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K-ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer

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ABSTRACT

BACKGROUND

Treatment with cetuximab, a monoclonal antibody directed against the epidermal growth factor receptor, improves overall and progression-free survival and preserves the quality of life in patients with colorectal cancer that has not responded to chemotherapy. The mutation status of the *K-ras* gene in the tumor may affect the response to cetuximab and have treatment-independent prognostic value.

METHODS

We analyzed tumor samples, obtained from 394 of 572 patients (68.9%) with colorectal cancer who were randomly assigned to receive cetuximab plus best supportive care or best supportive care alone, to look for activating mutations in exon 2 of the *K-ras* gene. We assessed whether the mutation status of the *K-ras* gene was associated with survival in the cetuximab and supportive-care groups.

RESULTS

Of the tumors evaluated for *K-ras* mutations, 42.3% had at least one mutation in exon 2 of the gene. The effectiveness of cetuximab was significantly associated with *K-ras* mutation status ($P=0.01$ and $P<0.001$ for the interaction of *K-ras* mutation status with overall survival and progression-free survival, respectively). In patients with wild-type *K-ras* tumors, treatment with cetuximab as compared with supportive care alone significantly improved overall survival (median, 9.5 vs. 4.8 months; hazard ratio for death, 0.55; 95% confidence interval [CI], 0.41 to 0.74; $P<0.001$) and progression-free survival (median, 3.7 months vs. 1.9 months; hazard ratio for progression or death, 0.40; 95% CI, 0.30 to 0.54; $P<0.001$). Among patients with mutated *K-ras* tumors, there was no significant difference between those who were treated with cetuximab and those who received supportive care alone with respect to overall survival (hazard ratio, 0.98; $P=0.89$) or progression-free survival (hazard ratio, 0.99; $P=0.96$). In the group of patients receiving best supportive care alone, the mutation status of the *K-ras* gene was not significantly associated with overall survival (hazard ratio for death, 1.01; $P=0.97$).

CONCLUSIONS

Patients with a colorectal tumor bearing mutated *K-ras* did not benefit from cetuximab, whereas patients with a tumor bearing wild-type *K-ras* did benefit from cetuximab. The mutation status of the *K-ras* gene had no influence on survival among patients treated with best supportive care alone. (ClinicalTrials.gov number, NCT00079066.)

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A randomized trial (CO.17) conducted by the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) in collaboration with the Australasian Gastro-Intestinal Trials Group (AGITG) showed that among patients with colorectal cancer that had not responded to advanced chemotherapy, monotherapy with cetuximab, a monoclonal antibody directed against the epidermal growth factor receptor (EGFR), improved overall survival and progression-free survival and preserved the quality of life better than did best supportive care alone.[1] However, resistance to cetuximab was common: at the first assessment of disease response, the disease had progressed in more than 50% of treated patients.

K-ras, a small G-protein downstream of EGFR and an essential component of the EGFR signaling cascade, can acquire activating mutations in exon 2, thus isolating the pathway from the effect of EGFR[2] and rendering EGFR inhibitors ineffective.[3-11]

We undertook correlative analyses to determine whether the mutation status of the *K-ras* gene modified the effect of cetuximab on overall survival and progression-free survival in the CO.17 trial. We also assessed the association of *K-ras* mutation status with overall survival and progression-free survival among patients receiving best supportive care alone.

Methods

A protocol committee that included members of the NCIC CTG and the AGITG designed the study. The NCIC CTG collected and analyzed the data and maintained full, unrestricted rights to submit the study data for publication. One of the academic authors reviewed all the data, another conducted the statistical analyses, and a third wrote the first draft of the manuscript; these authors vouch for the completeness and accuracy of the data presented. The initial draft of the manuscript was reviewed and commented on by all the authors as well as by employees of Bristol-Myers Squibb, who did not make substantive changes. The relevant institutional review boards approved the study protocol, including the use of archived tumor tissue. Written informed consent for tumor tissue research was obtained from the majority of patients. For use of tissue from those in whom a clinical decline prevented consent, approval was granted by the ethics board at the patient's institution on the basis of the patient's consent to the original research.

Patients and Trial Design

The trial design and eligibility criteria have been reported previously.[1] The primary objective of the phase 3 CO.17 study was to examine the effect of cetuximab on survival among patients with advanced colorectal cancer in whom all chemotherapy for colorectal cancer had failed and for whom no other standard anticancer therapy was available. Eligible patients were enrolled in the trial between December 2003 and August 2005. None of the patients had received previous therapy directed against EGFR. After enrollment, patients were randomly assigned to receive cetuximab plus supportive care or supportive care alone. Cetuximab was given as an intravenous loading dose of 400 mg per square meter of body-surface area, administered over a period of 120 minutes, on day 1 of treatment, followed by an infusion of 250 mg per square meter, administered over a period of 60 minutes, once a week. Patients in both groups were evaluated for tumor response or progression every 8 weeks by means of radiologic imaging. Cetuximab therapy was continued until the disease progressed or until the patient could not tolerate the toxic effects.

Tumor Collection and Processing

Formalin-fixed, paraffin-embedded samples of tumor tissue from archival specimens collected at the time of diagnosis were stored at a central tumor bank located at Queen's University in Kingston, Ontario, Canada. If tumor blocks were unavailable, unstained slides were retrieved. Assays of tissue samples for *K-ras* mutations were performed in a blinded fashion by members of the Department of Clinical Biomarkers–Oncology at Bristol-Myers Squibb, Hopewell, New Jersey. All available tissue samples were classified as having mutated or wild-type *K-ras*.

Nucleotide Sequence Analysis

Mutation analysis of *K-ras* was performed by extraction of genomic DNA from formalin-fixed, paraffin-embedded tissue slides or sections with the use of the QIAamp DNA Mini Kit (Qiagen). For *K-ras* analyses, the following nested primer sets for exon 2 were used: huKRAS2 ex2F, 5′GAATGGTCCTGCACCAGTAA3′; huKRAS2 ex2R, 5′GTGTGACATGTTCTAATATAGTCA3′; huKRAS ex2Fint, 5′GTCCTGCACCAGTAATATGC3′; and huKRAS2 ex2Rint, 5′ATGTTCTAATATAGTCACA-

TTTTC3′. Each 25-μl polymerase-chain-reaction (PCR) cocktail contained at least 15 ng of genomic DNA, 2.5 mM magnesium chloride, 300 mM deoxynucleotide triphosphates, and 2.5 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems). PCR conditions were as follows: 1 cycle at 94°C for 10 minutes; 45 cycles at 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds; followed by 1 cycle at 72°C for 7 minutes. Primer extension sequencing was performed with the use of the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Reactions were run on a 3730x1 DNA Analyzer (Applied Biosystems). Analyses of the DNA sequences were performed with the use of Mutation Surveyor v2.61 (SoftGenetics), along with visual inspection of each sample trace in both forward and reverse directions. Appropriate positive and negative controls were included for *K-ras*. The persons who performed the mutation analyses had no knowledge of the clinical outcome.

STATISTICAL ANALYSIS

All statistical analyses were performed by the NCIC CTG in accordance with a protocol for statistical analysis that was written before the assessment of *K-ras* mutation was performed. All randomly assigned patients for whom data on *K-ras* mutation status were available were included in the analysis. Overall survival, the primary end point, was defined as the time from randomization until death from any cause. The secondary end points were progression-free survival, defined as the time from randomization until the first objective evidence of disease progression or death from any cause; response rates, defined according to the Response Evaluation Criteria in Solid Tumors (RECIST); and quality of life, assessed with the use of the European Organisation for Research and Treatment of Cancer (EORTC) quality-of-life questionnaire (QLQ-C30). Wilcoxon tests were used to compare the two treatment groups with respect to the mean change from baseline in scores on the global quality-of-life scale. A difference of more than 10 points was considered to indicate clinical significance.[12]

The survival of patients in each *K-ras*–mutation group and treatment group was summarized with the use of Kaplan–Meier curves, and the difference between these groups was compared with the use of the log-rank test, with the hazard ratio and its 95% confidence intervals calculated from a Cox regression model with a single covariate. To assess whether *K-ras* was an independent prognostic factor for patients receiving supportive care, a multivariate Cox regression model was fitted to data for patients receiving supportive care alone; it included the following covariates specified by the protocol: Eastern Cooperative Oncology Group (ECOG) performance status (0 or 1 vs. 2, on a scale of 0 to 5, with lower scores representing a higher level of functioning), sex (male vs. female), age (≥65 years vs. <65 years), baseline lactate dehydrogenase level (higher than the upper limit of the normal range vs. the upper limit or lower), baseline alkaline phosphatase level (higher than the upper limit of the normal range vs. the upper limit or lower), baseline hemoglobin (lower than the lower limit of the normal range vs. the lower limit or higher), number of disease sites (>2 vs. ≤2), number of previous chemotherapy drug classes (>2 vs. ≤2), primary tumor site (rectum only vs. colon), and presence of liver metastases (yes vs. no). We used the Cox model, with treatment, *K-ras*–mutation status, and their interaction as covariates, to assess the interaction between treatment and *K-ras*–mutation status. All reported P values are two-sided and were not adjusted for multiple testing.

RESULTS

CHARACTERISTICS OF THE PATIENTS

Of 572 patients who underwent randomization, 287 were assigned to receive cetuximab plus supportive care, and 285 were assigned to receive supportive care alone. A total of 394 tumor specimens (198 from the cetuximab group and 196 from the supportive-care group) were examined for *K-ras*–mutation status (accounting for 68.9% of the total study population). Tumor specimens from the remaining patients could not be retrieved despite our best efforts; the most common reasons were lack of patient consent, insufficient tissue, and refusal or inability of the laboratory of origin to release the tissue for research. A *K-ras* mutation was detected in 40.9% of tumor specimens in the cetuximab group and in 42.3% of tumor specimens in the supportive-care group. Table 1 shows the *K-ras* mutations we identified and the distribution according to the treatment group. Table 2 summarizes the baseline demographic and disease characteristics of the total study population and of the patients who were evaluated for the *K-ras* mutation. The group of patients with *K-ras* mutations and the group with wild-type *K-ras* were similar with respect to baseline characteristics,

Table 1. Distribution of *K-ras* Mutations According to Treatment Group.

Mutation*	All Patients (N=164)	Patients Receiving Cetuximab plus Best Supportive Care (N=81)	Patients Receiving Best Supportive Care Alone (N=83)
		no. of mutations (%)	
G12A	11 (6.4)	7 (8.3)	4 (4.6)
G12C	9 (5.3)	6 (7.1)	3 (3.4)
G12D	61 (35.7)	28 (33.3)	33 (37.9)
G12R	2 (1.2)	1 (1.2)	1 (1.1)
G12S	17 (9.9)	7 (8.3)	10 (11.5)
G12V	48 (28.1)	28 (33.3)	20 (23.0)
G13A	1 (0.6)	0	1 (1.1)
G13C	1 (0.6)	0	1 (1.1)
G13D	20 (11.7)	7 (8.3)	13 (14.9)
G13V	1 (0.6)	0	1 (1.1)

* Seven patients had more than one mutation type.

including ECOG performance status and the other variables associated with survival in the multivariate analysis. The distribution of these characteristics among the patients who were evaluated for *K-ras* mutations was similar to that in the total study population. In the supportive-care group, 13 patients had protocol violations and crossed over to cetuximab treatment. Four of them received cetuximab before progression of the disease, and nine received cetuximab after progression. All of the patients who had been assigned to the cetuximab group and who were included in the *K-ras*–mutation analysis did receive cetuximab.

OVERALL SURVIVAL

Among patients with mutated *K-ras* tumors, the median overall survival was 4.5 months in the group receiving cetuximab and 4.6 months in the group receiving supportive care only, with 1-year overall survival rates of 13.2% and 19.6%, respectively (hazard ratio for death in the cetuximab group as compared with the supportive-care group, 0.98; 95% confidence interval [CI], 0.70 to 1.37; P=0.89) (Fig. 1A). Among patients with wild-type *K-ras* tumors, the median overall survival was 9.5 months in the cetuximab group as compared with 4.8 months in the supportive-care group, with 1-year overall survival rates of 28.3% and 20.1%, respectively (hazard ratio, 0.55; 95% CI, 0.41 to 0.74; P<0.001). In a multivariate Cox regression model, this difference remained significant after adjustments for potential prognostic factors specified in the protocol (hazard ratio, 0.62; 95% CI, 0.44 to 0.87; P=0.006) (Fig. 1B). The effect of cetuximab on overall survival was significantly greater among the patients with wild-type *K-ras* tumors than among those with mutated *K-ras* tumors (P=0.01 for the interaction between *K-ras*–mutation status and the assigned treatment).

PROGRESSION-FREE SURVIVAL

Among patients with mutated *K-ras* tumors, the median progression-free survival was 1.8 months in both the cetuximab and supportive-care groups (hazard ratio for progression or death in the cetuximab group as compared with the supportive-care group, 0.99; 95% CI, 0.73 to 1.35; P=0.96) (Fig. 2A). The precipitous drop in progression-free survival at 8 weeks was consistent with the tumor-imaging schedule mandated by the protocol. For patients with wild-type *K-ras* tumors, the median progression-free survival was 3.7 months in the cetuximab group and 1.9 months in the supportive-care group (hazard ratio for progression or death in the cetuximab group as compared with the supportive-care group, 0.40; 95% CI, 0.30 to 0.54; P<0.001) (Fig. 2B). This difference remained significant after adjustment for potential prognostic factors specified in the protocol (adjusted hazard ratio, 0.42; 95% CI, 0.30 to 0.58; P<0.001). The effect of cetuximab on progression-free survival was significantly greater among the patients with wild-type *K-ras* tumors than among those with mutated *K-ras* tumors (P<0.001 for the interaction between *K-ras*–mutation status and the assigned treatment).

RESPONSE TO TREATMENT

None of the patients in the supportive-care group had an objective tumor response. In the cetuximab group, the response rate among patients with wild-type K-ras tumors was 12.8%, whereas only one patient with a mutated *K-ras* tumor (1.2%) had a response. Significantly more patients with wild-type *K-ras* tumors than patients with mutated *K-ras* tumors had a rash during cetuximab treatment or within 30 days after completion of the treatment (94.9% vs. 84.0%, P=0.02).

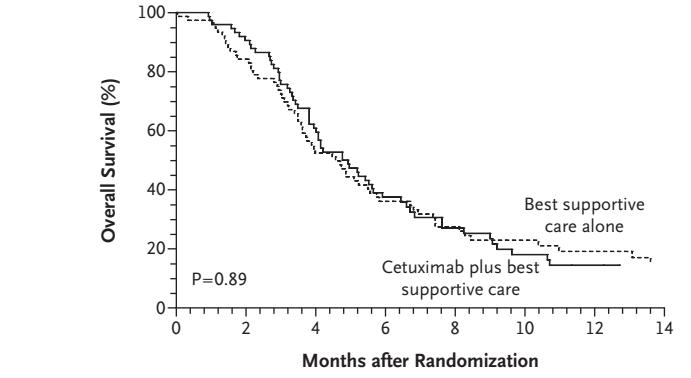
QUALITY OF LIFE

Among patients with wild-type *K-ras* tumors, those in the cetuximab group had an improvement in global health status at 8 weeks, whereas those in the supportive-care group had a deterioration in global health status (mean change in score, 3.2 vs.

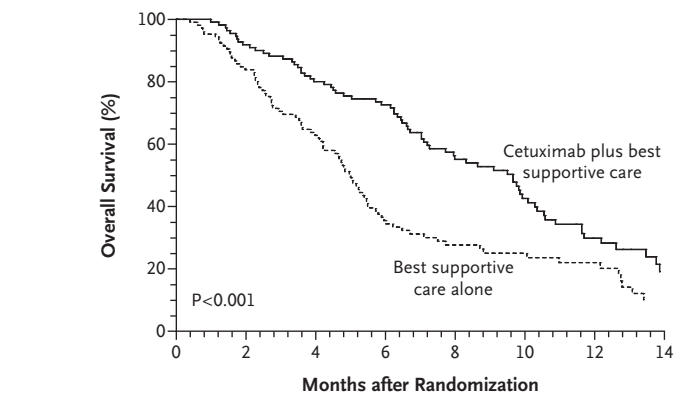
Table 2. Characteristics of Patients Included in the Analysis for *K-ras* Mutation.*

Characteristic	All Patients Who Underwent Randomization (N=572)	Patients with Mutated *K-ras* (N=164)	Patients with Wild-Type *K-ras* (N=230)	P Value
Age				
Median — yr	63.2	62.0	63.5	0.57
Range — yr	28.6–88.1	37.4–88.1	28.6–85.9	
<65 yr — no. (%)	335 (58.6)	99 (60.4)	133 (57.8)	
≥65 yr — no. (%)	237 (41.4)	65 (39.6)	97 (42.2)	
Sex — no. (%)				0.20
Female	204 (35.7)	63 (38.4)	74 (32.2)	
Male	368 (64.3)	101 (61.6)	156 (67.8)	
ECOG performance status — no. (%)				0.70
0	136 (23.8)	34 (20.7)	56 (24.3)	
1	302 (52.8)	94 (57.3)	127 (55.2)	
2	134 (23.4)	36 (22.0)	47 (20.4)	
Site of primary cancer — no. (%)				0.41
Colon only	332 (58.0)	108 (65.9)	137 (59.6)	
Rectum only	133 (23.3)	32 (19.5)	50 (21.7)	
Colon and rectum	107 (18.7)	24 (14.6)	43 (18.7)	
Any previous radiotherapy — no. (%)	202 (35.3)	50 (30.5)	77 (33.5)	0.53
Previous chemotherapy — no. (%)				
Adjuvant therapy	211 (36.9)	57 (34.8)	83 (36.1)	0.79
No. of regimens				0.70
1 or 2	104 (18.2)	27 (16.5)	46 (20.0)	
3	217 (37.9)	69 (42.1)	86 (37.4)	
4	159 (27.8)	46 (28.0)	63 (27.4)	
≥5	92 (16.1)	22 (13.4)	35 (15.2)	
Thymidylate synthase inhibitor	572 (100.0)	164 (100.0)	230 (100.0)	
Irinotecan	550 (96.2)	161 (98.2)	219 (95.2)	0.12
Oxaliplatin	559 (97.7)	163 (99.4)	222 (96.5)	0.06
Sites of disease — no. (%)				
Liver	463 (80.9)	129 (78.7)	189 (82.2)	0.38
Lung	368 (64.3)	98 (59.8)	144 (62.6)	0.57
Nodes	247 (43.2)	64 (39.0)	103 (44.8)	0.25
Ascites	86 (15.0)	23 (14.0)	38 (16.5)	0.50
No. of sites of disease — no. (%)				0.27
1	93 (16.3)	27 (16.5)	40 (17.4)	
2	153 (26.7)	45 (27.4)	63 (27.4)	
3	173 (30.2)	42 (25.6)	75 (32.6)	
≥4	153 (26.7)	50 (30.5)	52 (22.6)	
Treatment — no. (%)				0.77
Cetuximab plus supportive care	287 (50.2)	81 (49.4)	117 (50.9)	
Supportive care alone	285 (49.8)	83 (50.6)	113 (49.1)	

* P values, which are for the comparison of patients who had mutated *K-ras* tumors with patients who had wild-type *K-ras* tumors, were calculated with the use of the chi-square test for categorical variables and Student's t-test for continuous variables. ECOG denotes Eastern Cooperative Oncology Group.



No. at Risk								
Cetuximab plus best supportive care	75	67	45	26	15	10	7	4
Best supportive care alone	76	64	39	26	19	12	10	7



No. at Risk								
Cetuximab plus best supportive care	110	101	88	75	48	31	19	8
Best supportive care alone	105	88	65	34	23	17	12	5

Figure 1. Kaplan–Meier Curves for Overall Survival According to Treatment.
Panel A shows results for patients with mutated *K-ras* tumors, and Panel B for patients with wild-type *K-ras* tumors. Cetuximab as compared with best supportive care alone was associated with improved overall survival among patients with wild-type *K-ras* tumors but not among those with mutated *K-ras* tumors. The difference in treatment effect according to mutation status was significant (test for interaction, P=0.01).

–7.7 points; difference, 10.9; 95% CI, 4.2 to 17.6; P=0.002). The patients in the cetuximab group also had less deterioration at 16 weeks than did those in the supportive-care group (mean change in score, –0.2 vs. –18.1 points; difference, 17.9; 95% CI, 7.6 to 28.2; P<0.001). Among patients with mutated *K-ras* tumors, there was no significant difference in global health status between the cetuximab group and the supportive-care group at 8 weeks (mean change in score, –4.7 and –9.6 points, respectively; difference, 4.9; 95% CI, –4.2 to 14.0; P=0.53) or at 16 weeks (mean change in score, –9.5 and –13.9 points, respectively; difference, 4.4; 95% CI, –9.2 to 17.9; P=0.62).

EFFECT OF MUTATION STATUS IN THE SUPPORTIVE-CARE GROUP

In the supportive-care group, there was no significant difference in overall survival between patients with wild-type *K-ras* tumors and those with mutated *K-ras* tumors. As seen above, the median overall survival among the patients with mutated *K-ras* tumors was 4.6 months, as compared with 4.8 months among those with wild-type *K-ras* tumors, with 1-year overall survival rates of 19.6% and 20.1%, respectively (hazard ratio for death among patients with *K-ras* mutations, 1.01; 95% CI, 0.74 to 1.37; P=0.97) (Fig. 3). The median progression-free survival was 1.8 months and 1.9 months for patients with mutated and wild-type *K-ras* tumors, respectively (hazard ratio for progression, 1.12; 95% CI, 0.84 to 1.49; P=0.46). The differences remained nonsignificant after adjustment for other factors specified by the protocol (adjusted hazard ratio for death, 1.33; 95% CI, 0.95 to 1.86; P=0.10; adjusted hazard ratio for progression, 1.24; 95% CI, 0.90 to 1.70; P=0.19).

DISCUSSION

Our findings show that the mutation status of the *K-ras* gene is associated with overall survival among patients with advanced colorectal cancer who are being treated with cetuximab after previous chemotherapy has failed. Treatment with cetuximab as compared with supportive care alone was associated with almost a doubling of the median overall and progression-free survival among patients with wild-type *K-ras* tumors. There was no significant survival benefit from cetuximab, however, among patients with tumors that had *K-ras* mutations. Several small, retrospective studies have shown an association between *K-ras*–mutation status and responsiveness of a colorectal tumor to cetuximab.[5-7,13] Treatment with another monoclonal antibody directed against EGFR, panitumumab, has been compared with supportive care in a phase 3 trial.[14] A *K-ras* analysis showed, as with our findings, that the benefit associated with

panitumumab was limited to patients with wild-type *K-ras* tumors.[3] The group with wild-type *K-ras* tumors had a progression-free survival benefit from treatment with panitumumab, although an overall survival benefit was not shown.

We also examined the treatment-independent effect of *K-ras*–mutation status. The clinical significance of *K-ras* mutations has varied in reported studies, most of which have been compromised by a small sample size. In the largest reported series, *K-ras* mutations, as compared with wild-type *K-ras*, were associated with a risk of death that was increased by 26%. In particular, a mutation in *K-ras* codon 12 with substitution of valine for glycine (G12V), which was found in 8.6% of all patients, had a significant effect on failure-free and overall survival.[8,15] Mutations in *K-ras*, B-type Raf kinase (*BRAF*), or phosphatidylinositol 3-kinase (*PI3K*) genes were also significantly associated with shorter survival in another study, which involved 586 patients.[16] Less favorable clinical outcomes, particularly shorter survival, have been associated with the presence of *K-ras* mutations at codon 13.[11] No association with the outcome was found in a study of stage 3 colon cancer, but only 55 cases were examined.[17] In the randomized trial of panitumumab therapy as compared with supportive care, multivariate analysis confirmed that wild-type *K-ras* was significantly associated with overall survival, but since most patients in the supportive-care group crossed over and received the monoclonal antibody when their disease progressed, the significance of *K-ras*–mutation status was ultimately uncertain.[3]

We examined the association of *K-ras*–mutation status with survival among patients in the supportive-care group, in whom no effect of cetuximab would be expected. Such an analysis represents the best method for evaluating the significance of a variable, since its potential predictive effect is not relevant. We did not observe a significant difference in survival according to *K-ras*–mutation status in the supportive-care group.

Although *K-ras* mutations may be a common genetic aberration involved in carcinogenesis, mutations of other genes, such as phosphatase and tensin homologue (*PTEN*), *BRAF*, or *PI3K*, that can lead to unrestricted growth of cancer cells may also be useful biomarkers. Loss of PTEN activity, for example, has been associated with lack of efficacy of cetuximab in 11 patients with colorectal cancer.[7] Further upstream in the signaling pathway, high expression of EGFR ligands, particularly amphiregulin and epiregulin, has been observed in tumors that respond to cetuximab, and these ligands may be targets for new treatments.[18] Immunohistochemical studies suggest that EGFR,

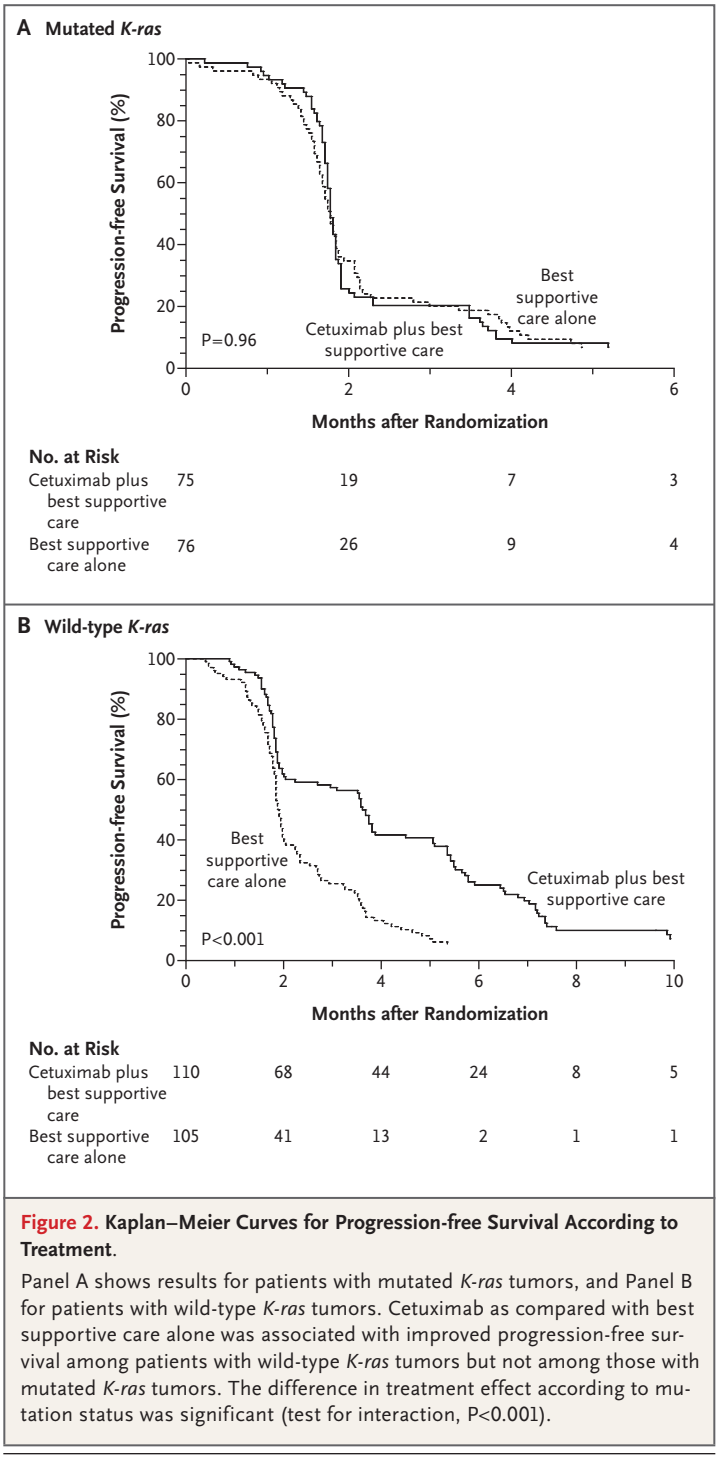


Figure 2. Kaplan–Meier Curves for Progression-free Survival According to Treatment.

Panel A shows results for patients with mutated *K-ras* tumors, and Panel B for patients with wild-type *K-ras* tumors. Cetuximab as compared with best supportive care alone was associated with improved progression-free survival among patients with wild-type *K-ras* tumors but not among those with mutated *K-ras* tumors. The difference in treatment effect according to mutation status was significant (test for interaction, P<0.001).

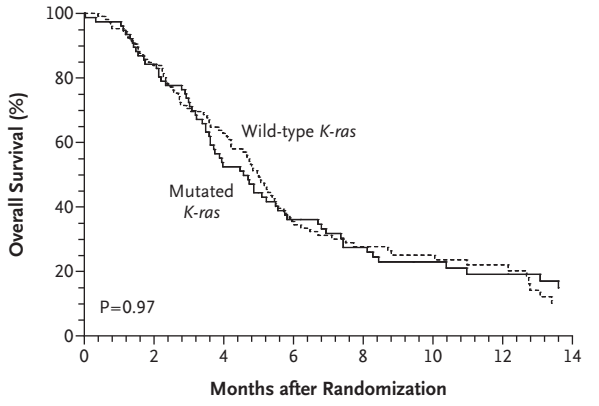


Figure 3. Kaplan–Meier Curves for Overall Survival According to *K-ras*–Mutation Status among Patients Receiving Supportive Care Alone.

the principal target of cetuximab, is not a useful predictive factor[19-22]; responses to cetuximab have been reported in patients with advanced colorectal cancers that do not have immunohistochemical evidence of EGFR expression.[23,24] The problems with immunohistochemical assessment include inaccuracy of and variations in the procedure, heterogeneity of EGFR expression in the tumor, and variable affinity of EGFR for cetuximab.[25,26] Assessment of the EGFR copy number may be more reliable, but the findings have been inconsistent.[27-29] These discrepant results highlight the need for standardization of the measurement of EGFR expression and activity.

The inactivation of the effect of cetuximab by *K-ras* mutation is biologically plausible and is supported by previous studies. A lack of activity of therapy directed against EGFR, particular tyrosine kinase inhibitors, has also been shown in patients who have non–small-cell lung cancer with *K-ras* mutations.[30-32] Our analysis was focused on *K-ras* and was based on a statistical plan that was specified before the assessment of *K-ras* status was performed. Evaluation of multiple correlative biomarkers was not performed.

Treatment with cetuximab, which is relatively expensive, would be most cost-effective if it were given to patients with the highest chance of benefiting from it. Our analysis identified a biomarker that would effectively exclude a clinically significant proportion of patients with colorectal cancer — those with tumors bearing *K-ras* mutations (42%) — from receipt of a therapy offering little prospect of a benefit. Nevertheless, there were also patients with wild-type *K-ras* tumors who did not have a response to cetuximab and in whom the tumor rapidly progressed. Additional reliable and easily measured biomarkers are clearly needed to improve the identification of patients who will benefit from treatment with cetuximab.

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REFERENCES

1. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007;357:2040-8.
2. Baselga J. The EGFR as a target for anticancer therapy — focus on cetuximab. Eur J Cancer 2001;37:Suppl 4:S16-S22.
3. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:1626-34.
4. Fransén K, Klintenäs M, Osterström A, Dimberg J, Monstein HJ, Söderkvist P. Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas. Carcinogenesis 2004;25:527-33.
5. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol 2008;19:508-15.
6. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. Br J Cancer 2007;96:1166-9.
7. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 2007;97:1139-45.
8. Andreyev HJ, Ross PJ, Cunningham D, Clarke PA. Antisense treatment directed against mutated Ki-ras in human colorectal adenocarcinoma. Gut 2001;48:230-7.
9. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 2007;67:2643-8.
10. Esteller M, Gonzalez S, Risques RA, et al. K-ras and p16 aberrations confer poor prognosis in human colorectal cancer. J Clin Oncol 2001;19:299-304.
11. Bazan V, Agnese V, Corsale S, et al. Specific TP53 and/or Ki-ras mutations as independent predictors of clinical outcome in sporadic colorectal adenocarcinomas: results of a 5-year Gruppo Oncologico

dell'Italia Meridionale (GOIM) prospective study. Ann Oncol 2005;16:Suppl 4:iv50-iv5.
12. Osoba D, Rodrigues G, Myles J, Zee B, Pater J. Interpreting the significance of changes in health-related quality-of-life scores. J Clin Oncol 1998;16:139-44.
13. Lièvre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 2008;26:374-9.
14. Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007;25:1658-64.
15. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst 1998;90:675-84.
16. Barault L, Veyrie N, Jooste V, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer 2008;122:2255-9.
17. Bleeker WA, Hayes VM, Karrenbeld A, et al. Impact of KRAS and TP53 mutations on survival in patients with left- and right-sided Dukes' C colon cancer. Am J Gastroenterol 2000;95:2953-7.
18. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25:3230-7.
19. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337-45.
20. Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 2004;22:1201-8.
21. Zhang W, Gordon M, Press OA, et al. Cyclin D1 and epidermal growth factor polymorphisms associated with survival in patients with advanced colorectal cancer treated with cetuximab. Pharmacogenet Genomics 2006;16:475-83.
22. Wierzbicki RD, Jonker DJ, Moore MJ, et al. A phase II multicenter study of cetuximab monotherapy in patients with EGFR-undetectable refractory metastatic colorectal carcinoma (mCRC). J Clin Oncol 2008;26:Suppl:15S. abstract.
23. Hebbar M, Wacrenier A, Desauw C, et al. Lack of usefulness of epidermal growth factor receptor expression determination for cetuximab therapy in patients with colorectal cancer. Anticancer Drugs 2006;17:855-7.
24. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005;23:1803-10.
25. Penault-Llorca F, Cayre A, Arnould L, et al. Is there an immunohistochemical technique definitively valid in epidermal growth factor receptor assessment? Oncol Rep 2006;16:1173-9.
26. Francoual M, Etienne-Grimaldi MC, Formento JL, et al. EGFR in colorectal cancer: more than a simple receptor. Ann Oncol 2006;17:962-7.
27. Lièvre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66:3992-5.
28. Moroni M, Veronese S, Benvenuti S, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. Lancet Oncol 2005;6:279-86.
29. Lenz HJ, Van Cutsem E, Khambata-Ford S, et al. Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. J Clin Oncol 2006;24:4914-21.
30. van Zandwijk N, Mathy A, Boerrigter L, et al. EGFR and KRAS mutations as criteria for treatment with tyrosine kinase inhibitors: retro- and prospective observations in non-small-cell lung cancer. Ann Oncol 2007;18:99-103.
31. Massarelli E, Varella-Garcia M, Tang X, et al. KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. Clin Cancer Res 2007;13:2890-6.
32. Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med 2005;2(1):e17.