The Clinical Benefit of Bevacizumab in Metastatic Colorectal Cancer Is Independent of K-ras Mutation Status: Analysis of a Phase III Study of Bevacizumab with Chemotherapy in Previously Untreated Metastatic Colorectal Cancer

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Key Words. Bevacizumab • Vascular endothelial growth factor • Colorectal cancer • Survival • Fluorouracil • Angiogenesis inhibitors

Disclosures

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ABSTRACT

Purpose. Mutations of the K-ras gene were identified as a prognostic marker in metastatic colorectal cancer (mCRC). In addition, emerging data suggest that K-ras mutations are a negative predictor of clinical benefit from anti–epidermal growth factor receptor treatment in mCRC. Previously reported data suggest that the longer overall survival (OS) observed with bevacizumab treatment in mCRC is independent of alterations in the Ras/Raf/Mek/Erk pathway. We conducted additional analyses to better describe the clinical benefit of bevacizumab treatment in mCRC relative to K-ras mutation status.

Patients and Methods. Additional statistical analyses were done with data from K-ras mutation analyses in 230 patients who were treated with irinotecan, fluorouracil, and leucovorin (IFL) in combination with either bevacizumab or placebo in a randomized phase III study. Following microdissection, tissue was subject to DNA sequencing to identify K-ras mutations in codons 12 and 13. Hazard ratios for the bevacizumab group relative to the control group were estimated from an unstratified Cox regression model. The median progression-free survival (PFS), OS times, and objective response rates were compared.

Results. K-ras status was assessed in 230 patients (28.3%). The median PFS was significantly longer in bevacizumab-treated patients with wild-type (wt)- (13.5 versus 7.4 months; hazard ratio 0.44, p < .0001) and mutant (m)-K-ras (9.3 versus 5.5 months; hazard ratio...
0.41, \( p = .0008 \)). A significantly higher response rate for IFL plus bevacizumab was observed only in wt-K-ras patients (60.0\% versus 37.3\%, \( p = .006 \)) compared with 43.2\% versus 41.2\% in the m-K-ras group.

**Introduction**

Bevacizumab was shown to improve the overall survival (OS) time, progression-free survival (PFS) time, and objective response rate (RR) in a placebo-controlled phase III trial when added to irinotecan, fluorouracil, and leucovorin (IFL) chemotherapy in the first-line treatment of metastatic colorectal cancer (mCRC) [1]. An exploratory analysis of this study suggested that the OS benefit of the addition of bevacizumab was independent of the mutation status of K-ras, B-raf, or p53 [2]. The selection of K-ras for these analyses was based upon evidence that K-Ras regulates vascular endothelial growth factor (VEGF) and other angiogenic factors [3, 4], as well as numerous reports that K-ras is a negative prognostic factor in patients with mCRC [5–8].

Mutations in K-ras strongly predict for a lack of response to anti–epidermal growth factor receptor (EGFR) antibodies [9–12]. For this reason, K-ras testing is required by the European Medical Authority for the use of panitumumab and cetuximab. The role of ras mutations in predicting response to traditional cytotoxic agents in advanced CRC has not been well studied.

The impact of ras mutations on OS was previously reported for the addition of bevacizumab to first-line IFL chemotherapy. To better describe the clinical benefit of bevacizumab according to K-ras mutation status in this patient population, we performed additional analyses of other measures of clinical benefit, including the PFS time and RR.

**Methods**

**Patients and Study Design**

The details of study AVF2107 (registered at http://www.ClinicalTrials.gov, ID number NCT00109070), including patient eligibility criteria, study design, treatment, and assessments, have been reported previously [1]. Only patients with sufficient tumor tissue for molecular assessment of K-ras were included in the exploratory analyses contained in this report.

**Ethics**

The institutional review boards of the investigative centers approved the study protocol, and the trial was conducted in accordance with the Declaration of Helsinki, U.S. Food and Drug Administration Good Clinical Practices, and local ethical and legal requirements. All patients provided written informed consent for their study participation.

**Molecular Testing of Tumor Tissue**

The details of the available tissue samples, laser capture microdissection, polymerase chain reaction (PCR) primers and conditions, and direct sequencing of PCR products have been described previously [2]. The selected primers covered codons 12 and 13.

**Statistical Analyses**

The efficacy analysis was based on randomized patients with known K-ras mutation status. The PFS time was defined as the time from randomization to disease progression or death resulting from any cause during first-line treatment. Median PFS times were estimated using the Kaplan–Meier method. An unstratified Cox regression model was used to estimate the hazard ratio (HR) for the bevacizumab group relative to the control group. A two-sided log-rank test (\( \alpha = 0.05 \)) was used to compare the differences between the bevacizumab and the placebo group. Because of sample size considerations, this test was unstratified. The objective RR was defined as the rate of a complete or partial response determined on two consecutive occasions at least 4 weeks apart during first-line treatment. The \( \chi^2 \) test was used to compare the objective RRs. Any differential effect of treatment between the K-ras mutation status groups was evaluated using a Cox model with K-ras mutation status, treatment, and the K-ras-by-treatment interaction term in the model.

**Results**

Tissue samples from 230 of 813 patients (28\%) were available for molecular analysis of the selected K-ras codons. Of these 230 patients, 129 had been randomly assigned to receive IFL chemotherapy plus bevacizumab (IFL + BV) and 101 had been randomly assigned to receive IFL chemotherapy plus placebo (IFL + placebo). Demographic and baseline disease characteristics for the K-ras subgroup and for the overall phase III study population are summarized in Table 1. The median ages in the groups were 62.0 and 58.0 years, respectively. The demographic and baseline characteristics were similar in the subset of patients with available tumor tissue and the entire study population [1]. A higher incidence of patients with an Eastern Cooperative Oncol-
ogy Group performance status score of zero in the placebo arm (61.4% versus 55.2%) and a shorter mean time from diagnosis in both arms of the subpopulation (12.4 months for IFL + placebo and 13.2 months for IFL + BV in the tissue available subgroup versus 16.1 months for IFL + placebo and 15.2 months for IFL + BV in the study as a whole) were observed. Additional characteristics not included in Table 1, that is, gender, race/ethnicity, body weight and surface area, and serum lactate dehydrogenase, were comparable with those in the entire population. K-ras mutations were detected in 78 of the 230 patients (34%).

Measures of clinical benefit were generally comparable in the subgroup with available tissue and in the overall population. Comparing the subgroup with available tissue with the overall population, the HRs for OS were similar (HR, 0.60; 95% confidence interval [CI], 0.40–0.91; p = .01 versus HR, 0.66; 95% CI, 0.54–0.81; p < .0001, respectively), although a modest difference in the median OS time was noted (25.1 versus 17.5 months compared with 20.3 versus 15.6 months, respectively). Modest differences between the subgroup with available tissue and the overall population were noted for PFS (HR, 0.44; 95% CI, 0.32–0.61; p < .0001 versus HR, 0.54; 95% CI, 0.45–0.66; p < .0001; median, 11.3 months versus 6.3 months, compared with 10.6 months versus 6.2 months, respectively).

**Table 1.** Baseline demographic and disease characteristics of randomized K-ras patients and overall phase III study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>K-ras subgroup</th>
<th>Overall</th>
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<tbody>
<tr>
<td></td>
<td>IFL + placebo (n = 101)</td>
<td>IFL + BV (n = 129)</td>
</tr>
<tr>
<td></td>
<td>IFL + placebo (n = 411)</td>
<td>IFL + BV (n = 402)</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>Mean (SD) 58.3 (10.8)</td>
<td>60.4 (10.9)</td>
</tr>
<tr>
<td></td>
<td>Median 58.0</td>
<td>62.0</td>
</tr>
<tr>
<td></td>
<td>Range 27–83</td>
<td>24–80</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 54 (53.5%)</td>
<td>75 (58.1%)</td>
</tr>
<tr>
<td></td>
<td>Female 47 (46.5%)</td>
<td>54 (41.9%)</td>
</tr>
<tr>
<td>ECOG performance status (baseline)</td>
<td>0 62 (61.4%)</td>
<td>78 (60.5%)</td>
</tr>
<tr>
<td></td>
<td>1 39 (38.6%)</td>
<td>51 (39.5%)</td>
</tr>
<tr>
<td></td>
<td>2 0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Mean (SD) duration of disease, mos</td>
<td>12.4 (16.27)</td>
<td>13.2 (22.84)</td>
</tr>
<tr>
<td>Location of primary tumor</td>
<td>Colon 83 (82.2%)</td>
<td>101 (78.3%)</td>
</tr>
<tr>
<td></td>
<td>Rectum 18 (17.8%)</td>
<td>28 (21.7%)</td>
</tr>
<tr>
<td>Mean (SD) serum albumin (baseline),</td>
<td>3.7 (0.51)</td>
<td>3.7 (0.53)</td>
</tr>
<tr>
<td>Mean (SD) serum alkaline phosphatase,</td>
<td>172.6 (139.62)</td>
<td>181.0 (163.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>163.1 (148.89)</td>
</tr>
</tbody>
</table>

Baseline ECOG performance status, weight, and laboratory test results are the last available values as reported on the case report form on or before the first day of treatment with study drug or chemotherapy. Location of primary tumor obtained from interactive voice response system when not reported on case report form. Abbreviations: BV, bevacizumab; ECOG, Eastern Cooperative Oncology Group; IFL, irinotecan, fluorouracil, and leucovorin; SD, standard deviation.

**Figure 1.** Progression-free survival by treatment for randomized patients with known K-ras status. Abbreviations: BV, bevacizumab; IFL, irinotecan, fluorouracil, and leucovorin.
results of the analyses of OS, PFS, and RR according to K-ras mutation status are listed in Table 2. Kaplan–Meier curves for OS and PFS are shown in Figure 2 and Figure 3, respectively. In both the wt-K-ras and m-K-ras groups, the addition of bevacizumab to IFL chemotherapy resulted in a statistically significant longer PFS time, with comparable HRs for progression. In the wt-K-ras group, the median PFS duration was 13.5 months for IFL + BV versus 7.4 months for IFL + placebo (HR, 0.44; 95% CI, 0.29–0.67; p < .0001). For the m-K-ras group, the median PFS duration was 9.3 months for IFL + BV versus 5.5 months for IFL + placebo (HR, 0.41; 95% CI, 0.24–0.70; p = .0008). As previously reported [2], comparable findings were noted for OS. In the wt-K-ras group, the median OS time was 19.9 months for IFL + BV versus 13.6 months for IFL + placebo (HR, 0.58; 95% CI, 0.34–0.99; p = .04). For the m-K-ras group, the median OS time was 17.6 months for IFL + BV versus 5.5 months for mutant (m)-K-ras patients (HR, 0.66; 95% CI, 0.41–0.98; p = .09 for wt-K-ras versus m-K-ras).

Table 2. PFS, overall survival, and objective response rate for K-ras patients randomized to IFL + placebo or IFL + BV

<table>
<thead>
<tr>
<th>Efficacy variable</th>
<th>Wild-type</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFL + placebo</td>
<td>IFL + BV</td>
</tr>
<tr>
<td>Median survival duration, mos</td>
<td>(n = 67)</td>
<td>(n = 85)</td>
</tr>
<tr>
<td>p = .04; HR, 0.58 (0.3–1.0)</td>
<td>17.6</td>
<td>27.7</td>
</tr>
<tr>
<td>Median PFS duration during first-line therapy, mos</td>
<td>p &lt; .0001; HR, 0.44 (0.3–0.7)</td>
<td>7.4</td>
</tr>
<tr>
<td>Objective response, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>25 (37.3%)</td>
<td>51 (60.0%)</td>
</tr>
<tr>
<td>Partial</td>
<td>2 (3.0%)</td>
<td>3 (3.5%)</td>
</tr>
</tbody>
</table>

*For survival and PFS, p-value is from an unstratified log-rank test; HR is relative to the placebo group and estimated by Cox regression. For objective response, p-value is from a Pearson χ² test.

Abbreviations: BV, bevacizumab; CI, confidence interval; HR, hazard ratio; IFL, irinotecan, fluorouracil, and leucovorin; PFS, progression-free survival.

Figure 2. Duration of overall survival by K-ras status for randomized patients with known K-ras status.

Abbreviations: BV, bevacizumab; IFL, irinotecan, fluorouracil, and leucovorin.

respectively) and for RR (44.8% versus 34.8% compared with 54.3% versus 38.6%, respectively).

Prognostic Value of K-ras Mutations in mCRC

To assess the prognostic importance of K-ras, PFS was compared according to K-ras status for both the IFL + placebo and the IFL + BV groups (Fig. 1). For patients treated with IFL + placebo, the median PFS duration was 7.4 months for wild-type (wt)-K-ras patients and 5.5 months for mutant (m)-K-ras patients (HR, 0.69; 95% CI, 0.44–1.08; p = .11 for wt-K-ras versus m-K-ras). In patients treated with IFL + BV, the median PFS was 13.5 months for wt-K-ras patients and 9.3 months for m-K-ras patients (HR, 0.66; 95% CI, 0.41–1.08; p = .09 for wt-K-ras versus m-K-ras).

Predictive Value of K-ras Mutations for Treatment with Bevacizumab in mCRC

Results of the analyses of OS, PFS, and RR according to K-ras mutation status are listed in Table 2. Kaplan–Meier curves for OS and PFS are shown in Figure 2 and Figure 3, respectively. In both the wt-K-ras and m-K-ras groups, the addition of bevacizumab to IFL chemotherapy resulted in a statistically significant longer PFS time, with comparable HRs for progression. In the wt-K-ras group, the median PFS duration was 13.5 months for IFL + BV versus 7.4 months for IFL + placebo (HR, 0.44; 95% CI, 0.29–0.67; p < .0001). For the m-K-ras group, the median PFS duration was 9.3 months for IFL + BV versus 5.5 months for IFL + placebo (HR, 0.41; 95% CI, 0.24–0.70; p = .0008). As previously reported [2], comparable findings were noted for OS. In the wt-K-ras group, the median OS time was 19.9 months for IFL + BV versus 13.6 months for IFL + placebo (HR, 0.58; 95% CI, 0.34–0.99; p = .04). For the m-K-ras group, the median OS time was 17.6 months for IFL + BV versus 5.5 months for mutant (m)-K-ras patients (HR, 0.66; 95% CI, 0.41–0.98; p = .09 for wt-K-ras versus m-K-ras).
Bevacizumab and K-ras in mCRC

Table 3. n (%) of patients with selected adverse events during first-line therapy for treated patients with known K-ras status

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Wild-type</th>
<th>Mutant</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IFL + placebo (n = 67)</td>
<td>IFL + BV (n = 85)</td>
</tr>
<tr>
<td>On-study death from any cause</td>
<td>5 (7.5)</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>Any adverse event, grade 3–4</td>
<td>50 (74.6)</td>
<td>78 (91.8)</td>
</tr>
<tr>
<td>Arterial thromboembolic event, any grade</td>
<td>1 (1.5)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>GI perforation, grade 3–4</td>
<td>0 (0)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Bleeding, grade 3–4</td>
<td>3 (4.5)</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>Hypertension, grade 3–4</td>
<td>2 (3.0)</td>
<td>8 (9.4)</td>
</tr>
<tr>
<td>Proteinuria, grade 3–4</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diarrhea, grade 3–4</td>
<td>14 (20.9)</td>
<td>21 (24.7)</td>
</tr>
</tbody>
</table>

Grading of adverse events used National Cancer Institute–Common Toxicity Criteria.
*aGI perforation was defined as GI abscess, perforation, or fistula.
*bReported adverse events included GI hemorrhage, hematuria, hemorrhage, hemotherax, melena, and rectal hemorrhage.

Abbreviations: BV, bevacizumab; GI, gastrointestinal; IFL, irinotecan, fluorouracil, and leucovorin.

Several reports have indicated that K-ras mutations are negative prognostic markers and portend a poorer outcome in mCRC [7, 8, 17]. The retrospective analysis presented in this report appears to support the prognostic significance of K-ras mutations in mCRC. This finding is consistent with previously reported data involving cytotoxic chemotherapies that generally support the concept that K-Ras is not a predictive marker for benefit, or lack of benefit, from traditional cytotoxic chemotherapy [5–7]. More recent data [10–12], however, suggest that K-ras status is not generally prognostic and may have a modest but distinct predictive importance for oxaliplatin versus irinotecan regimens. Thus, the question of the prognostic significance of mutated K-ras in mCRC appears to remain open.

Independent of whether K-ras status is prognostic in mCRC, the data in the current report strongly suggest that K-ras status does not predict clinical benefit from the addition of bevacizumab to first-line IFL chemotherapy. The relative benefits in terms of PFS and OS associated with the addition of bevacizumab to IFL chemotherapy were comparable in the m-K-ras and wt-K-ras groups.

Whereas patients with wt-K-ras in this study appeared to have a greater RR with the addition of bevacizumab to IFL, no such benefit was found in patients with m-K-ras.

Multiple preclinical studies have demonstrated that K-ras mutations upregulate VEGF and numerous other angiogenic factors in tumor cells [3, 4, 18]. Given these potential mechanisms of resistance, the same clinical benefit from bevacizumab in patients with m-K-ras and wt-K-ras tumors supports the role of VEGF as the primary angiogenic factor in CRC.

Discourse

A growing body of evidence suggests not only that mutations of the K-ras oncogene have prognostic significance in mCRC, but also that K-ras mutations may serve as predictive markers that can be used to guide the use of anti-EGFR therapy. Multiple phase II and III studies have demonstrated that patients with mCRC harboring mutations in K-ras do not appear to derive clinical benefit from anti-EGFR monoclonal antibody treatment, when used either as monotherapy or in combination with cytotoxic chemotherapy [9–16].

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Whereas patients with wt-K-ras in this study appeared to have a greater RR with the addition of bevacizumab to IFL, no such benefit was found in patients with m-K-ras.

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This analysis has several limitations. First, it is retrospective, so tissue was not available for the assessment of K-ras for a significant proportion of the study population; thus, an unintentional selection bias for the subset of patients included in this analysis is possible. However, the patient and tumor characteristics for the subgroup of patients with available tissue were comparable with those of the overall study population and the frequency and type of K-ras mutations in this study were similar to those from other reports [7–10, 17]. The relatively small sample size in this study also precludes a definitive assessment of the presence or absence of an interaction. A definitive study that could detect an HR of 0.6 in the wt-K-ras group and an HR of one in the m-K-ras group with 80% power would require 540 events and potentially >1,000 subjects.

These data have implications for the appropriate management of patients with mCRC as well as the design and interpretation of clinical trials in this disease. Our analyses suggest that the clinical benefit from the anti-VEGF therapy bevacizumab, unlike EGFR-targeted antibodies, appears to be independent of K-ras status. At a practical level, K-ras testing is unnecessary to determine which patients should receive bevacizumab. These data also highlight the complexity of K-ras biology in mCRC. Clinical trial populations need to be defined regarding the selection, or lack of selection, for K-ras mutation status in order to allow for appropriate interpretation.

Lastly, these data also highlight the distinction between prognostic and predictive markers, a topic that has been extensively reviewed [19]. As diagnostics and therapeutics related to key oncopgenes become available, these distinctions will take on even greater clinical significance.

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AUTHOR CONTRIBUTIONS
Conception/design: Herbert I. Hurwitz, William F. Novotny, Oliver Rosen
Administrative support: Oliver Rosen
Provision of study materials: Herbert I. Hurwitz
Collection/assembly of data: William Ince, William F. Novotny, Oliver Rosen
Data analysis: Herbert I. Hurwitz, Jing Yi, William F. Novotny, Oliver Rosen
Manuscript writing: Herbert I. Hurwitz, Jing Yi, Oliver Rosen
Final approval of manuscript: Herbert I. Hurwitz, Jing Yi, William Ince, William F. Novotny, Oliver Rosen

The listed authors take full responsibility for the content of the paper, but wish to also thank Linda Phillips, Ph.D., from Genentech for her assistance in organizing the published literature and editing the manuscript.

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