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Analysis of KRAS and NRAS mutations in Greek patients with metastatic Colorectal Cancer (mCRC) on the registry of the Gastro-intestinal Cancer Study Group (GIC-SG)

Research Article

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Abstract: *Introduction:* Several studies show that mutational profiles could influence treatment decisions in patients with metastatic CRC (mCRC). *KRAS* mutational status was the first step in biomarkers development in the era of molecular targeted therapies. Recently, NRAS mutational status was identified as an independent prognostic factor for the response to treatment with anti-EGFR moAbs. The aim of this observational study was to assess the feasibility of the KRAS/NRAS mutational analysis in patients with metastatic colorectal cancer in Greece and to identify any correlations with known clinical characteristics and histopathologic features. *Methods:* From January 2014 until September 2014 all patients registered to the GIC-SG database with newly diagnosed metastatic disease from colon or rectal cancer were included and tumor samples were analyzed for kras/nras mutations in 9 different certified laboratories in Greece. *Results:* Samples from 510 patients were analyzed. Mutations' distribution was as follows: 173 (33,9%) *KRAS* exon 2, 10 (2%) *KRAS* exon 3, 25 (4,9%) *KRAS* exon 4, 22 (4,3%) *NRAS* exon 2, 11 (2,2%) *NRAS* exon 3 and 3 (0,6%) *NRAS* exon 4. The only factor significantly associated with *RAS* mutational status was primary tumor location, with right sided tumors exhibiting higher rates of mutations. *Discussion:* The incidence and distribution of *KRAS* or *NRAS* exon 2-4 mutations are in accordance with those reported in the literature. The most significant clinical or pathological parameter revealed from the analysis is the location of the primary tumor.

Keywords: *KRAS-NRAS mutation • colorectal cancer • metastatic disease*

1. Introduction

Mounting knowledge of colorectal cancer (CRC) molecular genetics has substantially changed our approach towards the treatment of the disease, particularly in the metastatic setting. Up to date, several studies have shown that mutation profiles could influence treatment decisions in patients with metastatic CRC (mCRC). The determination of *KRAS* mutational status was the first step in the biomarkers development in the era of molecular targeted therapies. It has been proven that patients with mutations in exon 2, codons 12 and 13

of the KRAS oncogene gain no benefit from treatment with monoclonal antibodies (moAbs) against Epidermal Growth Factor Receptor (EGFR), cetuximab and panitumumab [1, 2]. But, beyond *KRAS* exon 2 mutations, the clinical relevance of other proposed mechanism of resistance to anti-EGFR moAbs, such as, other *KRAS* mutations outside codons 12 and 13 and mutations in other *RAS* gene family members, such as *NRAS*, have been also established later as biomarkers for selection of patients having the higher probability of response to this kind of treatment. Since most available data come from retrospective studies, validation in prospective trials

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is imperative. Although anti-EGFR moAbs cetuximab and panitumumab were initially registered for patients whose tumors were found to immunohistochemically express the EGFR protein, it soon became clear that this methodology was neither enough nor adequate to predict treatment efficacy. The activation of a growth factor receptor induces the recruitment of K-ras, which initiates the activation of a cascade of serine-threonine kinases leading to the signal transduction from the cell surface to the nucleus. *KRAS* mutations are present in more than one third of CRCs and in more than 90% of the cases they are located in exon 2, (codons 12 and 13) [1, 2]. *KRAS* mutations lead to the activation of one of the most important pathways for cell proliferation, the Ras/MAPK pathway, by inducing cyclin D1 synthesis. Consequently, in the presence of a *KRAS* mutation this activation pathway cannot be significantly inhibited by an anti-EGFR moAb (cetuximab or panitumumab) which acts upstream of the K-ras protein.

There is substantial evidence that specific *KRAS* mutations in hotspots located in codons 12 and 13 were predictors of resistance to anti-EGFR moAbs therapy and were associated with reduced efficacy and shorter progression free and overall survival [3, 4]. Apart from the fact that the majority of *KRAS* mutations which are located in exon 2 (codons 12 and 13), others exist, accounting approximately for 10% of the *KRAS* mutated cases and found mainly in exon 3 (codon 61) and 4 (codon 146). Preclinical data indicate that the above mentioned mutations result in phenotypes similar to the hotspot mutations leading to the RAS-RAF-MAPK kinase pathway activation. In contrast, other *KRAS* mutations, such as those found in codon 19 and 164 are phenotypically equivalent to wt *KRAS* [5, 6].

Similarly, *NRAS* encodes for a protein – member of the RAS superfamily of GTPases, and, like the rest of them plays a key role in the critical MAPK signaling pathway. Besides CRC, *NRAS* mutations are found in solid tumors like melanoma and thyroid cancer [7, 8]. Approximately 80% of the mutations reported in the NRAS gene are located in codon 61 [7, 9]. Again, in retrospective analysis, it was shown that patients with *KRAS* and *BRAF* wt but *NRAS* mutated tumors had significantly reduced response rates when treated with cetuximab in the salvage treatment setting [6]. Furthermore, in the retrospective analysis of panitumumab registration trial it was shown that panitumumab had no effect in *KRAS* wt but *NRAS* mutated patients [10]. The analysis of *NRAS* mutations is now mandatory for the administration of anti-EGFR moAbs for the treatment of patients with metastatic CRC.

The aim of this observational study was to assess the feasibility of the KRAS/NRAS exons 2 (codons 12, 13), 3(codon 61), 4 (codons 117 and 146) mutational analysis in patients with metastatic colorectal cancer in Greece as part of the daily clinical practice and to identify any correlations of *RAS* mutational status with known clinical characteristics and histopathologic features.

2. Methods

From January 2014 until September 2014 all patients registered to the GIC-SG database with newly diagnosed metastatic disease from colon or rectal cancer were included in the analysis. *KRAS* exon 2 (codons 12, 13) exon 3 (codon 61) and exon 4 (codons 117 and 146) and *NRAS* exon 2 (codons 12, 13) exon 3 (codon 61) and exon 4 (codons 117 and 146) mutational analysis has been performed in 9 certified laboratories across Greece. Each laboratory was responsible for the mutational analysis of a specific geographical area, in order to reduce the time required for the analysis as well as the cost. The mutational analysis and report was systematically performed according to the consensus guidelines of the Hellenic Society of Medical Oncology (FCO 2014).

Samples' registration and all the logistics of the study were performed by the Hellenic Society of Medical Oncology (HeSMO). Data management and analysis were performed by the GastroIntestinal Cancer Study Group (GIC-SG). Amgen Hellas supported the cost for the mutational analysis through a research grant to the GIC-SG.

Patients with histologically confirmed metastatic CRC aged above 18 years were enrolled in a web-based registry developed by the GIC-SG, which included all clinical parameters and relevant histopathological features of the tumors. All patients in order to be eligible for the registry had to sign an informed consent.

3. Statistics

Due to the descriptive nature of the study no formal sample size calculation was undertaken. Using the normal approximation method a sample size of 500 (95% ci: 467-528) patients was considered to be sufficient for the analysis of the primary and secondary endpoints. The prevalence of mutations in the study population was anticipated to be the follow:

KRAS exon 2 (codon 12,13) 42% (95% CI: 36.8%-47.6%)

KRAS exon 3 (codon 61) 2.2% (95% CI: 1.4%-3.2%)

KRAS exon 4 (codon 117 and 146) 4.8% (95% CI: 3.5%-6.4%)

NRAS exon 2 (codon 12,13) 2.1% (95% CI: 1.4%-3.3%)
KRAS exon 3 (codon 61) 2.5% (95% CI: 1.7%-3.3%)
KRAS exon 4 (codon 117 and 146) 0.8% (95% CI: 0.0%-1.4%)

Descriptive statistics are used for summaries of study variable (means, medians, percentages etc) and the results are presented in tables as appropriate. Associations between baseline characteristics and response were compared with two-sided Fisher's exact test or the Chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables.

4. Results

Five hundred and fourteen patients were enrolled in the study. Appropriate tumor samples after pathology review were submitted for analysis of *KRAS-NRAS* mutations. Samples from four patients were not appropriate for analysis. At the end, samples from 510 patients were analyzed. The median time from the registration to analysis was 10 calendar days (range 5-13 days) or 7 working days (5-12 days), while the cost of the analysis for all 6 exons was 250€.

Patients and disease features are presented in Table 1. In brief 329 male patients (65%) and 181 females (35%) were enrolled in the study. The median age for the whole study population was 64 years, while a hundred and thirty patients were above 70 years old at the time of the analysis (25,5%) whereas the other 380 (74,5%) were below that age. The distribution of the primary tumors along the bowel was as follows: 169 (33%) patients had their tumor at the right colon, 215 (42%) at the left colon and 126 (24%) at the rectum. As far as the localization of the metastatic disease is concerned, 379 (74%) patients had metastases in the liver, lung metastases were found in 193 (37%) patients and peritoneal disease was evident in 98 (19%). Two hundred and eighty-eight patients (56%) had synchronous metastatic disease and the rest 222 (43%) developed metastases metachronously. The grading of the tumors analyzed was unknown for 75 (14%) patients whereas 323 (63%) patients had a well- or moderate-differentiated tumor and the rest 112 (22%) were poorly differentiated. Lastly, 85 (16%) patients had mucinous tumors whereas 351 (68%) were not mucinous. In 74 (14%) patients it was not possible to identify correctly the mucinous part so their status was allocated as unknown.

Mutations to the *RAS* family genes were identified in tumors from 244 patients (47.8%), whereas the rest 266

Table 1: Patients and disease characteristics.

Feature		Patients #	%
Gender		**510**	**100**
	Male	329	65
	Female	181	35
Tumor Location			
	Right Colon	169	33
	Left Colon	215	42
	Rectum	126	25
Tumor Differentiation			
	Well/Moderate	323	63
	Undifferentiated	112	22
	Unknown	74	14
Mucinous	*Yes*	351	69
	No	85	17
	Unknown	74	14
Metastatic sites			
	Liver	379	74
	Lung	193	38
	Peritoneum	98	19
Mutation			
	Present	244	48
	Undetectable	256	52

(52.2%) had tumors with undetectable *RAS* mutations (Figures 1 and 2). Mutations were distributed among the different exons of the *KRAS-NRAS* gene family as follows: 173 (33,9%) *KRAS* exon 2, 10 (2%) *KRAS* exon 3, 25 (4,9%) *KRAS* exon 4, 22 (4,3%) *NRAS* exon 2, 11 (2,2%) *NRAS* exon 3 and 3 (0,6%) *NRAS* exon 4. There was no statistical difference for the detection rates among the 9 different laboratories where the analyses were performed.

There was no significant association between the *RAS* mutational status and the gender (p=0.095) or the age of the patients (p=0.360). The histopathological characteristics of the tumors (mucinous tumors, grade) did not exhibit any statistically significant association with the mutational status of the *RAS* gene family. On the other hand, there was a significant association between

Table 2: Correlation of RAS mutation with clinical and pathological characteristics.

| Feature No (%)			RAS mutation		p value
		Total	Wild Type	Mutant	
Age	≤ 70 years	380 (75)	203 (53.4)	177 (46.6)	0.360
	> 70 years	130 (25)	63 (48.5)	67 (51.5)	
Tumor Differentiation	Low grade	323 (74)	159 (49.2)	164 (50.8)	0.444
	High grade	112 (26)	60 (53.6)	52 (46.4)	
Tumor Location	Right	169 (33)	108 (63.9)	61 (36.9)	<0.001
	Left	341 (67)	136 (39.9)	205 (77.1)	
Mucinous	Yes	85 (17)	49 (57.6)	36 (42.4)	0.187
	No	351 (83)	173 (49.3)	178 (50.7)	
Gender	Male	389 (65)	148 (45)	181(55)	0.095
	Female	181 (35)	96 (53)	85 (45)	
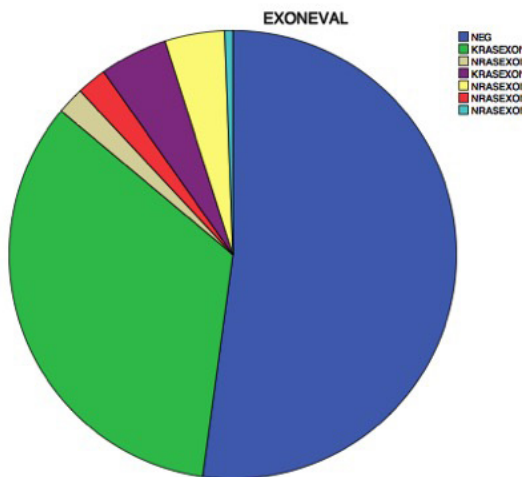
Figure 1: Distribution of KRAS and NRAS mutations by exon in all 510 included patients.
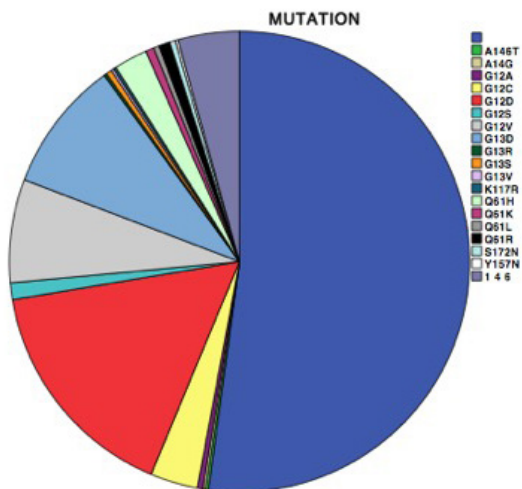

Figure 2. Distribution of KRAS and NRAS mutations by specific mutation in all 510 included patients.

RAS mutational status and the location of the primary tumor in the bowel (p<0.0001) with right-sided tumors exhibiting significantly higher rates of mutations (108 out of 169 – 63,9% patients samples with detectable mutations), whereas left-sided tumors (left colon and rectum) showed significantly lower rates (136 out of 341 – 39,9% patients samples with detectable mutations). Multifactorial analysis revealed also the location of the primary tumor as the only independent factor for the detection of a *RAS* mutation (*p* value < 0.0001). No difference between *KRAS* or *NRAS* mutations detection was observed regarding tumor location (interactive *p* value= 0.326).

5. Discussion

Firstly, the present study represents a successful example for the fruitful collaboration between a national research group, a national association of medical oncology and a pharmaceutical company. With this collaboration it became possible to tackle a significant problem in the daily clinical practice in the middle of severe economic crisis.

In details, the significant predictive role of beyond exon 2 *KRAS* mutations as well as those of the other member of the family *NRAS,* for the selection of patients with mCRC who will benefit the most from the anti-EGFR moAbs treatment, was first reported and published in June 2013 [10]. That led to the reconsideration of the reimbursement requirements from both cetuximab and panitumumab from EMEA and FDA, making the analysis of exon 2-4 *KRAS* and *NRAS* mutations mandatory for the prescription of these two drugs. Although the oncologic community had accept this step forward with great enthusiasm, the national authorities were

unprepared to provide an effective and reliable network for the systemic analysis of exon 2-4 *KRAS* and *NRAS* mutations.

The GIC-SG took the initiative to conduct a prospective analysis of *RAS* mutations in patients with newly diagnosed metastatic colorectal cancer, registered in a database after the 1st January 2014. The GIC-SG was able to include 510 patients in a period of 8 months, reflecting the increase interest of the Greek oncologic community for the upfront analysis for RAS mutations at the time of presentation of metastatic disease in patients with colorectal cancer. The study along with a consensus meeting conducted by the HeSMO has led to the approval of the reimbursement of *RAS* testing for patients with metastatic colorectal cancer in Greece among other genetic tests required for the reimbursement of targeted therapies (i.e. *EGFR* mutations in Non-Small Cell Lung Cancer).

The incidence and distribution of *KRAS* or *NRAS* exon 2-4 mutations are in accordance with those reported in the literature [10]. The most significant clinical or pathological parameter revealed from the analysis is the location of the primary tumor[11]. Indeed other studies recently emphasize the importance of tumor location for the selection of the most appropriate treatment [12]. According to these findings tumors located in the right colon (caecum, ascending colon and right flexure) bear more frequently *KRAS-NRAS* mutations, all well as *BRAF* mutations, in comparison with those located in the left colon (sigmoid colon, descending colon and left flexure) and this affects the response to anti-EFGR moAbs [12]. In contrast other clinical (such as gender, age, number of affected organs) and pathological parameters (such as histological grade and mutinous features) have no significant association with specific mutational detection rate.

The major weakness of the present study was the fact that mutational analysis was undertaken by different laboratories using different methods. On the other hand, all laboratories included in the study were certified for the *KRAS* and *NRAS* analysis and followed strict criteria for the analysis and report of predictive biomarkers as those were described in a consensus meeting of HeSMO. Despite that the findings of the study should be used as a hypothesis, the study generated results which may lead to further clinical and translational investigations and this is exactly the future plan of the GIC-SG.

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