Short Communication

Cancer Epidemiology, Biomarkers & Prevention

Real-Life Distribution of *KRAS* and *NRAS* Mutations in Metastatic Colorectal Carcinoma from French Routine Genotyping

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Abstract

In metastatic colorectal cancer, *KRAS* and *NRAS* genotyping is mandatory before prescription of panitumumab or cetuximab. In order to perform such molecular tests, the French National Cancer Institute has set up a nationwide network of molecular centers. We report here the percentage of these mutations according to a prospective nonselected cohort of incident metastatic colorectal carcinoma patients. A total of 6,803 patients were tested between July 1, 2013, and December 31, 2013. Overall, 49.06% of patients harbored a mutation in either *KRAS* or *NRAS*. Mutations of *NRAS* exons 3 and 4 were very rare. No *NRAS* exon 3 at c.59 or exon 4 at c.117 mutations were retrieved, and only 1 *NRAS* exon 4 at c.146 mutation was detected. This present cohort is likely to represent most of the incident cases of metastatic colorectal adenocarcinomas arising in France over 6 months and is to our knowledge the largest population set genotyped for these genes in this condition. This is a unique opportunity to observe the frequency of *RAS* mutations regardless of most inclusion bias. *Cancer Epidemiol Biomarkers Prev; 24(9); 1416–8. ©2015 AACR.*

Introduction

In metastatic colorectal cancer, analysis for *KRAS* and *NRAS* exons 2, 3, and 4 guides the use of anti-EGFR monoclonal antibody therapy, as patients benefit from such therapy only if their tumor does not harbor *KRAS*- or *NRAS*-activating mutation (1). Indeed, mutations in these genes lead to constitutive activation of the RAS–MAPK pathway, conferring resistance to anti-EGFR therapies (2). Thus, *KRAS* and *NRAS* genotyping of tumor is mandatory before prescription of panitumumab or cetuximab in metastatic colorectal cancer.

In order to perform such molecular tests, since 2006, the French National Cancer Institute (INCa) has set up a nationwide network of 28 regional molecular genetic centers, allowing a nationwide mutation databank (3). Based on formalin-fixed paraffin-embedded (FFPE) tissue samples, a broad range of techniques is used, depending on local expertise and available instruments. In spite of this heterogeneity of mutation detection methods, reproducibility is almost perfect across the platforms (4). In 2012, 18,568 *KRAS* molecular tests were performed (5). According to the incidence of colorectal adenocarcinoma in France (roughly 42,000 new cases in 2012) and to the proportion of metastatic stage (40% to 60%), it appears that in France, most metastatic patients benefited from molecular characterization of their tumor (5). Thus, this organization allows a nationwide mutation databank, unique in its kind.

Current knowledge on mutation distribution of *KRAS* and *NRAS* exons 2, 3, and 4 is mainly based on clinical trials in which patient samples are not perfectly representative of the wider population. Besides, observed mutation frequencies are not neutral in terms of testing guidelines, and distribution of mutations in *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 is not well described in the general population as genotyping of these regions has only been compulsory since August 2014.

We report here the percentage of these mutations according to a prospective cohort of 6,803 incident metastatic colorectal carcinoma patients.

Materials and Methods

Patients with metastatic colorectal cancer were tested between July 1, 2013, and December 31, 2013, in the 28 French regional centers. Serial sections of FFPE primary colorectal tumors were cut from a paraffin block representative of tumor and placed on glass slides: a 4-μm-thick section was stained with hematoxylin & eosin (H&E) for histopathologic examination. The following section was processed for tumor DNA preparation. The microtome razor blade was changed between each FFPE tumor sample, and the paraffin sections were processed individually to avoid cross-contamination.

H&E preparation enabled tumor area delimitation and visual estimation of tumor cell percentage by a senior pathologist. The delimited area contained more than 20% of tumor cells. The tumor area was macrodissected on a 10-μm-thick section placed on a glass slide using a single-use sterilized scalpel. Alternatively, a core biopsy was performed on the paraffin block in the area where most tumor cells were visualized on the H&E section. DNA was extracted from this material manually or automatically, depending on the regional center.

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doi: 10.1158/1055-9965.EPI-15-0059

Table 1. Distribution of *KRAS* and *NRAS* mutations in a French population of 6,803 incident metastatic colorectal carcinoma patients between July 1, 2013, and December 31, 2013

Mutation hotspot	Number of patients	Number of mutations detected	Percentage of genotyped patients at this nucleotide harboring the mutation
***KRAS* exon 2 c.12**	6,803	2,066	30.37
***KRAS* exon 2 c.13**	6,803	545	8.01
***KRAS* exon 3 c.59**	2,752	5	0.18
***KRAS* exon 3 c.61**	3,248	61	1.88
***KRAS* exon 4 c.117**	2,966	18	0.61
***KRAS* exon 4 c.146**	3,019	96	3.18
***NRAS* exon 2 c.12**	3,195	58	1.82
***NRAS* exon 2 c.13**	3,195	20	0.63
***NRAS* exon 3 c.59**	2,828	0	0.00
***NRAS* exon 3 c.61**	3,212	77	2.33
***NRAS* exon 4 c.117**	1,956	0	0.00
***NRAS* exon 4 c.146**	2,191	1	0.05

NOTE: The difference in number of genotyped patients for each nucleotide is explained by the fact that genotyping of *KRAS* exons 3 and 4 and *NRAS* was not implemented across all the platforms during the time period.

Mutation detection methods were diverse and sometimes combined: Sanger method of DNA sequencing, next-generation sequencing (NGS), allele-specific oligonucleotide hybridization (ASO), high-resolution melt analysis (HRM), TaqMan Real-Time PCR Assays, SNaPshot multiplex genotyping, Pyrosequencing, Sequenom MassARRAY genotyping, peptide nucleic acids based methods (PNA), and Cobas KRAS Mutation Test kit. In some centers, the strategy chosen was to perform sequential molecular testing (mainly *KRAS* exon 2 genotyping first, then, if no mutation was retrieved, *KRAS* exons 3 and 4 and *NRAS* genotyping; Table 1), whereas in others, all patients were tested simultaneously, without sequential strategy. In addition, *KRAS* exons 3 and 4 and *NRAS* genotyping were not implemented at the same time in all the regional platforms during the study period, which explains the discrepancy between the number of patients screened at the different nucleotides.

Results and Discussion

The distribution of *KRAS* and *NRAS* mutations is listed in Table 1. Overall, 6,803 patients were tested in platforms, and 49.06% of them harbored a somatic mutation in either *KRAS* or *NRAS*, with 38.38% harboring a mutation in *KRAS* exon 2. In addition, around 5.85% harbored a mutation in *KRAS* exons 3 or 4, and 4.83% in *NRAS* exons 2, 3, or 4. Mutations of *NRAS* exons 3 and 4 were very rare, harbored by only 2.38% of the tumors. Table 2 summarizes the protein effects of identified mutations. Only 6 patients presented double mutations which involved *KRAS* exon 2, and, interestingly, all of these cases involved a G13D mutation paired with a mutation at codon 12 (3 cases involving a G12C and a G13D mutation, 1 involving a G12A and a G13D, 1 involving a G12D and a G13D, and 1 involving a G12R and a G13D). Because of the sequential strategy mostly chosen to screen these 2 genes, double mutations involving *KRAS* exon 2 and *KRAS* exons 3 and 4 or *NRAS* exons 2, 3, and 4 may not have been detected. However, no double mutation was noted in *KRAS* exons 3 and 4 or *NRAS* exons 2, 3, and 4.

Frequencies of mutations in *KRAS* exons 3 and 4 and in *NRAS* were similar between subjects from centers where sequential strategy was used and those genotyped for all loci independent of *KRAS* exon 2 genotypes. Therefore, selection bias due to sequential strategy has to be excluded.

As a result of this organization model for tumor genotyping headed by INCa, this present cohort is likely to represent most of the incident cases of metastatic colorectal adenocarcinomas arising in France over 6 months and is to our knowledge the largest population set genotyped to date for these genes in this condition. This is a unique opportunity to observe the frequency of somatic

Table 2. Details of protein effects of identified mutations according to the international nomenclature for the description of sequent variants

	G12A	G12C	G12D	G12F	G12N	G12R	G12S	G12T	G12V	G12Y	G12*
***KRAS* exon 2 c.12**	167	230	827	4	1	37	130	8	644	15	3
***NRAS* exon 2 c.12**	2	7	33			4	4		8		
	G13C	**G13D**	**G13E**	**G13F**	**G13H**	**G13R**	**G13S**	**G13V**			
***KRAS* exon 2 c.13**	20	514	2		1	7		1			
***NRAS* exon 2 c.13**		7		1		9	1	2			
	A59G	**A59T**	**A59del**								
***KRAS* exon 3 c.59**	1	3	1								
***NRAS* exon 3 c.59**											
	Q61E	**Q61H**	**Q61K**	**Q61L**	**Q61R**						**Q61***
***KRAS* exon 3 c.61**	1	30	6	4	11						9
***NRAS* exon 3 c.61**		6	31	18	22						
	K117N										
***KRAS* exon 4 c.117**	18										
***NRAS* exon 4 c.117**											
	A146E	**A146P**	**A146T**	**A146V**							
***KRAS* exon 4 c.146**	1	9	71	15							
***NRAS* exon 4 c.146**			1								

mutations of *KRAS* and *NRAS* in a nationwide population, regardless of inclusion bias such as socioeconomic factors as the tests were free of charge for the patient.

The distribution of the *KRAS* and *NRAS* mutations reported in the present study was similar to data in the literature (6–10). No *NRAS* exon 3 at c.59 or exon 4 at c.117 mutations was retrieved, and only 1 mutation of *NRAS* c.146 (exon 4) was detected, representing only 0.05% of the whole incident population. Taken together, these data suggest that it may not be relevant to look for molecular alterations at *NRAS* c.59, c.117, and c.146 nucleotides in routine-based practice. Nevertheless, high-throughput technologies like NGS that allow detection of all mutations in selected exons with no significant increase in cost or genotyping time will exempt us from this recommendation in the near future.

Disclosure of Potential Conflicts of Interest

N. Piton has travel expenses reimbursed by Pfizer and meal expenses paid by AstraZeneca to disclose. J.-C. Sabourin has a consulting or advisory role for Merck Serono, Boehringer Ingelheim, and Roche, research funding by Roche, and travel, accommodations, or expenses paid or reimbursed by Merck Serono, Boehringer Ingelheim, and Roche to disclose. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Nowak, J.-C. Sabourin

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Piton, E. Lonchamp, F. Nowak

Writing, review, and/or revision of the manuscript: N. Piton, E. Lonchamp, F. Nowak, J.-C. Sabourin

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Lonchamp

Acknowledgments

The authors are indebted to the *KRAS* group: Mougin Christiane, Gaub Marie-Pierre, Merlio Jean Philippe, Dubus Pierre, Soubeyran Isabelle, Dechelotte Pierre, Penault-Llorca Frédérique, Hardouin Agnès, Martin Laurent, Chapusot Caroline, Lizard Sarab, Mosser Jean, Le Marechal Cédric, Pages Jean-Christophe, Baala Lekbir, Delvincourt Chantal, Beaudoux Olivia, Clavel Christine, Dalstein Véronique, Ferrand Christophe, Lamy Aude, Schischmanoff Olivier, Fabre Emmanuelle, Theou-Anton Nathalie, Soufir Nadem, Lamoril Jérôme, Clauser Eric, Vidaud Dominique, Laurent-Puig Pierre, Blons Hélène, Emile Jean-François, Leroy Karen, Lemoine-Corbel Antoinette, Soubrier Florent, Coulet Florence, Lascols Olivier, Barbu Véronique, De The Hugues, Lehmann-Che Jacqueline, De Cremoux Patricia, Bernaudin Jean-François, Lacave Roger, Bieche Ivan, Bidart Jean Michel, Lacroix Ludovic, Scoazec Jean-Yves, Maudelonde Thierry, Lamy Pierre Jean, Lumbroso Serge, Labrousse François, Merlin Jean Louis, Brousset Pierre, Selves Janick, Porchet Nicole, Buisine Marie-Pierre, Boisdron-Celle Michèle, Bezieau Stéphane, Karayan-Tapon Lucie, Pedeutour Florence, Milano Gérard, Ouafik L'Houcine, Xerri Luc, Richard Marie-Jeanne, De Fraipont Florence, Joly Marie-Odile, Wang Qing, Haddad Véronique, Peoc'h Michel, Hofman Paul, Gubler Brigitte, Sobol Hagay. They also thank Nikki Sabourin-Gibbs, Rouen University Hospital, for assistance with language editing.

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Received January 23, 2015; revised July 8, 2015; accepted July 8, 2015; published OnlineFirst July 19, 2015.

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