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The *KRAS* mutation detection within the initial management of patients with metastatic colorectal cancer: A status report in France in 2011

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Abstract ***Background:*** The detection of *KRAS* mutations is mandatory to initiate an anti-epidermal growth factor receptor (EGFR) antibody in the treatment of metastatic colorectal carcinoma (mCRC).

Patients and methods: This observational retrospective study was performed in 160 French centres during a 2-week period in 2011. Its main objective was to evaluate the rate of *KRAS* testing in patients with mCRC having initiated their first-line therapy. Secondary objectives included time of process, techniques used and reasons for non-prescription.

Results: Five hundred and thirty eight mCRC patients (67.1 ± 11.3 years, synchronous metastases: 69.9%) were enrolled in the study. *KRAS* testing was prescribed in 81.1% of patients, in a median of 15 days after the diagnosis of metastases, and of 15 days prior to the initiation of

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the first-line metastatic chemotherapy. *KRAS* status was available for 87% of patients, after 23.6 ± 28.2 days, but after the choice of the first-line therapy in 56.6% of patients. Heterogeneity of reception time was noteworthy within regions (8.3 ± 7 days to 38.8 ± 101 days). *KRAS* testing was not prescribed mainly due to the planned non-prescription of an anti-EGFR antibody.

Conclusion: This study confirmed that *KRAS* testing is definitely part of the management of most of mCRC patients, despite discrepancies observed in the rate of prescription and the time of results.

1. Introduction

Colorectal cancer (CRC) is the most frequent cancer and the second cause of cancer death in Europe.[1,2] Nearly half the patients with a CRC develop metastases during the disease progression and 22% are already metastatic at the time of diagnosis; their 5-year survival rate remains low, around 55%.[3,4] Advances in standard chemotherapy, combining 5-fluorouracil and folinic acid, with or without irinotecan or oxaliplatin, and development of targeted therapies allowed a survival improvement of metastatic colorectal carcinoma (mCRC) patients in the last 20 years. Given the role of the epidermal growth factor receptor (EGFR) in colorectal carcinogenesis and its frequent overexpression in colorectal tumours, monoclonal antibodies targeting EGFR (cetuximab and panitumumab) have been developed and were shown to improve tumour response rate and patient survival.[5–7] Nevertheless, their efficiency is limited to the subset of patients without tumour *KRAS* mutation.[8–11] Indeed, *KRAS* mutations, which are present in 30–40% of CRC are responsible for an acquired activation of the Ras/MAPK (mitogen-activated protein kinase) and the PI3K/AKT phosphoinositide 3-kinase/AKT pathways independently of the ligand-induced activation of the EGFR and therefore induce a resistance to anti-EGFR monoclonal antibodies.

The identification of *KRAS* mutations as a strong predictive biomarker of resistance to anti-EGFR antibodies has opened the way to an individualised treatment of mCRC patients, resulting in an improved outcome of *KRAS* wild-type patients receiving these targeted therapies. In this context, the determination of *KRAS* mutation status has become an essential parameter in the therapeutic strategy of mCRC, especially if a treatment with an anti-EGFR antibody is discussed, as it is specified by main societies of clinical oncology that recommend that cetuximab or panitumumab should not be used in patients with a *KRAS* mutated tumour.[12,13] These data also led the European Medicines Agency (EMA) to restrict the use of anti-EGFR antibodies to mCRC patients with a wild-type *KRAS* tumour.[14]

Consequently, genetic testing to confirm the absence of *KRAS* mutation is now supposed to be performed in clinical routine before starting a treatment with an anti-EGFR monoclonal antibody and more generally at the beginning of the management in a perspective of optimal therapeutic strategy.

Epidemiological data on *KRAS* testing are limited.[15–17] The aim of this observational and retrospective study was to evaluate in France the rate of *KRAS* testing prescribed in patients recently diagnosed with mCRC and receiving first-line therapy.

2. Patients and methods

2.1. Patients

The Flash-*KRAS* study was a French multicentre observational retrospective study conducted from 28th March 2011 to 08th April 2011 in 160 hospital centres (including 54 general and 36 university hospitals, 44 private centres and 8 cancer centres). The 'retrospective' feature was intentionally chosen in order to avoid any incitation of prescription. Inclusion criteria were patients older than 18 years with mCRC who initiated first-line therapy prior to the study during the first trimester of 2011. Physicians retrospectively filled out a questionnaire for each patient seen in consultation during the 2 week study period and who fulfilled the inclusion criteria. Following data were collected retrospectively from the medical file of each patient: patient and tumour characteristics, initial management, process of prescription of *KRAS* testing and first-line chemotherapy. When available, reports of the *KRAS* testing were added to the questionnaire.

2.2. Objectives

The primary objective of the study was to evaluate the current rate of prescription of *KRAS* testing in 2011 in newly diagnosed mCRC patients. Secondary objectives were to analyse reasons for the non-prescription of genotyping, steps and timing of the process of *KRAS* testing from the prescription to the reception of its results, techniques used to determine *KRAS* status, as well as the impact of *KRAS* status on therapeutic strategy.

2.3. Statistics

Based on an expected percentage of patients with a prescription of *KRAS* testing of 50%, the number of

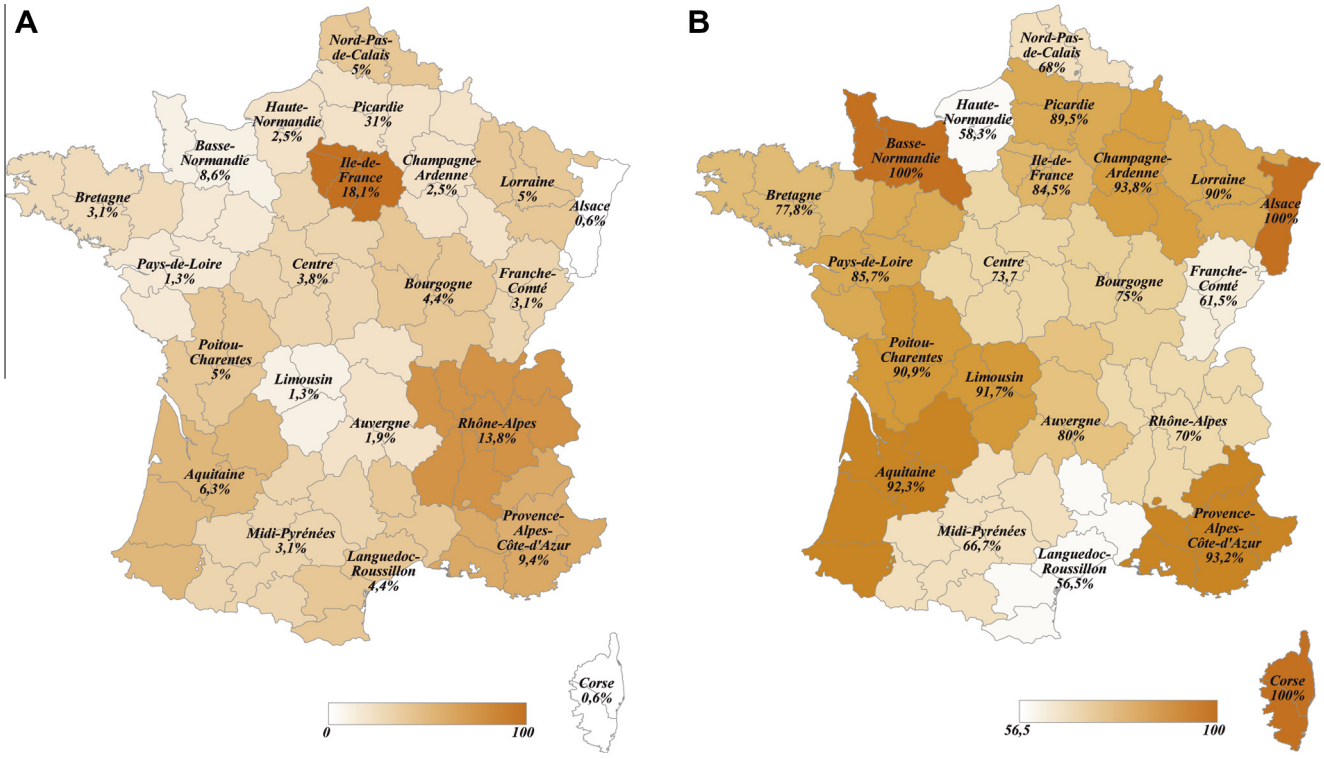

Fig. 1. Location of oncogenetics platforms and rate of participation to the study (A) and rate of *KRAS* genotyping prescribed on the national French territory (B).

patients needed was initially estimated to be 1000, with a confidence interval (CI) at 95% by an extension of 6.2%, i.e. a precision at 3.1%. Given the results of the study and the number of patients finally included ($n = 548$), the precision remained at 3%.

Statistical analysis was performed using SAS software, version 8.2. Descriptive statistics used for quantitative parameters were mean, standard deviation, minimum, maximum, median and missing values; those used for qualitative parameters were frequency and percentage.

A logistic regression was done on factors that could promote the prescription of the *KRAS* testing (type of centres, age of investigators and area of the exercise site ...). Explicative data were selected according to their discriminant power at the time of the univariate analyses ($P < 0.2$). According to the French law, this study received the authorisation from the National Committee on Informatics and Freedom (CNIL) and the French Medical Council's approval.

3. Results

3.1. Centres and patients

Overall, 366 out of 1751 approached physicians (160 centres across 23 metropolitan French Regions) participated in the Flash-*KRAS* study. Distribution of the centres was not homogeneous on the national territory (Fig. 1). Physicians' median age was 42 years (28–65) and most of them were men (63.8%). Out of the 548 patients recruited, 10 (1.8%) were excluded from the analysis due to at least one major deviation from the protocol: lack of information given to the patient ($n = 7$), consultation beyond the study period ($n = 1$) and second-line therapy ($n = 2$).

A total of 538 subjects were included. Patients and tumour characteristics are summarised in Table 1. Metastases were synchronous in 69.9% of the patients. The first-line therapy was initiated 1.1 months (median) after the diagnosis of mCRC.

3.2. KRAS testing request

KRAS testing was prescribed in 433 (81.1%) out of the 538 patients (CI at 95%: [77.8%; 84.4%] (Fig. 2). Accordingly, 101 patients (18.9%) had no demand of *KRAS* testing. The main reason given was the planned non-prescription of an anti-EGFR monoclonal antibody ($n = 58$; 57.4%). *KRAS* testing was mainly requested by oncologists ($n = 195$; 45.5%) and gastroenterologists ($n = 133$; 31.0%).

The rate of prescription varied according to the type of centre: 72.9% in university hospitals, 81.6% in general hospitals, 83.3% in cancer centres, 87.7% in private centres. The difference was significant when comparing university hospitals and private centres ($p = 0.031$). Of note, the patient's age interfered significantly: patients aged 80 years or more had 2.2 less chance to have a prescription of *KRAS* testing than younger patients ($p = 0.017$).

Table 1
Patient and tumour characteristics.

	Population	N[a]
Total patients, *N*	538	538
Mean age, *years*	67.1 ± 11.3	533
Male gender, *N* (%)	319 (59.4)	537
Site of the primary tumour, *N* (%)		535
Colon, *N* (%)	408 (76.3)	
Rectum, *N* (%)	125 (23.4)	
Colon and rectum, *N* (%)	2 (0.4)	
TNM stage at the time of the diagnosis of CRC		488
Stage I, *N* (%)	5 (1.0)	
Stage II, *N* (%)	44 (9.0)	
Stage III, *N* (%)	98 (20.1)	
Stage IV, *N* (%)	341 (69.9)	
Neo-adjuvant therapy, *N* (%)	77 (14.8)	519
Adjuvant therapy, *N* (%)	147 (28.5)	516
Median time between diagnosis of mCRC and initiation of the first-line therapy, *months*	1.1	538

[a] Number of patients with available data.

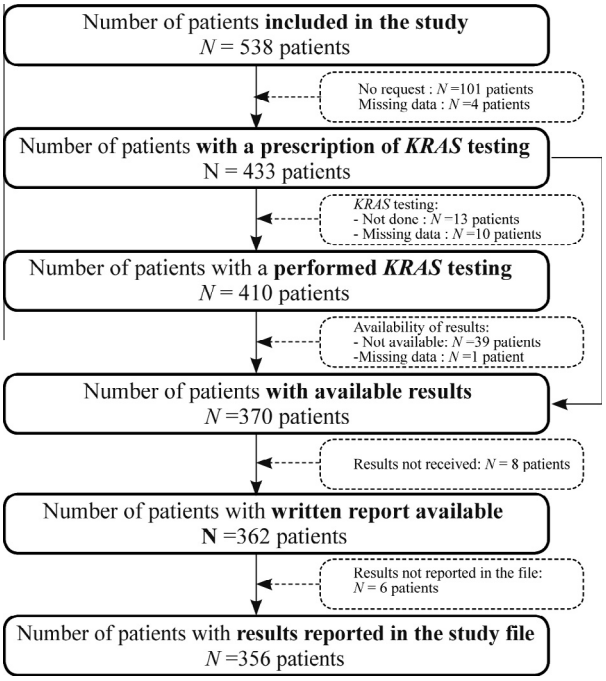

Fig. 2. Flow-chart of the study patients.

In 78.9% of patients, *KRAS* testing was requested within the 3 months following the diagnosis of metastases, including 40% within the first month (Table 2, Fig. 3). The time of the prescription of *KRAS* testing was hence around 15 days (median) (mean: 1.5 ± 5.0 months) prior to the introduction of the first-line therapy ($n = 418$).

The material used for the *KRAS* testing was mainly issued from the primary tumour ($n = 365$; 86.1%) (Table 2). It was addressed to the genetics platform by the pathologist in most cases (91%). When specified ($n = 303$) techniques most frequently used for the KRAS testing were sequencing (sequencing/pyrosequencing/snapshot) ($n = 158$; 52.1%), allelic discrimination ($n = 88$; 29.0%), high resolution melting ($n = 28$; 9.2%), or others ($n = 29$; 9.6%).

3.3. Results of the KRAS testing

Once genotyping was prescribed, the *KRAS* status was determined in 410 subjects (94.7%). Results were available at the time of the study for 370 patients (90.2%) after 23.6 ± 28.2 days (median at 19) (Fig. 2).

The mean time between the prescription of the *KRAS* testing and the sending of the tumour material to the oncogenetics platform was 9.7 ± 14.3 days [1; 121] with a median of 6 days. The time between this shipment to the reception of the results was 14.0 ± 11.0 days (median: 11 days). Consequently, the global time of the overall process was 23.6 ± 28.2 days (median: 19 days) (Fig. 4). Result of the *KRAS* testing was known prior to the choice of the first-line metastatic chemotherapy for 158 patients (43.4%) only. The time between this shipment to the reception of the results and the time of reception of the results were significantly prolonged when the oncogenetics platform was located outside the centre of patient care (14.4 ± 10.4 days versus 12.5 ± 13.6; $p = 0.006$ and 20.3 ± 16.4 versus 24.8 ± 31.2 days; $p = 0.007$). These delays varied markedly according to the region (1.5 ± 3.0 to 23.3 ± 15.6 days and 8.3 ± 7.2 to 38.8 ± 101.8 days, respectively).

Genotyping revealed a *KRAS* mutation in 133 patients (37.4%) out of 356 patients with a given result. According to the physicians interviewed, the result of *KRAS* testing had an impact on therapeutic strategy in 150 patients (42.8%). Of note, the therapeutic impact was significantly more marked ($n = 108$; 49.1%) in the 223 patients with a wild type KRAS tumour than in the 133 mutated patients ($n = 42$; 32.3%) ($p = 0.002$) and resulted in the majority of the cases in the prescription of an anti-EGFR monoclonal antibody (97 among the 108 patients; 89.9%).

4. Discussion

This study was the first observational, retrospective study evaluating *KRAS* testing in the initial management of mCRC patients regardless of treatment strategy, allowing a snapshot of practices regarding *KRAS* testing in France in 2011. Characteristics of the study population and participating centres are close to those expectable in patients with mCRC and support therefore the representativeness of the management observed in the daily practice in France.

Our data showed that determining the tumour *KRAS* status, which is recommended prior to initiate an EGFR

Table 2
KRAS genotyping characteristics.

	Population	N[a]
KRAS genotyping		534
Prescribed, *N* (%)	433 (81.1)	
Not prescribed, *N* (%)	101 (18.9)	
Median time of the *KRAS* genotyping prescription regarding the introduction of a first-line metastatic therapy (months)	0.5	418
First-line metastatic cytotoxic therapy		538
5-Flurouracil/leucovorin IV, *N* (%)	36 (6.7)	
Capecitabine monotherapy, *N* (%)	17 (3.2)	
FOLFIRI (5-fluorouracil, levofolinate, irinotecan), *N* (%)	228 (42.4)	
FOLFOX (5-fluorouracil, levofolinate, oxaliplatin), *N* (%)	220 (40.9)	
XELOX (capecitabine, oxaliplatin), *N* (%)	5 (0.9)	
XELIRI (capecitabine, irinotecan), *N* (%)	4 (0.7)	
FOLFIRINOX (5-fluorouracil, levofolinate, irinotecan, oxaliplatin), *N* (%)	17 (3.2)	
Other, *N* (%)	28 (5.2)	
Data not available, *N*	5	
First-line metastatic therapy associated with a targeted therapy, *N* (%)	289 (54.3)	532
Request performed		421
Prior to the diagnosis of the first metastases, *N* (%)	61 (14.5)	
Within 3 months, *N* (%)	332 (78.9)	
Beyond 3 months after the diagnosis of the first metastases, *N* (%)	28 (6.7)	
Nature of the material sent for *KRAS* testing		424
Primary tumour, *N* (%)	365 (86.1)	
Metastasis, *N* (%)	56 (13.2)	
Primary tumour and metastasis, *N* (%)	3 (0.7)	

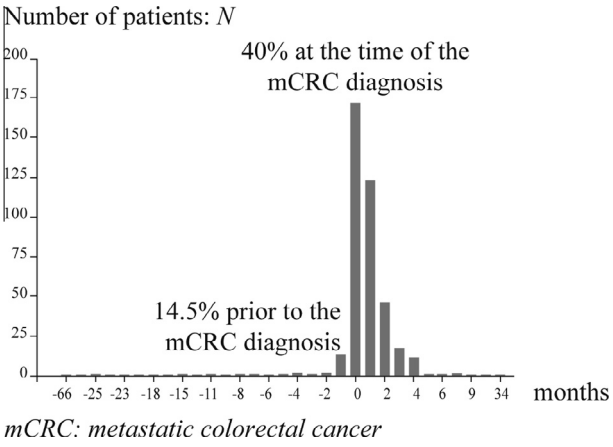

Fig. 3. Time of prescription of the *KRAS* testing with respect to the diagnosis of CRC metastases.

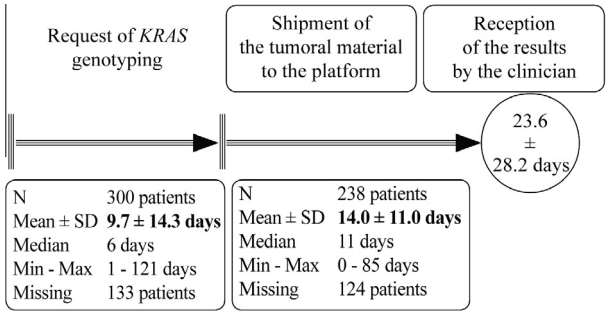

Fig. 4. Duration of the whole process of KRAS testing.

inhibitor[12,18] is now part of the clinical practice, with a prescription rate of 81.1% in first-line therapy. This reflects a real improvement in the approach of personalised care. Our results are in concordance with those of a previous study conducted in 14 countries in Europe, Latin America and Asia reporting an increased frequency of *KRAS* testing from 3% in 2008 to 69% in 2010 for all line-therapies, and more accurately from 54% to 78% for patients who had started or were about to start first-line therapy in Europe.[16]

Our results are consistent with European Society for Medical Oncology and French National Thesaurus of Digestive Oncology clinical guidelines[13,18,19] which both support the determination of the *KRAS* status as a key factor in the selection of the best combined regimen for the first-line therapy of mCRC. In 2009, the EMA (European Medicines Agency) and the US FDA (United States Food and Drug Administration) updated the labels of cetuximab and panitumumab by restricting their prescription to mCRC without *KRAS* mutation.[14,20] Considering this issue, the French National Cancer Institute (INCa) set on a network of genetics platforms, in order to make the *KRAS* testing easier, ensure a quality control and provide *KRAS* status within a time matching with the requirements of the daily routine in France.[21] The French network of genetic platforms initiated in 2006 is already mentioned as a reference in terms of organisation and efficiency; 28 centres have been involved in the *KRAS* testing for more than 16,000 patients in France in 2010 and will be solicited exponentially in the future considering the 40,250 new CRC diagnosed in 2011 and the rate of such cancers with metastases (40–60%).[21–25]

As *KRAS* testing has been introduced in clinical practice, its rapidity but also and especially its quality must be ensured for all patients. Indeed, the correctness of the KRAS testing result is of great importance for good patient care and must avoid false-negative and false positive findings, which both might have deleterious consequences for the patient. To guarantee a high reliability of the test, external quality assessment (EQA) programmes are necessary and have been conducted in Europe. The results of the first european joined regional quality assessment round performed in 2009 in 59 laboratories from eight different European countries, including France, revealed that only 70% of laboratories correctly identified the KRAS mutational status in all samples analysed (genotyping errors: 22%, technical failures: 8%).[26] The authors concluded that the overall

quality of KRAS testing could be improved, notably by participating regularly in EQA schemes assessing the whole testing process (i.e. the pathology review, the molecular test itself and the report). Since then, several EQA programmes to which a high number of laboratories subscribed have been implemented, showing a high rate ($\geqslant$90%) of laboratories providing correct KRAS testing results in United Kingdom (UK),[27] Germany[28] and also in Italy where the educational programme of scientific societies with the publication of guidelines followed by their presentation in several national meetings might have improved KRAS testing over the time.[29] However, mistakes persist and some laboratories provide results below the standards set by the EQA providers. They are encouraged to continue to participate in one of the several accessible EQA programmes. Guideline on the requirements of EQA programmes have been recently developed and published to improve reliability of analyses in molecular pathology, including KRAS testing.[30]

In our study, *KRAS* testing was prescribed early after the diagnosis of the metastases (40% within the first month). It is usually not performed prior to the diagnosis of metastases although this might be justified in advanced stage tumours with a high risk of relapse in order to have *KRAS* status at diagnosis of metastases without wasting time. This situation has concerned 14.5% of the patients included in our study. This attitude, however, cannot be recommended routinely.

We report a mean time from the prescription of the *KRAS* testing to the reception of its results was 23.7 ± 28.2 days. This delay appears consistent with data reported in rare studies that have specifically analysed this time.[17,31] In the survey reported by Ciardiello et al., *KRAS* testing results were available within 15 days for 85%, 51% and 98% of the 1679 European, 679 Latin-Americans and 271 Asian tested patients.[16] In another recent observational French study, results were obtained with a mean delay of 33.4 ± 39.8 days although this study was performed in chemoresistant patients treated with panitumumab.[17] The delay reported in our study was compatible with the time of initiation of a first-line therapy. Of note, the time of the process was the reason for a non-prescription of *KRAS* testing in only 2% of patients. According to rare published data, no marked progress was observed in the time to obtain *KRAS* mutation tests results compared to former data[16] the rate of results available within 15 days was 70% of tested patients in 2009 with a marginal increase at 82% in 2010 in Europe. This might probably be due to fixed periods such as those required to get archived tumour tissues and to perform the histological pre-analytical phase. It could be nevertheless shorten by a stronger implication and coordination of all actors (oncologist, surgeon, pathologist, biologist,etc) implied in the whole diagnosis and management process.

Our data corroborate the good collaboration with the platforms previously reported.

However, it is unfortunate that results of the *KRAS* testing were available for only 56.6% of patients prior to the initiation of first-line chemotherapy. However, lack of *KRAS* testing request observed in our study was most often (57.4%) related to an intentional decision of physicians not to prescribe an anti-EGFR antibody and/or to a planned surgery for resectable metastases regardless of the *KRAS* status. The non-availability of *KRAS* status had henceforth no real deleterious impact on the therapeutic management considering this former decision to withdraw the anti-EGFR therapy.

Our study highlights numerous techniques for *KRAS* testing available to date. Sequencing according to the Sanger's method, previously considered as 'gold standard', is progressively substituted by other techniques (pyrosequencing/snapshot, allelic discrimination, high-resolution melting) with higher sensitivity.[32–36] These techniques showed they allow the identification of *KRAS* mutations in tumours previously identified as wild-type.[37,38] Considering their equivalence, no method is official or specifically recommended.[39] KRAS quality assessment did not reveal differences in the ability to detect mutations between techniques used by different laboratories,[29] but combining different mutation testing techniques greatly reduce the probability to get false-negative or false-positive results and was also shown to significantly decrease the delay for reporting results.[40] However, pre-analytical procedure (paraffin embedding, fixative and fixation time) needs to be strictly controlled as DNA degradation was found to be a major cause of non-interpretable results.[40] The percentage of tumour cells is also of critical importance for the reliability of the determination of KRAS mutational status and it was pointed out in a recent study that a decrease in correct mutation rate proportionally with decreasing percentage of tumour cells was seen for all techniques used.[41] In our study, the low rate of missing *KRAS* status due to a not readable material was positively noteworthy (4%) and close to data published in the literature.[42]

The research of *KRAS* mutations in plasma[43–45] offers perspectives for future regarding its progressive sensitivity improvement, and its ability to provide more rapidly and easily a *KRAS* status without known concerns of availability and quality of tumoural tissue.

Our study has some limitations: the patients' recruitment based on willing physicians and the short study's duration might have induced some bias, altering the representativeness of the reality. Heterogeneity within the national territory was observed with wide variations of prescription rates and delays in obtaining results and opened perspectives of improvement.

In conclusion, the Flash-*KRAS* study shows that (i) *KRAS* testing is definitely part of the therapeutic approach of mCRC in France, as soon as the initial step

of its management, (ii) time from its prescription to its results availability is already consistent with the requirements of treatment decision making in clinical practice but could be still improved (iii) this observance is the result of efficient platforms that allow an equal access to a target therapy to all patients in the national French territory (iv) *KRAS* testing result has a relevant impact on treatment decision and therefore represents an essential parameter in the pre-therapeutic assessment of mCRC patients.

Conflict of interest statement

A.L.; advisoryboard and lecture honoraria for Merck Serono, advisory board for Sanofi, and lecture honoraria for Roche; P.L.P.: advisory boards in particular related to anti EGFR therapy (Merck-Serono; Amgen; Boerhinger Ingleheim); J.L.M.: honoraria and member of advisory board from Merck Serono; J.C.S.: advisory boards for Merck Serono, Roche and Boehringer-Ingelhem; M.D.: advisory boards and lectures in symposium. All remaining authors have declared no conflicts of interest.

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