictor of outcomes, intermediate timepoints could provide additional insights into ctDNA kinetics and further optimize response evaluation. In this sense, some authors have suggested that ctDNA dynamics assessed at earlier timepoints—such as 1-2 weeks following treatment initiation—may already provide indications of treatment efficacy, particularly in the setting of chemotherapy and targeted agents.[26] Future studies should explore earlier and more frequent sampling strategies to refine molecular response classification further and enhance its predictive accuracy.

Conclusions

This study confirms the clinical validity of LB-RECIST as a reliable tool for assessing early molecular response in mCRC patients receiving first-line standard systemic treatment. Our findings provide a foundation for integrating LB-RECIST into prospective interventional randomized clinical trials in mCRC, where it could be used to guide treatment modifications. Ultimately, the implementation of LB-RECIST in clinical practice has the potential to improve patient outcomes and enhance survival in mCRC.

FUNDING

This work was supported by Instituto de Salud Carlos III FEDER [grant number PI21/00045] and Fundacion CRIS Excellence [grant number 19-30] (to CM).

DISCLOSURE

The authors have declared no conflicts of interest.

REFERENCES

1. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol*. 2014;32(6):579-586.
2. Alix-Panabières C, Pantel K. Liquid biopsy: from discovery to clinical application. *Cancer Discov*. 2021;11(4):858-873.
3. Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2022;33(8):750-768.
4. Alese OB, Cook N, Ortega-Franco A, Ulanja MB, Tan L, Tie J. Circulating tumor DNA: an emerging tool in gastrointestinal cancers. *Am Soc Clin Oncol Educ Book*. 2022;42:1-20.
5. Dasari A, Morris VK, Allegra CJ, et al. ctDNA applications and integration in colorectal cancer: an NCI Colon and Rectal-Anal Task Forces whitepaper. *Nat Rev Clin Oncol*. 2020;17(12):757-770.
6. Bachet JB, Bouché O, Taieb J, et al. RAS mutation analysis in circulating tumor DNA from patients with metastatic colorectal cancer: the AGEO RASANC prospective multicenter study. *Ann Oncol*. 2018;29(5):1211-1219.
7. Meric-Bernstam F, Hurwitz H, Raghav KPS, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol*. 2019;20(4):518-530.
8. Nakamura Y, Okamoto W, Kato T, et al. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer: a phase 2 trial. *Nat Med*. 2021;27(11):1899-1903.
9. Montagut C, Argilés G, Ciardiello F, et al. Efficacy of Sym004 in patients with metastatic colorectal cancer with acquired resistance to anti-EGFR therapy and molecularly selected by circulating tumor DNA analyses: a phase 2 randomized clinical trial. *JAMA Oncol*. 2018;4(4):e175245.
10. Cremolini C, Rossini D, Dell'Aquila E, et al. Rechallenge for patients with RAS and BRAF wild-type metastatic colorectal cancer with acquired resistance to first-line cetuximab and irinotecan: a phase 2 single-arm clinical trial. *JAMA Oncol*. 2019;5(3):343-350.

11. Van Cutsem E, Huijberts S, Grothey A, et al. Binimetinib, encorafenib, and cetuximab triplet therapy for patients with BRAF V600E-mutant metastatic colorectal cancer: safety lead-in results from the phase III BEACON colorectal cancer study. *J Clin Oncol*. 2019;37(17):1460-1469.
12. Sartore-Bianchi A, Pietrantonio F, Lonardi S, et al. Circulating tumor DNA to guide rechallenge with panitumumab in metastatic colorectal cancer: the phase 2 CHRONOS trial. *Nat Med*. 2022;28(8):1612-1618.
13. Vivas CS, Barrull JV, Rodriguez CF, et al. 511MO Third line rechallenge with cetuximab (Cet) and irinotecan in circulating tumor DNA (ctDNA) selected metastatic colorectal cancer (mCRC) patients: the randomized phase II CITRIC trial. *Ann Oncol*. 2024;35:S433-S434.
14. Cervantes A, Adam R, Rosello S, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34(1):10-32.
15. Wyatt AW, Litiere S, Bidard FC, et al. Plasma ctDNA as a treatment response biomarker in metastatic cancers: evaluation by the RECIST working group. *Clin Cancer Res*. 2024;30(22):5034-5041.
16. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45(2):228-247.
17. Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol*. 2017;18(3):e143-e152.
18. Tie J, Kinde I, Wang Y, et al. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. *Ann Oncol*. 2015;26(8):1715-1722.
19. Thompson JC, Carpenter EL, Silva BA, et al. Serial monitoring of circulating tumor DNA by next-generation gene sequencing as a biomarker of response and survival in patients with advanced NSCLC receiving pembrolizumab-based therapy. *JCO Precis Oncol*. 2021;5:510-524.
20. Tan L, Tran B, Tie J, et al. A phase Ib/II trial of combined BRAF and EGFR inhibition in BRAF V600E positive metastatic colorectal cancer and other cancers: the EVICT (erlotinib and vemurafenib In combination trial) study. *Clin Cancer Res*. 2023;29(6):1017-1030.
21. Zhang Q, Luo J, Wu S, et al. Prognostic and predictive impact of circulating tumor DNA in patients with advanced cancers treated with immune checkpoint blockade. *Cancer Discov.* 2020;10(12):1842-1853.
22. Paik PK, Felip E, Veillon R, et al. Tepotinib in non-small-cell lung cancer with MET exon 14 skipping mutations. *N Engl J Med*. 2020;383(10):931-943.
23. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer.* 2017;17(4):223-238.
24. Postel M, Roosen A, Laurent-Puig P, Taly V, Wang-Renault SF. Droplet-based digital PCR and next generation sequencing for monitoring circulating tumor DNA: a cancer diagnostic perspective. *Expert Rev Mol Diagn*. 2018;18(1):7-17.
25. Gouda MA, Huang HJ, Piha-Paul SA, et al. Longitudinal monitoring of circulating tumor DNA to predict treatment outcomes in advanced cancers. *JCO Precis Oncol*. 2022;6:e2100512.
26. Gouda MA, Janku F, Wahida A, et al. Liquid biopsy response evaluation criteria in solid tumors (LB-RECIST). *Ann Oncol*. 2024;35(3):267-275.
27. Vidal J, Fernandez-Rodriguez MC, Casadevall D, et al. Liquid biopsy detects early molecular response and predicts benefit to first-line chemotherapy plus cetuximab in metastatic colorectal cancer: PLATFORM-B study. *Clin Cancer Res*. 2023;29(2):379-388.
28. Shitara K, Muro K, Watanabe J, et al. Baseline ctDNA gene alterations as a biomarker of survival after panitumumab and chemotherapy in metastatic colorectal cancer. *Nat Med*. 2024;30(3):730-739.
29. Stintzing S, Klein-Scory S, Fischer von Weikersthal L, et al. Baseline liquid biopsy in relation to tissue-based parameters in metastatic colorectal cancer: results from the randomized FIRE-4 (AIO-KRK-0114) Study. *J Clin Oncol*. 2025;43(12):1463-1473.
30. Andersen L, Kisistok J, Henriksen TV, et al. Exploring the biology of ctDNA release in colorectal cancer. *Eur J Cancer.* 2024;207:114186.
31. Elez E, Chianese C, Sanz-Garcia E, et al. Impact of circulating tumor DNA mutant allele fraction on prognosis in RAS-mutant metastatic colorectal cancer. *Mol Oncol*. 2019;13(9):1827-1835.
32. Ciardiello D, Boscolo Bielo L, Napolitano S, et al. Comprehensive genomic profiling by liquid biopsy captures tumor heterogeneity and identifies cancer vulnerabilities in patients with RAS/BRAF(V600E) wild-type metastatic colorectal cancer in the CAPRI 2-GOIM trial. *Ann Oncol.* 2024;35(12):1105-1115.
33. Schulze M, Wang X, Hamad J, et al. Real-world genomic landscape of colon and rectal cancer. *FEBS Open Bio*. 2025;15(4):674-685.
34. Rolfo CD, Madison RW, Pasquina LW, et al. Measurement of ctDNA tumor fraction identifies informative negative liquid biopsy results and informs value of tissue confirmation. *Clin Cancer Res*. 2024;30(11):2452-2460.