Real-Life Distribution of \textit{KRAS} and \textit{NRAS} Mutations in Metastatic Colorectal Carcinoma from French Routine Genotyping

Nicolas Piton$^1$, Etienne Lonchamp$^2$, Frédérique Nowak$^2$, and Jean-Christophe Sabourin$^1$, on behalf of the \textit{KRAS} group

Abstract

In metastatic colorectal cancer, \textit{KRAS} and \textit{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 \textit{KRAS} or \textit{NRAS}. Mutations of \textit{NRAS} exons 3 and 4 were very rare. No \textit{NRAS} exon 3 at c.59 or exon 4 at c.117 mutations were retrieved, and only 1 \textit{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 \textit{RAS} mutations regardless of most inclusion bias. Cancer Epidemiol Biomarkers Prev; 24(9); 1416–8. ©2015 AACR.

Introduction

In metastatic colorectal cancer, analysis for \textit{KRAS} and \textit{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 \textit{KRAS}- or \textit{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, \textit{KRAS} and \textit{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 database (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 \textit{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.

Corresponding Author: Nicolas Piton, Rouen University Hospital, Bâtiment Déborah, CHU C. Nicole, 1 rue de Germont, 76031 Rouen Cedex, France. Phone: 33-232-888-121; Fax: 33-232-888-646; E-mail: nicolas.piton@chu-rouen.fr

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

©2015 American Association for Cancer Research.
KRAS and NRAS Mutations in Colorectal Carcinoma

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

<table>
<thead>
<tr>
<th>Mutation hotspot</th>
<th>Number of patients</th>
<th>Number of mutations detected</th>
<th>Percentage of genotyped patients at this nucleotide harboring the mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS exon 2 c.12</td>
<td>6,803</td>
<td>2,066</td>
<td>30.37</td>
</tr>
<tr>
<td>KRAS exon 2 c.13</td>
<td>6,803</td>
<td>545</td>
<td>8.01</td>
</tr>
<tr>
<td>KRAS exon 3 c.59</td>
<td>2,752</td>
<td>5</td>
<td>0.78</td>
</tr>
<tr>
<td>KRAS exon 3 c.61</td>
<td>3,248</td>
<td>61</td>
<td>1.88</td>
</tr>
<tr>
<td>KRAS exon 4 c.117</td>
<td>2,966</td>
<td>18</td>
<td>0.61</td>
</tr>
<tr>
<td>KRAS exon 4 c.146</td>
<td>3,019</td>
<td>96</td>
<td>3.18</td>
</tr>
<tr>
<td>NRAS exon 2 c.12</td>
<td>3,195</td>
<td>58</td>
<td>1.82</td>
</tr>
<tr>
<td>NRAS exon 2 c.13</td>
<td>3,195</td>
<td>20</td>
<td>0.63</td>
</tr>
<tr>
<td>NRAS exon 3 c.59</td>
<td>2,828</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>NRAS exon 3 c.61</td>
<td>3,212</td>
<td>77</td>
<td>2.33</td>
</tr>
<tr>
<td>NRAS exon 4 c.117</td>
<td>1,956</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>NRAS exon 4 c.146</td>
<td>2,191</td>
<td>1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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 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, 1 involving a G12A and a G13D, 1 involving a G12V 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...
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 incidence 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 technol-

graphy 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

References


Acknowledgments


The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 23, 2015; revised July 8, 2015; accepted July 8, 2015; published OnlineFirst July 19, 2015.
Real-Life Distribution of KRAS and NRAS Mutations in Metastatic Colorectal Carcinoma from French Routine Genotyping

Nicolas Piton, Etienne Lonchamp, Frédérique Nowak, et al.


Updated version  Access the most recent version of this article at: doi:10.1158/1055-9965.EPI-15-0059

Cited articles  This article cites 9 articles, 4 of which you can access for free at: http://cebp.aacrjournals.org/content/24/9/1416.full.html#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.