original reports

Undetectable *RAS*-Mutant Clones in Plasma: Possible Implication for Anti-EGFR Therapy and Prognosis in Patients With *RAS*-Mutant Metastatic Colorectal Cancer

Mohamed Bouchahda, MD[1-4]; Raphael Saffroy, PhD[5]; Abdoulaye Karaboué, MD[2,6]; Jocelyne Hamelin, PhD[5]; Pasquale Innominato, MD, PhD[2,7,8]; Faouzi Saliba, MD, PhD[9]; Francis Lévi, MD, PhD[1,2,8]; Nelly Bosselut, MD[5]; and Antoinette Lemoine, PharmD, PhD[5]

abstract

PURPOSE Combining cetuximab with chemotherapy provides clinical benefit to 60% of the patients with *RAS* wild-type (*RAS*-wt) metastatic colorectal cancer (mCRC). This pilot study investigated the efficacy of cetuximab-based chemotherapy in a sample of patients (40%) with *RAS* mutation (*RAS*-mt) in their primary tumor whose circulating tumor DNA (ctDNA) was *RAS*-wt.

MATERIALS AND METHODS The occurrence of Kirsten rat sarcoma viral oncogene homolog (*KRAS*), neuroblastoma rat sarcoma viral oncogene homolog (NRAS), V-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), and *PI3KCA* mutations was determined in ctDNA by using a new ultrasensitive analysis based on mass spectrometry detection. All consenting patients with confirmed *RAS*-mt mCRC had disease progression on previous chemotherapy that contained no anti–epidermal growth factor receptor (EGFR). The patients with *RAS*-wt ctDNA received cetuximab + fluorouracil, leucovorin, and irinotecan (FOLFIRI), whereas those with *RAS*-mt ctDNA were treated with the oncologist's choice of therapy.

RESULTS Of 16 registered patients, 11 were male and five female. They were age 48 to 81 years, and they had unresectable metastatic adenocarcinoma from the colon (n = 11) or rectum (n = 5), with a median of two metastatic sites. They had received a median number of three previous chemotherapy protocols. Plasma genotyping identified *RAS*-mt in seven patients (44%) and *RAS*-wt in nine patients (56%). In the patients with wt ctDNA, objective tumor response rate was 50.0%, including one complete response and four partial responses after a median number of 6 courses of cetuximab + FOLFIRI (range, 1 to 16 courses). Two of the nine patients had stable disease, and two had progressive disease. No grade 3 to 4 toxicities were encountered. One-year survival rates were 60.0% for the patients with *RAS*-wt ctDNA and 17.9% for those with *RAS*-mt ctDNA. Median overall survival times were not reached and 4.7 months, respectively.

CONCLUSION Patients with *RAS*-mt mCRC whose plasma biopsies contained *RAS*-wt could benefit from cetuximab-based therapy, a hypothesis to be tested in a prospective randomized trial.

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INTRODUCTION

The addition of anti-epidermal growth factor receptor (EGFR) to chemotherapy has been shown to be more effective than chemotherapy only for the treatment of metastatic colorectal cancer (mCRC) in several randomized phase III trials. This was particularly the case for the combination of cetuximab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) or fluorouracil, leucovorin, and oxaliplatin (FOLFOX).[1,2] However, several studies have shown that the survival of those patients with tumor *RAS* mutations (*RAS*-mt) was shorter than for those with *RAS*-wild-type (*RAS*-wt) tumors.[3,4]

Since 2014, it has been recommended that only patients with *RAS*-wt mCRC should receive an anti-EGFR targeted agent.[5] *RAS* mutations have been found in 30% to 50% of patients with mCRC,[6-9] which makes these patients ineligible for EGFR-targeted therapies. Recent studies have demonstrated that the analysis of circulating tumor DNA (ctDNA) in blood samples, through its ability to recapitulate tumor heterogeneity, is a remarkable surrogate of tumor biopsy for detecting mutations.[10-12] This technique has the advantage of being less invasive than a tissue biopsy and can be easily repeated over time. Thus, extensive research on liquid biopsy has recently led to significant achievements

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

To determine whether the lack of *RAS* mutation in a liquid biopsy supports the administration of an anti–epidermal growth factor receptor (EGFR) antibody, despite earlier documentation of a pathogenic *RAS* mutation in the primary colorectal cancer (CRC).

Knowledge Generated

Nearly half the patients in this pilot study received cetuximab-based chemotherapy, because no *RAS* mutation was detected in the liquid biopsy, despite such mutations having been found in the primary tumor. The progression-free survival and the overall survival of these heavily pretreated patients largely exceeded those in patients whose liquid biopsy revealed *RAS* mutation and who received chemotherapy only.

Relevance

Tracking the gain or loss of *RAS*-mutated cancer clones through liquid biopsies along the course of CRC disease may have a profound impact on its therapeutic management. This is achieved through enabling the administration of anti-EGFR that was initially rejected on the basis of previous molecular testing of tissue.

in the characterization of the dynamics of acquired resistance to anti-EGFR therapies.[13] To date, studies with liquid biopsy have been selectively focused on the early detection of the appearance of *RAS*-mt clones in tumor deposits by analyzing ctDNA in blood samples from patients with *RAS*-wt primary CRC[13] as biomarkers of an increasing resistance to anti-EGFR agents. In addition, *RAS* mutations were not detected in the plasma of a low proportion of patients with *RAS*-mt detected in tissue genotyping.[14] We conducted this pilot study as a proof of concept for the efficacy and safety of anti-EGFR–targeted therapy added to chemotherapy in patients with unresectable mCRC with *RAS*-wt ctDNA but *RAS*-mt primary tumor.

MATERIALS AND METHODS

The design of this pilot study was based on the hypothesis that there are dynamic changes in tumor *RAS* mutational status and that they are assessable by liquid biopsy in routine oncology.

Patient Selection

The study outline and the patient information and consent form for this pilot salvage protocol were approved by an institutional review board. A liquid biopsy that showed *RAS*-wt was considered adequate to allow administration of anti-EGFR. Indeed, no specification in the European Marketing Authorisation for Cetuximab mentions that tumor RAS testing should be determined on solid tumor tissue in order to allow for anti-EGFR administration. This is also the case for the recommendations by the French High Health Authority regarding cetuximab use.

All consecutive patients treated between August 2017 and February 2019 at one of three participating centers in France were screened for inclusion in this pilot study. Inclusion required histologic or cytologic proof of colorectal adenocarcinoma, with a Kirsten rat sarcoma viral oncogene homolog (*KRAS*) or neuroblastoma rat sarcoma viral oncogene homolog (*NRAS*) mutation (*NRAS*-mt) from a tissue biopsy. Metastases had to be measurable according to Response Evaluation Criteria in Solid Tumors (RECIST).[15] Patients had to have received at least one previous chemotherapy regimen and to have documented progressive disease on imaging or doubling of serum levels of carcinoembryonic antigen (CEA) or cancer antigen 19.9 (CA19.9) over the previous 90 days or fewer.[5] Other eligibility criteria were WHO performance status of 0 or 1 and a signed informed consent form. Exclusion criteria included previous severe toxicity from irinotecan or fluorouracil or a history of participation in another interventional trial for CRC.

ctDNA *RAS* Mutational Analysis

Total circulating DNA was extracted from 3 mL of plasma by using a QIAsymphony DSP Circulating DNA kit with a QIAsymphony instrument, according to manufacturer's protocols (QIAGEN, Courtaboeuf, France). Molecular profiling was performed by using an ultrasensitive panel for detecting targeted mutations in *RAS* genes. This panel was developed using Massarray Ultraseek technology and Massarray online design tools (Agena Bioscience, Hamburg, Germany). The panel included the main mutation sites in *KRAS* (codon 12-13-61-146), *NRAS* (codon 12-13-61), *BRAF* (V600E), *EGFR* (S492R), and *PIK3CA* (codon 542-545-546-1047). The Massarray Ultraseek procedure involves a 3-step process consisting of the initial polymerase chain reaction, inactivation of unincorporated nucleotides by shrimp alkaline phosphatase, and a single-base primer extension according to the manufacturer's protocol. The products were then nano-dispensed onto a matrix-loaded silicon chip (SpectroChipII, Agena Bioscience), and the mutations were detected by matrix-assisted laser desorption-ionization–time of flight mass spectrometry. Data were analyzed by using MassArrayTyper Analyzer software 4.0.4.20 (Agena Bioscience), which helps visualization of data patterns as well as the raw spectra.

The sensitivity of this technique is similar to that of digital polymerase chain reaction. It simultaneously analyses

several gene mutations with high accuracy. For our study, the sensitivity for the detection of clinically relevant *RAS* gene mutations in ctDNA was 88% for patients with CRC liver metastases, in good agreement with Bettegowda et al.[16] We currently use these highly sensitive mass spectrometry ctDNA analyses for monitoring treatment of patients with non–small-cell lung cancer or breast cancer in routine oncology practice.

Study Treatment

All patients had *RAS*-mt tissue biopsies. The study population was divided in two groups according to the results of the ctDNA mutational analysis: group 1 included the patients with *RAS*-mt also found in plasma; group 2 included the patients with *RAS*-wt ctDNA. Patients in group 1 received the chemotherapy regimen decided upon at a multidisciplinary staff meeting according to the expertise of the center and the choice of the oncologist. Patients in group 2 received the experimental regimen (ie, cetuximab + FOLFIRI once every 2 weeks (Fig 1).[17] Treatments were administered until disease progression, occurrence of major toxicity, secondary surgery, or death.

Assessments

Before each treatment course, complete blood cell counts were performed and renal and hepatic serum biochemistry and plasma CEA and CA19.9 were determined. Performance status and adverse events were graded according to WHO and the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0, respectively. A thoraco-abdomino-pelvic computed tomography scan was performed within 4 weeks before inclusion and subsequently after every third treatment course. Positron emission tomography scans with 18-fluorodeoxyglucose and/or magnetic resonance imaging scans were performed whenever they were deemed necessary. The sum of the largest diameters of the target lesions was computed on the inclusion imaging and used as baseline for the quantification of tumor downsizing and response categorization according to RECIST. Response was classified as complete response, partial response, stable, or progressive disease.[15]

Statistical Consideration

This pilot exploratory study included consecutive patients, and no sample size was defined a priori. Objective response rates (ORRs) were computed for each group. The durations of progression-free survival (PFS) and overall survival (OS) were measured from inclusion until the date of progression or death, respectively, or that of last follow up, with the database locked on May 25, 2019. Both PFS and OS were computed using the Kaplan-Meier method and compared with log-rank tests. All analyses were performed with intent-to-treat using SPSS v18.0 software (SPSS, Chicago, IL).

RESULTS

Patients

Sixteen patients with unresectable mCRC and *RAS*-mt in a cancer tissue were registered at one of three centers in this pilot study. Initial *RAS* mutational status was determined in the resected primary tumor for 11 patients (69%) or in a tumor biopsy for 5 patients (31%). Patients' main clinical characteristics at baseline are reported in Table 1. There were 11 males (69%) and 5 females (31%) age 48 to 81 years (median, 69 years). They had one to four organs involved with metastases, and a median of two metastatic sites. All the patients receiving chemotherapy had PD upon inclusion in the study. Two or more chemotherapy protocols that did not contain anti-EGFR failed for 13 (81.3%) of the 16 participating patients. All the patients had received previous chemotherapy, including fluorouracil (100% of the patients), irinotecan (69%), oxaliplatin (81%), bevacizumab (69%), and aflibercept (56%). Both irinotecan and oxaliplatin had been given to 50% of the patients. The majority of patients had received

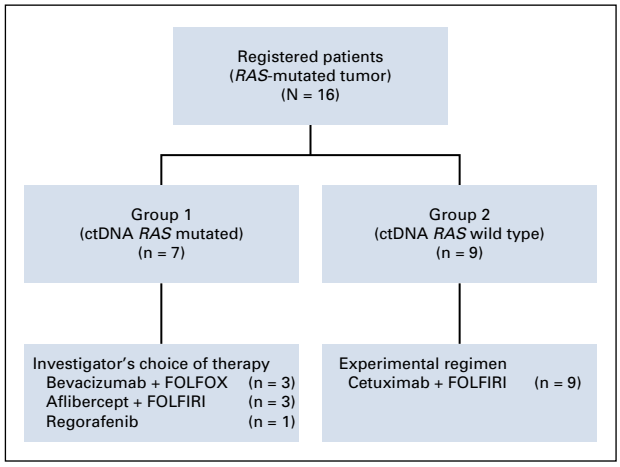

FIG 1. Study flow diagram. ctDNA, circulating tumor DNA; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX, fluorouracil, leucovorin, and oxaliplatin.

TABLE 1. Patient Baseline Characteristics

Characteristic	All Patients (N = 16)			Group 1 (n = 7)			Group 2 (n = 9)		
	No.	%	Median (range)	No.	%	Median (range)	No.	%	Median (range)
Age, years			69 (48-81)			70 (48-81)			64 (51-77)
Sex									
Male	11	69		4	57		7	78	
Female	5	31		3	43		2	22	
Site of primary tumor									
Rectum	5	31		3	43		2	22	
Right colon	4	25		2	29		2	22	
Left colon	7	44		2	29		5	56	
Synchronous metastases									
Yes	11	69		6	86		5	56	
No	5	31		1	14		4	44	
Metastasis sites			2 (1-4)			1 (1-4)			2 (1-3)
Main locations of metastases									
Liver only	3	19		3	43		0	0	
Liver plus other sites	8	50		3	43		5	56	
Other sites only	5	31		1	14		4	44	
Degree of liver replacement, %									
≤ 25	5	33		1	14		4	44	
25-50	8	50		4	57		4	44	
> 50	3	13		2	28		1	11	
No. of previous lines of chemotherapy			2 (1-3)			2 (1-3)			3 (1-3)
Drugs previously received									
Fluorouracil	16	100		7	100		9	100	
Oxaliplatin	13	81		6	86		7	78	
Irinotecan	11	69		4	57		7	78	
Both irinotecan and oxaliplatin	8	50		3	43		5	56	
Bevacizumab	11	69		6	86		5	56	
Aflibercept	9	56		4	57		5	56	

(Continued on following page)

TABLE 1. Patient Baseline Characteristics (Continued)

Characteristic	All Patients (N = 16)			Group 1 (n = 7)			Group 2 (n = 9)		
	No.	%	Median (range)	No.	%	Median (range)	No.	%	Median (range)
Median time elapsed between *RAS* determinations on solid tissue *v* liquid biopsy, months			23.2 (11.6-35.6)[a]			25.8 (14.0-59.9)[a]			21.1 (9.9-33.3)[a]
Baseline CEA, ng/mL									
≤ 5	9	56		4	57		4	44	
> 5	7	43		3	43		5	56	
Baseline CA19.9, UI/mL									
≤ 37	9	56		4	57		5	56	
> 37	7	43		3	43		4	44	

NOTE. Group 1, circulating tumor DNA *RAS* mutated; group 2, circulating tumor DNA *RAS* wild type.
Abbreviations: CA19.9, cancer antigen 19.9; CEA, carcinoembryonic antigen.
[a]Interquartile range.

two or more chemotherapy regimens for metastatic disease.

Two groups of patients were identified according to the results of ctDNA mutational analysis at inclusion. Group 1 included seven patients (44%) in whom ctDNA mutational analysis revealed *RAS*-mt. Group 2 consisted of nine patients (56%) with ctDNA *RAS*-wt, despite an earlier tissue genotyping that showed *RAS*-mt. One patient in group 1 also had *KRAS* and *PIK3CA* mutations in both the solid tumor specimen and in the liquid biopsy. The medians and interquartile ranges (IQRs) in the interval durations between tissue and liquid biopsies were 25.8 months (IQR, 14.0 to 59.9 months) in group 1 and 21.1 months (IQR, 9.9 to 33.0 months) in group 2. Group 1 received the investigator's choice of chemotherapy regimens, which included bevacizumab + FOLFOX for three patients, aflibercept + FOLFIRI for three patients, and regorafenib for one patient. All patients in group 2 received cetuximab + FOLFIRI (Fig 1). Baseline characteristics were similar in the 2 groups (Table 1).

Safety Data for the Nine Patients With ctDNA *RAS*-wt (group 2)

Overall, the cetuximab + FOLFIRI regimen was well tolerated. There were no deaths as a result of toxicity or any grade 3 or 4 toxicities. No patients stopped treatment because of toxicity. The main grade 1 or 2 clinical toxicities were fatigue, diarrhea, nausea or vomiting, mucositis, acneiform rash, and alopecia (6 [67%] of 9 for all). Additional grade 1 or 2 toxicities were anorexia (5 [56%] of 9), allergic reaction (5 [56%] of 9), hand-foot skin reaction (4 [44%] of 9), abdominal pain (3 [33%] of 9), and peripheral sensory neuropathy (3 [33%] of 9). Grade 1 or 2 hematologic toxicities included neutropenia (7 [78%] of 9), leukopenia (8 [89%] of 9), thrombopenia (8 [89%] of 9), and anemia (9 [100%] of 9).

Efficacy

Median follow-up time was 5.6 months (range, 0.3 to 20.8 months). Considering the entire study population, median PFS was 6.4 months (95% CI, 4.5 to 8.3 months) and OS was 7.4 months (95% CI, 5.0 to 9.8 months). The ORR was 50% (95% CI, 25.5% to 74.5%) and disease control rate (DCR) was 81% (95% CI, 62.2% to 100%; Table 2). At the time of analysis, 11 patients (68.8%) had progressed and seven (43.8%) had died.

Assessing the two groups separately, group 1 (*RAS*-mt ctDNA) had an ORR of 42.9% and a DCR of 85.7%, whereas in group 2 (*RAS*-wt ctDNA), the ORR was 55.6% and DCR was 77.8% (Table 2). Nonetheless, PFS was nearly three-fold shorter in group 1 (3.5 months; 95% CI, 0.8 to 6.1 months) compared with that in group 2 (9.0 months; 95% CI, 4.7 to 13.3 months; Fig 2). Such differences translated into major differences between groups in median OS: 4.7 months (95% CI, 1.1 to 8.3 months) for group 1 and not reached for group 2 (Fig 3).

Case Report of Objective Response

A male patient (No. 12) age 72 years old had *KRAS*-mt rectal adenocarcinoma (exon 3, codon 61). His disease had progressed on three previous chemotherapy lines when he was included into the study. He then presented with multiple lung metastases and metastatic pelvic lymph nodes. CEA was 185.2 ng/mL and CA19.9 was 273.4 UI/mL. ctDNA was *RAS*-wt in the blood sample. After six courses of cetuximab + FOLFIRI, an objective response

TABLE 2. Main Efficacy Parameters in All Patients (study population) and Separately in the Subgroups Defined by Circulating DNA Mutational Status

	Group 1 (n = 7)			Group 2 (n = 9)			All (N = 16)		
Efficacy Parameters	**No.**	**%**	**Median (95% CI)**	**No.**	**%**	**Median (95% CI)**	**No.**	**%**	**Median (95% CI)**
CR	0			1	11.1		1	6.3	
PR	3	42.9		4	44.4		7	43.8	
SD	3	42.9		2	22.2		5	31.3	
PD	1	14.3		2	22.2		3	18.8	
Objective response									
CR + PR	3	42.9		5	55.6		8	50.0	
Disease control									
CR + PR + SD	6	85.7		7	77.8		13	81.3	
PFS, months			3.5 (0.8 to 6.1)			9.0 (4.7 to 13.3)			6.4 (4.5 to 8.3)
OS, months			4.7 (1.1 to 8.3)			Not reached			7.4 (5.0 to 9.8)

NOTE. Group 1, circulating tumor DNA *RAS* mutated; group 2, circulating tumor DNA *RAS* wild-type.

Abbreviations: CR, complete response; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

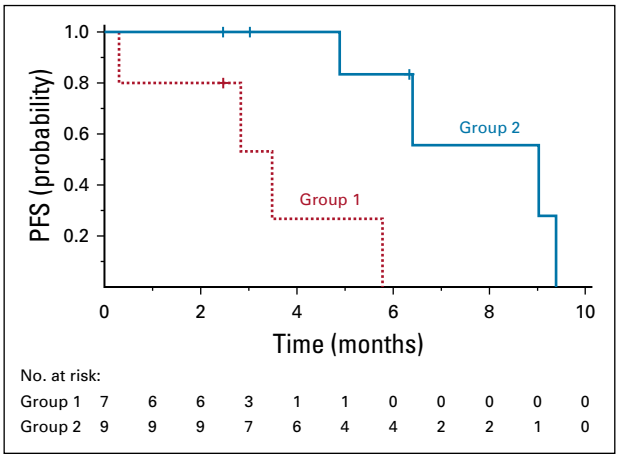

FIG 2. Kaplan-Meier curves of progression-free survival (PFS) according to circulating tumor DNA (ctDNA) *RAS* mutational status. Median PFS was 3.5 months (95% CI, 0.8 to 6.1 months) for the patients with *RAS*-mutated ctDNA (group 1) compared with 9.0 months (95% CI, 4.7 to 13.3 months) for those with *RAS*-wild-type ctDNA (group 2).

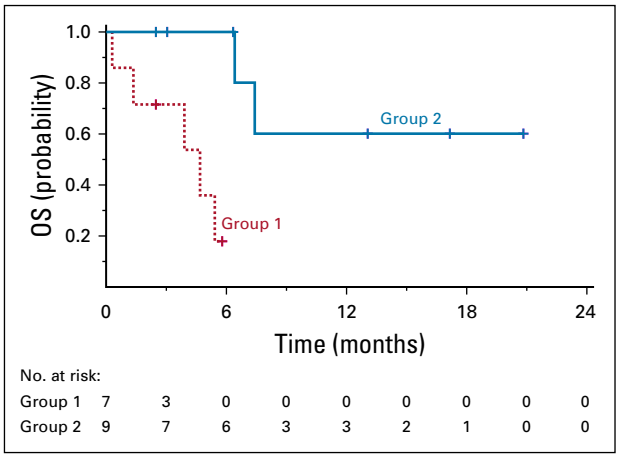

FIG 3. Kaplan-Meier curves of overall survival (OS) according to circulating tumor DNA (ctDNA) *RAS* mutational status. Median OS was 4.7 months (95% CI, 1.1 to 8.3 months) for the patients with *RAS*-mutated ctDNA (group 1), but it was not reached at 18.7 months for those with *RAS*-wild-type ctDNA (group 2).

was achieved. This efficacy was confirmed after six additional courses of the same protocol, as illustrated on serial PET scans (Fig 4). After 12 courses, tumor markers had normalized, with CEA dropping to 3.0 ng/mL and CA19.9 dropping to 18.4 UI/mL. This fourth-line protocol was well tolerated, with the most severe toxicities being grade 2 leukopenia, neutropenia, fatigue, and acneiform rash.

DISCUSSION

All the patients in this study had advanced and chemotherapy-resistant metastatic disease, without any imbalance in apparent overall tumor burden that could reasonably influence the liquid biopsy results. Thus, no evidence supports the possibility that the differences in detection of ctDNA *RAS* mutation would be related to between-patient variations in tumor burden.

The identification of a mutation in *KRAS* or *NRAS* from a cancer tissue biopsy precludes the use of anti-EGFR treatment in association with chemotherapy against mCRC, based on consistent evidence.[5] Our group first reported the occurrence of acquired *KRAS* mutations along the progression of colorectal metastases in patients treated with cetuximab. We then hypothesized that the late acquisition of *KRAS* mutations could represent a possible mechanism of secondary resistance to anti-EGFR antibodies.[18] However, the assumption of persisting *RAS*-mt genotype for patients who received chemotherapy has not been challenged before now because of the expected evolutionary advantage of *RAS*-mt clones.[19] Yet in this pilot study, we found that nearly half the patients with mCRC displayed no detectable *RAS*-mt in ctDNA, although their cancer tissue genotyping had demonstrated *RAS*-mt. This finding raised several questions.

First, is there a concordance between the *RAS* mutational status of tumor tissues and that of ctDNA? Several studies have highlighted discrepant results in *RAS* mutational status for 10%-15% of the patients tested, depending on the method used.[11,14,20] Vidal et al explained such plasma-versus-tissue *RAS* discrepancies with spiral and temporal heterogeneity in *RAS*-mt tumor clones within the tumor tissue.[11] According to Thierry et al,[20] such discrepancies could relate to the use of biopsies. Other discrepancies that seemed to affect concordance were long intervals between assessments of the *RAS* status in tumor tissue and that in the blood sample, resection of the tumor at the time of blood draw, tumor site, and type of tissue analyzed. Grasselli et al[14] ascribed the discrepancies in *RAS* status to differences in technical sensitivity of the methods used for analysis or to heterogeneity.

Second, is there a change in *RAS* status over time and/or during treatment? This question does not yet have a clear answer. We were the first to report 1 case of acquired *KRAS* mutation in metastases after progression under combined anti-EGFR and doublet chemotherapy in 12 patients with *KRAS*-wt mCRC.[18] Other authors have highlighted the concurrent detection of sensitive and resistant clones to anti-EGFR antibodies within tumor deposits from different locations in the same patient.[21-24] This multiclonality could explain the dissociated antitumor responses that are frequently encountered in clinical practice. Such tumor heterogeneity assumes the existence of wt clones sensitive to anti-EGFR treatment alongside resistant mutant clones. Interestingly, the team of Raimondi et al[25] recently reported the disappearance of *RAS*-mt clones in ctDNA after tumor progression while receiving bevacizumab and chemotherapy in 4 patients with *RAS*-mt mCRC. In their study however, the mutational status of ctDNA was not assessed before treatment was initiated. In our study, a median interval of 23.2 months was found between the determination of tumor tissue *RAS*-mt and the subsequent liquid biopsies

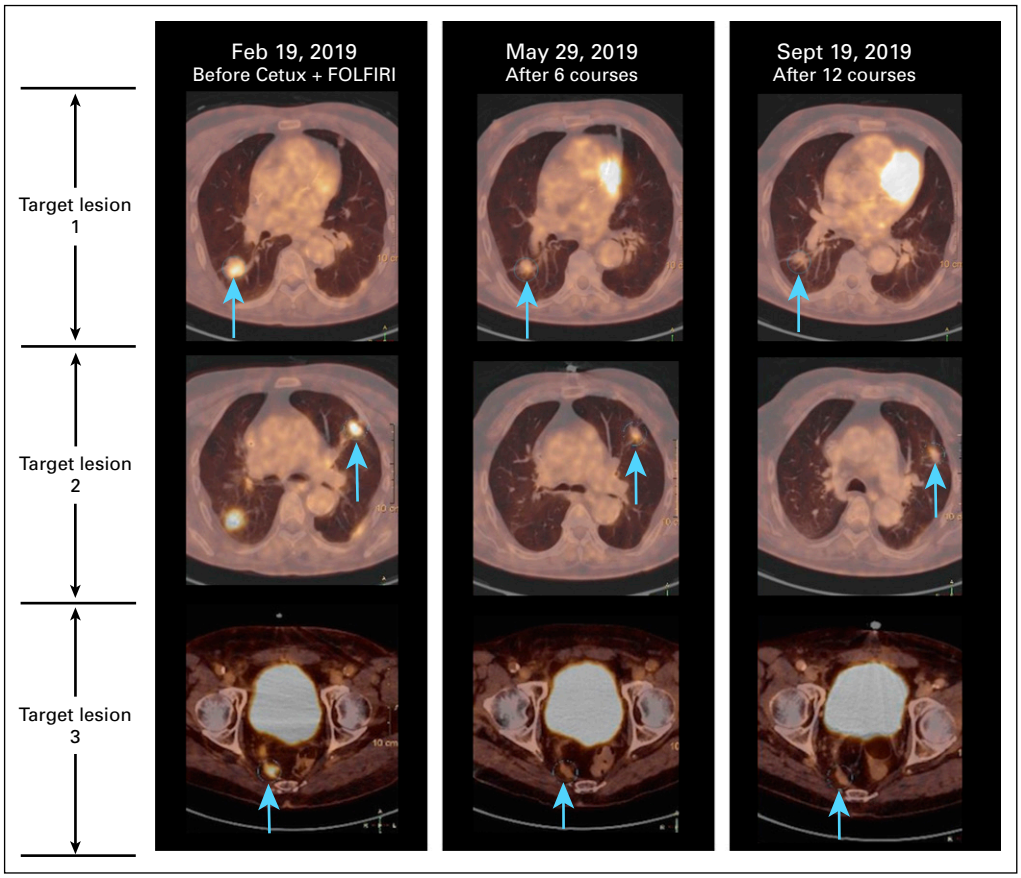

FIG 4. Case report illustrating exceptional efficacy of cetuximab + fluorouracil, leucovorin, and irinotecan (Cetux + FOLFIRI) as fourth-line chemotherapy received by a 72-year-old patient with *KRAS* mutation in tumor tissue but *KRAS* wild-type in liquid biopsy, upon inclusion in the study. From left to right, repeated imaging findings at baseline, after six courses, and after 12 courses. An objective response of lung and pelvic lymph node metastases was documented with repeat positron emission tomography using 18-fluorodeoxyglucose, and confirmed with the normalization of both cancer antigen 19.9 (CA19.9) and carcinoembryonic antigen (CEA).

that assessed *RAS* mutational status in the same patients. Our findings of *RAS*-wt in the ctDNA of 56% of the patients with an earlier *RAS* mutation in solid tumor tissue supported possible loss of such tumor *RAS* mutation over time in heavily pretreated patients.

And third, is the efficacy of anti-EGFR treatment more strongly associated with the mutational status of *KRAS*, as determined in tumor tissues or in the ctDNA? Several studies have shown that anti-EGFR treatments were indeed more effective in *RAS*-wt compared with *RAS*-mt as determined in the primary tumor tissue.[26,27] This finding has established the relevance of anti-EGFR treatment of patients whose tumor tissue does not reveal *RAS*-mt.[28-32] Conversely, discrepancies in *RAS* mutational status between primary colon tumor and metastases in the same patient have been reported.[33]

Our pilot study clearly showed a clinical benefit of anti-EGFR treatment added to cytotoxics for those patients with metastatic *RAS*-mt CRC who display *RAS*-wt ctDNA and have received cetuximab-based chemotherapy as second-line to fourth-line therapy. Indeed, the observed median PFS and OS in these patients were similar to those reported for patients with *RAS*-wt tumor tissue who received this combination as first-line treatment for mCRC. No a priori sample size was defined because we aimed to achieve clinical evidence of efficacy in the absence of undue toxicity in this pilot study. Our internal steering committee proposed to stop the pilot study, once the information was adequate for designing a randomized trial for testing the hypothesis further on the basis of an apparent three-fold increase in the median PFS of the experimental treatment compared with cetuximab-free chemotherapy.

The intriguing and encouraging results of this exploratory trial need to be confirmed in randomized clinical trials. Such validation steps are particularly relevant because all our patients with *RAS*-wt ctDNA received cetuximab; thus, we cannot differentiate between a prognostic and a predictive role of *RAS*-wt ctDNA for outcomes of patients receiving cetuximab-based chemotherapy. *RAS*-mt in tumor tissue is considered to be predictive of anti-EGFR resistance rather

than prognostic of an aggressive tumor biology.[34] Such consideration would further support the hypothesis of an added benefit from cetuximab in this subgroup of patients. Yet recent evidence suggests that the proportion of *RAS*-mt in ctDNA was a prognostic indicator for both OS and PFS in patients with mCRC.[35] This finding raised the question of a potential divide on the clinical significance between tumor and ctDNA genotyping, which this study cannot respond to. The fact that ORRs exceeded 40% and were similar in both treatment groups suggested that all the patients included in the study were not completely resistant to chemotherapy and that those with *RAS*-mt in the liquid biopsy could have a worse prognosis, as proposed by Elz et al.[35] However, single-agent cetuximab achieved only a 10% ORR in patients with chemotherapy refractory mCRC,[36] despite prolonging PFS by 4 months. Taken together, the literature results support a much greater value for PFS prolongation compared with ORR as an efficacy end point, which supports the hypothesis that the liquid biopsy results have a predictive value.

The lack of a prespecified sample size and the low number of patients in each group constitute the main limitations of our pilot salvage study. The three-fold increase in median PFS in the absence of any apparent bias does suggest that anti-EGFR–based chemotherapy could represent a promising option for nearly half the patients with initially documented *RAS*-mt on tumor tissue at a later stage of their disease.

Our study was in line with the CHRONOS study (ClinicalTrials.gov identifier: NCT03227926),[37] whose design is based on the concept that CRC genome adapts dynamically to intermittent anti-EGFR drug schedules. That study uses iterative ctDNA determinations for guiding treatment adjustments. A follow-up on our study would involve a randomized clinical trial in which eligible patients with *RAS*-mt on solid tumor tissue undergo *RAS* mutational status assessment in ctDNA upon progression on chemotherapy. Although those with *RAS*-mt in the ctDNA would receive chemotherapy only (reference group), those with *RAS*-wt ctDNA would be randomly assigned to receive chemotherapy only (controls) or anti-EGFR-based chemotherapy (experimental group).

AFFILIATIONS

[1]Medical Oncology Department, Paul Brousse Hospital, Villejuif, France
[2]French National Institute for Health and Medical Research (INSERM), Unit 935, Villejuif, France
[3]Medical Oncology Unit, Clinique Saint Jean L'Ermitage, Melun, France
[4]Medical Oncology Unit, Clinique du Mousseau, Evry, France
[5]Oncogenetics Department, Assistance Publique-Hôpitaux de Paris, Paul Brousse Hospital, Université Paris-Saclay, Villejuif, France
[6]Medical Oncology Unit, Groupe Hospitalier Intercommunal Le Raincy-Montfermeil, Montfermeil, France
[7]North Wales Cancer Centre, Ysbyty Gwynedd, Betsi Cadwaladr University Health Board, Bangor, United Kingdom
[8]Cancer Chronotherapy Team, Cancer Research Centre, Division of Biomedical Sciences, Warwick Medical School, Coventry, United Kingdom
[9]Centre Hépato Biliaire, Assistance Publique-Hôpitaux de Paris, Hôpital Paul Brousse, Villejuif, France

CORRESPONDING AUTHOR

Mohamed Bouchahda, MD, Medical Oncology Department, Paul Brousse Hospital, 12 Avenue Paul Vaillant Couturier, 94800 Villejuif, France; e-mail: bouchahda.m.mb@gmail.com.

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AUTHOR CONTRIBUTIONS

Conception and design: Mohamed Bouchahda, Raphael Saffroy, Abdoulaye Karaboué, Francis Lévi, Antoinette Lemoine
Provision of study materials or patients: Mohamed Bouchahda
Collection and assembly of data: Mohamed Bouchahda, Antoinette Lemoine
Data analysis and interpretation: Mohamed Bouchahda, Abdoulaye Karaboué, Jocelyne Hamelin, Pasquale Innominato, Faouzi Saliba, Francis Lévi, Nelly Bosselut
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Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Faouzi Saliba
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Francis Lévi
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