Clinical Outcome of Japanese Metastatic Colorectal Cancer Patients Harbouring the KRAS p.G13D Mutation Treated with Cetuximab + Irinotecan

Hideaki Bando1, Takayuki Yoshino1, Satoshi Yuki2, Eiji Shinozaki3, Tomohiro Nishina4, Shigenori Kadowaki5, Kentaro Yamazaki6, Shinya Kajiura7, Katsuya Tsuchihara8, Satoshi Fujii9, Takeharu Yamanaka10 and Atsushi Ohtsu1,10

1Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Chiba, 2Department of Gastroenterology, Hokkaido University Graduate School of Medicine, Hokkaido, 3Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, 4National Hospital Organization Shikoku Cancer Center, Ehime, 5Division of Gastroenterology, Saitama Cancer Center, Saitama, 6Division of Gastrointestinal Oncology, Shizuoka Cancer Center, Shizuoka, 7The Third Department of Internal Medicine, University of Toyama, Toyama, 8Division of Translational Research, Research Center for Innovative Oncology, National Cancer Center Hospital East, Chiba, 9Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Chiba and 10Research Center for Innovative Oncology, National Cancer Center Hospital East, Chiba, Japan

*For reprints and all correspondence: Takayuki Yoshino, Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan. E-mail: tyoshino@east.ncc.go.jp

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Objective: Metastatic colorectal cancer with KRAS codon 12 or 13 mutations is not currently treated with anti-epidermal growth factor antibodies. A recent retrospective study in Western countries raised the possibility that KRAS p.G13D mutation may not be absolutely predictive of non-response compared with other KRAS mutations from the findings of longer overall survival and progression-free survival following cetuximab treatment. We retrospectively investigated the relationship between KRAS status and cetuximab efficacy among Japanese patients.

Methods: Data of 109 patients from nine institutions in Japan were retrospectively analysed. All patients were refractory or intolerant to fluoropyrimidine, oxaliplatin and irinotecan, and they were treated with a cetuximab + irinotecan regimen. The response rate, disease control rate, progression-free survival and overall survival were compared according to KRAS status.

Results: Overall, 76 (70%), 7 (6%) and 26 (24%) patients had KRAS wild-type, KRAS p.G13D and other KRAS mutations. Their various parameters were as follows: response rate: 30% (23/76), 14% (1/7) and 0% (0/26); disease control rate: 71% (54/76), 71% (5/7) and 54% (14/26); median progression-free survival: 4.6 months (95% confidence interval, 2.8–6.3), 4.1 months (0–9.9) and 2.1 months (1.5–2.8); and median overall survival: 11.2 months (6.4–16.0), 8.5 months (5.3–11.8) and 6.8 months (4.1–9.6), respectively.

Conclusions: Although no statistically significant difference in progression-free survival or overall survival was observed between KRAS p.G13D-mutant and other mutant tumours, the disease control rate was higher in KRAS p.G13D-mutant patients and a partial response was observed in one such patient. Our study suggested that cetuximab showed some activity in KRAS p.G13D-mutant colorectal cancer patients. Further research is warranted.

Key words: KRAS protein – human – point mutation – epidermal growth factor – cetuximab
INTRODUCTION

Retrospective subset analyses and prospective randomized phase III clinical trials have suggested that anti-epidermal growth factor antibodies do not provide any benefit for metastatic colorectal cancer patients harbouring KRAS codon 12 or 13 mutations (1–6). On the basis of these findings, regulatory authorities in Europe, the USA and Japan have recommended compulsory KRAS mutation testing.

An in vitro experiment revealed that cancer cells with KRAS codon 13 mutations had a weaker level of resistance to apoptosis than those with KRAS codon 12 mutations (7). Furthermore, although growth inhibition was observed for KRAS wild-type and KRAS p.G13D-mutant colorectal cancer cells following cetuximab treatment, no significant inhibition was observed for KRAS p.G12V-mutant cells (8). Clinical experience has shown that colorectal cancer patients with KRAS occasionally respond to cetuximab. A glycine (G) to aspartate (D) transition mutation (p.G13D mutation) is observed in the tumours of patients predominantly with codon 13 mutations as well as in all responders with codon 13 mutations (9–11).

According to a pooled analysis of randomized controlled studies in Western countries, patients with KRAS p.G13D-mutant tumours treated with only best supportive care have no significant differences in overall survival (OS) and progression-free survival (PFS) compared with other KRAS-mutant tumours or KRAS wild-type tumours by a multivariate analysis. In contrast, patients with KRAS p.G13D-mutant tumours treated with cetuximab had longer OS and PFS than those with other KRAS-mutant tumours (8). These findings suggest that the KRAS p.G13D mutation in colorectal cancer patients may not be a prognostic factor, but rather a predictive factor of response to cetuximab treatment.

In Japan, cetuximab was approved by the regulatory authority in July 2008. Nevertheless, KRAS mutation testing was not approved until April 2010. During the intervening period, cetuximab was administered to Japanese colorectal cancer patients regardless of their KRAS mutation status. Therefore, we collected clinical data from colorectal cancer patients with KRAS wild-type as well as KRAS codon 12 and 13 mutations who were treated with cetuximab-containing regimens. Patient data were collected from nine major cancer centres in Japan and were retrospectively analysed to determine whether there was a relationship between clinical outcomes and KRAS mutation status.

PATIENTS AND METHODS

PATIENTS

We screened and selected patients treated between July 2008 and April 2010. All selected patients were treated with a cetuximab + irinotecan combination regimen, and their KRAS mutation status was evaluated using direct sequencing or amplification refractory mutation system (ARMS)-Scorpion or Luminex assays (12–14). In the combination treatment regimen, cetuximab was administered at an initial dose of 400 mg/m² followed by weekly infusions of 250 mg/m², and irinotecan was administered in bi-weekly infusions of 150 mg/m² in accordance with the package insert instructions for irinotecan in Japan. A treatment cycle in our study comprised twice weekly cetuximab plus bi-weekly irinotecan infusions.

Patients who met all of the following inclusion criteria were retrospectively included in the analyses: (i) age ≥20 years; (ii) histologically confirmed adenocarcinoma of the colon or rectum with evaluated KRAS status; (iii) the presence of unresectable metastatic disease; (iv) baseline computed tomography (CT) scan performed within 28 days of initial cetuximab administration; (v) initial evaluation by CT scan within 3 months of initial cetuximab administration; (vi) documented prior refractory response to or intolerance of fluoropyrimidines, oxaliplatin and irinotecan; (vii) Eastern Cooperative Oncology Group performance status score ≤2 and (viii) adequate haematological, hepatic and renal function. The study was conducted with the approval of the review board of each institution.

KRAS TESTING

KRAS status was evaluated using direct sequencing or ARMS-Scorpion or Luminex assays. At the National Cancer Center Hospital East, KRAS testing using the ARMS-Scorpion method was performed by its associate research institution (Research Center for Innovative Oncology) (12,13). At the Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Luminex assays were used. At all other institutions, direct sequencing was used for evaluations. All KRAS testing by Luminex assay and direct sequencing was performed by clinical testing companies (MBL and SRL, Japan) (14). The sensitivity of KRAS testing by ARMS-Scorpion, Luminex and direct sequencing has been reported as 1, 10 and 10%, respectively.

STATISTICAL ANALYSIS

The therapeutic response rate (RR) was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECISTs) (version 1.0) criteria. PFS was defined as the time from the first cetuximab + irinotecan administration to either the first objective evidence of disease progression or death due to any cause. OS was defined as the time from the first administration of cetuximab + irinotecan to death from any cause. The RR, the disease control rate (DCR), PFS, and OS of all patients were reevaluated by the principal investigators at each institution. The relative dose intensity was defined as the ratio of the actual dose administered to the planned dose. Fisher’s exact and Kruskal–Wallis tests were used to compare the patient characteristics and relative dose intensity. PFS and OS data were plotted as Kaplan–Meier curves, and the differences among the groups categorized by KRAS status were compared using the log-rank test. All analyses
were performed by a biostatistician (Takeharu Yamanaka) using IBM SPSS® Statistics 18 package software (SPSS Inc., Tokyo, Japan).

RESULTS

PATIENT CHARACTERISTICS

From September 2008 to April 2010, 376 patients were treated with a cetuximab + irinotecan combination regimen at nine institutions; 109 of these patients met the inclusion criteria and were selected for analysis. The date of data cut-off was 31 July 2011. Of the patients selected, 76 (70%), 7 (6%) and 26 (24%) had KRAS wild-type, KRAS p.G13D-mutant and other KRAS-mutant tumours, respectively. The 26 other KRAS mutations included 10 p.G12D (glycine to aspartate), 9 p.G12V (glycine to valine) and 7 p.G12C (glycine to cysteine) mutations. No p.G12A (glycine to alanine) or p.G12S (glycine to serine) mutations were found in our data set. No double mutations consisting of KRAS p.G13D and another KRAS mutation were detected.

Although only seven patients had the KRAS p.G13D mutation, baseline characteristics between the groups were not significantly different (Table 1).

RESPONSE TO TREATMENT

The RR of the patients with KRAS wild-type, KRAS p.G13D-mutant and other KRAS-mutant tumours was 30% (23/76), 14% (1/7) and 0% (0/26), respectively. Although a single partial response (PR) occurred among the seven patients with KRAS p.G13D-mutant tumours, no PR was observed among the patients with other KRAS-mutant tumours (Table 2). In contrast, the DCR for patients with the KRAS wild-type, KRAS p.G13D-mutant and other KRAS-mutant tumours (including PR and stable disease) was 71% (54/76), 71% (5/7) and 54% (14/26), respectively (Table 2).

SURVIVAL

The median PFS values among patients with KRAS wild-type, KRAS p.G13D-mutant and other KRAS-mutant tumours were 4.6 months (95% confidence interval, 2.8–6.3), 4.1 months (0–9.9) and 2.1 months (1.5–2.8), respectively; moreover, the median OS values were 11.2 months (6.4–16.0), 8.5 months (5.3–11.8) and 6.8 months (4.1–9.6), respectively (Table 2). No statistically significant differences in OS and PFS were identified between KRAS status and the clinical effectiveness of cetuximab; data from a large pooled analysis performed previously were collected from European countries and Canada (1–6).

The KRAS mutation frequency among Japanese patients is reportedly 37.0–44.0% (14,15). In this study, several participating institutions had used KRAS testing to decide on cetuximab administration on the basis of KRAS status. This could

DISCUSSION

To the best of our knowledge, this study is the first to use data from Asian (Japanese) patients to verify the relationship between KRAS status and the clinical effectiveness of cetuximab; data from a large pooled analysis performed previously were collected from European countries and Canada (1–6).
be the reason why the frequency of patients with KRAS-mutant tumours was slightly lower than that of other Western and Asian study populations (1,2,14). However, there were no remarkable differences in the mutation spectrum of KRAS codon 12 or 13 mutations except for p.G12A and p.G12S mutations.

To minimize the selection bias among KRAS wild-type and KRAS-mutant tumours in a retrospective manner, we set up strict patient selection criteria and ensured that the baseline characteristics among groups were consistent with those previously reported. We believe that there were no significant differences in baseline characteristics between the groups despite the small sample size.

The RR of the patients with KRAS wild-type, KRAS p.G13D-mutant and other KRAS-mutant tumours was 32, 14 and 0%, respectively, and these values are consistent with those from the pooled analysis of randomized controlled trials (KRAS wild-type 26.4%, KRAS p.G13D mutation 6.4% and other KRAS mutations 1.6%) (8). DCRs of these patients were 71, 71 and 54%, respectively; these values were higher than expected, although DCRs were not reported in the previous pooled analysis (8). Notably, the DCR of patients with KRAS p.G13D-mutant tumours was higher than that of

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**Table 2. Efficacy in the test population according to the KRAS status**

<table>
<thead>
<tr>
<th></th>
<th>KRAS wild-type (n = 76)</th>
<th>KRAS p.G13D mutants (n = 7)</th>
<th>Other KRAS mutants (n = 26)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Partial response</td>
<td>22</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>32</td>
<td>4</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Progressive disease</td>
<td>21</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>7</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Response rate (%)</td>
<td>30</td>
<td>14</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Disease control rate (%)</td>
<td>71</td>
<td>71</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Progression-free survival [median (95% CI) (months)]</td>
<td>4.6 (2.8–6.3)</td>
<td>4.1 (0–9.9)</td>
<td>2.1 (1.5–2.8)</td>
<td></td>
</tr>
<tr>
<td>Overall survival [median (95% CI) (months)]</td>
<td>11.2 (6.4–16.0)</td>
<td>8.5 (5.3–11.8)</td>
<td>6.8 (4.1–9.6)</td>
<td></td>
</tr>
</tbody>
</table>

**Relative dose intensity**

<table>
<thead>
<tr>
<th></th>
<th>Irinotecan [median (range) (%)]</th>
<th>Cetuximab [median (range) (%)]</th>
<th>Number of treatment cycles [median (range)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>82.0 (13.0–100)</td>
<td>86.0 (10.0–100)</td>
<td>10 (2–86)</td>
</tr>
<tr>
<td></td>
<td>75.0 (47.2–100)</td>
<td>84.5 (60.6–100)</td>
<td>7 (2–17)</td>
</tr>
<tr>
<td></td>
<td>80.0 (48.5–100)</td>
<td>87.3 (11.1–100)</td>
<td>6 (1–22)</td>
</tr>
</tbody>
</table>

**P value**

|                       | 0.188*                          | 0.856*                          | 0.048*                                      |

CI, confidence interval.

*Kruskal–Wallis test.
patients with other KRAS-mutant tumours, and was similar to that of the patients with KRAS wild-type tumours. In an in vitro and in vivo study recently reported, growth inhibition was observed for both KRAS wild-type and KRAS p.G13D-mutant colorectal cancer cells following cetuximab treatment. The higher DCR in the patients with KRAS p.G13D-mutant tumours might have contributed to the inhibition of growth and led to similar outcomes to those observed for the patients with KRAS wild-type tumours.

The median PFS and OS of the patients with KRAS wild-type colorectal cancers were 4.6 and 11.2 months, respectively, and these values are consistent with those from the pooled analysis of randomized controlled trials (median PFS 4.2 months, median OS 10.1 months) (8).

According to the pooled analysis of European patients, patients with KRAS p.G13D-mutant tumours treated with cetuximab had significantly longer PFS and OS than patients with other KRAS-mutant tumours (median PFS: KRAS p.G13D mutation 4.0 months, other mutations 1.9 months, P = 0.02; median OS: KRAS p.G13D mutation 7.6 months, other mutations: 5.7 months, P = 0.003) (8). In our study, the differences in PFS and OS between patients with KRAS p.G13D-mutant tumours and those with other KRAS-mutant tumours were not statistically significant (median PFS: KRAS p.G13D mutation 4.1 months, other KRAS mutations 2.1 months, P = 0.668; median OS: KRAS p.G13D mutation 8.5 months, other KRAS mutations: 6.8 months, P = 0.477). Cox analysis showed similar results in our study after adjusting for baseline factors (data not shown).

There was a hint of activity as one patient with a KRAS p.G13D-mutant tumour achieved a PR and there was a higher DCR in patients with KRAS p.G13D-mutant tumours. Although our study was retrospective in nature with a small sample size, the presence of a single responder and a higher DCR would contribute to a difference in both PFS and OS if the sample size was larger.

It has been estimated that ~6–7% of all colorectal cancer patients have a KRAS p.G13D mutation; that is, ~2400 patients (6% of the 40,000 who die of colorectal cancer) per year in Japan and 36,000 patients (6% of the 600,000) per year worldwide could be treated with anti-epidermal growth factor antibody therapies. At the same time, it is difficult for Japanese investigators alone to accumulate a large enough sample size to confirm the clinical significance of cetuximab in patients with KRAS p.G13D-mutant tumours. Thus, an international phase III randomized trial with a larger sample size that includes patients from both Japan and Western countries is necessary to confirm the benefit of cetuximab for patients with a KRAS p.G13D mutation.

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Conflict of interest statement

None declared.

References


Appendix

Research group members: Hideaki Bando, Takayuki Yoshino, Katsuya Tsuichiha, Satoshi Fujii, Takeharu
Yamanaka, Atsushi Ohtsu (National Cancer Center Hospital East), Satoshi Yuki, Takahide Sasaki (Hokkaido University), Eiji Shinozaki (Cancer Institute Hospital of Japanese Foundation for Cancer Research), Tomohiro Nishina (Shikoku Cancer Center), Kensei Yamaguchi, Shigenori Kadowaki, Masako Asayama (Saitama Cancer Center), Kentaro Yamazaki (Shizuoka Cancer Center), Shinya Kajiura (University of Toyama), Tetsuo Kimura, Takahiro Goushi (The University of Tokushima Graduate School) and Yasuo Hamamoto (Keio University).