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

Nicolas Piton¹, Etienne Lonchamp², Frédérique Nowak², and Jean-Christophe Sabourin¹, on behalf of the KRAS group

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 KRAS 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 database, 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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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>0.18</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, SNuPshot 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
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 were 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

References


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

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