case report

EGFR Inhibitor as Second-Line Therapy in a Patient With Mutant *RAS* Metastatic Colorectal Cancer: Circulating Tumor DNA to Personalize Treatment

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INTRODUCTION

RAS mutations are found in 30% to 50% of metastatic colorectal cancer (mCRC) and determine the ineligibility of patients for epidermal growth factor receptor (*EGFR*)–targeted therapies. *RAS* mutations are associated with inferior progression-free survival and overall survival of patients with mCRC compared with patients with nonmutated tumors.[1] It is conceivable that *RAS* mutations are negative prognostic factors per se; however, the availability of only one actionable molecular target, that is, the inhibition of angiogenesis, in patients with mutated tumors certainly affects survival in this subgroup of patients. Therefore, genotyping colorectal cancer tissue is mandatory in routine practice to personalize the therapeutic approach. 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 mutation detection.[2] Extensive research on liquid biopsy led to significant achievements in the comprehension of the biologic reasons behind acquired resistance to anti-*EGFR* therapies.[3] To date, studies with liquid biopsy have been selectively concentrated on the appearance of *RAS*-mutant clones in the blood of patients with *RAS* wild-type primary CRC, as biomarkers of anti-*EGFR* therapy resistance. Conversely, the clinical implications of the selective pressure exerted by antiangiogenic treatments in patients with *RAS*-mutant CRC have been seldom investigated. We here report that ctDNA analysis, over the course of antiangiogenic treatment, might reveal a therapeutically exploitable window of opportunity, characterized by the relative prevalence of *RAS* wild-type clones over time. As proof of concept, we here describe the case of a patient with a primary *NRAS*–mutated colorectal cancer, who received second-line treatment with anti-*EGFR* after failure of first-line triplet chemotherapy plus bevacizumab, according to the absence of any clinically relevant mutation of *RAS* genes in blood, achieving a partial response.

CASE REPORT

A 47-year-old male patient presented in March 2016 to our unit with a palpable painless left supraclavicular mass. A whole-body contrast-enhanced computed tomography (CT) scan revealed a left supraclavicular lymphadenopathy, transverse colon thickening (3 cm), multiple chest and abdominal lymphadenopathies, and peritoneal carcinomatosis. Colonoscopy revealed a bleeding area at 15 cm from the anal verge; biopsy was performed, and the result was negative for a primary cancer. Supraclavicular lymph node excision revealed a papillary adenocarcinoma, with immunohistochemistry positive for CDK20 and CDX2 and negative for TTF1 and CK7. Mutational analysis of the lymph node metastasis performed through real-time polymerase chain reaction (PCR; Real-time OncoScreen *NRAS*) revealed exon 4 *NRAS* A146T mutation at 29 cycles. A whole-body fluorodeoxyglucose positron emission tomography scan revealed several metabolically active lymph nodes (standardized uptake value [SUV], 1.7 to 4.5) and a focal uptake in the rectosigmoid junction (SUV, 5). Given the intestinal immunohistochemistry profile, he was started on a colon-specific regimen, with combined triplet chemotherapy of fluoropyrimidines, oxaliplatin,

and irinotecan plus an anti-VEGF monoclonal antibody (April to August 2016). Serum tumor markers significantly decreased, with rapid negativization (carcinoembryonic antigen [CEA], 1.3→1.23; CA 19-9, 71.8→39; CA-125, 82→26). After eight cycles of therapy, fluorodeoxyglucose positron emission tomography scan showed a partial response. From September 2016 to October 2016, the patient received fluorouracil plus bevacizumab maintenance chemotherapy. After 2 months of therapy, serum levels of CA 19-9 started increasing again (131), and irinotecan was reintroduced together with fluorouracil and bevacizumab. Clinical and radiologic reassessment revealed stable disease until June 2017. At that time, serum tumor markers increased again (CA 19-9, 530; CEA, 9), with clinical evidence of bilateral axillary lymph node enlargement. A whole-body CT scan confirmed the progression of disease, revealing pleural effusion associated with pulmonary lymphangitic carcinomatosis. Concurrently, the patient was enrolled in an observational study open at our institution, which aimed to serially monitor the mutational status of ctDNA in patients with *RAS*-mutant mCRC disease, before starting any new line of treatment and in course of therapy until progression.

METHODS

Blood samples were prospectively collected after obtaining informed consent with ethical committee approval. Blood draws (8 mL) were performed starting from the failure of first-line therapy and serially repeated every 3 months until December 2017. Idylla (Biocartis, NV, Mechelen, Belgium), a fully automated, real-time PCR-based molecular diagnostics system, was used to investigate *RAS* mutational profile from plasma. Specifically, Idylla ct*KRAS* Mutation Assay and Idylla ct*NRAS/BRAF/EGFR* Mutation Assay were used. The first allows the detection of 21 mutations in codons 12, 13, 59, 61, 117, and 146 of the *KRAS* gene, and the second allows the detection of 18 mutations in codons 12, 13, 59, 61, 117, and 146 of the *NRAS* gene, five mutations in codon 600 of the *BRAF* gene, and two mutations in codon 492 of the *EGFR* gene from 1 mL of plasma.

Among the parameters describing the generated PCR curves, the ΔCq value is calculated as the difference between the quantification cycle value (Cq) of the gene control signal and the Cq of the mutant signal. A sample is classified as mutation positive if the parameters of the PCR curve generated are within the validated range. Otherwise the sample is reported as being mutation negative, that is, wild type. According to the manufacturer's instructions, a Cq value of the *NRAS* control of 35.5 or higher indicates that a low amount of cell-free DNA is present in the sample. In such cases, low-frequency mutations may not be detected.

RESULTS

In July 2017, a ctDNA mutational analysis was performed and revealed the absence of any clinically relevant mutation in *KRAS*, *NRAS*, and *BRAF* genes. The Cq of *NRAS* total was 28, to indicate that input was sufficient. This new insight into the biology of the disease convinced us to take this window of therapeutic opportunity, which was not guaranteed to remain open indefinitely; in July 2017, the treatment was changed to the second-line combination of irinotecan and cetuximab. As early as after the first cycle of chemotherapy, an objective complete clinical response of the bilateral axillary adenopathy was documented, and this was confirmed at ultrasound assessment. Restaging CT scan after eight cycles of therapy showed a partial response, with significant reduction of pleural effusion and pulmonary lymphangitic carcinomatosis and stability of peritoneal metastases (Fig 1). A second ctDNA analysis was performed on November 2017, confirming the *RAS* genes wild-type status (Cq of *NRAS* total: 35.5, which might be due to a reduction in ctDNA amount). Accordingly, the patient continues to receive fluorouracil, leucovorin, and irinotecan plus cetuximab with the same schedule. The patient's therapeutic history is illustrated in Figure 2. Unpublished preliminary results, obtained in a series of patients with mCRC treated at our institution and serially monitored through plasma ctDNA analysis, show that 46% of patients harboring a *RAS* mutation in tumor tissue at the time of diagnosis switch to a nonmutated/wild-type *RAS* status during the course of chemotherapy plus antiangiogenic drugs (Table 1).

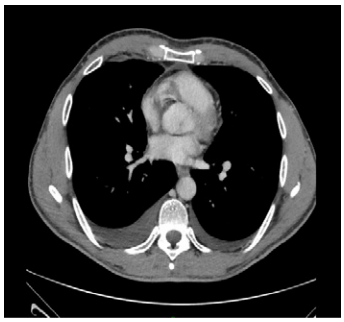
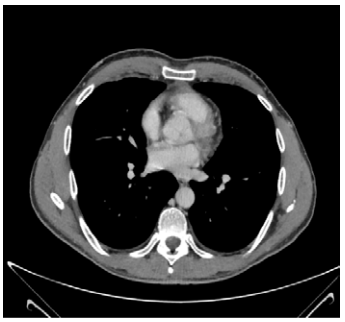
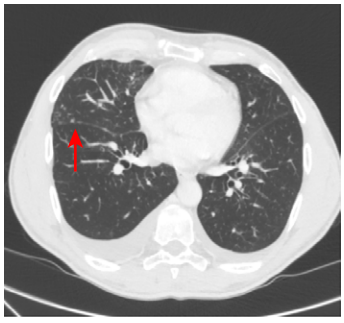
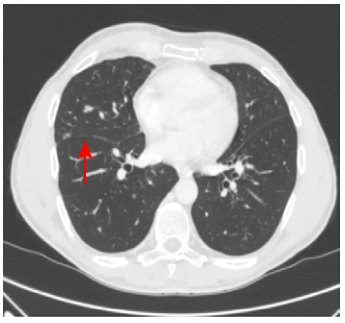
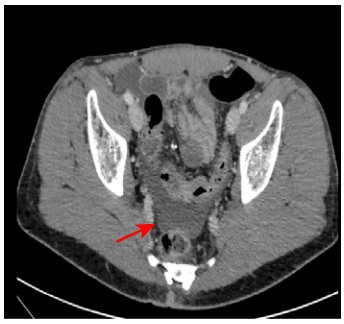
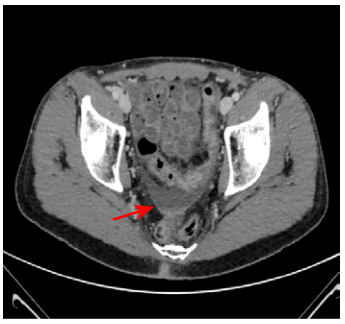
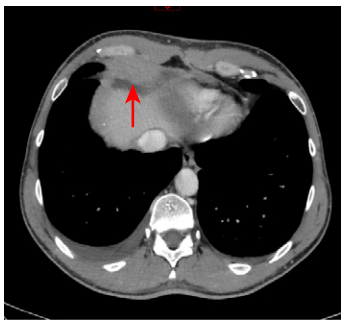
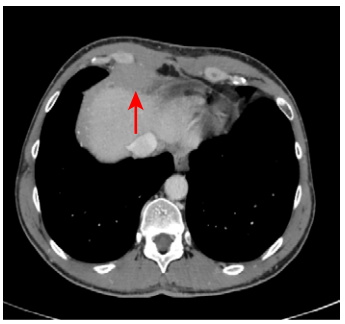
	June 2017 (before FOLFIRI-cetuximab)	October 2017 (after 3 months of FOLFIRI-cetuximab)
Pleural effusion		
Pulmonary lymphangitic carcinomatosis		
Malignant ascites		
Peritoneal carcinosis		

Fig 1. Axial computed tomography scan after eight cycles of fluorouracil, leucovorin, and irinotecan (FOLFIRI) plus cetuximab shows a partial response, with significant reduction of pleural effusion and pulmonary lymphangitic carcinomatosis and stability of peritoneal metastases. Red arrows indicate target lesions.

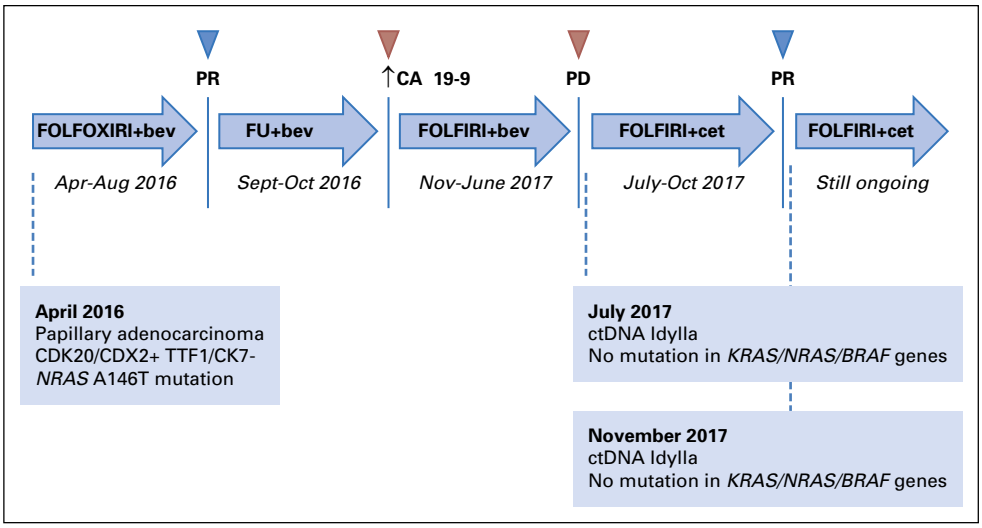


Fig 2. Diagnostic and therapeutic timeline. Sequence of treatments, clinical and radiologic response to the different schedules adopted (partial response [PR] is indicated by blue arrow, and progressive disease [PD] is indicated by red arrow), and molecular tests performed on tumor tissue and plasma at different time points. Bev, bevacizumab; cet, cetuximab; ctDNA, circulating tumor DNA; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOXIRI, fluorouracil, leucovorin, oxaliplatin, and irinotecan; FU, fluorouracil.

DISCUSSION

It is recognized that the *NRAS* A146T mutation that we found in tumor tissue at the time of diagnosis is rare, with a reported prevalence of *NRAS* exon 4 mutations (c117 and c146) around 0.5% across studies. Nevertheless, A146T results in increased *NRAS* activity and downstream signaling and is transforming in cell culture resulting from increased *NRAS* guanosine diphosphate/guanosine triphosphate exchange rate,[4] and consequently patients who harbor mutations in *NRAS* also have significantly inferior anti-*EGFR* treatment outcomes benefit compared with those without any *RAS* mutations.[5] It is recommended that patients with CRC being considered for anti-*EGFR* therapy must receive *RAS* mutational testing, including *KRAS* and *NRAS* codons 12 and 13 of exon 2, 59, and 61 of exon 3 and 117 and 146 of exon 4, and that anti-*EGFR* should only be prescribed for patients with mCRC who are wild type for all known *RAS*-activating mutations.[6] In patients with *RAS*-mutant primary colorectal cancers, ineligible for *EGFR* inhibitors, the importance of the *EGFR* pathway, which indeed sustains the disease, is counterintuitive. International guidelines currently recommend treatment of *RAS* mutant mCRC with bevacizumab first line, followed by chemotherapy backbone change or aflibercept/ramucirumab at disease progression.[7] Preclinical and clinical data, however, demonstrate that tumor angiogenesis inhibition induces biologic changes in tumor-stroma interactions, mainly derived from hypoxia. Preclinical observations suggest that hypoxia exerts a negative selection against *RAS*-mutant clones through a mechanism known as secretory senescence, in which *RAS*-mutant senescent cells operate in a paracrine manner to support the growth of surrounding *RAS* wild-type clones, leading to their relative prevalence over time.[8] We here report that ctDNA analysis, under

Table 1. Patients With *RAS*-Mutated Metastatic Colorectal Cancer Who Converted to a Nonmutated/Wild-Type *RAS* Status in Plasma During the Course of Therapy Serially Monitored Through Circulating Tumor DNA Analysis

Patient ID	Tissue Point Mutation (baseline)	Plasma Point Mutation (baseline)	Plasma Point Mutation (2-3 months)	Plasma Point Mutation (4-6 months)	Plasma Point Mutation (6-8 months)
1	*KRAS* A146T	*KRAS* A146T	WT	WT	WT
2	*KRAS* Q61H	*KRAS* Q61H	*KRAS* Q61H	WT	*
3	*KRAS* G12C	*KRAS* G12C	WT	WT	WT
4	*KRAS* G12C	*KRAS* G12C	WT	*KRAS* G12C	*KRAS* G12C
5	*KRAS* G13D	*KRAS* G13D	*KRAS* G13D	WT	WT
6	*KRAS* G12V	*KRAS* G12V	WT	WT	WT
7	*KRAS* G12D	*KRAS* G12D	WT	WT	*KRAS* G12C

Abbreviation: WT, wild type.
*Death.

antiangiogenic treatment, might reveal a therapeutically exploitable window of opportunity, characterized by the relative prevalence of *RAS* wild-type clones, which can be converted in a clinically meaningful benefit for patients. Targeting this window with *EGFR* inhibitors might represent an exploitable second-line option in *RAS*-mutant CRC. To date, the adaptation of a *RAS*-mutant colorectal cancer genome to angiogenesis inhibition has never been exploited for therapeutic purpose. Our planned KAIROS trial (Keeping the Advantage of the Impermanent *RAS*–Wild Type Window Offering Second-Line *EGFR* Inhibitors) is expected to help determine whether the response to *EGFR* inhibition, in patients with *RAS*-mutant primary cancers converted to *RAS* wild type in the course of first-line antiangiogenic treatments, might become the rule rather than the exception.

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