Sensitive cell-free DNA assay for accurate detection of RAS and BRAF mutations in liquid biopsies of patients with metastatic colorectal cancer: Final results of the multicentric ColoBEAM study

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Abstract. The multicentric prospective ColoBEAM study (NCT02751177) aimed to assess the feasibility of using liquid biopsy for the detection of *KRAS*, *NRAS* and BRAF gene mutations in patients with metastatic colorectal cancer (mCRC) in a real-world setting. The study involved eight medical centres. Tumour DNA from formalin-fixed paraffin embedded tissue was analysed using either PCR or next-generation sequencing as the gold standard, while circulating tumour DNA from plasma was evaluated using the beads emulsion and magnetics digital PCR technique. Blood sampling was conducted without strict timing constraints, allowing for a real-world reflection of clinical practice. Discrepancies between tissue and blood results were re-evaluated externally to ensure the reliability. A total of 202 patients had *KRAS* and *NRAS* (*RAS*) status available for analysis, and 198 patients had both RAS and BRAF statuses available. Overall, the study confirmed the feasibility of liquid biopsy, achieving a concordance rate of 83.2% for RAS mutations and 82.3% for RAS and BRAF mutations when compared with tissue biopsy. The sensitivity of liquid biopsy for detecting RAS mutations was 77.3%, with a specificity of 94.3%. For RAS and BRAF mutations combined, the sensitivity was 77.0% and the specificity was 93.7%. Further analysis based on patient characteristics at the time of blood sampling showed higher sensitivity in chemotherapy-naive patients (sensitivity, 86.1%; specificity, 91.3%) and in patients with liver metastases (sensitivity, 88.6%; specificity, 89.7%). Sensitivity was also higher when the primary tumour was present (sensitivity, 88.6%) and in cases of disease progression or recurrence (sensitivity, 82.8%). In conclusion, liquid biopsy was a feasible and valuable method for detecting RAS and BRAF mutations in patients with mCRC, offering a less invasive alternative to traditional tissue biopsy in real-world settings. The trial registration number is 2015-A01272-47/NCT02751177 (registered March 3, 2016).

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Abbreviations: BEAMing, beads emulsion and magnetics digital PCR; cfDNA, cell-free DNA; ctDNA, circulating tumour DNA; FFPE, formalin-fixed paraffin-embedded; INCa, Institut National du Cancer (French National Cancer Institute); mCRC, metastatic colorectal cancer; NCT, National Clinical Trial; NGS, next-generation sequencing

Key words: metastatic colorectal cancer, liquid biopsy, KRAS, NRAS, BRAF

Introduction

Colorectal cancer (CRC) is the third most diagnosed cancer and the third leading cause of cancer-related death worldwide for both sexes combined (1). In the U.S, it is the second most common cancer in men under 50 years of age, with an estimated total in 2023 of 153,020 diagnosed cases and 52,550 deaths, including 19,550 cases and 3,750 deaths in individuals younger than 50 years (2). In the E.U., CRC accounts for 520,000 new cases (12.9% of all cancer diagnoses) and 250,000 deaths (12.6% of cancer-related mortality) (3). The management of metastatic CRC (mCRC) has advanced, particularly with monoclonal antibodies targeting the epidermal growth factor receptor (anti-EGFR mAb) combined with 5FU-based chemotherapy. Anti-EGFR mAb therapy requires the absence of somatic mutations in exons 2, 3, and 4 of KRAS and NRAS genes (4). *BRAF* gene mutations are routinely assessed for their prognostic significance and their association with new therapeutic strategies combining anti-EGFR mAbs and BRAF kinase inhibitors (5).

Tissue-based biopsy remains the gold standard method for molecular analysis of cancer, but its invasiveness and potential complications limit its frequent use (6).

In France, somatic mutations in *KRAS*, *NRAS*, and *BRAF* are primarily analysed on INCa (Institut National du Cancer-French Cancer Institute) certified molecular genetics platforms (INCa platform) using formalin-fixed paraffin embedded (FFPE) tissue from biopsies of primary or secondary lesions or surgically excised primary tumours (7). Given the limitations of tissue-based biopsies and the dynamic genetic evolution of tumours, there is a growing demand for a less invasive and more precise alternative like liquid biopsy. In real-world settings, challenges such as insufficient sample quantity or quality for genotyping, or the inability to retrieve specimens from external centres, further underscore this need. Monocentric prospective biomarker studies have demonstrated the clinical utility of circulating tumour DNA (ctDNA) as a valuable marker in first-line mCRC treatment (8) and in primary CRC at surgery and during post-surgery follow-up (9). However, without standardized workflows, liquid biopsy must be validated against standard-of-care tissue testing in real-world settings for routine tumour molecular profiling and identification of treatment response biomarkers, such as *KRAS*, *NRAS*, and *BRAF* mutations in CRC.

Several ctDNA analysis techniques exist, with studies confirming the feasibility of liquid biopsy for KRAS and BRAF genotyping (10,11). Yet, few prospective real-world studies have been conducted. The BEAMing (Beads, Emulsion, Amplification and Magnetics) technique, a reference method used in re-analyses of historical trials like CRYSTAL (12) and OPUS (13), and studies, such as FIRE3 (14).

The ColoBEAM protocol evaluated the real-world feasibility of liquid biopsy as a standard for detecting *KRAS*, *NRAS*, and *BRAF* mutations, comparing genotyping results from blood samples with those from routine FFPE tissue specimens.

Materials and methods

Patients. A total of 278 patients, aged 18 or older, with pathologically confirmed mCRC were enrolled in this study from March 2016 to May 2017 at 8 medical centres (Institut de Cancérologie de Lorraine, Vandœuvre-lès-Nancy, France; Polyclinique de Gentilly, Nancy, France; Hôpital Belle-Isle-Metz, Metz, France; Centre Paul Strauss, Strasbourg, France; CHU Reims Hôpital Robert Debré, Reims, France; CH Auxerre, Auxerre, France; CH Chalon Sur Saône-William Morey, Chalon sur Saône, France; CH Besançon-Hôpital Jean Minjoz, Besançon, France). Eligible patients were adults (≥18 years) diagnosed with metastatic colorectal cancer. Inclusion required that a molecular analysis of *KRAS*, *NRAS*, and *BRAF* mutations be clinically indicated as part of routine disease management. All participants provided written informed consent prior to enrolment, and were covered by the French national health insurance system. Patients with metastatic colorectal cancer (mCRC) were eligible for inclusion if they had not received prior anti-EGFR monoclonal antibody therapy. This criterion was established to ensure that the evaluation of RAS/BRAF mutational status by BEAMing in plasma would reflect the untreated molecular profile. As prior exposure to anti-EGFR agents may induce clonal selection and alter the ctDNA landscape, excluding previously treated patients was necessary to avoid potential confounding effects on concordance analyses between tissue and liquid biopsy results (15).

Exclusion criteria included non-metastatic CRC at the time of initial tissue biopsy, local recurrence, exclusive nodal metastases, contraindications to a 30 ml blood draw, receipt of a blood transfusion within 15 days prior to blood collection, other malignant tumours within the past 5 years, and pregnancy or breastfeeding, and prior receipt of anti-EGFR therapy (n=4). Of the included patients, 25 had no detectable metastases at the time of blood sampling due to prior resection of metastatic lesions (e.g. liver or lung metastases). Blood samples were collected without strict timing constraints, including from patients who had received chemotherapy or radiotherapy, to reflect real-world clinical conditions.

The research protocol was approved by the Ethics Committee (CPP Est III, Nancy, France; number 15.09.09), and written informed consent was obtained from all patients. The protocol was registered at ClinicalTrials.gov (NCT02751177).

Procedures. DNA extracted from FFPE tissue, collected at the time of the initial biopsy, was analysed for *KRAS*, *NRAS* and *BRAF* mutations using PCR or next-generation sequencing (NGS) assays at INCa platform, as per standard-of-care guidelines. At inclusion, three 10 ml blood samples were collected in DNA BCT tubes (Streck, La Vista, NE, USA). Sample collection lacked strict timing constraints and, in some instances, occurred long after the initial biopsy to reflect real-world conditions. The samples were shipped at room temperature to the Biopathology Department of the Institut de Cancérologie de Lorraine for cell-free DNA (cfDNA) extraction and centralized analysis, hereafter referred to as 'ICL analyses' (Fig. 1). Plasma was obtained through double centrifugation (10 min at 1,600 x g, followed by 10 min at 6,000 x g) and stored at -80°C until ctDNA analysis. cfDNA was then extracted from 3-4 ml of plasma using the QIAamp circulating nucleic acid kit (Qiagen, Hilden, Germany) and analysed with the OncoBEAM™ assay (Sysmex, Norderstedt, Germany). Plasma-derived RAS mutation results were then compared with tissue-based results.

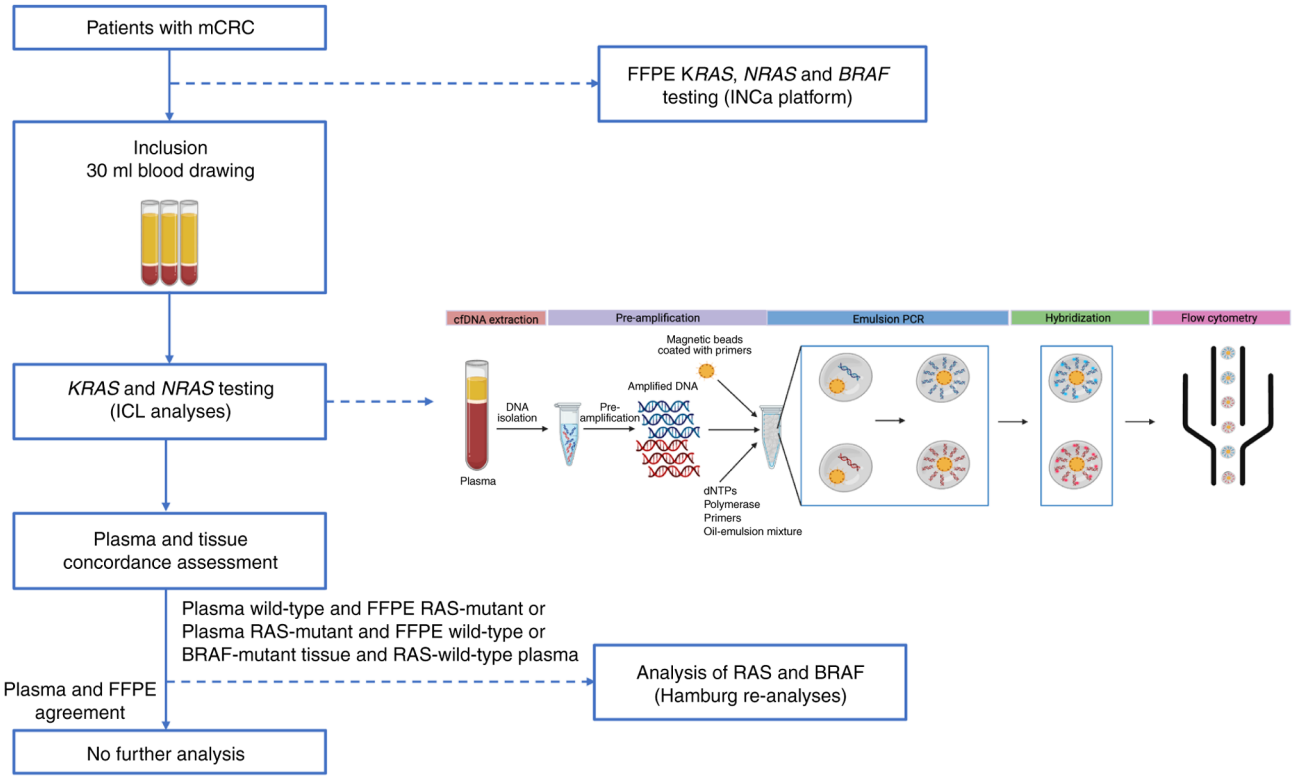

Figure 1. Study and sample analysis workflow. cfDNA, cell-free DNA; FFPE, formalin-fixed paraffin-embedded; ICL, Institut de Cancérologie de Lorraine; INCa, Institut National du Cancer (French National Cancer Institute); mCRC, metastatic colorectal cancer.

To ensure the robustness of mutational status assignment and to resolve discordant cases, an independent blinded re-analysis was conducted by the Sysmex service laboratory in Hamburg-hereafter referred to as the 'Hamburg re-analyses.' This laboratory served a dual role: it provided an external confirmation of BRAF status (V600E mutation) for all relevant samples, and acted as a fully blinded reference centre for the assessment of RAS mutations, enabling an unbiased comparison with initial local results. None of the named authors on the paper are affiliated with Sysmex Inostics Inc., and the company did not have any input into the planning or design of the experiments. The re-analysis focused specifically on samples displaying discrepancies between tissue and plasma results, including cases where i) the tumour tissue was RAS-mutant but the corresponding plasma was wild-type (potential false negatives), ii) the tumour was BRAF-mutant while plasma was wild-type (potential false negatives), and iii) the tissue was RAS wild-type but the plasma showed a RAS mutation (potential false positives). For each of these cases, DNA was re-extracted from the original FFPE tumour samples and ctDNA was isolated from a second blood sample collected at the time of patient inclusion. Both DNA sources were analysed in Hamburg using the BEAMing technology for RAS and BRAF mutations. In the event of new discrepancies between the initial analyses ('ICL analyses') and the Hamburg re-analyses, the latter were considered the reference due to their blinded nature and standardized quality procedures. The reconciled mutation calls derived from this process were used as the final BEAMing results in all statistical analyses, with FFPE tumour genotyping from the INCa-certified platform consistently considered the gold standard for tissue mutation status. This rigorous, multi-source validation framework, based on blinded external testing with a reference technology, ensured the reliability and clinical relevance of the final dataset, particularly in evaluating the performance of liquid biopsy in routine practice.

In accordance with routine clinical management of metastatic colorectal cancer (mCRC), treatment response was evaluated after three months using radiological imaging and clinical assessment.

Data collection. All clinical and biological data at inclusion, along with *RAS* and *BRAF* testing results, were recorded in an electronic case report form (CleanWeb, Telemedicine Technologies, Boulogne-Billancourt, France).

Samples analysis workflow. The sample analysis strategy is outlined in Fig. 1. All FFPE samples were analysed using standard-of-care protocols established by INCa-certified platforms, employing PCR-based or NGS-based assays (16-18). *RAS* and *BRAF* results obtained from FFPE samples were blinded to laboratory personnel during data analysis.

The BEAMing assay was used to analyse cfDNA samples, targeting 34 mutations in codons 12, 13, 59, 61, 117, and 146 of *KRAS* and *NRAS* gene. In brief, cfDNA underwent pre-amplification, followed by emulsion PCR and hybridization, with prepared samples analysed by flow cytometry per the manufacturer's protocol.

Statistical analysis. Final BEAMing results for plasma samples were determined after reconciling ICL analyses with Hamburg re-analyses. FFPE tissue results from

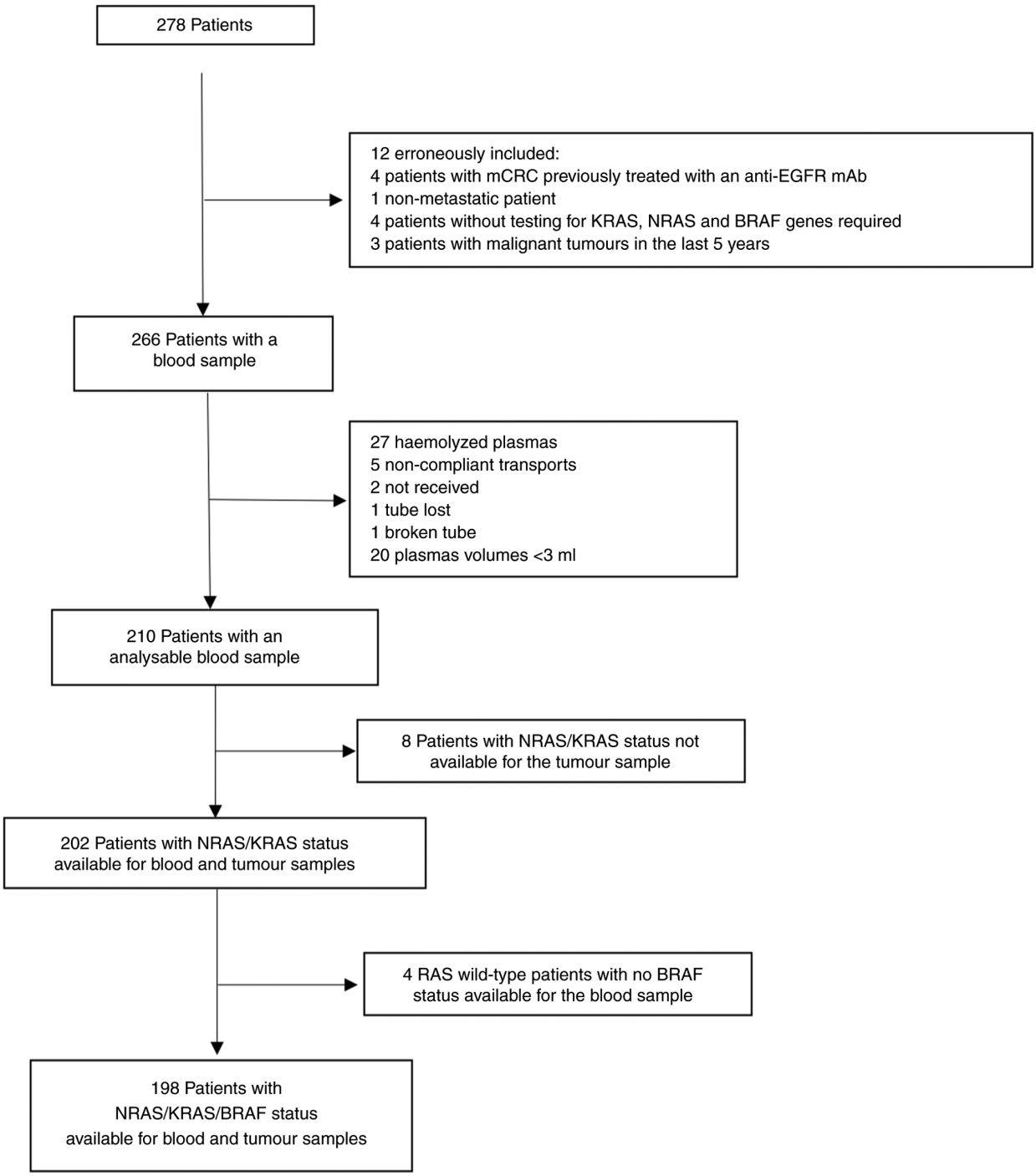

Figure 2. Patients and samples available for analysis. mAb, monoclonal antibody; mCRC, metastatic colorectal cancer.

INCa-certified platforms served as the gold standard. Mutation carriers were defined as patients with at least one detected mutation in the *KRAS*, *NRAS*, or *BRAF* genes. The sensitivity of the BEAMing test was calculated as the proportion of mutation carriers identified by the BEAMing test relative to those identified in FFPE samples, reported with a 95% confidence interval.

Similarly, the specificity of the BEAMing assay was calculated as the proportion of patients classified as non-mutation carriers in plasma relative to those classified as non-mutation carriers in FFPE samples, also reported with a 95% confidence interval.

Results

Patient inclusion and sample availability. A total of 278 patients were initially enrolled in the study. Twelve patients were excluded due to erroneous inclusion (Fig. 2), resulting in a corrected cohort of 266 patients. Among them, 56 blood samples were not analysable due to failure to meet quality or processing criteria. Of the remaining 210 patients with analysable blood samples, KRAS/NRAS status from tumour tissue was unavailable in 8 cases. Consequently, 202 patients had complete RAS genotyping data available for both blood and tumour samples. Within

Table I. Study population characteristics at the time of blood sampling of the 202 analysed patients.

Variable	Value
Median age, years[a]	67.0 (58.0-76.0)
Weight, kg (median)[a]	72.0 (62.0-83.0)
Missing	5
Location, n (%)	
Colon	110 (54.5)
Left	18 (16.4)
Right	50 (45.5)
Hinge	34 (30.9)
Transverse	8 (7.3)
Rectosigmoid junction	30 (14.9)
Rectum	59 (29.2)
Other	3 (1.5)
Presence of metastasis at the time of blood sampling, n (%)	
No	25 (12.4)
Yes	177 (87.6)
Number of metastatic sites, n (%)	
0	25 (12.4)
1	78 (38.6)
>1	99 (49.0)
Metastasis location (non-exclusive), n (%) (n=177)	
Liver	127 (62.9)
Pulmonary	94 (46.5)
Peritoneal	45 (22.3)
Ganglionary	36 (17.8)
Bone	10 (5.0)
Brain	3 (1.5)
Other	15 (7.4)
Chemotherapy[b], n (%)	
Naive	59 (29.2)
Undergoing	94 (46.5)
Finished	49 (24.3)
Chemotherapy administration, n (%) (n=143)	
Within 15 days of blood collection	29 (20.9)
>15 days from blood collection	110 (79.1)
Missing	4
Primary tumour, n (%)	
Present	70 (34.7)
Eradicated after treatment	132 (65.3)

[a]Values are presented as the median (minimum-maximum); [b]ll patients were treated in the first-line metastatic setting.

this subgroup, BRAF mutation status in plasma was unavailable for 4 patients, resulting in 198 patients with complete RAS and BRAF data for both sample types. In summary, 202 patients were included in the final RAS concordance analysis and 198 patients in the combined RAS/BRAF analysis (Fig. 2).

Discordant cases and external re-analysis. After local testing ('ICL analyses'), discordances between plasma and tumour samples were identified in 50 patients for NRAS status (refer to Data S1, and Tables SI, SII and SIII). Among these, 46 patients had RAS mutations detected in tumour tissue but classified as wild-type in plasma (false negatives), and 4 patients had wild-type RAS in tissue but were called mutated in plasma (false positives). Additionally, 16 patients initially classified as RAS wild-type in plasma by ICL were reclassified as RAS-mutated based on Hamburg re-analyses.

Table II. Tumour sample description (n=202).

Variable	Value
Nature of tissue, n (%)	
Biopsy	89 (44.1)
Surgery specimen	113 (55.9)
Tumour type, n (%)	
Primary tumour	164 (81.2)
Metastasis	38 (18.8)
Tumour cell content, %[a]	50 (30-70)
Routine results from INCa platform, n (%)	
Known mutation status (FFPE tissue)	202 (100)
KRAS or NRAS gene mutation	
Mutated	132 (65.4)
Wild-type	70 (34.6)
BRAF gene mutation	
Mutated	5 (2.5)
Wild-type	197 (97.5)
KRAS/*NRAS*/*BRAF* gene mutation	
Mutated	137 (67.8)
Wild-type	65 (32.2)

[a]Values are presented as the median (minimum-maximum). FFPE, formalin-fixed paraffin-embedded; INCa, Institut National du Cancer (French National Cancer Institute).

Regarding the five BRAF-mutated tumour samples, BRAF mutation status in plasma was confirmed in two cases, while data were not available for two others (refer to Data S1).

Sample and tumour characteristics. Patient characteristics at the time of blood sampling are detailed in Table I, and the tumour sample availability is summarized in Table II. Among the 202 patients analysed, the timing of blood collection relative to the initial tumour biopsy was distributed as follows: 15.8% (n=32) within one month, 10.9% (n=22) between 1 and 3 months, 14.4% (n=29) between 3 months and 1 year, 21.8% (n=44) between 1 and 2 years, 18.8% (n=38) between 2 and 3 years, and 18.3% (n=37) three or more years after biopsy.

Primary endpoint and overall performance. The comparison of BEAMing plasma genotyping results with tumour tissue genotyping from INCa-certified platforms is presented in Table III. The study's primary objective-evaluating the accuracy of BEAMing for detecting KRAS, NRAS, and BRAF mutations using tumour genotyping as the reference-was achieved. For RAS mutation detection, the sensitivity was 77.3%, specificity was 94.3%, and overall concordance was 83.2%. For combined RAS and BRAF mutations, sensitivity was 77.0%, specificity 93.7%, and concordance 82.3%. Despite high specificity (>93%) across analyses, overall sensitivity remained moderate when evaluating tumour vs. plasma mutation status globally.

Subgroup analyses. Subgroup analyses are shown in Fig. 3 and account for key clinical parameters at the time of blood sampling, including tumour presence, metastatic status and location, chemotherapy exposure, and treatment response.

Metastatic status and tumour presence. Sensitivity improved significantly in patients with liver metastases (sensitivity, 88.6%; specificity, 89.7%) and in those with a visible primary tumour (sensitivity, 88.6%; specificity, 88.5%). In contrast, patients without metastases showed limited sensitivity (50.0%), while those with metastases had improved sensitivity (75.0%), which increased with the number of metastatic sites. Metastatic site analysis revealed higher sensitivity for liver metastases (~88%) than for pulmonary metastases (77%).

Tumour location. Sensitivity also varied with primary tumour location: 100% for transverse colon, 95% for sigmoid, 69% for left colon, and 63% for right colon tumours.

Chemotherapy exposure. BEAMing sensitivity was highest in chemotherapy-naive patients (sensitivity, 86.1%; specificity, 91.3%) and declined with recent chemotherapy. Sensitivity was acceptable (79.0%) when chemotherapy was administered more than 15 days before sampling but dropped to 61.0% when treatment occurred within 15 days.

Treatment response. Sensitivity was highest in patients with progressive or recurrent disease (82.8%), intermediate in those with stable disease or partial response (74.5%), and lowest in patients achieving complete response (50.0%). Regardless of response status, specificity remained excellent (91.2 to 100%).

Discussion

The ColoBEAM study was designed as a real-world study to evaluate the feasibility of using liquid biopsy to detect *KRAS*, *NRAS*, and *BRAF* mutations in patients with mCRC, potentially replacing tissue biopsy. Each patient underwent routine tissue analysis, with plasma derived from whole blood analysed using the OncoBEAM™ assay (19,20). Compared to previous studies, such as the prospective multicentre real-world comparison of OncoBEAM-based liquid biopsy and tissue analysis for RAS mutations in mCRC (21), or a multi-institutional study (22), the ColoBEAM study provides additional insights by simultaneously assessing *RAS* and *BRAF* mutations in ctDNA from mCRC patients.

Of the 278 enrolled patients, data from 202 patients with *RAS* status and 198 patients with both *RAS* and *BRAF* status were analysed (4 patients with wild-type *RAS* lacked *BRAF* status in plasma samples, as shown in Fig. 2) after exclusions. To assess concordance, results were compared, with discordant cases sent to Sysmex in Germany for blinded external re-analysis. Some samples were excluded due to quality issues, such as haemolysis during transport.

This study evaluated BEAMing for detecting KRAS, NRAS, and BRAF mutations in plasma from 202 CRC patients, achieving a sensitivity of 77.3%, specificity of 94.3%, and concordance of 83.2% for RAS mutations, with similar metrics for combined RAS/BRAF analysis (sensitivity 77.0%, specificity 93.7%, concordance 82.3%). Compared to studies using various liquid biopsy methods, our sensitivity is slightly lower than Bettegowda *et al* (23) at 87.2% with digital PCR in CRC, likely due to our mixed cohort including non-metastatic cases, and Thierry *et al* (24) at 85% in metastatic CRC (mCRC). Focusing on BEAMing-specific studies,

Table III. *KRAS*, *NRAS* and *BRAF* mutational status in plasma samples determined by beads emulsion and magnetic digital PCR, compared with tumour genotyping performed on formalin-fixed paraffin-embedded samples at the French National Cancer Institute platform, used as the reference standard.

Mutations	No. of patients	TP, n	TN, n	FN, n	FP, n	Sensitivity, %	Specificity, %	Concordance, %	Kappa (95% CI)
RAS	202	102	66	30	4	77.3	94.3	83.2	0.658 (0.557-0.759)
RAS + *BRAF*	198	104	59	31	4	77.0	93.7	82.3	0.634 (0.529-0.740)

TP, true positive (presence of the mutation in the plasma and the tumour); TN, true negative (wild-type in plasma and tumour); FP, false positive (presence of the mutation in the plasma and wild-type in the tumour); FN, false negative (wild-type in the plasma and presence of the mutation in the tumour).

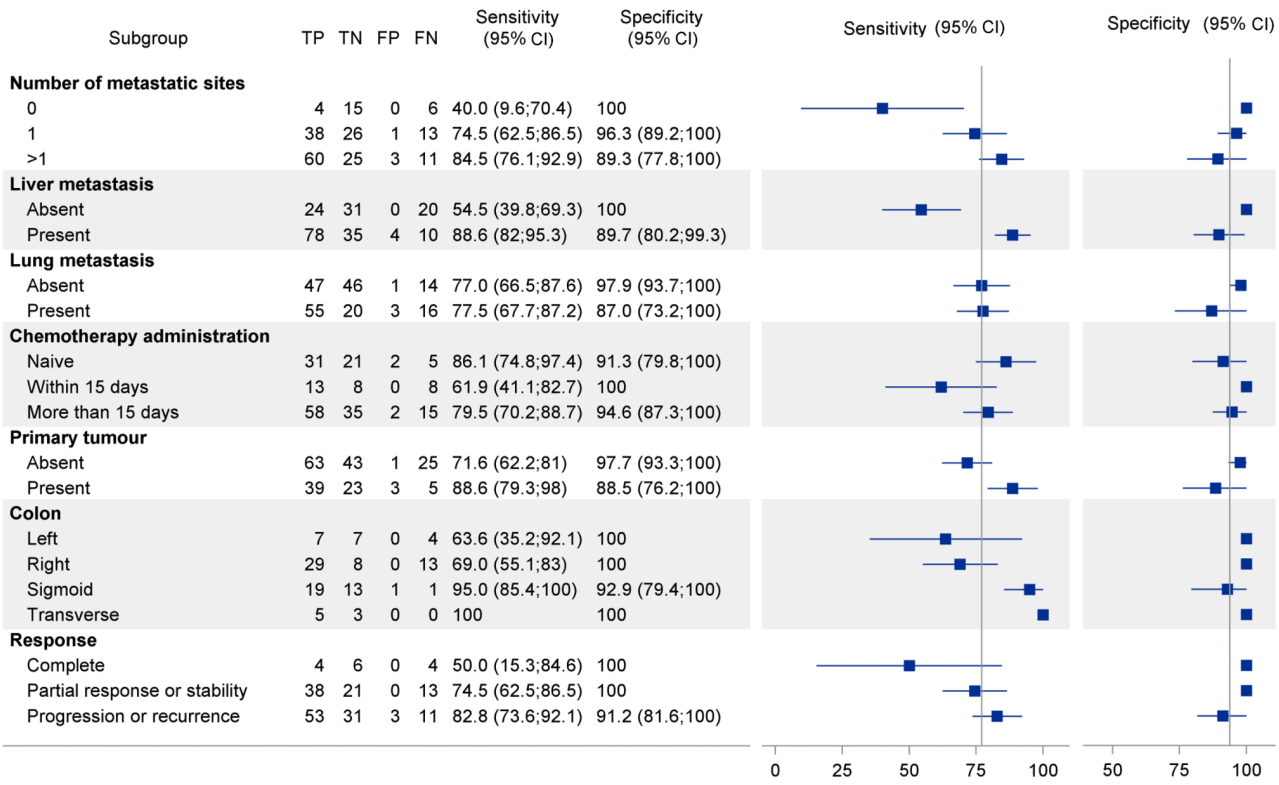

Figure 3. Sensitivity and specificity of mutational status detection in plasma samples across patient subgroups compared with tumour genotyping performed on formalin-fixed paraffin-embedded samples. TP, true positive; TN, true negative; FP, false positive; FN, false negative.

García-Foncillas *et al* (21) reported 93.3% concordance for OncoBEAM RAS testing in mCRC, and Vivancos *et al* (25) found 89% concordance (kappa 0.770) in 236 mCRC patients, with re-analysis improving to 92%; our lower sensitivity in non-metastatic cases (50.0%) and post-chemotherapy settings echoes Grasselli *et al* (26) on reduced BEAMing sensitivity with lower tumour burden. The ColoBEAM study provides unique value by simultaneously evaluating *KRAS*, *NRAS*, and *BRAF* mutations in a real-world setting with a diverse patient cohort, including non-metastatic cases, which may explain the slightly lower concordance (83.2% for RAS mutations) compared to Vidal *et al* (27) (93%) and García-Foncillas *et al* (21) (89%). These studies primarily focused on metastatic patients, whereas our broader inclusion criteria likely introduced variability in ctDNA shedding, impacting sensitivity, as noted in our subgroup analyses (e.g., 50.0% sensitivity in non-metastatic cases vs. 88.6% in patients with liver metastases). The high specificity (>93%) supports BEAMing's reliability for ruling out mutations, as seen in Bando *et al* (28) with 90.4% positive agreement, reinforcing its utility for guiding anti-EGFR therapy in mCRC.

Subgroup analysis revealed that the presence of metastases aligns with existing literature, which indicates that patients with disease progression exhibit higher ctDNA levels, enhancing the sensitivity and effectiveness of ctDNA detection (29-31). In our study, the concordance between *KRAS*, *NRAS* and *BRAF* mutation status in ctDNA and FFPE tissue was higher in patients with progressive disease or liver metastases (sensitivity, 82.8%; specificity, 91.2%), consistent with prior findings (21), reporting greater concordance in patients with liver metastases (94.5-94.8%) comparted to those without (83.8%; P=0.040). The 'lung metastasis' subgroup, consisting of patients with unique

lung metastases only, showed the lowest concordance rate of 68.8%. The inclusion of 25 patients with no detectable metastases at the time of blood sampling, due to prior resection of metastatic lesions, likely contributed to the lower overall concordance (83.2% for RAS mutations) compared to studies focused exclusively on patients with active metastases (27). These patients exhibited a reduced sensitivity of 50.0% for detecting KRAS, NRAS, and BRAF mutations in plasma (Fig. 3), likely due to decreased circulating tumour DNA (ctDNA) shedding in the absence of metastatic burden, as supported by prior studies (32). This finding highlights the influence of metastatic status on the performance of liquid biopsy and underscores the need to consider disease stage when implementing the BEAMing assay in real-world clinical settings.

These findings have significant implications, supporting the strategic timing of blood-based mutation testing, ideally before treatment initiation as per current guidelines, to maximize sensitivity and clinical utility. The differential sensitivity in chemotherapy-naive patients vs. those receiving chemotherapy suggests a dynamic interplay between treatment and ctDNA release or clearance.

Although ctDNA offers high specificity, cfDNA profiling has broader applications, reflecting diverse cellular process in the body. This precision medicine strategy supports liquid biopsy not only as a potential alternative to tissue biopsy but also as a complementary tool that captures the temporal and spatial heterogeneity of tumours. As demonstrated in lung and breast cancer, ctDNA mutation tracking has proven effective for guiding therapeutic adjustments throughout disease progression (33,34).

Our findings are consistent highlighting the utility of circulating tumour DNA (ctDNA) as a highly specific biomarker for cancer detection, disease progression monitoring, adjuvant therapy guidance, and potentially reducing unnecessary toxicity (35). Notably, studies such as CIRCULATE-Japan have established a foundation for understanding liquid biopsy in cancer treatment and recurrence monitoring (36).

Despite the promising results, this study acknowledges limitations, including the influence of recent chemotherapy and metastatic burden on test sensitivity. Another notable limitation of our study was the exclusion of 56 out of 266 blood samples (21%) due to preanalytical issues, such as haemolysis or insufficient blood volume, despite the use of DNA BCT tubes designed to stabilize cfDNA. These issues were likely exacerbated by initial sample transport at room temperature and, in some instances, accidental freezing during shipment, which compromised sample integrity. These challenges, observed in our multicentre real-world setting, highlight the critical need for standardized preanalytical protocols, including controlled transport conditions to prevent temperature fluctuations, to ensure reliable ctDNA analysis. Future studies should prioritize optimized sample collection, handling, and transport workflows to minimize such issues and enhance the clinical applicability of liquid biopsy techniques like the BEAMing assay.

A notable finding in our study was the reclassification of 16 patients from RAS wild-type to RAS-mutated in plasma following centralized re-analysis in Hamburg, highlighting differences in sensitivity between local (ICL) and centralized analyses. Several factors may explain this discrepancy. First, the Hamburg laboratory, as a centralized facility with extensive experience in OncoBEAM™ assay implementation, likely benefited from optimized workflows and greater technical expertise, enhancing mutation detection. Second, the Hamburg re-analysis may not have strictly followed the same procedure as the local ICL analyses, with potential differences in assay optimization or quality control measures. These findings underscore the importance of centralized laboratory expertise and standardized procedures to maximize the sensitivity of ctDNA analysis in real-world settings. Another limitation of our study is that only discrepant cases underwent re-analysis using BEAMing. It is therefore possible that additional discrepancies would have been identified if all samples had been retested, particularly in cases classified as *RAS* or *BRAF* wild-type in tissue. The higher sensitivity of BEAMing may have revealed ultra-low frequency subclonal mutations, potentially leading to a reclassification and further highlighting the complexity of tumour heterogeneity.

The timing of blood collection relative to initial tissue biopsy may also influence the concordance between plasma and tissue-based mutation detection, as tumour evolution or changes in circulating tumour DNA (ctDNA) shedding could occur over time. In our study, blood sampling occurred at varying intervals post-biopsy (15.8% within one month, 21.8% between 1 and 2 years, and 18.3% three or more years, as shown in Table I), reflecting the real-world setting of the ColoBEAM study. While we did not perform a specific statistical analysis of this variable's impact, longer intervals may contribute to discordance due to tumour heterogeneity or disease progression, particularly in patients with mCRC. This observation aligns with prior studies suggesting that temporal discrepancies between tissue and plasma sampling can affect ctDNA detection (15). Future studies should quantify the impact of sampling timing on concordance to optimize liquid biopsy protocols, particularly in real-world clinical settings.

Integrating ctDNA analysis with other biomarkers, such as microsatellite instability (MSI) status (6), could facilitate the development of innovative checkpoint inhibitor therapies (37,38). This synergy may advance the identification of a comprehensive set of routine biomarkers to support personalized medicine.

In conclusion, our study demonstrates that blood-based testing for detecting mutations in patients with mCRC is feasible. Although the test exhibited high accuracy in identifying mutations compared to tissue biopsies, factors such as recent chemotherapy and tumour location may reduce its sensitivity. However, adherence to guideline recommendations (4), may mitigate these limitations, as testing is ideally performed before treatment initiation to minimize the impact of factors like recent chemotherapy. ctDNA analysis is now included in guidelines for CRC progression after therapy, at diagnosis in patients with limited access to tissue biopsies, or when tissue specimens are inadequate due to insufficient quantity or quality or are unavailable for molecular analysis (39). Despite these limitations, our results indicate that blood-based mutation testing provides a less invasive and valuable option in real-world settings.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

AH, AL and JLM wrote the original protocol, analysed the data and wrote the manuscript. CG was the principal investigator. CG, OB, MBA, JP, DS, AB, FG, ALV and CB were investigators. CG, OB, MBA, JP, DS, AB, FG, ALV, CB and PG contributed to data acquisition and critically revised the manuscript for important intellectual content. MR and MH analysed the samples. JS analysed the data and all statistics. AH and JS confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All patients gave their informed and written consent to participate. The study is registered under number NCT02751177 and has been approved by the ethical committee Comité de Protection des Personnes Est (CHRU de Nancy, Hôpital de Brabois, Vandoeuvre-Lès-Nancy, France) under the number 15.09.09, ID RCB 2015-A01272-47.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools (OpenAI Chat GPT 4.0) were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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