RAS mutation testing in patients with metastatic colorectal cancer in French clinical practice: A status report in 2014

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Abstract (194/200 words)

Background: RAS (NRAS+KRAS) mutation testing is required in addition to simple *KRAS* testing prior to initiating anti-epidermal-growth-factor-receptor (EGFR) antibodies (MAb) as in metastatic colorectal cancer (mCRC).

Aims: To assess prescription and implementation rates of *RAS/KRAS* mutation testing. To describe the *RAS/KRAS* mutation test procedure and its impact on therapeutic strategy.

Patients and Methods: Observational retrospective study conducted from June to September 2014 in all consecutive patients with newly diagnosed mCRC.

Results: Data from 375 patients (male: 57.8%; mean age, 65.7±11.7 years) were analysed. *RAS*/*KRAS* mutation testing was prescribed in 90.1% of patients (338/375). The test was prescribed within 1 month around mCRC diagnosis and prior to first-line therapy in 73.1% (242/331) and 85.4% (280/328) of patients, respectively. Time from test request to receipt of results was 24.6±17.2 days. 59.7% of patients (190/318) had a mutation, mainly *KRAS* (47.9%; 152/317). Anti-EGFR MAb was prescribed in 90.9% of *RAS*-wild-type cases (60/66), consistent with the goal of genotyping-testing in this population.

Conclusion: In 2014, *RAS* genotyping-testing in addition to *KRAS* testing was routinely prescribed and performed in mCRC patients in France. Time to receive results remains long and must be reduced so as to match clinical practice.

Key words: *RAS*; *KRAS* mutations; genotyping; colorectal cancer (4/4 key words)

Introduction

Colorectal cancer (CRC) is the second most common cause of cancer death in Europe, with 446,800 new CRC diagnoses and 214,700 disease-related deaths in 2012 [1]. Approximately 20% of the patients have metastatic disease at initial diagnosis, and an additional 30% to 40% develop metastases during the course of the disease [2]. Twenty years ago, patients with metastatic colorectal cancer (mCRC) had a 5-year survival rate of only 11% [2]. Progress has been made through improvements in treatment, including targeted therapies. Anti-epidermal growth factor receptor monoclonal antibodies (anti-EGFR MAb) such as cetuximab and panitumumab have emerged as effective in a subset of mCRC patients and were initially approved in refractory mCRC, but then reported to be ineffective in tumours with mutations in codons 12 and 13 of exon 2 of the *KRAS* gene [3, 4]. In addition to the *KRAS* exon 2 mutation, in 2013 it was shown that mutations in *KRAS* exons 3 and 4, and in *NRAS* exons 2 to 4 had similar negative impact on anti-EGFR MAb efficacy [5], and anti-EGFR MAbs were therefore restricted to mCRC patients without so-called "*RAS* mutations" (*KRAS* or *NRAS* mutations), improving target population definition.

In 2011, we conducted the French national Flash-KRAS study, showing that *KRAS* testing (exon 2 exclusively) was well-established in the management of mCRC patients in clinical practice in France, despite some regional discrepancies [6]. In 2014, we conducted the present Flash-RAS observational study to assess whether the genotyping tests were performed in accordance with the intervening changes in approved indications for anti-EGFR MAbs. The primary objective was to evaluate the prescription and implementation rates of *RAS* mutation testing in newly diagnosed mCRC patients (approved indication). Secondary objectives comprised analysis of reasons for non-prescription of testing, timing of the *RAS* testing

process from prescription to results, techniques used to determine *RAS* status, and impact of *RAS* status on treatment strategy.

Patients & Methods

Patient selection

This national multicentre non-interventional retrospective study was conducted with oncologists, gastroenterologists, and radiotherapists treating patients with mCRC in mainland France. Participating physicians were to screen consecutive patients seen in daily practice satisfying the following criteria: age ≥18 years, with mCRC confirmed histologically after March 2014 (date at which the *NRAS* tests became available in France), seen in consultation between June 15th, 2014 and September 30th, 2014, and initiating or having initiated first-line therapy for mCRC during that period. The study complied with the principles of the Declaration of Helsinki and the international directives (ICH3) for non-interventional studies. All participants gave oral consent prior to inclusion.

Data collection

Data were collected from patients' medical files and recorded in a questionnaire collecting patient and tumour characteristics, first-line chemotherapy, details of the *RAS*/*KRAS* mutation test process (i.e., *NRAS+KRAS* or *KRAS* exons 2, 3 and 4, sometimes in association with *BRAF*) from test request to reception of results, reason(s) for request or non-request for mutation testing, and impact of results on therapeutic management. When available, an anonymized copy of the genotyping test report was added to the questionnaire.

Statistical Analysis

Patients' demographic and disease characteristics and treatments were reported as standard statistics: mean and standard deviation (SD), median, range, interquartile range for quantitative parameters, and number and percentage for qualitative parameters (excluding missing data). Statistical analysis used SAS® software, version 9.2, (SAS Institute Inc., Cary, NC, USA). The number and percentage of requests for *RAS* mutation testing were reported with their 95% confidence interval (95%CI). Analysable patients were all patients enrolled in the study and respecting selection criteria.

Results

Centres, patients and disease characteristics

2,700 oncologists, gastroenterologists, and radiotherapists, representative of the nationwide population of physicians treating CRC, were invited to participate in the study: 298 accepted, and 104 finally enrolled a total of 406 patients (median: 3 patients per physician). Mean age of physicians was 44.5 ± 7.1 years; the majority were male (61.2%), and most were practicing in private clinics/hospitals (41.2%), or general hospitals (30.6%).

Data for 375 of the 406 enrolled patients were analysed (Table 1). The most commonly used first-line chemotherapies were FOLFOX and/or XELOX (51.1%; 186/364) and FOLFIRI and/or IRINOTECAN (32.1%; 117/364). More than half of the patients (53.2%; 198/372) received targeted therapy. ***Desired location of Table 1***

RAS/*KRAS* mutation test requests

RAS/*KRAS* mutation testing was requested for 90.1% of patients (338/375) and a report of the results was available for 84.8% of patients (318/375) (Figure 1). Reasons for non-request were provided for 28 patients (9 missing data). The main reason for non-request was that no anti-EGFR MAb therapy was planned by the physician (57.1%; 16/28); other reasons included the patient's age or general condition (10.7%; 3/28), scheduled metastasis resection surgery (10.7%; 3/28), excessive delay in obtaining test results (5.4%; 2/28), multidisciplinary team decision (7.1%; 2/28), and "other" (7.1%; 2/28). ***Desired location of Figure 1***

RAS/*KRAS* mutation testing was mainly requested by oncologists (50.4%; 169/335) and gastroenterologists (24.2%; 81/335). In most cases (86.4%; 286/331), physicians requested testing of both *NRAS* and *KRAS* (*BRAF* testing being sometimes requested in association with *NRAS*/*KRAS*). Simple *KRAS* mutation testing was requested for 9.2% of patients for whom a test was requested (31/338) (Table 2). The most frequently used technique of enrichment of mutated *RAS* gene alleles was pyrosequencing/sequencing/snapshot (59.8%; 202/338).

Most patients (73.1%; 242/331) had a *RAS/KRAS* test request within 1 month around the diagnosis of metastatic disease. For 22.1% of patients (73/331), the test was requested more than 1 month after this diagnosis. It was requested before initiation of first-line therapy for 85.4% of patients for whom a test was requested and data were available (280/328) (Table 2).

Desired location of Table 2

The oncogenetics platform was located outside the mCRC treatment centre for 80.7% of patients for whom a test was requested and data were available (268/332). The mean time between request for *RAS*/*KRAS* mutation testing and receipt of the results report was 24.6 ± 17.2 days (median: 20 days). The duration of the overall process is detailed in Table 3. The median time for biomarker genotyping varied slightly according to analytic technique: 13

days for pyrosequencing and allelic discrimination, 10 days for high resolution melting, and 12 days for other techniques. No patient-, tumour- or practitioner-related factors were significantly associated with time to obtain results. ***Desired location of Table 3***

Mutation test results

59.7% of patients with available *RAS* (± *BRAF*) mutation results (190/318) had 1 mutation. *KRAS* mutations were identified in 47.9% of patients (152/317), *NRAS* mutations in 8.7% (20/231) and *BRAF* mutations in 10.0% (25/250). For 59.6% of patients (180/302), all 6 exons (*KRAS* exons 2, 3, 4 plus *NRAS* exons 2, 3, 4) were analysed. There were 44 patients with full *RAS* genotyping request for whom only *KRAS* exon 2 was in fact tested; in 41 of these cases, exon 2 was mutated, and this finding likely stopped the genotyping sequence.

Impact of *RAS* mutations on therapeutic management

According to the physicians, *RAS* mutation status had an impact on the therapeutic management of 94 of the 179 patients (52.5%) with 1 *RAS* mutation, and of 71 of the 120 (59.2%) with wild-type *RAS*. Treatment changes, known for 76 of the 94 *RAS*-mutated patients, were, in decreasing order: anti-VEGF MAb initiation (bevacizumab: 39.5%; 30/76), no anti-EGFR MAb prescription despite being initially considered (22.4%; 17/76), and chemotherapy regimen change (17.1%; 13/76). Changes, known for 66 of the 71 wild-type *RAS* patients, were mainly anti-EGFR MAb (cetuximab, panitumumab) initiation (90.9%; 60/66 patients) (Figure 2). An anti-EGFR MAb (cetuximab, panitumumab) was prescribed for 10 of the 179 patients (5.6%) with a *RAS* mutation, despite the change in marketing authorizations. ***Desired location of Figure 2***

Discussion

This study was the first to describe the prevalence and procedure of *RAS* (*KRAS+NRAS*) mutation testing in routine practice at national level in France, since the approved indication for anti-EGFR MAbs in patients without activating *RAS* mutations.

The study showed that *RAS* mutation testing was routinely performed as part of mCRC patient management in 2014 in France. Compared to 2011, the rate of genotyping testing requests increased from 81.1% in the 2011 Flash-K*RAS* study [6] to 90.1% in the present study, indicating clinical integration of anti-EGFR MAbs guidelines. For 9.2% of patients, mutation testing was requested for *KRAS* only, possibly because the study was conducted shortly after these recommendations were added to the cetuximab and panitumumab summaries of product characteristics [7, 8].

In 2014, as previously observed in the 2011 Flash-KRAS study [6], the test was prescribed early during mCRC patient management: within 1 month around the diagnosis of metastases in 73.1% of patients, and before initiation of first-line therapy in 85.4%. However, for 22.1% of the patients, the test was requested more than 1 month after diagnosis and, for 14.6%, after initiation of first-line therapy, which is not compatible with an informed choice of first-line treatment according to the patient's *RAS* status.

A mutation was identified in 59.7% of the patients, and mainly consisted in *KRAS* mutation. In more than half of the patients, all 6 exons were analysed (*KRAS* exons 2, 3, and 4 plus *NRAS* exons 2, 3, and 4). Genotyping was in some cases conducted sequentially, and in 44 patients only *KRAS* exon 2 was analysed despite a request for full *RAS* genotyping, almost always because of early discovery of a *KRAS* exon 2 mutation.

Regarding time to obtain results, the mean time for the whole test procedure (from test request to the results feedback) was 24.6 ± 17.2 days (median: 20 days) in 2014, similar to that reported in the 2011 Flash-KRAS study (23.6 ± 28.2 days; median: 19 days). The narrower standard deviation, however, indicates a trend for more uniform feedback times in 2014, suggesting greater uniformity and coordination of mCRC patient management in the deployment of these new tests. This time interval was slightly shorter than reported in a previous French retrospective study [9] but remains long and hardly acceptable in clinical practice. The interval exceeded the maximum 10 working days recommended by European EQA schemes in 2013 [10], the 7-to-10 working days recommended by the French National Cancer Institute (INCa) in 2010 [11], and the median 9 days (range: 4-21 days) specified in the summary of the activity of the French hospital molecular genetics platforms in 2012 [12]. In a large study (2,510 tumour samples) performed in Germany from 2014 to 2016, 72% of *RAS* results were reported within 6 working days (lab turnaround time) [13]. In Canada, median turnaround time for *EGFR* results in lung cancer was 18 days (range: 15–26 days) in one study [14], and 21 days in another in which *EGFR* and *ALK* mutation tests were performed after the first oncology consultation [15]. In the present study, time from dispatch to the technical platform to reception of the report was longer in 2014 (19.5 ± 15.8 days; median: 15 days) than 2011 (mean 14.0 ± 11.0 days; median: 11 days), probably due to the greater number of exons tested (1 in 2011 versus 6 in 2014).

The combination of pyrosequencing/sequencing/snapshot was the most frequent technique used for analysis of mutations, whatever the allele studied (*KRAS*, *NRAS*, or *BRAF*). This technique tended to replace sequencing by Sanger's method, previously considered as the "gold standard'. According to the literature, between 7% and 20% of CRC cases characterised as wild-type by Sanger sequencing or real-time PCR were found to harbour *KRAS* codon 12

or 13 mutations on pyrosequencing, locked nucleic acid PCR or mutant-enriched PCR techniques [16-19].

With regard to the impact on therapeutic management, the results of *RAS*/*KRAS* mutation testing showed that, when absence of mutation was confirmed, a majority of patients (90.9%: 60/66) were prescribed an anti-EGFR MAb, consistent with the goal of genotyping testing in this population. For a limited number of patients with a *RAS* mutation (5.6%: 10/179), an anti-EGFR MAb (cetuximab, panitumumab) was prescribed, despite the restriction laid down in the market approval, which may suggest that there is still room for improvement in therapeutic practices.

This study has some limitations owing to its observational nature, and possible selection bias for the physicians and patients participating. Only 104 of the 2,700 physicians contacted included at least 1 patient. Difficulties of recruitment led to a small number of included patients, which may limit extrapolation to a wider patient population. However, patient characteristics and distributions by centres and regions were representative of the population of mCRC patients, although 4 of the 22 French administrative Regions were overrepresented and 3 were not represented [20]. Missing data, especially concerning time between *RAS/KRAS* mutation test request and reception of the genotyping report, constituted another limitation.

Liquid biopsy, analysing circulating tumour DNA (ctDNA), has emerged as new non-invasive procedure for detecting gene mutations in cancer patients. In a recent study, this method detected ctDNA in 100% of patients and exhibited high specificity (98%) and sensitivity (92%) for 7 *KRAS* point mutations [21]. While liquid biopsy is not yet well established in routine clinical practice, it could advantageously replace tumour section analysis for detection of *RAS* mutations, reducing procedure time. Liquid biopsy could bypass the time-consuming and therefore limiting steps of unarchiving, selecting and dispatching samples to the

oncogenetics platform. This method may, therefore, expand the scope of personalized medicine for cancer patients.

In conclusion, this study showed that, in 2014, *RAS* mutation genotyping testing was a routine part of mCRC patient management in France. Compared to 2011, the rate of genotyping testing requests increased markedly. Overall, mCRC patient management was consistent with health authority guidelines, according to *RAS* status. However, the interval between test request and results feedback was longer than expected, and is not acceptable in clinical practice; this delay must be reduced. Standardization of assessment methods at European level could be a way to shorten this delay.

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AL: Merck, Baxalta, Ipsen (board member), Merck, Amgen, Roche, Lilly, Novartis (lecturer).

JLM: Merck, Amgen (board member and lecturer), Sanofi (lecturer)

JCS: Merck, Amgen, MSD, BMS, Roche, Astra Zeneca, Pfizer, Boehringer Ingelheim (board member)

PA: Roche, Merck (board member) Sanofi, Amgen, Lilly (lecturer)

ST and **LL** worked for Axonal, which received funding from Merck, at the time of the study.

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Figure 1: Study flowchart

[a]A given patient might show several major protocol deviations: diagnosis of mCRC before 2014 (N=11); refusal of data collection (N=10); no informed consent (N=4); >1 prior line of mCRC therapy (N=10); and/or participation in a concomitant interventional study (N=12).

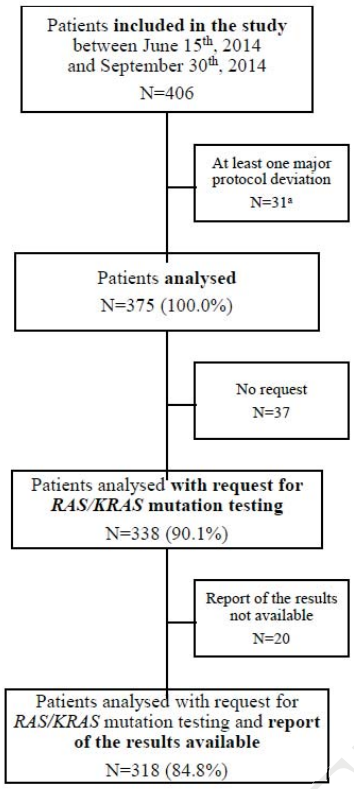

Figure 2: Impact of *RAS* mutations on therapeutic management

[a]318 of the 338 patients with at least 1 *RAS/KRAS* mutation test request (94.1%) had results available for *RAS* mutations.

RAS: *KRAS+NRAS* or *KRAS+NRAS+BRAF*

EGFR: epidermal growth-factor receptor; MAb: monoclonal antibody; VEGF: vascular endothelial growth factor

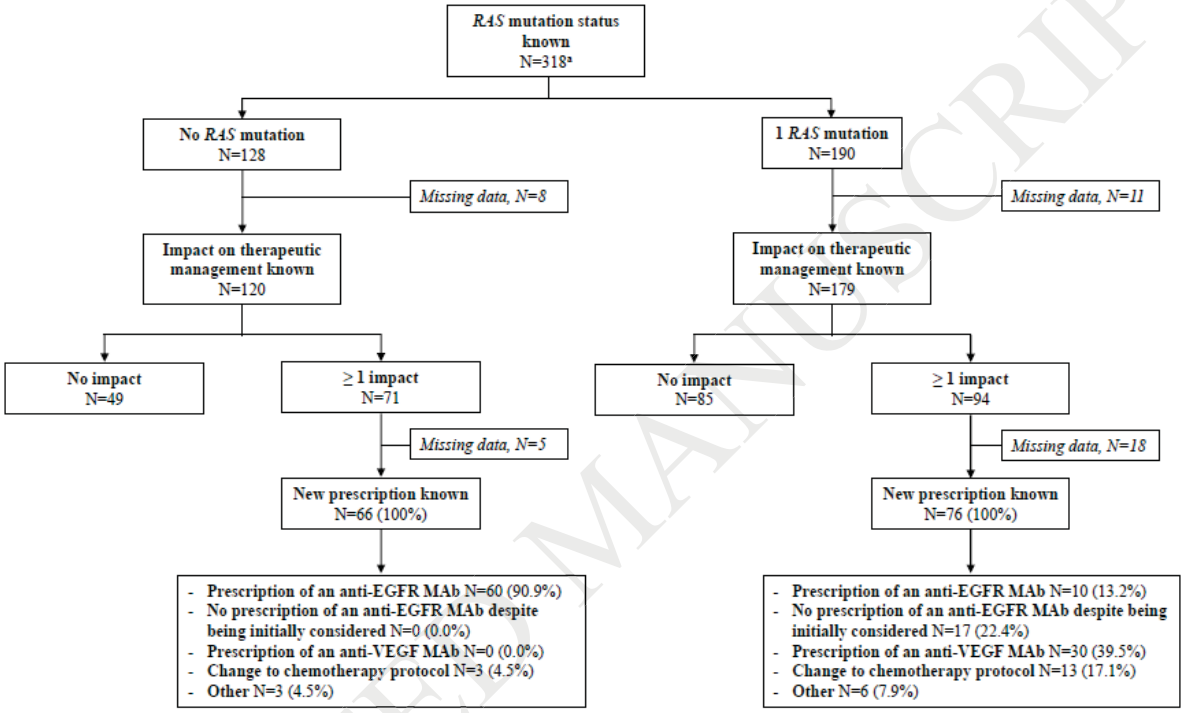

Table 1: Patient and tumour characteristics

	Total (N=375)
Gender, n (%)	**N=374**
Male	216 (57.8%)
Age in years	**N=371**
Mean ± SD	65.7 ± 11.7
Median	67
Q1-Q3	58-74
Location of primary tumour, n (%)	**N=370**
Colon	282 (76.2%)
Rectum	86 (23.2%)
Colon + Rectum	2 (0.5%)
TNM Stage at the time of the CRC diagnosis, n (%)	**N=367**
I-II	38 (10.4%)
III	59 (16.1%)
IV	270 (73.6%)
ECOG Performance status at study entry, n (%)	**N=350**
0	140 (40.0%)
1	158 (45.1%)
≥2	52 (14.9%)
Time (months) between diagnostic of mCRC and initiation of the first line chemotherapy	**N=368**
Mean ± SD	1.11 ± 0.70

Median	1
Q1-Q3	0.6-1.5
First-line metastatic chemotherapy, n (%)	**N=364**
FOLFOX and/or XELOX	186 (51.1%)
FOLFIRI and/or IRINOTECAN	117 (32.1%)
5 FU/LV IV and/or XELODA	40 (11.0%)
FOLFIRINOX	24 (6.6%)
Other chemotherapy	3 (0.8%)
Targeted therapy associated with first-line metastatic chemotherapy, n (%)	**N=372**
At least one targeted therapy prescribed	198 (53.2%)
Targeted therapy, n (%)	**N=197**
Cetuximab	29 (14.7%)
Bevacizumab	142 (72.1%)
Panitumumab	23 (11.7%)
Aflibercept	3 (1.5%)

5-FU/LV IV: 5- 5-fluorouracil/leucovorin Intravenous; CRC: colorectal cancer; ECOG: Eastern Cooperative Oncology Group; FOLFOX: 5-fluorouracil, levofolinate, oxaliplatin; FOLFIRI: 5-fluorouracil, levofolinate, irinotecan; FOLFIRINOX: 5-fluorouracil, levofolinate, irinotecan, oxaliplatin; mCRC: metastatic colorectal cancer; N: number of patients with available data; SD: Standard Deviation; XELODA: capecitabine; XELOX: capecitabine, oxaliplatin

Table 2: Characteristics of the *RAS*/*KRAS* mutation testing requests

	Total (N=375)
***RAS*/*KRAS* mutation testing, n (%)**	**N=375**
No mutation testing requested	37 (9.9%)
At least one mutation test request	338 (90.1%)
RAS[a]	292 (86.4%)
KRAS[b]	31 (9.2%)
Genotyping mCRC[c]	9 (2.7%)
Missing data	6 (1.8%)
Time between diagnosis of mCRC and *RAS*/*KRAS* mutation testing request, n (%)	**N=331**
>1 month prior to diagnosis of metastases	16 (4.8%)
≤1 month before and ≤1 month after diagnosis of metastases	242 (73.1%)
>1 month after diagnosis of metastases	73 (22.1%)
Time between *RAS*/*KRAS* mutation testing request and initiation of first-line metastatic therapy, n (%)	**N=328**
Before the introduction of the first-line therapy	280 (85.4%)
After the introduction of the first-line therapy	48 (14.6%)

[a]*RAS*=*RAS* or *KRAS*+*NRAS* or *KRAS*+*NRAS*+*BRAF*

[b]*KRAS*=*KRAS* or *KRAS*+*BRAF* or *KRAS*+*BRAF*+microsatellite instability (MSI) phenotype

[c]Genotyping mCRC=*BRAF*+MSI phenotype or MSI phenotype or Genotyping or CRC biomarker

Table 3: Duration in days of the time between test request and receipt of the genotyping report for *RAS*/*KRAS* mutation testing

	Duration in days from test request to dispatch of tumour material to platform (N=237)	**Duration in days from dispatch of tumour material to platform to receipt of the genotyping report (N=244)**	**Duration in days from test request to receipt of the genotyping report (N=280)**
Mean ± SD	7.7 ± 11.3	19.5 ± 15.8	24.6 ± 17.2
Median	4.0	15	20
Q1-Q3	0.0-9.0	10.0-23.0	14.0-29.0
Min; Max	0.0; 65.0	1.0; 112.0	1.0; 118.0

Population: all patients, whatever the wording of the request, for whom there was a result for both *KRAS* and *NRAS* plus requests for *RAS* tests, for which there was at least one result for *KRAS* gene (N=304)