Tumor KRAS Status Predicts Responsiveness to Panitumumab in Japanese Patients with Metastatic Colorectal Cancer

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Received August 10, 2010; accepted November 19, 2010

Objective: Mutation status of the KRAS gene in tumors has been shown to be a predictive biomarker of response to anti-epidermal growth factor receptor antibody therapy in patients with metastatic colorectal cancer. This retrospective analysis examined the association between efficacy and safety of the fully human anti-epidermal growth factor receptor antibody panitumumab and KRAS mutation status in Japanese metastatic colorectal cancer patients using data from two clinical trials with adherence to good clinical practices.

Methods: An exploratory, integrated analysis of data from KRAS evaluable patients enrolled in a Phase 1 study (Study 20040192) and a Phase 2 study (Study 20050216) was performed. Paraffin-embedded tumor samples were analyzed for KRAS status. Primary efficacy endpoint of this analysis was objective tumor response per modified response evaluation criteria in solid tumors; a key secondary endpoint was progression-free survival. Safety endpoints included incidence of adverse events.

Results: Tumor samples with known KRAS status were available from 8 of 13 (62%) metastatic colorectal cancer patients in the Phase 1 study and 16 of 53 patients (30%) in the Phase 2 study. Overall, 14 (58%) patients had wild-type KRAS tumors and 10 (42%) patients had mutated KRAS tumors. Four (17%) patients had a partial response; all responders had tumors with wild-type KRAS. Results of all secondary efficacy endpoints also favored patients with wild-type KRAS. Treatment-related adverse events were predominantly mild to moderate and skin related, and were similar between patients with tumors with wild-type and mutated KRAS in this small patient population.

Conclusions: Mutated KRAS status in tumors of Japanese patients with metastatic colorectal cancer is associated with lack of response to panitumumab therapy.

Key words: panitumumab – epidermal growth factor receptor – colorectal cancer – KRAS

INTRODUCTION

Expression of the epidermal growth factor receptor (EGFR) is frequently associated with malignant transformation in human cancers (1). This observation led to the development of anti-EGFR therapies for the treatment of EGFR-expressing tumors (2). While anti-EGFR antibody therapies have demonstrated efficacy in patients with metastatic colorectal cancer (mCRC) (3), the level of EGFR expression has not been shown to be associated with response to anti-EGFR antibodies (4,5). Biomarkers that can identify patients who are likely to respond to anti-EGFR therapy are needed. Downstream signaling pathways are activated when EGFR binds to its ligand. The KRAS gene codes for a protein that is a member of the ras family of small G-proteins involved in intracellular signaling. When EGFR binds to ligand, its tyrosine kinase function is activated, ultimately resulting in the activation of the Ras-Raf-MAP kinase signaling cascade (6). Ras is activated by binding to GTP and deactivation of
ras in normal cells is accomplished by hydrolysis of GTP. Mutations in the \textit{KRAS} gene that abolish the intrinsic GTPase activity result in constitutively active ras proteins that are oncogenic (7). It is possible that downstream signaling pathways are therefore constitutively activated and can become EGFR independent. In support of this hypothesis, mutations in \textit{KRAS} have been shown to predict non-responsiveness to anti-EGFR antibody therapies in patients with mCRC (8–11).

\textit{KRAS} mutations occur in ~30–50\% of all patients with colorectal cancers (8–10,12,13). \textit{KRAS} mutations have been reported in ~30\% of Japanese patients with colorectal cancers (14,15). Anti-EGFR antibody therapies are therefore likely to be ineffective in at least one-third of Japanese patients with mCRC, highlighting the importance of screening for these mutations in tumors.

Panitumumab is a fully human monoclonal antibody against EGFR that is indicated as monotherapy for treatment of EGFR-expressing mCRC in the USA and EGFR-expressing plus wild-type \textit{KRAS}-expressing mCRC in the European Union (EU) (16,17). In retrospective analyses of data from panitumumab clinical trials, including a Phase 3 trial comparing panitumumab monotherapy with best supportive care, the presence of a mutated \textit{KRAS} gene in tumors was associated with lack of response (18,19). This retrospective integrated analysis of data from two clinical trials (20,21) is the first study to examine the efficacy and safety of panitumumab monotherapy according to tumor \textit{KRAS} status in Japanese patients with mCRC.

**PATIENTS AND METHODS**

**STUDY DESIGN**

This was a retrospective, exploratory integrated analysis of data from two clinical trials (20,21) in Japanese patients with mCRC to examine the efficacy and safety of panitumumab monotherapy according to tumor \textit{KRAS} status.

Study 20040192 was a Phase 1 clinical trial of panitumumab monotherapy in Japanese patients with advanced solid tumors (20). Key objectives of this study were to evaluate the safety, pharmacokinetics, immunogenicity and clinical efficacy of panitumumab at various dose/dosing schedules in Japanese patients with advanced solid tumors. Patients with documented, advanced solid tumors that were refractory to standard chemotherapy or for which no standard therapy was available were eligible. Patients were sequentially enrolled into one of three panitumumab dosing cohorts: 2.5 mg/kg once weekly (QW), 6.0 mg/kg once every 2 weeks (Q2W) and 9.0 mg/kg once every 3 weeks (Q3W). These doses are all considered to reach clinically active panitumumab exposures. Objective responses were determined by the investigators using modified response evaluation criteria in solid tumors (RECIST) (22). Eighteen patients (six per cohort) were enrolled in the study. Only patients with mCRC were included in this analysis.

Study 20050216 was an open-label, single-arm, Phase 2 clinical trial of panitumumab monotherapy in Japanese patients with EGFR-expressing mCRC, who had developed disease progression while on or after prior fluoropyrimidine, irinotecan and oxaliplatin therapy (21), which were the same eligibility criteria as those used in the global Phase 3 trial (23). Key objectives of this study were to assess the effect of treatment with panitumumab on best overall objective response rate, progression-free survival, overall survival, safety and pharmacokinetics of Japanese patients with mCRC. Patients received panitumumab 6.0 mg/kg Q2W until disease progression or intolerance. Objective responses were determined by central radiographic review and by the investigators using modified RECIST, as defined in the pivotal trial (23). Fifty-three patients enrolled in the study and received at least one dose of panitumumab.

Participation in the biomarker analysis was optional in the two studies and additional informed consent was required to participate. Therefore, only the subset of patients with the additional informed consent enrolled in the studies were included in the analysis reported here. Overall, tumor samples from 28 of the total 66 patients with mCRC enrolled in the studies were available for biomarker analyses.

**STUDY ENDPOINTS**

The primary efficacy endpoint was the objective tumor response rate. A key secondary efficacy endpoint was progression-free survival time. An \textit{ad hoc} analysis of change in target lesions (sum of target lesion diameters) from baseline was also performed. Safety endpoints included the incidence of treatment-emergent adverse events. Pharmacokinetics of panitumumab were characterized for selected patients.

**TUMOR KRAS ASSESSMENTS**

A retrospective analysis of \textit{KRAS} mutation status (wild-type or mutated) was conducted using existing paraffin-embedded tumor tissues. Most specimens were from the primary tumor; three specimens were from metastatic sites. Samples were tested using the K-RAS Mutation kit (RUO KR-02) from DxS (Manchester, UK), which was the convenient, commercially available method used to detect \textit{KRAS} mutations in the pivotal panitumumab trial. DNA was extracted from paraffin-embedded tumor samples using the QIAamp \textsuperscript{\textregistered} DNeasy kit (QIAGEN, Inc., Valencia, CA, USA). All testing was performed by a central laboratory (HistoGeneX, Antwerp, Belgium); personnel performing the assays were blinded to the clinical outcomes.

The K-RAS Kit utilizes the amplification refractory mutation system (ARMS\textsuperscript{\textregistered}) (24) for mutation-specific amplification and Scorpions\textsuperscript{\textregistered} (25,26) technology to detect the mutations. ARMS technology is based on the observation that Taq DNA polymerase is ineffective at amplifying oligonucleotides with a mismatched 3’ residue. Primers for seven specific mutations in the \textit{KRAS} gene (with the mutations appearing at the 3’ end of the primers) are used to amplify...
mutated KRAS sequences in PCR reactions: Gly12Ala, Gly12Asp, Gly12Arg, Gly12Cys, Gly12Ser, Gly12Val and Gly13Asp. Scorpions, bifunctional molecules comprising a PCR primer covalently linked to a probe, are included in the PCR reaction. The Scorpion probe consists of a fluorophore and a quencher that are separated by the specific probe sequence. Complementary stem sequences flanking the specific probe sequence cause the Scorpion probe to form a hairpin structure in which the fluorophore and quencher are brought together, resulting in loss of fluorescence from the fluorophore. When the Scorpion probe is heat-denatured and then allowed to cool and bind to its target amplicon (a mutated KRAS sequence that has been amplified by an ARMS probe), the fluorophore and quencher are separated and fluorescence is increased. The fluorescence is measured in a LightCycler® 480 Instrument (Roche Applied Science, Indianapolis, IN, USA) using software version LCS480 1.2.9.11. This kit has the ability to detect ~1% mutated DNA in a background of wild-type genomic DNA. The failure rate of the KRAS assay was 4% in a large data set of patients with mCRC in a prior panitumumab clinical study (18).

**Statistical Analyses**

All analyses were descriptive evaluations to assess the relationship between clinical outcome and tumor KRAS mutation status in Japanese patients with mCRC who had measurable disease at baseline. Because of the small number of patients with samples available for KRAS testing, data from the two studies were pooled for these analyses, ignoring the potential heterogeneity in the analysis sets between the studies. All efficacy and safety analyses were performed on the KRAS analysis sets (enrolled patients who had: given consent for biomarker analysis; measurable disease at baseline; evaluable KRAS status and received at least one dose of panitumumab). Analyses were stratified by KRAS status; no other covariates were considered. No hypothesis testing was performed to compare endpoints between wild-type KRAS and mutated KRAS strata.

For continuous endpoints, the mean and standard deviation (SD) values are provided. The frequency and percentage distributions are provided for discrete data. The objective response rate and its two-sided 95% confidence interval (CI) were calculated; the 95% CI was based on the F distribution method (27). Kaplan–Meier estimates for progression-free survival time and 95% CIs were calculated; the 95% CI was based on a sign test (28). No imputation for missing or incomplete data was performed. All analyses were performed using SAS version 8.2 or higher (SAS Institute Inc., Cary, NC, USA) on the Sun/UNIX platform (Sun Microsystems, Inc; Santa Clara, CA, USA).

**RESULTS**

**Patients**

Participation in the biomarker analyses was optional in these studies and required additional written consent. Of the 66 patients with mCRC enrolled in the two studies, consent to participate in the study was obtained from 28 patients. Of these, 24 had known KRAS status. Patient demographics and disease characteristics at baseline are shown in Table 1.

At the time of data cut-off (12 April 2007), all patients had ended treatment because of disease progression (n = 28). All patients completed the protocol-specified safety follow-up. No patient included in this analysis withdrew from the studies. All patients had baseline measurable disease.

**KRAS Status**

KRAS status was determined in 24 (34%) patients, including 8 patients from Study 20040192 and 16 patients from Study 20050216. Of these, 14 (58%) had tumors with wild-type KRAS and 10 (42%) had tumors with mutated KRAS. The KRAS test failed for four patients: there was insufficient DNA in the tumor samples for three patients, and the tissue failed pathology review (i.e. no tumor sample) for one patient.

<table>
<thead>
<tr>
<th>Table 1. Patient demographics, disease characteristics and dose assignments at baseline</th>
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</thead>
<tbody>
<tr>
<td>Wild-type KRAS</td>
</tr>
<tr>
<td>Sex, n (%)</td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Primary diagnosis, n (%)</td>
</tr>
<tr>
<td>Colon cancer</td>
</tr>
<tr>
<td>Rectal cancer</td>
</tr>
<tr>
<td>Colorectal cancer*</td>
</tr>
<tr>
<td>ECOG performance status, n (%)</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>Assigned dose cohort in Study 20040192, n</td>
</tr>
<tr>
<td>N°</td>
</tr>
<tr>
<td>2.5 mg/kg QW</td>
</tr>
<tr>
<td>6.0 mg/kg Q2W</td>
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<tr>
<td>9.0 mg/kg Q3W</td>
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</tbody>
</table>

ECOG, Eastern Cooperative Oncology Group; SD, standard deviation; QW, once weekly; Q2W, every 2 weeks; Q3W, every 3 weeks.

*Specification of cancer site (colon vs. rectum) was not required in Study 20040192.

°Number of patients in Study 20040192 only.
Efficacy Outcomes

In the KRAS analysis set, four patients had a partial response to panitumumab therapy; all four responders had tumors expressing wild-type KRAS (Table 2). Six patients with tumors with wild-type KRAS had stable disease (median duration of 13.2 weeks; 95% CI: 11.1, 15.1). Of patients with tumors expressing mutated KRAS, none had a partial response and one had stable disease for 11.4 weeks. Patients with wild-type KRAS-expressing tumors had longer progression-free survival (median 13.2 weeks) than patients with mutated KRAS (median 7.3 weeks) (Fig. 1).

In an ad hoc analysis, the maximum decrease in the sum of target lesion diameters was determined for all patients. Of the 14 patients with tumors expressing wild-type KRAS, the sum of target lesion diameters was decreased in 10 patients; 4 of these patients had a partial response and 6 patients had stable disease (Fig. 2). The remaining four patients with wild-type KRAS in their tumors had an increase in the sum of target lesion diameters and had progressive disease. All patients with mutated KRAS in their tumors had an increase in the sum of their target lesion diameters.

Safety Outcomes

All 24 patients in the KRAS analysis set experienced an adverse event (related or unrelated to panitumumab therapy) during the study. Six (25%) patients had an adverse event with the worst grade of 3; 3 (13%) patients had an event with the worst grade of 4 and 7 (29%) patients had a serious adverse event. Treatment-related adverse events also occurred in all patients, including two (8%) patients with an adverse event with a worst grade of 3 and one (4%) patient with a serious adverse event. All skin-related events were Grade 1 or 2 in severity. Hypomagnesemia was reported in five (36%) patients with tumors expressing wild-type KRAS and in four (40%) patients with tumors expressing mutated KRAS. Adverse events occurring in 20% or more of the patients are shown in Table 3. No patient had an investigator-reported adverse event with a preferred term indicative of an infusion reaction or a reaction to panitumumab. No deaths or withdrawals due to adverse events were reported in the KRAS analysis set. No marked difference in adverse events was observed based on KRAS mutation status.

Table 2. Best objective response and progression-free survival

<table>
<thead>
<tr>
<th></th>
<th>Panitumumab</th>
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<tbody>
<tr>
<td></td>
<td>Wild-type KRAS</td>
<td>Mutated KRAS</td>
<td>All patients</td>
</tr>
<tr>
<td></td>
<td>(n=14)</td>
<td>(n=10)</td>
<td>(n=24)</td>
</tr>
<tr>
<td>Best objective response, (n) (%)</td>
<td>(n)</td>
<td>(n)</td>
<td>(n)</td>
</tr>
<tr>
<td>Partial response</td>
<td>4 (29)</td>
<td>0</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>6 (43)</td>
<td>1 (10)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>4 (29)</td>
<td>9 (90)</td>
<td>13 (54)</td>
</tr>
<tr>
<td>Objective response rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with partial response, (n)</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Rate, %</td>
<td>28.6</td>
<td>0</td>
<td>16.7</td>
</tr>
<tr>
<td>95% CI</td>
<td>8.4–58.1</td>
<td>0–30.9</td>
<td>4.7–37.4</td>
</tr>
<tr>
<td>Progression-free survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median weeks</td>
<td>13.2</td>
<td>7.3</td>
<td>7.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>8.0–23.1</td>
<td>7.1–7.6</td>
<td>7.4–11.4</td>
</tr>
</tbody>
</table>

CI, confidence interval.

Figure 1. Progression-free survival. The proportion of patients with progression-free survival over time (weeks) is shown.
This pharmacokinetic analysis only included patients who received panitumumab at 6 mg/kg Q2W. Panitumumab pharmacokinetic data were available for 10 patients with tumors expressing mutated KRAS (from 2 patients in Study 20040192 and 8 patients in Study 20050216) and 9 patients with tumors expressing wild-type KRAS (from 1 patient in Study 20040192 and 8 patients in Study 20050216). The pharmacokinetic profiles of panitumumab were similar between patients with wild-type and mutated KRAS status. On the basis of the population pharmacokinetic model (29), the mean (SD) areas under the curves at steady-state were 1110 (385) and 863 (240) μg·day/ml, the mean maximum concentrations were 168 (38.8) and
137 (27.0) μg/ml and the mean minimum concentrations were 38.4 (21.8) and 27.9 (13.2) μg/ml for patients with wild-type and mutated KRAS status, respectively.

**DISCUSSION**

The results of this exploratory integrated analysis of safety and efficacy of panitumumab monotherapy in Japanese patients by KRAS status are consistent with those of other studies of anti-EGFR antibodies in patients with mCRC (9–11,18,19). The distribution of KRAS status (wild-type vs. mutated) was also similar to those seen in these other studies. Results of efficacy endpoints were favorable in patients with tumors expressing wild-type KRAS, and mutated tumor status was associated with lack of response to anti-EGFR therapy. In this limited analysis, response to panitumumab therapy was seen only in patients with tumors expressing wild-type KRAS. Patients with tumors with wild-type KRAS showed a trend toward longer progression-free survival.

Technical issues in sample processing resulted in the loss of four samples for testing, including lack of sufficient material for testing (n = 3) and KRAS assay failure (n = 1). Assay failure can be caused by inappropriate tissue fixation at the time of tissue collection. It is important, therefore, for investigators enrolling patients in clinical trials to ensure availability of proper materials and procedures for tissue collection. These precautions should enhance the quality and quantity of data obtained in clinical trials for these types of analyses.

The safety profile of panitumumab in this study was also consistent with prior studies of panitumumab monotherapy in patients with mCRC (23,30,31). Skin toxicities and hypomagnesemia are known effects of EGFR inhibition but are generally manageable. Because of the small sample size and the varying doses of panitumumab received by patients in the 20040192 study, it is not possible to draw meaningful conclusions regarding potential differences in the incidence or severity of adverse events between patients with tumors expressing wild-type or mutated KRAS.

Panitumumab exhibits pharmacokinetics that are consistent with target-mediated drug disposition, involving saturable binding to EGFR and subsequent internalization and degradation inside the cells (32). In addition, panitumumab is cleared by the reticuloendothelial system, similar to other endogenous immunoglobulins. As it is unlikely that KRAS is involved in the clearance of panitumumab, it was not unexpected that pharmacokinetic profiles of panitumumab were similar between patients with tumors expressing mutated KRAS and patients with tumors expressing wild-type KRAS.

The results of this study also support the need for KRAS testing to assist in identification of patients who are unlikely to respond to panitumumab therapy. Expression of EGFR on tumors is a requirement of both the USA (16) and EU (17) labels, although EGFR expression has now been shown to have no predictive value with respect to response to anti-EGFR therapy (4,5). Data from our analysis support the suggestion that panitumumab therapy should be restricted to patients whose tumors express wild-type KRAS.

In conclusion, the efficacy of panitumumab in the treatment of mCRC is similar in Japanese patients and Western patients. Additionally, panitumumab efficacy according to KRAS status is similar in Japanese and non-Japanese patients.

**Acknowledgements**

The authors thank the patients and their families and friends for participation in the study and the clinical study staff at all participating institutions. We also thank Julia R. Gage, PhD, for writing assistance on behalf of Amgen Inc.

**Funding**

This study was supported by Amgen Inc.

**Conflict of interest statement**

Toshihiko Doi, Makoto Tahara, Takayuki Yoshino and Tomohide Tamura have no disclosures. Kentaro Yamazaki has been granted a contribution or research support from Taiho, Pfizer, Takeda and Chugai. Yasuhide Yamada has been a lecturer and received fees at lectures sponsored by Taiho, Chugai, Novartis, Bayer, Pfizer Japan and Yakult Honsha. Bing-Bing Yang and Kelly Smith Oliner are compensated employees and shareholders of Amgen Inc. Satoru Otani and Daisuke Asahi are compensated employees of Takeda Bio Development Center Limited.

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