Lack of Relationship Between Clinical Features and *KRAS* Mutations in Patients with Metastatic Colorectal Cancer

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Abstract. *Background/Aim: We previously identified three clinical predictive factors of efficacy of cetuximab-irinotecan. Here, we analyzed the clinical characteristics of patients with metastatic colorectal cancer (CRC) in order to detect potent correlations with KRAS mutations. Patients and Methods: We conducted a retrospective, multicenter study between 2008 and 2012. We included patients with metastatic colorectal adenocarcinomas, previously treated by irinotecan, and with an available KRAS mutation test. Results: We included 299 patients. The median age was 60 years; the median number of metastatic sites was 2. One hundred and eight patients (36.1%) had a previous objective response to irinotecan. The median interval between diagnosis and irinotecan discontinuation was 1.94 years. A KRAS mutation was detected in 133 patients (44.5%). In univariate and multivariate analyses, none of the assessed factors was associated with the presence of a KRAS mutation. Conclusion: No easily clinically assessable parameter was significantly associated with KRAS mutations in patients with colorectal cancer.*

In 2004, the epidermal growth factor receptor (EGFR) monoclonal antibody cetuximab showed its efficacy in association with irinotecan after failure of an irinotecan-based chemotherapy in patients with metastatic colorectal cancer (CRC) (1).

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Skin rash was the first identified predictive factor of response to cetuximab (2). However, this information comes *a posteriori* and cannot help select patients who will benefit from cetuximab. In a previous study, we developed a clinical score, predictive of response to cetuximab (3). This score was composed of three features: (i) prior objective response to irinotecan, (ii) only one metastatic site and (iii) more than 2 years between diagnosis of cancer and cetuximab administration. Four groups were determined with a score between 0 and 3 points (3). The median progression-free survivals (PFS) were 3, 3.8, 5.6 and 8 months, respectively.

In 2008, Lievre *et al*. showed that tumoral *KRAS* mutations were a strong predictive factor of resistance to anti-EGFR antibodies (4). Moreover, in the mutated population, the overall survival (OS) was worse with cetuximab plus FOLFOX than with FOLFOX alone (5). This is why the American Society of Clinical Oncology (ASCO) recommendations restricted cetuximab use to patient without tumoral *KRAS* mutations (6).

To date, there is no clear correlation between the presence of a *KRAS* mutation and the patients' clinical and tumoral profiles.

In the present study, we systematically analyzed baseline clinical characteristics of patients with metastatic CRC in order to detect potent correlations with tumoral *KRAS* mutational status. We specifically assessed the three features composing the score described above.

Patients and Methods

Patients. We conducted a retrospective, multicenter study in five oncology centers in Northern France between 2008 and 2012, when *KRAS* mutational status determination was mandatory before anti-EGFR use. We included patients with a histologically documented metastatic colorectal adenocarcinoma, previously treated by

irinotecan, and with an available *KRAS* mutation test. Irinotecan was previously administrated every two weeks at a dose of 180 mg/m^2 in association with 5-fluorouracil 400 mg/m^2 bolus followed by 2,400 mg/m^2/46 h infusion (FOLFIRI regimen).

Different features were collected: demographical (gender, age at the time of *KRAS* mutation test); tumoral (date of initial and metastatic diagnosis, number of metastatic sites at the end of first irinotecan administration).

Response to therapy was evaluated by computed tomography (CT) scan every two months. Best response (objective response, stability, progression) was defined according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (7).

The *KRAS* mutation status was searched on exon 2 (codons 12 and 13) by pyrosequencing and confirmed by Snapshot on one centralized regional biomolecular platform (University Hospital, Lille, France).

Statistical analysis. The main objective was to determine if the clinical score proposed previously was predictive of *KRAS* mutation (3). The secondary objective was to determine if baseline clinical features were predictive of *KRAS* mutations.

The clinical score was calculated with 1 point for each following criteria: (a) only one metastatic site, (b) interval between metastatic diagnosis and (c) irinotecan cessation ≥2 years, prior objective response on irinotecan.

The population was split in 4 groups with 0 to 3 points. With an identical distribution than in the previous study (3), we expected 15% patients with 0 point, 45% with 1 point, 30% with 2 points and 10% with 3 points.

Assuming an odds ratio (OR) of 2, a significance level fixed at 0.05 and a statistical power of 0.90, 120 patients at the minimum had to be included in each group (with or without *KRAS* mutations). With a *KRAS* mutation occurring in about 40% of patients, we had to include 300 patients.

Univariate analysis with Chi^2 test was conducted on all features and on the score. Multivariate analysis, including all significant features at the level of 0.20, was conducted by logistic regression. This study was approved by the consulting committee for information treatment to healthy research (CCTIRS) (N° 15.272).

Results

We included 299 patients. The median age was 60 years. One hundred and sixty-eight patients (56.2%) were female. Patients had a median of two metastatic sites (1-4), 119 patients (39.8%) had only one metastatic site, metastases were synchronous in 187 patients (62.5%). Prior response on irinotecan was: objective response for 108 patients (36.1%), stable disease for 108 patients (36.1%) and progression for 83 (27.8%). The median interval between diagnosis and cessation of irinotecan was 1.94 years.

A *KRAS* mutation was detected in 133 patients (44.5%). The most common mutations were Gly12Asp (40.6%), Gly12Val (24.1%), Gly13Asp (13.5%) and Gly12Cys (9.8%) (Table I).

In univariate analysis, the Chi^2 test showed no significant results for interval between diagnosis and irinotecan cessation (p=0.560), prior objective response to irinotecan (p=0.801) or one metastatic site (p=0.285). The clinical score was not associated with *KRAS* mutation (p=0.576) (Table II).

Table I. *Population description.*

	Median (min-max)	Number (%)
Age (years)	60 (26-86)	
Interval before cessation of irinotecan (years)	1.94 (0-12.4)	
Female gender		131 (43.8)
Synchronous metastases		187 (62.5)
Previous response on irinotecan		
Objective response		108 (36.1)
Stability		108 (36.1)
Progression		83 (27.8)
Number of metastatic sites	2 (1-4)	
Mutations		133 (44.5%)
Gly12Asp		54 (40.6%)
Gly12Val		32 (24.1%)
Gly13Asp		18 (13.5%)
Gly12Cys		13 (9.8%)
Gly12Ala		10 (7.5%)
Gly12Ser		5 (3.7%)
Gly13Cys		1 (0.7%)

We tested the association between the most frequent mutations (Gly12Asp, Gly12Val, Gly13Asp, Gly12Cys and Gly12 Ala) and the clinical score. No correlation was found (Table III).

None of the other assessed factors was associated with the *KRAS* mutations in multivariate analyses (Table II).

Discussion

It would be of interest to identify easily available predictive factor of *KRAS* mutations. In some cases, it is difficult to obtain a biopsy to quickly perform an assessment of the mutational status. Secondly, we do not know if *KRAS* mutations are associated with a particular tumor profile, as it is the case for *EGFR* mutations in patients with non-small cell lung cancer (8).

We previously identified three predictive factors of response to irinotecan-cetuximab combination: previous objective response to irinotecan, only one metastasis site and more than two years between cancer diagnosis and cetuximab administration. This was confirmed in the larger MABEL study, which indicated that a prior response to irinotecan and only one metastatic site were predictive factors of response to irinotecan-cetuximab (9). A relationship between these factors and the presence of an intra-tumoral *KRAS* mutation may be suggested.

In the present study, none of the three features was significantly associated with *KRAS* mutational status.

Table II. *Univariate analysis with Chi² test.*

Features	Mutation N=133 (44.5%)	*p*-Value
Female	65 (49.6%)	0.114
Age (years)		0.134
<50	20 (41.7%)	
50-60	51 (48.6%)	
60-70	37 (43.0%)	
70-80	21 (37.5%)	
>80	4 (100%)	
Synchronous metastases	85 (45.4%)	0.662
Interval to irinotecan cessation ≥ 2 years	67 (46.2%)	0.560
Objective response	47 (43.5%)	0.801
One metastatic site	48 (40.7%)	0.285
Clinical score		0.576
0 point	31 (46.3%)	
1 point	57 (46.3%)	
2 points	49 (62.0%)	
3 points	15 (50.0%)	

Table III. *Univariate analysis between KRAS mutations and the clinical score.*

Location of mutation	*p*-Value (Chi² test)
Gly12Asp	0.682
Gly12Val	0.460
Gly13Asp	0.532
Gly12Cys	0.367
Gly12Ala	0.170

The frequency of *KRAS* mutations was 44.5%, that is consistent with previous studies indicating a 35.5 to 43.1% mutations rate (6). Moreover, the repartition of the mutations type in our study was the same than in literature with 28.7 to 59.6% of Gly12Asp, 13.5 to 25% of Gly12Val, 11.4 to 21.4 of Gly13Asp and 6.2 to 11.4% of Gly12Cys (10-13).

In univariate analysis, female gender was a predictive factor of *KRAS* mutation but this was not confirmed in the multivariate analysis. In two large previous studies, female gender was a predictive factor, whereas, in two others, it was not (1-17). Nonetheless, Watanabe *et al*. found an OR of 1.21 (range=1.08-1.36), which is not clinically relevant (15).

On the other hand, these studies showed a correlation between age and *KRAS* mutations but without agreement on the cut-off. There is a high prevalence of mutations between 40 and 60 years for Ferreira *et al*., after 40 years for Breivik *et al*. and before 50 years for Patil *et al*. (14, 16, 18). In the Watanabe *et al*. study, the proportion of mutations increased with age (15).

The last clinical feature most often described is the location of primitive tumor. Watanabe *et al*., Barault *et al*. and Zlobec *et al*. described an association between proximal tumors and presence of *KRAS* mutations but, conversely, Samowitz *et al*. found an association between the mutation and a distal tumor (15, 17, 19, 20). Besides, three others studies did not find any correlation between the presence of a mutation and the primary location. In our study, we did not have the precise tumor location.

Some patients with *KRAS* wild-type status will not benefit from anti-EGFR antibodies. This is why some studies are conducted to find other mutations to better select responder patients. *KRAS* mutations on exon 3 and NRAS mutations were currently tested before anti-EGFR therapy (21). In 2015, Van Cutsem showed that, on the patients of the CRYSTAL study, a better selection of the patients with *RAS* mutations increased the objective response rate from 57.3% to 66.3% and the median overall survival from 23.5 to 28.4 months (22).

To explain the persistent 35% of patients who were resistant to anti-EGFR therapy, other mutations, such as *BRAF* and *PTEN*, or *EGFR* amplification were studied. Laurent-Puig *et al*., showed that *EGFR* amplification would be predictive of anti-EGFR sensibility but *BRAF* and *PTEN* mutations would be bad prognostic factors (23).

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