EGFR Antagonists in Cancer Treatment

Fortunato Ciardiello, M.D., Ph.D., and Giampaolo Tortora, M.D., Ph.D.

Cancer cells may acquire the capacity for autonomous and dysregulated proliferation through the uncontrolled production of specific molecules that promote cell growth (growth factors) or through abnormal, enhanced expression of specific proteins (growth factor receptors) on the cell membranes to which growth factors selectively bind. Both processes trigger a series of intracellular signals that ultimately lead to the proliferation of cancer cells, induction of angiogenesis, and metastasis. The majority of human epithelial cancers are marked by functional activation of growth factors and receptors of the epidermal growth factor receptor (EGFR) family. Given this phenomenon, EGFR was the first growth factor receptor to be proposed as a target for cancer therapy. After 20 years of drug development, four EGFR antagonists are currently available for the treatment of four metastatic epithelial cancers: non–small-cell lung cancer, squamous-cell carcinoma of the head and neck, colorectal cancer, and pancreatic cancer. Less information is available about the use of EGFR antagonists in the treatment of earlier stages of cancer. This article summarizes the mechanisms of action of EGFR inhibitors, presents the clinical evidence of their anticancer activity, and considers the current, and controversial, clinical issues with respect to their optimal use in the treatment of patients with cancer.

EGFR in Human Carcinogenesis

EGFR is a transmembrane receptor belonging to a family of four related proteins (Fig. 1). Ten different ligands can selectively bind to each receptor. After a ligand binds to a single-chain EGFR, the receptor forms a dimer that signals within the cell by activating receptor autophosphorylation through tyrosine kinase activity. Autophosphorylation triggers a series of intracellular pathways that may result in cancer-cell proliferation, blocking apoptosis, activating invasion and metastasis, and stimulating tumor-induced neovascularization.

Development of EGFR Antagonists for Anticancer Therapy

The first anti-EGFR drugs were developed in the 1980s. Two classes of EGFR antagonists have been successfully tested in phase 3 trials and are now in clinical use: anti-EGFR monoclonal antibodies and small-molecule EGFR tyrosine kinase inhibitors (Tables 1 and 2).

Anti-EGFR monoclonal antibodies, such as cetuximab, bind to the extracellular domain of EGFR when it is in the inactive configuration, compete for receptor binding by occluding the ligand-binding region, and thereby block ligand-induced EGFR tyrosine kinase activation. Small-molecule EGFR tyrosine kinase inhibitors, such as erlotinib and gefitinib, compete reversibly with ATP to bind to the intracellular catalytic domain of EGFR tyrosine kinase and, thus, inhibit EGFR autophosphorylation and downstream signaling. Anti-EGFR monoclonal antibodies recognize EGFR...
exclusively and are therefore highly selective for this receptor. In addition, various small-molecule EGFR tyrosine kinase inhibitors can block different growth factor receptor tyrosine kinases, including other members of the EGFR family, or the vascular endothelial growth factor receptor. Various irreversible EGFR tyrosine kinase inhibitors are now in early stages of clinical development.\textsuperscript{4,5,12} The mechanism (or mechanisms) of action, pharmacologic effects, and spectrum of activity of anti-EGFR monoclonal antibodies and small-molecule EGFR tyrosine kinase inhibitors have differences that may be relevant for clinical activity (Table 1 and Fig. 2 and 3).\textsuperscript{13}
More than 10 EGFR-targeting agents are in advanced clinical development for the treatment of various human cancer types.\textsuperscript{5,10,11,12} Two anti-EGFR monoclonal antibodies (cetuximab and panitumumab) and two small-molecule, reversible EGFR tyrosine kinase inhibitors (gefitinib and erlotinib) have been approved in several countries for the treatment of metastatic non–small-cell lung cancer, colorectal cancer, squamous-cell carcinoma of the head and neck, and pancreatic cancer (Table 2).\textsuperscript{20-24} (For relevant clinical studies supporting the use of anti-EGFR drugs in the first three conditions, see Tables 1, 2, and 3 in the Supplementary Appendix, available with the full text of this article at www.nejm.org.)

### Table 2. Functional and Pharmacologic Characteristics of EGFR Inhibitors.\textsuperscript{*}

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Blocking Monoclonal Antibodies</th>
<th>Small-Molecule Tyrosine Kinase Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of administration</td>
<td>Intravenous (generally once a week or every 2 wk)</td>
<td>Oral (generally daily continuous dosing)</td>
</tr>
<tr>
<td>Structure</td>
<td>Recombinant immunoglobulins (150–180 kD)</td>
<td>Low-molecular-weight compounds (400–600 kD)</td>
</tr>
<tr>
<td>Target selectivity</td>
<td>Exclusively specific for EGFR</td>
<td>Relatively specific for EGFR; may inhibit only one or all EGFR family receptors; some EGFR tyrosine kinase inhibitors also inhibit other growth factor receptors (e.g., dual inhibitors of EGFR and VEGFR)</td>
</tr>
<tr>
<td>Mechanism of interference with EGFR activation</td>
<td>Bind extracellular portion of receptor, preventing ligand binding and receptor dimerization by occluding ligand region (cetuximab)</td>
<td>Bind intracellular portion of receptor within tyrosine kinase domain, generally by competing with ATP and inhibiting receptor autophosphorylation; most are reversible; irreversible EGFR tyrosine kinase inhibitors are in clinical development</td>
</tr>
<tr>
<td>Cellular effects of EGFR inhibition</td>
<td>Inhibit cancer-cell proliferation (G1 phase arrest), angiogenic growth factor production (VEGF) and tumor-induced angiogenesis, and cancer-cell invasion; potentiate antitumor activity of cytotoxic drugs and radiotherapy</td>
<td>Inhibit cancer-cell proliferation (G0–G1 phase arrest), angiogenic growth factor production (VEGF) and tumor-induced angiogenesis, and cancer-cell invasion; potentiate antitumor activity of cytotoxic drugs and radiotherapy</td>
</tr>
<tr>
<td>Induction of EGFR internalization, down-regulation, and degradation</td>
<td>Yes</td>
<td>No (although irreversible EGFR tyrosine kinase inhibitors can cause EGFR degradation and subsequent EGFR down-regulation)</td>
</tr>
<tr>
<td>Inhibition of EGFR-dependent intracellular signaling</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Activity against mutant EGFR proteins</td>
<td>Probably yes, for mutations of EGFR tyrosine kinase domain, since anti-EGFR monoclonal antibodies bind to EGFR extracellular domain; not completely known for mutations of EGFR extracellular domain</td>
<td>Yes, for most mutations of EGFR tyrosine kinase domain (mutation in codons 746–750 in exon 19 and L858R in exon 21), since these EGFR mutant proteins bind with higher-affinity small-molecule EGFR tyrosine kinase inhibitors, such as erlotinib or gefitinib; no, for gefitinib- or erlotinib-acquired EGFR resistance mutation (T790M in exon 20), although several new-generation EGFR tyrosine kinase inhibitors that are active against mutant EGFR proteins are in early clinical development</td>
</tr>
<tr>
<td>Activation of host immune response</td>
<td>Yes — antibody-dependent cytotoxicity may significantly contribute to anticancer activity of some anti-EGFR monoclonal antibodies; such as cetuximab; however, no antibody-dependent cytotoxicity has been reported for panitumumab</td>
<td>No</td>
</tr>
</tbody>
</table>

* EGFR denotes epidermal growth factor receptor, VEGF vascular endothelial growth factor, and VEGFR VEGF receptor.
metastatic, chemoresistant non–small-cell lung cancer.\textsuperscript{25-29} Dose-dependent and reversible diarrhea and acneiform rash have been the most prominent side effects (maximum tolerated dose, 750 mg per day for gefitinib and 150 mg per day for erlotinib). The histologic characteristics of the rash (a neutrophilic infiltrate in perifollicular areas within the basal layer of the skin) differ from those seen in typical acne and are common to all EGFR-targeted drugs, including anti-EGFR monoclonal antibodies.\textsuperscript{30} Skin toxicity is generally observed within 2 to 3 weeks after the start of treatment and gradually resolves in most patients, even when anti-EGFR treatment is continued. The maximum tolerated dose of erlotinib (150 mg per day), based on side effects, was chosen for further study, whereas for gefitinib, relatively low doses (patients were randomly assigned to receive 250 mg or 500 mg per day), given the maximum tolerated doses, were chosen.

Gefitinib was the first anti-EGFR agent that was shown, in two randomized phase 2 studies, to have clinically important antitumor activity in patients with non–small-cell lung cancer who had not had a response to one or more chemotherapy regimens, including platinum-based and docetaxel-based therapies.\textsuperscript{30-32} The two doses of gefitinib (250 mg and 500 mg) had similar antitumor activity, but toxicity was greater at the higher dose. Therefore, the lower dose was selected for further clinical studies. These trials led the Food and Drug Administration (FDA) in May 2003 to approve gefitinib as third-line therapy for patients with locally advanced or metastatic non–small-cell lung cancer after failure of both platinum-based and docetaxel-based chemotherapies.

However, a placebo-controlled, randomized phase 3 trial (the Iressa Survival Evaluation in Lung Cancer [ISEL] trial) failed to show that gefitinib was effective in improving survival.\textsuperscript{33} Neither median survival nor the rate of survival at 1 year differed significantly between patients receiving gefitinib and those receiving placebo in either the overall study population or a subgroup with a history of adenocarcinoma.

**Table 2. EGFR Inhibitors Currently Approved for Cancer Treatment.\textsuperscript{34}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molecular Properties</th>
<th>Approved Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erlotinib</td>
<td>Reversible EGFR tyrosine kinase inhibitor (quinazoline-derivative molecule)</td>
<td>Erlotinib has been approved by several regulatory agencies worldwide, including the FDA and the EMEA in the European Union, as monotherapy for the treatment of non–small-cell lung cancer that is refractory to platinum-based chemotherapy. More recently, erlotinib has been approved by the FDA and the EMEA for use in combination with gemcitabine as first-line treatment for advanced pancreatic cancer.</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>Reversible EGFR tyrosine kinase inhibitor (quinazoline-derivative molecule)</td>
<td>Gefitinib has been approved in various countries for use as third-line treatment of non–small-cell lung cancer that is refractory to platinum-based and docetaxel-based chemotherapy regimens. After an accelerated approval process, it was approved by the FDA in May 2003 but has been withheld from the U.S. market since June 2005, as a result of the release of preliminary results of the ISEL trial, which assessed its use in patients with non–small-cell lung cancer that was refractory to previous platinum-based chemotherapy. Gefitinib has never been approved in the European Union but is currently on the market in Japan, Korea, China, and several other Asian countries. It is currently an investigational drug in the United States and the European Union.</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Human–mouse chimeric monoclonal antibody (IgG1 subtype)</td>
<td>Cetuximab has been approved by several regulatory agencies worldwide, including the FDA and the EMEA, for the treatment of advanced colorectal cancer that is refractory to irinotecan-based chemotherapy (alone or in combination with irinotecan in the United States but only in combination with irinotecan in the European Union). Cetuximab in combination with radiotherapy is also approved for the treatment of locally advanced squamous-cell carcinoma of the head and neck.</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>Fully human monoclonal antibody (IgG2k subtype)</td>
<td>Panitumumab has been approved by several regulatory agencies worldwide, including the FDA, as monotherapy for third-line treatment of colorectal cancer that is refractory to irinotecan-based chemotherapy, and the EMEA, for the treatment of advanced colorectal cancer that is refractory to previous platinum-based and docetaxel-based chemotherapies. Panitumumab was approved by the EMEA for use in patients with colorectal cancer who carry a normal, wild-type K-RAS gene.</td>
</tr>
</tbody>
</table>

\textsuperscript{34} EGFR denotes epidermal growth factor receptor, EMEA European Medicines Evaluation Agency, FDA Food and Drug Administration, and ISEL Iressa Survival Evaluation in Lung Cancer.
planned subgroup analysis showed a significant survival benefit only in patients of Asian origin and in those who had never smoked. In June 2005, on the basis of the lack of a survival benefit in the ISEL study, the FDA restricted the use of gefitinib to patients participating in a clinical trial or continuing to benefit from treatment already initiated. Currently, gefitinib is marketed in several countries in eastern Asia but is not available in the United States or the European Union.

More recently, two randomized phase 3 trials evaluated the effectiveness of gefitinib monotherapy as compared with that of standard chemotherapy (docetaxel) as second-line treatment for chemotherapy-refractory non–small-cell lung cancer. The V-15-32 trial, conducted in Japan, failed to demonstrate the noninferiority of gefitinib in terms of overall survival, which was the primary end point. However, in a large multicenter trial, this end point was achieved with gefitinib after platinum-based therapy had failed. In addition, the side-effect profile appeared to favor gefitinib.

In a phase 2 study, the antitumor activity of erlotinib as a single agent in heavily pretreated non–small-cell lung cancer was similar to that of gefitinib. More important, in the BR.21 trial, a phase 3, randomized, double-blind, placebo-controlled study involving patients with pretreated non–small-cell lung cancer, erlotinib increased median survival by approximately 2 months as compared with placebo (Table 3). Responses were significantly more frequent in women, in patients with adenocarcinoma, and in patients with no history of smoking. However, a significant survival advantage was observed in all patient subgroups after treatment with erlotinib as compared with placebo. Quality-of-life analysis supported the palliative benefit of erlotinib in extending the time during which patients were free of symptoms (cough, dyspnea, and pain). On the basis of these results, erlotinib was approved by the FDA in November 2004 and by the European Medicines Evaluation Agency (EMEA) in October 2005 for second- and third-line treatment of chemotherapy-resistant, advanced non–small-cell lung cancer. Several hypotheses have been proposed as to why the efficacy seems different for gefitinib and erlotinib in the similar BR.21 and ISEL phase 3 studies. One possible explanation is dosing: erlotinib was used at the maximum tolerated dose, whereas gefitinib was provided at a much lower dose.

On the basis of preclinical data demonstrating that anti-EGFR drugs potentiate the antitumor activity of cytotoxic drugs, four phase 3, double-blind, placebo-controlled, randomized clinical trials examined the combination of erlotinib or gefitinib with chemotherapy as first-line treatment for non–small-cell lung cancer. Two standard platinum-based, dual-drug regimens were used in combination with erlotinib or gefitinib. Neither a survival advantage nor a benefit with respect to the response rate or time to progression was seen with the addition of gefitinib or erlotinib to chemotherapy in any of these trials. One possible reason that these trials failed to demonstrate any advantage of gefitinib or erlotinib is that they were conducted in...
unselected patients with non–small-cell lung cancer.\textsuperscript{44} Since only a subgroup of EGFR-positive patients with non–small-cell lung cancer have tumors that are dependent on the EGFR pathway, few patients with this type of cancer would have a clinical benefit from the addition of an anti-EGFR drug to chemotherapy.\textsuperscript{44} In addition, a retrospective subgroup analysis suggested that the addition of erlotinib to carboplatin and paclitaxel significantly prolonged survival only in the subgroup of patients who had never smoked.\textsuperscript{42}

Cetuximab treatment is said to have relatively few side effects. The most common adverse events include skin toxicity (flushing, an acnelike rash, and folliculitis), fever and chills, asthenia, transient elevations in aminotransferase levels, and nausea.\textsuperscript{45} Approximately 1.5% of patients have infusion reactions, which include allergic reactions requiring discontinuation of therapy; this rate is in keeping with the use of a chimeric human–mouse monoclonal antibody. Whereas cetuximab is marginally active as a single agent in advanced non–small-cell lung cancer, most phase 2 studies suggest that adding cetuximab to platinum-based therapies is of clinical benefit.\textsuperscript{46–50} A large, multicenter, randomized, phase 3 study in which cetuximab was added to standard platinum-based chemotherapy (cisplatin and vinorelbine) has recently been completed (ClinicalTrials.gov number, NCT00148798). A more thorough evaluation of the role of cetuximab in the treatment of advanced non–small-cell lung cancer awaits publication of the results of this trial.

**COLORECTAL CANCER**

Cetuximab has been evaluated in both chemotherapy-refractory and untreated metastatic colorectal...
paring the use of cetuximab with best supportive therapy. Recently, a randomized phase 3 trial com-
treatment after the failure of irinotecan mono-
half the patients crossed over to cetuximab
seen in overall survival, probably because almost
achieved when cetuximab was used in combina-
toxicity and diarrhea are the most common side effects of this
agent. A randomized phase 3 clinical trial com-
pared the use of panitumumab with the best
supportive care for patients in whom all available drugs,
including fluoropyrimidines, oxaliplatin, and irino-
tocan, had failed showed that cetuximab increased
progression-free survival, overall survival, and
goodness of life (Table 4).
Cetuximab appears to be the only drug that does so with colorectal
cancer who have had unsuccessful courses of all currently
available chemotherapies.

Phase 2 studies indicate that cetuximab
A multicenter, randomized, phase 2 trial evalu-
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The cetuximab–irinotecan combination was sig-
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cancer, either in combination with irino-
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irinotecan.

A multicenter, randomized, phase 3 trial ex-
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who cannot tolerate irinotecan). The EMEA has
approved cetuximab only in combination with
irinotecan.
The combination of cetuximab and radiotherapy was initially tested in patients with previously untreated, locally advanced squamous-cell carcinoma of the head and neck in a randomized, phase 3 clinical trial. Patients were treated with radiotherapy alone or in combination with cisplatin in the treatment of this disease.

Several phase 2 studies evaluated cetuximab alone or in combination with cisplatin in the treatment of platinum-resistant squamous-cell carcinoma of the head and neck. In the first-line treatment of recurrent or metastatic squamous-cell carcinoma of the head and neck, a cancer in which no specific therapy has been effective; such patients have a very short life expectancy. The overall response rate with cetuximab monotherapy was 10 to 13%, with a disease-control rate of approximately 50%.

Radiotherapy in showing a survival benefit for a novel treatment as compared with platinum-based chemotherapy and best supportive care. On the basis of these results, panitumumab was approved by the FDA in September 2006 as monotherapy for the treatment of metastatic colorectal cancer with disease progression after chemotherapy regimens consisting of a fluoropyrimidine, oxaliplatin, and irinotecan.

Table 4. Efficacy of Cetuximab in Chemotherapy-Refractory Colorectal Cancer.†

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cetuximab (N=111)†</th>
<th>Cetuximab plus Irinotecan (N=218)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
<th>Best Supportive Care (N=285)</th>
<th>Cetuximab (N=287)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOND trial‡</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall response rate (%)§</td>
<td>10.8</td>
<td>22.9</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median time to progression (mo)</td>
<td>1.5</td>
<td>4.1</td>
<td>0.54 (0.42–0.71)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Median overall survival (mo)</td>
<td>6.9</td>
<td>8.6</td>
<td>0.91 (0.68–1.21)</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCIC-CO.17 trial¶</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall response rate (%)§</td>
<td>0</td>
<td>8</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median progression-free survival (mo)</td>
<td>1.8</td>
<td>1.9</td>
<td>0.68 (0.57–0.80)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Median overall survival (mo)</td>
<td>4.6</td>
<td>6.17</td>
<td>0.77 (0.64–0.92)</td>
<td>0.005</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

† CI denotes confidence interval, and NS not significant.
‡ Crossover to cetuximab plus irinotecan was allowed after progression in 56 patients (50%) treated initially with cetuximab alone.
§ The Bowel Oncology with Cetuximab Antibody (BOND) trial was a randomized phase 2 trial. Patients with metastatic, irinotecan-refractory colorectal cancer were treated either with cetuximab alone (intravenous loading dose of 400 mg per square meter of body-surface area, followed by weekly intravenous doses of 250 mg per square meter) or with cetuximab (loading dose of 400 mg per square meter followed by 250 mg per square meter weekly) plus irinotecan until disease progression. Approximately two thirds of the patients had also received a line of treatment for metastatic disease with an oxaliplatin-based therapy before study entry. Data are from Cunningham et al.54
¶ The NCIC-CO.17 trial was a randomized phase 3 trial. Patients with metastatic colorectal cancer that was refractory to fluorouracil, irinotecan, and oxaliplatin were treated either with cetuximab alone (intravenous loading dose of 400 mg per square meter, followed by weekly intravenous doses of 250 mg per square meter) or with best supportive care until disease progression. Crossover to cetuximab was not allowed after progression in the group that received best supportive care. Data are from Jonker et al.55
Table 5. Efficacy of Cetuximab in Squamous-Cell Carcinoma of the Head and Neck.*

<table>
<thead>
<tr>
<th>Study</th>
<th>Radiotherapy (N = 213)</th>
<th>Radiotherapy plus Cetuximab (N = 211)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
<th>Chemotherapy (N = 220)</th>
<th>Chemotherapy plus Cetuximab (N = 222)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonner et al.;‡</td>
<td>Overall response rate (%)‡</td>
<td>64</td>
<td>74</td>
<td>0.57 (0.36–0.90)</td>
<td>0.02</td>
<td>Overall response rate (%)‡</td>
<td>19.5</td>
<td>35.6</td>
</tr>
<tr>
<td></td>
<td>Median locoregional control</td>
<td>14.9</td>
<td>24.4</td>
<td>0.68 (0.52–0.89)</td>
<td>&lt;0.005</td>
<td>Median progression-free survival (mo)</td>
<td>12.4</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>Median overall survival (mo)</td>
<td>29.3</td>
<td>49.0</td>
<td>0.74 (0.57–0.97)</td>
<td>&lt;0.03</td>
<td>Median overall survival (mo)</td>
<td>7.4</td>
<td>10.1</td>
</tr>
<tr>
<td>EXTREME trial§</td>
<td>Overall response rate (%)‡</td>
<td>19.5</td>
<td>35.6</td>
<td>0.54 (0.43–0.67)</td>
<td>&lt;0.001</td>
<td>Median progression-free survival (mo)</td>
<td>3.3</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>Median overall survival (mo)</td>
<td>7.4</td>
<td>10.1</td>
<td>0.80 (0.64–0.98)</td>
<td>&lt;0.001</td>
<td>Median overall survival (mo)</td>
<td>7.4</td>
<td>10.1</td>
</tr>
</tbody>
</table>

* CI denotes confidence interval.
† This study was a randomized phase 3 trial of radiotherapy alone or radiotherapy plus cetuximab in locally advanced disease. Patients with locally advanced squamous-cell carcinoma of the head and neck were treated either with radiotherapy alone or with radiotherapy plus cetuximab (intravenous loading dose of 400 mg per square meter of body-surface area, followed by weekly intravenous doses of 250 mg per square meter for the duration of radiotherapy).
‡ The overall response rate includes complete and partial responses.
§ The Erbitux in First-Line Treatment of Recurrent or Metastatic Head and Neck Cancer (EXTREME) trial was a randomized phase 3 trial of chemotherapy alone or chemotherapy plus cetuximab as first-line treatment in metastatic disease. Patients with metastatic squamous-cell carcinoma of the head and neck were treated with platinum–fluorouracil chemotherapy or with the same chemotherapy plus cetuximab (intravenous loading dose of 400 mg per square meter, followed by weekly intravenous doses of 250 mg per square meter) until disease progression. Crossover to cetuximab was not allowed after progression in the group that received chemotherapy alone. Data are from Vermorken et al.⁶²

Since only a subgroup of patients with cancer who have a clinical benefit from treatment with EGFR inhibitors, there is an urgent need for identification and validation of useful criteria for selecting patients for such treatment. A series of studies suggests that considering certain clinicopathological characteristics might help to identify patients whose cancers could be either sensitive or resistant to anti-EGFR therapy. Although the increase in survival could be considered modest in absolute terms, it showed that there is a significant advantage in the treatment of metastatic pancreatic cancer — a unique finding.
The discovery that certain somatic mutations within the tyrosine kinase, ATP-binding domain of the EGFR gene are associated with a response to EGFR tyrosine kinase inhibitors in non–small-cell lung cancer suggested that the selection of patients through molecular screening might be feasible. Approximately 90% of EGFR mutations affect small regions of the gene within exons (18 to 24) that code for the EGFR tyrosine kinase domain.

**SOMATIC EGFR GENE MUTATIONS**

The most common mutations are an in-frame deletion in exon 19 around codons 746 to 750 (accounting for 45 to 50% of EGFR mutations) and a missense mutation leading to a substitution of arginine for leucine at codon 858 (L858R) in exon 21 (35 to 45% of EGFR mutations). Somatic EGFR mutations are found in approximately 5 to 15% of unselected white patients and in 25 to 35% of unselected Asian patients with non–small-cell lung cancer. These mutations seem to be limited to non–small-cell lung cancer, since they have rarely been detected in other types of human cancer. Somatic mutations in the EGFR gene are most frequently detected in a subpopulation of patients with this form of cancer who have one or more of the following characteristics: histologic features of adenocarcinoma and, in particular, nonmucinous bronchioloalveolar carcinoma; an absence of a history of smoking; an absence of K-RAS gene mutations; Asian ethnicity; and female sex. The likelihood of EGFR mutations decreases as the exposure to tobacco smoke increases, leading to the hypothesis that lung adenocarcinoma in patients who have never smoked is a distinct form of non–small-cell lung cancer with a high frequency of EGFR mutations and increased sensitivity to EGFR tyrosine kinase inhibitors. The association between EGFR mutations and a response to erlotinib or gefitinib has been retrospectively confirmed in several clinical studies. It has been also suggested that this association translates into improved survival. However, in larger randomized studies, such as the BR.21 trial, a similar survival advantage was observed for patients treated with erlotinib, independently of the presence of EGFR mutations or of a wild-type EGFR gene, indicating that the presence of EGFR mutations is not the only biomarker for predicting a survival benefit of treatment with small-molecule EGFR tyrosine kinase inhibitors in patients with non–small-cell lung cancer.

**INCREASED EGFR COPY NUMBER**

The EGFR gene is rarely amplified in human cancers. However, fluorescence in situ hybridization (FISH) shows an increased EGFR copy number with balanced polysomy in a high proportion of cancer cells in approximately 25 to 40% of patients with non–small-cell lung cancer, squamous-cell carcinoma of the head and neck, or colorectal cancer. A single-group, phase 2 trial of...
treatment with gefitinib in advanced, chemotherapy-refractory non–small-cell lung cancer was the first to show that patients with FISH-positive tumors had significantly higher rates of response and survival than patients with FISH-negative tumors. In the BR.21 trial, patients with FISH-positive tumors (approximately 40% of the patients) who were randomly assigned to receive erlotinib had significantly longer survival as compared with patients with FISH-positive tumors who received placebo. In the patients with FISH-negative tumors, there was no significant difference in survival. Similar results were observed in the ISEL trial, which confirmed that patients with FISH-positive tumors who were treated with gefitinib had higher response rates and longer survival than patients receiving placebo. No difference in survival was seen in FISH-negative patients, irrespective of treatment. However, FISH analysis failed to demonstrate any difference in progression-free survival or overall survival in a phase 3 trial that compared gefitinib with docetaxel as second-line therapy for advanced non–small-cell lung cancer (the Iressa–Non–Small-Cell Cancer Trial Evaluating Response and Survival against Taxotere [INTEREST]).

The predictive role of increased EGFR copy numbers has also been evaluated in patients with metastatic colorectal cancer in a series of retrospective studies. The first report on the correlation between positive results for EGFR and a response to therapy with cetuximab or panitumumab involved a small cohort of patients (31 patients) with advanced, chemotherapy-refractory colorectal cancer. Recently, a FISH analysis of EGFR in tumor samples from patients enrolled in the phase 3 study comparing the use of panitumumab with best supportive care was reported. In the group treated with panitumumab, patients with normal EGFR copy numbers had a shorter median progression-free survival and overall survival than patients with high EGFR copy numbers. Moreover, in the group treated only with best supportive care, no correlation between EGFR copy numbers and survival was observed, suggesting a predictive rather than a prognostic role of this genetic feature in patients with metastatic colorectal cancer who are treated with anti-EGFR monoclonal antibodies.

RESISTANCE TO EGFR ANTAGONISTS

INTRINSIC RESISTANCE

Activating mutations in the K-RAS gene, which result in EGFR-independent activation of the mitogen-activated protein kinase pathway, are found in approximately 15 to 30% of patients with non–small-cell lung cancer and 40 to 45% of patients with colorectal cancer, and their presence generally correlates with a worse prognosis with respect to the outcome of the cancer. K-RAS mutations occur in patients with a history of substantial cigarette use. These mutations are most frequently recorded in codons 12 and 13 in the exon 2 of the K-RAS gene and are generally mutually exclusive with EGFR mutations. In several studies, K-RAS mutations have been significantly associated with lack of response to EGFR tyrosine kinase inhibitors in patients with non–small-cell lung cancer and with lack of response to cetuximab or to panitumumab in patients with advanced, chemotherapy-refractory colorectal cancer. Both findings suggest that EGFR-independent, constitutive activation of the K-RAS signaling pathway could impair the response to anti-EGFR drugs. However, no correlation between K-RAS mutations and efficacy was reported in the INTEREST trial, which compared docetaxel and gefitinib as second-line treatments for non–small-cell lung cancer. In contrast, the results of the phase 3 trial comparing the use of panitumumab with best supportive care in chemotherapy-refractory colorectal cancer have confirmed that the efficacy of panitumumab is limited to patients whose tumors carry the wild-type K-RAS gene.

ACQUIRED RESISTANCE

In patients with non–small-cell lung cancer that initially responds to gefitinib or erlotinib, an acquired resistance to EGFR inhibitors, resulting in treatment failure, is associated with the development of an additional EGFR mutation. The most extensively studied of such EGFR mutations occurs in exon 20, resulting in a substitution of methionine for threonine in codon 790 (T790M). This mutation causes a change in the tridimensional structure of the tyrosine kinase domain and prevents both erlotinib and gefitinib from binding to EGFR. According to a recent report, amplifi-
cation of the MET proto-oncogene could be involved in acquired resistance to EGFR tyrosine kinase inhibitors in patients with non–small-cell lung cancer.\textsuperscript{104} MET amplification leads to EGFR-independent activation of the PI3K–AKT pathway through activation of HER3-dependent signaling. In a gefitinib-sensitive lung-cancer cell line that developed resistance to gefitinib as a result of MET amplification, inhibition of MET signaling restored sensitivity to gefitinib.\textsuperscript{104} A pilot study of 18 tumor specimens from patients with non–small-cell lung cancer that had previously responded to gefitinib but that subsequently developed clinical resistance showed MET amplification in 4 of the tumors.\textsuperscript{104}

**Future Directions**

Appropriate selection of patients is a major challenge for the clinical use of EGFR antagonists. In fact, although long-lasting therapeutic responses have been observed even in patients with heavily pretreated, metastatic cancer, responses are observed in only 10 to 20\% of patients receiving these drugs.\textsuperscript{105} Cancer cells must express functional EGFRs to respond to these agents. An optimal response to EGFR antagonists also requires the EGFR-activated intracellular signal-transduction machinery to be intact. In addition, EGFR-dependent cancer cells may escape from EGFR-targeted growth inhibition by using alternative growth factor receptor pathways or by constitutively activating downstream intracellular signaling effectors,\textsuperscript{106–109} indicating the need for therapeutic strategies designed to overcome resistance to EGFR inhibitors.\textsuperscript{110–112} Several molecular predictors have been detected for identifying patients who would be most likely to benefit from treatment with anti-EGFR drugs. However, most available clinical data are from retrospective studies and subgroup analyses; there is an urgent need to validate these observations in properly designed prospective studies. Another clinical issue is the need to determine the most effective sequences and combinations of EGFR inhibitors to use with chemotherapy, radiotherapy, or both in order to optimize cytotoxicity potentiation. In fact, the schedules that have been tested so far have been based on the empirical association of a standard chemotherapy regimen with the continuous administration of an EGFR-targeting drug rather than derived from molecular, pharmacokinetic, and pharmacodynamic studies.

Supported in part by grants from Associazione Italiana per la Ricerca sul Cancro.

Dr. Ciardiello reports receiving consulting fees from Merck Serono, Roche, and AstraZeneca and lecture fees from Merck Serono, AstraZeneca, and GlaxoSmithKline; and Dr. Tortora, consulting and lecture fees from Merck Serono, Roche, and GlaxoSmithKline. Roche is the manufacturer of erlotinib (Tarceva). No other potential conflict of interest relevant to this article was reported.

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87. Panitumumab antitumor activity in pa-


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American Society of Clinical Oncology Provisional Clinical Opinion: Testing for KRAS Gene Mutations in Patients With Metastatic Colorectal Carcinoma to Predict Response to Anti–Epidermal Growth Factor Receptor Monoclonal Antibody Therapy


Abstract

Purpose

An American Society of Clinical Oncology (ASCO) provisional clinical opinion (PCO), offers timely clinical direction to ASCO’s oncologists following publication or presentation of potentially practice-changing data from major studies. This PCO addresses the utility of KRAS gene mutation testing in patients with metastatic colorectal carcinoma to predict response to anti–epidermal growth factor receptor (anti-EGFR) monoclonal antibody (MoAb) therapy with cetuximab or panitumumab (see Note).

Clinical Context

Recent results from phase II and III clinical trials demonstrate that patients with metastatic colorectal cancer benefit from therapy with monoclonal antibodies directed against the EGFR, when used either as monotherapy or combined with chemotherapy. Retrospective subset analyses of the data from these trials strongly suggest that patients who have KRAS mutations detected in codon 12 or 13 do not benefit from this therapy.

Recent Data

Five randomized controlled trials of cetuximab or panitumumab have evaluated outcomes for patients with metastatic colorectal carcinoma in relation to KRAS mutational status as no mutation detected (wild type) or abnormal (mutated). Another five single-arm studies have retrospectively evaluated tumor response according to KRAS status.

Provisional Clinical Opinion

Based on systematic reviews of the relevant literature, all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment.

NOTE. ASCO’s provisional clinical opinions (PCOs) reflect expert consensus based on clinical evidence and literature available at the time they are written, and are intended to assist physicians in clinical decision-making and identify questions and settings for further research. Due to the rapid flow of scientific information in oncology, new evidence may have emerged since the time a PCO was submitted for publication. PCOs are not continually updated and may not reflect the most recent evidence. PCOs cannot account for individual variation among patients, and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge of the patient, to determine the best course of treatment for the patient. Accordingly, adherence to any PCO is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient’s individual circumstances. ASCO PCOs describe the use of procedures and therapies in clinical practice and cannot be assumed to apply to the use of these interventions in the context of clinical trials. ASCO assumes no responsibility for any injury or damage to persons or property arising out of or related to any use of ASCO’s PCOs, or for any errors or omissions.

J Clin Oncol 27:2091-2096. © 2009 by American Society of Clinical Oncology
INTRODUCTION
The American Society of Clinical Oncology (ASCO) has established a rigorous, evidence-based approach—the provisional clinical opinion (PCO)—to offer a rapid response to emerging data in clinical oncology. The PCO is intended to offer timely clinical direction to ASCO’s oncologists following publication or presentation of potentially practice-changing data from major studies (Appendix).

This PCO addresses only the utility of testing for mutations in codons 12 or 13 of the KRAS gene in patients with metastatic colorectal carcinoma to predict response to anti–epidermal growth factor receptor (anti-EGFR) monoclonal antibody (MoAb) therapy, that is, cetuximab or panitumumab.

STATEMENT OF THE CLINICAL ISSUE
Results from phase II and III clinical trials of the anti-EGFR MoAbs cetuximab and panitumumab when used either as monotherapy or in combination with chemotherapy, have shown that patients with metastatic colorectal carcinoma may benefit from these therapies and both agents are approved by the US Food and Drug Administration (FDA) for treatment of metastatic colorectal cancer. Stratified analyses of data from these trials by KRAS mutational status—not detected (“wild type”) or abnormal (mutated)—indicated that patients with KRAS mutation in codon 12 or 13 did not derive benefit from treatment with cetuximab or panitumumab.

ASCO’S PROVISIONAL CLINICAL OPINION
Based on a systematic review of the relevant literature, all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR monoclonal antibody therapy should have their tumors tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR monoclonal antibody therapy as part of their treatment.

LITERATURE REVIEW AND ANALYSIS
The review of the evidence on which this PCO is based consists primarily of the rigorous systematic review of the literature conducted by the Blue Cross and Blue Shield Association (BCBSA) Technology Evaluation Center (TEC). Details of the BCBSA TEC assessment can be found in the full TEC report, which is available at www.bcbs.com/blueresources/tec/press/KRAS-mutations-epidermal.html. In summary, the TEC searched MEDLINE through October 2008 to identify all relevant articles on anti-EGFR MoAb therapy and KRAS mutation analysis. The TEC supplemented its review by searching for relevant abstracts from the ASCO 2008 Annual Meeting via the online database (www.asco.org/vm). Studies were included in the TEC assessment if they were peer-reviewed, full-length, English-language articles and investigated response to anti-EGFR MoAbs among patients with metastatic colorectal cancer with respect to KRAS mutational status. The TEC also included phase II and III randomized clinical trials from the 2008 ASCO Annual Meeting if the full presentation slides were available online.

The TEC review identified post hoc analyses on subsets from five randomized, controlled trials (RCTs) of cetuximab or panitumumab that evaluated outcomes for patients with metastatic colorectal cancer in relation to KRAS mutational status (Table 1); and five single-arm studies that retrospectively evaluated treatment response according to KRAS status (Table 2). Two broad findings emerged from the TEC assessment of these studies: (1) a consistent correlation between presence of a KRAS mutation in codon 12 or 13 and lack of response to anti-EGFR MoAb therapy in patients with metastatic colorectal cancer; and (2) evidence of improved tumor response, progression-free, and/or overall survival in response to anti-EGFR MoAb therapy only in those patients with no mutation in codon 12 or 13 (wild type) versus abnormal (mutated) KRAS tumors in analyses from four of five RCTs.

Oncologists should understand that both the PCO and the TEC review are based on assays that detect mutations in codons 12 and 13 of KRAS only. Mutations also occur, although uncommonly, at codons 61 and 146, and these also activate KRAS. In addition, the PCO and the TEC review do not evaluate the differences in sensitivity and specificity among the various assays that are available for KRAS mutation testing. (See excerpt from the College of American Pathologists [CAP] Perspectives on Emerging Technology [POET] Report on KRAS mutation testing, next paragraph.) The oncologist is encouraged to discuss these issues with the Director of his/her clinical laboratory. Finally, this PCO is limited to the current state of knowledge about the treatment of metastatic colorectal carcinoma and does not address the use of anti-EGFR MoAbs for adjuvant therapy in colorectal carcinoma, the use of small molecule tyrosine kinase inhibitors in metastatic colorectal carcinoma, or assays for other alterations that have been reported to affect response to anti-EGFR MoAbs (eg, mutation in BRAF, PI3K, PTEN, or PI3K) or loss of expression of PTEN that may indicate resistance; amplification of EGFR, lack of amplification of PTEN, and expression of epiregulin or amphiregulin that may indicate response). These subjects are either the focus of current research, or there are insufficient data to justify an opinion at present.

SUMMARY OF THE COLLEGE OF AMERICAN PATHOLOGISTS POET REPORT ON KRAS MUTATION TESTING
Under a reciprocal arrangement with the CAP, ASCO is reprinting key elements of a CAP POET report on KRAS mutation testing for colorectal cancer as a service to the ASCO membership. The CAP POET report was developed by an ad hoc panel and offers state-of-the-art information on KRAS testing for the CAP membership. The full CAP document can be found at http://www.cap.org/POET.

CAP KRAS Specimen and Test Information
Acceptable sample types. The samples should be specifically chosen by a pathologist to include predominantly tumor cells without significant necrosis or inflammation.
Freshly extracted from patient, provided fresh or in RNA preservation solution such as RNA later.

Freshly extracted from patient and rapidly frozen and stored frozen.

Neutral buffered formalin fixed and paraffin embedded, area of interest selected specifically by the pathologist.

Acceptable assay types. In all cases, DNA is first extracted by laboratory specific and standardized protocols that incorporate standards to assure adequate and specific extraction.

Real-time polymerase chain reaction. In real-time polymerase chain reaction, fluorescent probes specific for the most common mutations in codons 12 and 13 are utilized.

Table 1. Randomized Clinical Trial Evidence on Relationship of KRAS Mutation Status to Efficacy of Anti-EGFR Monoclonal Antibodies in Patients With Metastatic Colorectal Cancer

<table>
<thead>
<tr>
<th>Study and Population</th>
<th>Treatments by Arm</th>
<th>Variable</th>
<th>KRAS WT</th>
<th>KRAS Mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Antibody Arm</td>
<td>Control Arm</td>
<td>Antibody Arm</td>
</tr>
<tr>
<td>van Cutsem et al, 2008¹; CRYSTAL trial of first line therapy</td>
<td>FOLFIRI + cetuximab</td>
<td>No. of patients</td>
<td>172</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>59.3</td>
<td>43.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>51.6 to 66.7</td>
<td>35.8 to 50.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.0025</td>
<td>.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, months</td>
<td>9.9</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.088</td>
<td>.017</td>
</tr>
<tr>
<td>Bokemeyer et al, 2008³; OPUS trial of first line therapy</td>
<td>FOLFOX + cetuximab</td>
<td>No. of patients</td>
<td>61</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>60.7</td>
<td>37.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>47.3 to 72.9</td>
<td>26.0 to 49.1</td>
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<tr>
<td></td>
<td></td>
<td>P</td>
<td>.011</td>
<td>.106</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OR</td>
<td>2.54</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>1.24 to 5.23</td>
<td>0.22 to 1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, months</td>
<td>7.7</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.057</td>
<td>.0192</td>
</tr>
<tr>
<td>Punt et al, 2008⁵; CAIRO2 trial of first line therapy</td>
<td>(Capecitabine + oxaliplatin + bevacizumab) + cetuximab</td>
<td>No. of patients</td>
<td>153</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, months</td>
<td>10.5</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.10</td>
<td>.043</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median OS, months</td>
<td>22.2</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.49</td>
<td>.35</td>
</tr>
<tr>
<td>Amado et al, 2008¹; chemotherapy-refractory disease</td>
<td>Panitumumab v best supportive care</td>
<td>No. of patients</td>
<td>124</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, weeks</td>
<td>12.3</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>0.45</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>0.34 to 0.59</td>
<td>0.73 to 1.36</td>
</tr>
<tr>
<td>Karapetis et al, 2008⁶; second-or subsequent-line therapy</td>
<td>Cetuximab v best supportive care</td>
<td>No. of patients</td>
<td>117</td>
<td>113</td>
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<tr>
<td></td>
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<td>Response rate, %</td>
<td>12.8</td>
<td>0</td>
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<tr>
<td></td>
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<td>Median PFS, months</td>
<td>3.7</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>0.40</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>0.30 to 0.54</td>
<td>0.73 to 1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>&lt;.001</td>
<td>.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median OS, months</td>
<td>9.5</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.01 (for interaction, KRAS mutation status x treatment arm)</td>
<td>.01 (for interaction, KRAS mutation status x treatment arm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OS at 1 year, %</td>
<td>28.3</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
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<td>HR (death)</td>
<td>0.55</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>0.41 to 0.74</td>
<td>0.70 to 1.37</td>
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<tr>
<td></td>
<td></td>
<td>P</td>
<td>&lt;.001</td>
<td>.89</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; WT, wild type; HR, hazard ratio; OR, odds ratio; PFS, progression-free survival; FOLFIRI, folinic acid, fluorouracil, and irinotecan; FOLFOX, folinic acid, fluorouracil, and oxaliplatin; CRYSTAL, Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; OPUS, Oxaliplatin and Cetuximab in First-Line Treatment of mCRC; CAIRO2, Capecitabine, Irinotecan, and Oxaliplatin in Advanced Colorectal Cancer (2).

When a mutation is present, the probe binds and fluorescence is detected.

- Direct sequencing analysis. KRAS mutations can also be identified using a direct sequencing method of exon 1 in the KRAS gene. This technique identifies all possible mutations in the exon.
- At this time, there are no FDA-approved tests for KRAS testing, but KRAS testing can be performed using laboratory-developed tests. Outside the United States, a United Kingdom-based company, DxS, offers a kit (TheraScreen) for its KRAS mutations assay. DxS and other vendors are expected to seek US Food and Drug Administration approval for their assays.
- Choice of assay defined by which assay the laboratory has validated and routinely uses. Oncologist should consult with laboratory about specific test name to order.

**Assay Reporting**

**KRAS normal.** No mutation was identified. Report will specify assay type and controls used.

**KRAS abnormal.** Treatment with anti-EGFR monoclonal antibody therapy is not recommended based on the ASCO PCO. Mutation was found. Report will specify what mutation was found, what assay was done, and what controls were used.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a “U” are those for which no compensation was received; those relationships marked with a “C” were compensated. For a detailed description of the disclosure categories, or for more information about ASCO’s conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

**Employment or Leadership Position:** Daniel F. Hayes, Eli Lilly & Co

**Consultant or Advisory Role:** None

**Stock Ownership:** None

**Honoraria:** None

**Research Funding:** None

**Expert Testimony:** None

**Other Remuneration:** None

---

**Table 2. Single-Arm Studies of Treatment of Metastatic CRC With Anti-EGFR Monoclonal Antibodies and KRAS Mutational Status**

<table>
<thead>
<tr>
<th>Study and Population</th>
<th>Treatments by Arm</th>
<th>Variable</th>
<th>KRAS WT</th>
<th>KRAS Mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lievre et al, 2008&lt;sup&gt;2&lt;/sup&gt;; second-line therapy</td>
<td>Cetuximab</td>
<td>No. of patients</td>
<td>65</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PFS, weeks</td>
<td>31.4</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>19.4 to 36</td>
<td>8 to 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OS, months</td>
<td>14.3</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>9.4 to 20</td>
<td>5.1 to 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.026</td>
<td></td>
</tr>
<tr>
<td>De Roock et al, 2008&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Cetuximab alone with irinotecan</td>
<td>No. of patients</td>
<td>57</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P (cetuximab + irinotecan)</td>
<td>.000001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P (cetuximab alone)</td>
<td>.126</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PFS cetuximab + irinotecan, weeks</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>28.5 to 40.0</td>
<td>5.4 to 18.7</td>
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<tr>
<td></td>
<td></td>
<td>P</td>
<td>.016</td>
<td></td>
</tr>
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<td></td>
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<td>PFS cetuximab, weeks</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>4.2 to 20.0</td>
<td>7.0 to 17.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.351</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OS cetuximab + irinotecan, weeks</td>
<td>44.7</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>28.4 to 61.0</td>
<td>9.5 to 45.0</td>
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<td>P</td>
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<td>95% CI</td>
<td>8.9 to 45.1</td>
<td>0.0 to 70.0</td>
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<td></td>
<td></td>
<td>P</td>
<td>.330</td>
<td></td>
</tr>
<tr>
<td>Kambata-Ford et al, 2007&lt;sup&gt;7&lt;/sup&gt;</td>
<td>Cetuximab; second- or third-line treatment</td>
<td>No. of patients</td>
<td>50</td>
<td>30</td>
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<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Di Fiore et al, 2007&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Cetuximab plus chemotherapy</td>
<td>No. of patients</td>
<td>43</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Benvenuti et al, 2007&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Panitumumab or cetuximab, or cetuximab plus chemotherapy</td>
<td>No. of patients</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>31</td>
<td>6</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; CRC, colorectal cancer; WT, wild type; PFS, progression-free survival; OS, overall survival. Adapted with permission<sup>11</sup>: BlueCross BlueShield Association. Technology Evaluation Center. KRAS Mutations and Epidermal Growth Factor Receptor Inhibitor Therapy in Metastatic Colorectal Cancer TEC Assessments 2008; volume 23, tab 6. Copyright © 2008, BlueCross BlueShield Association.
The ad hoc panel is grateful to Monica Bertagnolli, MD, Douglas Blayney, MD, Nancy Davidson, MD, Jeffrey Meyerhardt, MD, Lisa Newman, MD, Richard Theriault, DO, and Sandra Wong, MD, for reviewing earlier drafts of the manuscript on behalf of the ASCO Board of Directors and Health Services Committee; to Kaitlin Einhaus, Karen Hagerty, Shauniece Morris, Jerome Seidenfeld, and Sarah Temin for their assistance in manuscript preparation; to the BlueCross and BlueShield Association Technology Evaluation Center’s Heather Brown, Naomi Aronson, and other staff; and to the College of American Pathologists’ Saeed Ahmad and Noel Adachi, other staff, and physician volunteers for their collaboration.

**Appendix**

**Overview of the Provisional Clinical Opinion Development Process**

*PCO topic selection.* The American Society of Clinical Oncology (ASCO) Health Services Committee (HSC) leadership is responsible for accepting, reviewing, and approving proposed provisional clinical opinion (PCO) topics on behalf of the ASCO Board of Directors. The selection of this PCO topic was guided by the Topic Selection Algorithm that is used by the HSC to guide selection of topics for ASCO’s clinical practice guidelines (www.asco.org/guidelines/manual).

*PCO evidentiary basis.* Provisional clinical opinions are informed by expedient methodological assessments of the data in question. To this end, ASCO has established a relationship with the National Cancer Institute’s Physician Data Query (PDQ) Editorial Boards. The PDQ’s Editorial Boards are comprised of content experts in oncology and related specialties. On request from ASCO, the relevant PDQ Editorial Board will provide a written assessment of the new data.

For the present PCO, however, ASCO learned that the Blue Cross and Blue Shield Association (BCBSA) Technology Evaluation Center (TEC) was conducting a technology assessment, including a comprehensive systematic review of the available evidence, on the topic of KRAS mutations and anti-EGFR monoclonal antibody in metastatic colorectal cancer. BCBSA TEC made the technology assessment available to ASCO for use in developing this PCO.

*Ad hoc PCO panel.* The BCBSA TEC Assessment was forwarded to an ad hoc panel that was selected and charged by the HSC to draft the PCO. The ad hoc panel includes six content experts and a patient representative. The membership of the ad hoc panel was chosen in accordance with ASCO’s Conflict of Interest Management Procedures for Clinical Practice Guidelines (“COI Procedures”). The COI Procedures call for the
majority of ad hoc panel members to have no relationships with companies potentially affected by the PCO, and generally require ad hoc panel cochairs to be free from relationships with affected companies.

PCO review and approval. The PCO was approved by a unanimous vote of (1) the ad hoc panel members (2); the HSC leadership (Past-Chair, Chair, Chair-Elect, and selected content experts) and selected content experts drawn from the HSC membership; and (3) a subset of the ASCO Board (Past-President, President, President-Elect) and selected content experts drawn from the Board membership and appointed at the discretion of the President.
Chemotherapy, Bevacizumab, and Cetuximab in Metastatic Colorectal Cancer


Abstract

From Radboud University Nijmegen Medical Center, Nijmegen (J.T., M.K., J.R.D., M.E.V.-B., J.H.J.M.K., C.J.A.P.); the Netherlands Cancer Institute, Amsterdam (A.C., N.F.A., O.D.); Meander Medical Center, Amersfoort (C.J.R.); Catharina Hospital, Eindhoven (G.J.M.C.); Sparnae Hospital, Hoofddorp (J.G.S.); Maasland Hospital, Sittard (F.L.G.E.); Bernhoven Hospital, Oss (A.H.V.); the Free University Medical Center, Amsterdam (C.J.G.); Jeroen Bosch Hospital, ’s-Hertogenbosch (H.A.M.S.); the Amsterdam Medical Center, Amsterdam (D.J.R.); University Medical Center, Utrecht (E.E.V.); and the Comprehensive Cancer Center East, Nijmegen (L.M.) — all in the Netherlands. Address reprint requests to Dr. Punt at the Department of Medical Oncology, Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands, or at c.punt@onco.umcn.nl.

This article (10.1056/NEJMoa0808268) was updated on December 22, 2010, at NEJM.org.

The New England Journal of Medicine

Background

Fluoropyrimidine-based chemotherapy plus the anti–vascular endothelial growth factor (VEGF) antibody bevacizumab is standard first-line treatment for metastatic colorectal cancer. We studied the effect of adding the anti–epidermal growth factor receptor (EGFR) antibody cetuximab to a combination of capecitabine, oxaliplatin, and bevacizumab for metastatic colorectal cancer.

Methods

We randomly assigned 755 patients with previously untreated metastatic colorectal cancer to capecitabine, oxaliplatin, and bevacizumab (CB regimen, 378 patients) or the same regimen plus weekly cetuximab (CBC regimen, 377 patients). The primary end point was progression-free survival. The mutation status of the KRAS gene was evaluated as a predictor of outcome.

Results

The median progression-free survival was 10.7 months in the CB group and 9.4 in the CBC group (P=0.01). Quality-of-life scores were lower in the CBC group. The overall survival and response rates did not differ significantly in the two groups. Treated patients in the CBC group had more grade 3 or 4 adverse events, which were attributed to cetuximab-related adverse cutaneous effects. Patients treated with cetuximab who had tumors bearing a mutated KRAS gene had significantly decreased progression-free survival as compared with cetuximab-treated patients with wild-type–KRAS tumors or patients with mutated-KRAS tumors in the CB group.

Conclusions

The addition of cetuximab to capecitabine, oxaliplatin, and bevacizumab resulted in significantly shorter progression-free survival and inferior quality of life. Mutation status of the KRAS gene was a predictor of outcome in the cetuximab group. (ClinicalTrials.gov number, NCT00208546.)
FLUOROPYRIMIDINES (E.G., FLUOROURACIL and capecitabine), irinotecan, and oxaliplatin are the standard cytotoxic drugs used in treating metastatic colorectal cancer.\textsuperscript{1,2} The combination of capecitabine and oxaliplatin is similar to the combination of fluorouracil and oxaliplatin in efficacy and safety.\textsuperscript{3,4} Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF),\textsuperscript{5,7} combined with fluoropyrimidine-based chemotherapy is now the standard first-line treatment for metastatic colorectal cancer. Cetuximab, a chimeric IgG1 monoclonal antibody against epidermal growth factor receptor (EGFR), has efficacy as monotherapy and in combination with irinotecan in irinotecan-resistant patients.\textsuperscript{8,9} We prospectively evaluated the addition of cetuximab to capecitabine, oxaliplatin, and bevacizumab as first-line treatment in patients with metastatic colorectal cancer (the CAIRO2 trial).

METHODS

PATIENTS

Eligible patients were older than 18 years of age, had histologically proved colon or rectal carcinoma, metastatic disease not amenable to curative surgery, measurable tumor, no previous systemic chemotherapy for metastatic disease, World Health Organization (WHO) performance status 0 or 1, no adjuvant chemotherapy within 6 months before randomization, and adequate bone marrow, liver, and renal function. We excluded patients if they had higher than grade 1 sensory neuropathy, previous intolerance of adjuvant chemotherapy, symptomatic central nervous system metastases, bleeding diathesis, coagulation disorders, clinically significant cardiovascular disease, or other cancers within the previous 5 years, except for adequately treated squamous or basal-cell carcinoma of the skin or carcinoma in situ of the cervix.

STUDY DESIGN

This open-label, randomized, phase 3 trial was conducted in 79 centers in the Netherlands. Eligible patients were randomly assigned at a 1:1 ratio to receive treatment with or without cetuximab. Randomization was performed centrally by a minimization technique with stratification according to serum lactate dehydrogenase level (normal or abnormal, according to the cutoff values of each individual center), previous adjuvant chemotherapy (yes or no), number of affected organs (one or more than one), and treatment center. The study was approved by the Committee on Human-Related Research Arnhem–Nijmegen and by the local institutional review boards. An independent data and safety monitoring committee evaluated all serious adverse events. All patients provided written informed consent before study entry.

Bevacizumab was donated by Roche, and cetuximab was donated by Merck Serono. The sponsors of the study were informed of the results of the study but did not contribute to any phase of the study design; the collection, analysis, and interpretation of the data; or the writing of the manuscript.

TREATMENT AND TESTING

Treatment for the capecitabine–bevacizumab (CB) group consisted of 1000 mg of capecitabine per square meter of body-surface area, given orally twice daily on days 1 to 14; 130 mg of oxaliplatin per square meter, given intravenously on day 1; and 7.5 mg of bevacizumab per kilogram of body weight, given intravenously on day 1. Treatment for the capecitabine–bevacizumab–cetuximab (CBC) group consisted of the same regimen of capecitabine, oxaliplatin, and bevacizumab plus 400 mg of cetuximab per square meter, given intravenously on day 1 of the first treatment cycle, followed by 250 mg of cetuximab per square meter given weekly thereafter. All treatment cycles were administered every 3 weeks. In both treatment groups, oxaliplatin was administered for a maximum of six cycles to prevent serious peripheral sensory neurotoxicity, and from cycle 7 the dose of capecitabine was increased to 1250 mg per square meter.

Adverse effects were graded according to the National Cancer Institute Common Toxicity Criteria, version 3.0. Dose reductions because of adverse events were performed for each agent as specified in the study protocol. A cetuximab-related adverse cutaneous effect was defined as any adverse cutaneous effect with the exception of hand–foot syndrome. Central review was performed of the charts of all patients who died within 30 days after the last administration of the study drugs and whose death was accompanied by any event other than disease progression, regardless of the reported cause. The results of the central review were submitted to the independent data and safety
monitoring committee for final assessment. An interim analysis of safety in the first 400 patients has been published.10

Tumor response was assessed by the local investigators every 9 weeks with the use of computed tomographic scans, according to the Response Evaluation Criteria in Solid Tumors (RECIST).11 The overall response rate was defined as the rate of all responses, including complete and partial responses. Disease control was defined as complete response, partial response, or stable disease as the best response. Treatment was continued until the occurrence of disease progression, death, or unacceptable adverse event, whichever came first. Patients whose treatment was discontinued for reasons other than disease progression were evaluated for a response every 3 months. The relative dose intensity was defined as the ratio of the dose administered to the planned dose. Quality of life was assessed by the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire at baseline within 2 weeks before randomization and every 9 weeks thereafter until the end of the study treatment.

Formalin-fixed, paraffin-embedded tumor material was collected from patients for whom resected primary tumor tissue was available. DNA was extracted from tumor tissue for mutation analysis of the KRAS gene (see the Supplementary Appendix, available with the full text of this article at NEJM.org).

EGFR expression was determined by immunohistochemical assay on tissue microarrays with the use of the EGFR pharmDx Kit (Dako) according to the manufacturer’s instructions. Powervision (Immunologic) was used as a visualization method. In accordance with the pharmDx Kit guidelines, tumors showing more than 1% membranous EGFR stained cells were considered positive.

## Statistical Analysis

The primary end point was progression-free survival, which was defined as the interval from the date of randomization to the date of disease progression, death, or last follow-up, whichever occurred first. It was estimated that with 540 events (progression or death), a two-sided log-rank test at a significance level of 5% would have a power of 80% to detect a difference in median progression-free survival of 11 to 14 months (hazard ratio, 0.79). On the assumption of an accrual and follow-up period of 36 months, we planned to include approximately 750 patients in the study. The secondary end points were overall survival, safety, response rate, quality of life, and the influence of KRAS mutational status and expression of EGFR in tumor samples on the outcome. Ineligible patients or patients who withdrew informed consent were excluded from all analyses. Data from eligible patients were analyzed according to the intention-to-treat principle, and these patients remained in follow-up until disease progression occurred. Data from patients who were alive without recurrence at the time of analysis were censored. The progression-free and overall survival curves were estimated by the Kaplan–Meier method and compared by means of the log-rank test. We performed a multivariate analysis using a Cox proportional-hazards model with treatment group, serum lactate dehydrogenase level, number of affected organs, and previous adjuvant chemotherapy as covariables.

Patients who started treatment were evaluated for adverse events, and patients who completed at least three cycles were evaluated for response. The worst grade of adverse event was compared between the treatment groups with the use of the chi-square test. The correlation between cetuximab-related adverse cutaneous effects and survival was assessed in a landmark-type analysis. Patients who started treatment were grouped according to the worst grade of cetuximab-related adverse cutaneous effect reported during the first six cycles. The Wilcoxon rank-sum test was used to detect statistically significant differences between the treatment groups in the change in the mean quality-of-life score. Patients who completed the quality-of-life questionnaire at baseline and at least once during treatment were evaluated. All analyses were performed with the use of SAS software, version 9.1.

## Results

### Patients

Between June 2005 and December 2006, 755 patients underwent randomization: 378 to receive treatment without cetuximab (CB group) and 377 to receive treatment with cetuximab (CBC group). Nineteen patients (2.5%) were ineligible (Supplementary Appendix). The study was completed according to the protocol, and the estimated number of events occurred. The baseline characteristics of all 736 eligible patients (368 in each treatment...
group) were well matched between the two groups except for sex (Table 1). The median duration of follow-up at the time of this analysis was 23 months.

**TREATMENT**

Treatment was started in 732 eligible patients, 366 in each group. The Supplementary Appendix gives the median and mean numbers of treatment cycles, the median duration of treatment, and the median relative dose intensity.

The reasons for discontinuation of treatment in the CB group (313 patients) and the CBC group (334 patients) were progression of disease (169 patients [54.0%] and 162 patients [48.5%], respectively; \( P=0.16 \)), adverse events (81 [25.9%] and 99 [29.6%], \( P=0.28 \)), resection of metastases (13 [4.2%] and 18 [5.4%], \( P=0.46 \)), and declining of treatment by the patient (19 [6.1%] and 25 [7.5%], \( P=0.48 \)). All study drugs were discontinued in 12 patients in the CBC group for adverse events that appeared to be related exclusively to cetuximab. A total of 345 of all 736 eligible patients (46.9%) received further systemic treatment after disease progression: 168 in the CB group (45.7%) and 177 in the CBC group (48.1%). Of these patients, 48 received oxaliplatin (18 in the CB group and 30 in the CBC group), 278 received irinotecan (144 and 134, respectively), and 22 received cetuximab (15 and 7); cetuximab was usually administered in combination with irinotecan.

**Efficacy**

The primary end point was reached in 293 patients in the CB group and 316 patients in the CBC group. The addition of cetuximab significantly decreased the median progression-free survival (10.7 months in the CB group and 9.4 months in the CBC group, \( P=0.01 \)) (Table 2 and Fig. 1A).

![Table 1. Baseline Characteristics of the Patients.*](image)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CB Group (N = 368)</th>
<th>CBC Group (N = 368)</th>
<th>P Value</th>
</tr>
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<tr>
<td>Age — yr</td>
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<td>0.95</td>
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<tr>
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<td>62</td>
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<tr>
<td>Range</td>
<td>27–83</td>
<td>33–80</td>
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</tr>
<tr>
<td>Sex — no. (%)</td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Male</td>
<td>205 (55.7)</td>
<td>233 (63.3)</td>
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<tr>
<td>Female</td>
<td>163 (44.3)</td>
<td>135 (36.7)</td>
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<tr>
<td>WHO performance status — no. (%)</td>
<td></td>
<td></td>
<td>0.09</td>
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<tr>
<td>0</td>
<td>219 (59.5)</td>
<td>240 (65.2)</td>
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<tr>
<td>1</td>
<td>149 (40.5)</td>
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<td>No data</td>
<td>2 (0.5)</td>
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<tr>
<td>Serum lactate dehydrogenase level — no. (%)</td>
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<td>0.82</td>
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<tr>
<td>Normal</td>
<td>210 (57.1)</td>
<td>207 (56.2)</td>
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<tr>
<td>Above normal</td>
<td>158 (42.9)</td>
<td>161 (43.8)</td>
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<td>Previous adjuvant therapy — no. (%)</td>
<td>55 (14.9)</td>
<td>56 (15.2)</td>
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<td>Site of primary tumor — no. (%)</td>
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<td></td>
<td>0.50</td>
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<tr>
<td>Colon</td>
<td>164 (44.6)</td>
<td>172 (46.7)</td>
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<tr>
<td>Rectum</td>
<td>108 (29.3)</td>
<td>94 (25.5)</td>
<td></td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>96 (26.1)</td>
<td>102 (27.7)</td>
<td></td>
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<tr>
<td>No. of affected organs — no. (%)</td>
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<td></td>
<td>0.77</td>
</tr>
<tr>
<td>1</td>
<td>167 (45.4)</td>
<td>163 (44.3)</td>
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</tr>
<tr>
<td>&gt;1</td>
<td>201 (54.6)</td>
<td>205 (55.7)</td>
<td></td>
</tr>
</tbody>
</table>

* CB denotes capecitabine, oxaliplatin, and bevacizumab, CBC capecitabine, oxaliplatin, and bevacizumab plus cetuximab, and WHO World Health Organization. Because of rounding, not all percentages total 100.
hazard ratio for disease progression or death in the CBC group was 1.22 (95% confidence interval, 1.04 to 1.43). In a multivariate analysis, an elevated serum lactate dehydrogenase level (P<0.001) and treatment group (P=0.03) correlated significantly with progression-free survival. The median overall survival was 20.3 months in the CB group and 19.4 months in the CBC group (P=0.16) (Table 2 and Fig. 1B). A total of 407 patients died, 193 in the CB group and 214 in the CBC group. The rate of death from any cause at 60 days was 1.9% in the CB group and 2.7% in the CBC group. The overall response rate in the 649 patients who were evaluated was 50.0% in the CB group and 52.7% in the CBC group (P=0.49). Disease control was observed in 94.0% of the patients in the CB group and 94.6% of those in the CBC group (P=0.72).

**SUBGROUP ANALYSES**
The mutation status of the KRAS gene was evaluated in 528 tumors (Table 3). Eight samples were excluded because of discordance in the results of the two test methods. An activating KRAS mutation was found in 206 tumors (39.6%): 108 from patients in the CB group and 98 from patients in the CBC group. The baseline characteristics were well balanced between patients with wild-type–KRAS tumors and those with mutated-KRAS tumors (data not shown). Cetuximab-treated patients with mutated-KRAS tumors had significantly shorter progression-free survival than cetuximab-treated patients with wild-type–KRAS tumors (8.1 vs. 10.5 months, P=0.04) (Fig. 2A). As compared with patients with mutated-KRAS tumors in the CB group, cetuximab-treated patients with mutated-KRAS tumors had significantly shorter progression-free survival (8.1 vs. 12.5 months, P=0.003) and overall survival (17.2 vs. 24.9 months, P=0.03). Among patients with wild-type–KRAS tumors, there was no significant difference in progression-free survival between the two treatment groups. Among patients treated with cetuximab, the response rate was significantly lower in those with KRAS mutations than in those with wild-type–KRAS tu-

<table>
<thead>
<tr>
<th>Table 2. Efficacy of Study Treatment.*</th>
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<td><strong>Outcome</strong></td>
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<td>---------------------------------------</td>
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<tr>
<td>Progression-free survival (mo)</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>95% CI</td>
</tr>
<tr>
<td>Overall survival (mo)</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>95% CI</td>
</tr>
<tr>
<td>Response rate (%)†</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>Disease control rate (%)†</td>
</tr>
<tr>
<td>No. of treatment cycles</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>Range</td>
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<tr>
<td>Duration of treatment (mo)</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Primary reason for treatment discontinuation (%)</td>
</tr>
<tr>
<td>Disease progression</td>
</tr>
<tr>
<td>Adverse events</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>60-Day mortality (%)</td>
</tr>
</tbody>
</table>

* CB denotes capecitabine, oxaliplatin, and bevacizumab, CBC capecitabine, oxaliplatin, and bevacizumab plus cetuximab, and CI confidence interval.
† A total of 649 patients (332 in the CB group and 317 in the CBC group) were evaluated for response.
EGFR-positive and EGFR-negative patients (data not shown). Among EGFR-positive patients, the median progression-free survival was 12.2 months in the CB group and 9.8 months in the CBC group (P = 0.003). We did not observe a significant correlation between KRAS mutation status or EGFR expression and the incidence of cetuximab-related adverse cutaneous effects (data not shown).

As compared with women in the CBC group, women who were assigned to the CB group had significantly better progression-free survival (12.5 vs. 8.6 months, P < 0.001) and overall survival (20.1 vs. 18.8 months, P = 0.02). However, these differences were not observed in men. The distribution of baseline characteristics was similar in women and men. In a multivariate analysis, the interaction between sex and treatment group was statistically significant for progression-free survival (P = 0.005) but not for overall survival (P = 0.10).

The severity of cetuximab-related adverse cutaneous effects correlated significantly with progression-free survival (P < 0.001) (Fig. 2B). The median progression-free survival in patients with grade 0 or 1, patients with grade 2, and patients with grade 3 cetuximab-related adverse cutaneous effects was 7.8 months, 10.2 months, and 11.4 months, respectively. The progression-free survival did not differ significantly between patients in the CB group and patients with grade 3 cetuximab-related adverse cutaneous effects in the CBC group (P = 0.72).

SAFETY

Table 4 and the Supplementary Appendix list the most frequently observed grade 3 or 4 adverse events. The incidence of any grade 3 or 4 adverse event was 73.2% in the CB group and 81.7% in the CBC group (P = 0.006). When grade 3 cetuximab-related adverse cutaneous effects were excluded from this analysis, the incidence was similar: 73.2% in the CB group and 74.3% in the CBC group (P = 0.74).

QUALITY OF LIFE

A total of 532 patients (276 in the CB group and 256 in the CBC group) were evaluated for quality of life. Overall quality of life and global health status were similar in the two groups at baseline; during treatment, both measures improved significantly more in the CB group than in the CBC group (P = 0.007 and P = 0.03, respectively). The mean increase in global health status was 0.4
point in the CB group and 0.0 points in the CBC group (P=0.007). There were no significant differences between the treatment groups in the change from baseline in scores for pain, financial problems, and decrease in functioning (physical, emotional, cognitive, and social).

**DISCUSSION**

In this randomized trial conducted in previously untreated patients with metastatic colorectal cancer, the addition of cetuximab to treatment with capecitabine, oxaliplatin, and bevacizumab resulted in a significant decrease in progression-free survival and a poorer quality of life. The reduction in progression-free survival was unexpected, since preclinical as well as early clinical studies suggested a benefit from the combination of anti-VEGF and anti-EGFR antibodies. An increase in adverse events is an unlikely cause of the reduction in progression-free survival, since such events were manageable and the percentage of patients who discontinued treatment because of adverse events was similar in the two treatment groups. A similar result with anti-EGFR therapy was observed in the Panitumumab Advanced Colorectal Cancer Evaluation (PACCE) trial, in which previously untreated patients with metastatic colorectal cancer were randomly assigned to fluorouracil, leucovorin, bevacizumab, and irinotecan or oxaliplatin, with or without panitumumab, a human antibody against EGFR. The PACCE trial was prematurely discontinued because of decreased progression-free survival and increased adverse events in the panitumumab group, but the decrease in progression-free survival was observed only in patients who were treated with oxaliplatin. The Bowel Oncology with Cetuximab Antibody (BOND) 2 trial showed efficacy in treatment with irinotecan, bevacizumab, and cetuximab in patients with irinotecan-resistant colorectal cancer, a result that suggested a higher response rate and longer progression-free survival than was found in a previous trial (BOND) of irinotecan and cetuximab in similar patients. Preliminary results of chemotherapy with or without cetuximab in the first-line treatment of metastatic colorectal cancer indicate somewhat better progression-free survival with irinotecan than with oxaliplatin, but these comparisons should be interpreted with caution: whether cetuximab is more efficacious when given in combination with irinotecan than with oxaliplatin remains speculative.

The results of our trial might be due to a negative interaction between cetuximab and bevacizumab. Hypertension, a common side effect of bevacizumab treatment, was recently shown to correlate with clinical outcome in patients with colorectal cancer. Our observation that hypertension was less frequent in the CBC group suggests decreased efficacy of bevacizumab when administered in combination with cetuximab. In contrast, preclinical studies have suggested a positive interaction between VEGF- and EGFR-inhibiting agents. However, to our knowledge, the combination of cetuximab and bevacizumab has not been tested in this setting.

The severity of cetuximab-related adverse cutaneous effects correlated directly and significantly with progression-free survival, but the median progression-free survival among patients with the most severe cetuximab-related adverse cutaneous effects was not significantly better than that among patients treated without cetuximab.

Women treated with cetuximab had shorter progression-free survival than women treated without cetuximab.  

**Table 3.** Association of the Mutation Status of the KRAS Gene with Progression-free Survival, Overall Survival, and Response Rate.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wild-Type KRAS</th>
<th>Mutated KRAS</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>CB group 156, CBC group 158</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median progression-free survival (mo)</td>
<td>CB group 10.6, CBC group 10.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.6</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Median overall survival (mo)</td>
<td>CB group 22.4, CBC group 21.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.64</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Response rate (%)</td>
<td>CB group 50.0, CBC group 61.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.06</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

* CB denotes capecitabine, oxaliplatin, and bevacizumab, and CBC capecitabine, oxaliplatin, and bevacizumab plus cetuximab.
Among patients in the group receiving capecitabine, oxaliplatin, and bevacizumab plus cetuximab (CBC), the median progression-free survival was significantly decreased when their tumor harbored a KRAS mutation (8.1 vs. 10.5 months, P=0.04) (Panel A). Among patients with mutated-KRAS tumors, the median progression-free survival was significantly decreased in the CBC group as compared with the group receiving capecitabine, oxaliplatin, and bevacizumab (CB) (8.1 vs. 12.5 months, P=0.003). In the CBC group, the median progression-free survival in treated patients with grade 0 or 1, treated patients with grade 2, and treated patients with grade 3 cetuximab-related adverse cutaneous effects was 7.8 months, 10.2 months, and 11.4 months, respectively (P<0.001) (Panel B). The difference between patients with grade 3 adverse cutaneous effects in the CBC group and patients in the CB group was not statistically significant (P=0.72).

Figure 2. Kaplan–Meier Estimates of Progression-free Survival According to KRAS Mutation Status and Cetuximab-Related Adverse Cutaneous Effects.
without cetuximab, but this difference was not found in men. Women also had a lower incidence of grade 3 cetuximab-related adverse cutaneous effects, which might indicate a decreased efficacy of cetuximab in our study. Although the management of adverse cutaneous effects may be different in women than in men, with earlier discontinuation of cetuximab in women for cosmetic reasons, this would not explain the poorer results in the CBC group.

The KRAS genotype affects the response to anti-EGFR treatment: patients with wild-type–KRAS tumors have longer progression-free survival than those with mutated-KRAS tumors.\textsuperscript{22-26} The results of our study also confirm the role of the mutation status of the KRAS gene in the response to cetuximab when cetuximab is administered in combination with chemotherapy and bevacizumab as first-line treatment. We observed the worst result for progression-free survival in patients with mutated-KRAS tumors who were treated with cetuximab. A similar result was found in trials of chemotherapy with or without cetuximab as first-line treatment of metastatic colorectal cancer.\textsuperscript{25,26}

Many targeted agents are available or under development for use in a wide range of tumors. The inhibition of a single signal-transduction pathway is unlikely to provide optimal results, and therefore a combination of agents appears to be a valid strategy. Our results, however, argue against the combined use of anti-VEGF and anti-EGFR monoclonal antibodies with chemotherapy in cases of metastatic colorectal cancer.\textsuperscript{22-26}

**Table 4. Serious Adverse Events.**

<table>
<thead>
<tr>
<th>Event</th>
<th>CB Group (N=366)</th>
<th>CBC Group (N=366)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any grade 3 or 4 event</td>
<td>268 (73.2)</td>
<td>299 (81.7)</td>
<td>0.006</td>
</tr>
<tr>
<td>Adverse cutaneous effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any event</td>
<td>76 (20.8)</td>
<td>143 (39.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acneiform rash</td>
<td>2 (0.5)</td>
<td>93 (25.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hand–foot skin reaction</td>
<td>71 (19.4)</td>
<td>68 (18.6)</td>
<td>0.78</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>70 (19.1)</td>
<td>95 (26.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Nausea</td>
<td>31 (8.3)</td>
<td>23 (6.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>Vomiting</td>
<td>30 (8.2)</td>
<td>22 (6.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Fatigue</td>
<td>48 (13.1)</td>
<td>55 (15.0)</td>
<td>0.46</td>
</tr>
<tr>
<td>Sensory neuropathy</td>
<td>38 (10.4)</td>
<td>28 (7.7)</td>
<td>0.20</td>
</tr>
<tr>
<td>Infection</td>
<td>25 (6.8)</td>
<td>22 (6.0)</td>
<td>0.65</td>
</tr>
<tr>
<td>Neutropenic fever</td>
<td>5 (1.4)</td>
<td>3 (0.8)</td>
<td>0.48</td>
</tr>
<tr>
<td>Hypersensitivity reaction</td>
<td>15 (4.1)</td>
<td>18 (4.9)</td>
<td>0.59</td>
</tr>
<tr>
<td>Hypertension</td>
<td>54 (14.8)</td>
<td>34 (9.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Gastrointestinal perforation</td>
<td>1 (0.3)</td>
<td>6 (1.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Bleeding</td>
<td>6 (1.6)</td>
<td>2 (0.5)</td>
<td>0.16</td>
</tr>
<tr>
<td>Venous thromboembolic events</td>
<td>25 (6.8)</td>
<td>30 (8.2)</td>
<td>0.48</td>
</tr>
<tr>
<td>Arterial thromboembolic events</td>
<td>12 (3.3)</td>
<td>8 (2.2)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* CB denotes capecitabine, oxaliplatin, and bevacizumab, and CBC capecitabine, oxaliplatin, and bevacizumab plus cetuximab.

Supported by the Dutch Colorectal Cancer Group (DCCG). The DCCG received grants for data management and analysis from the Commissie Klinisch Toegestaan Onderzoek of the Dutch Cancer Foundation and unrestricted scientific grants from Roche, Merck Serono, Sanofi-Aventis, and DxS.

Dr. Tol reports receiving grant support from the Netherlands Organization for Health Research and Development; Dr. Cats, grant support from the Dutch Cancer Foundation, Roche, and Sanofi-Aventis and consulting fees from Merck Serono; Dr. Erdkamp, consulting fees from Sanofi-Aventis and Merck Serono; Dr. Voest, consulting fees from Sanofi-Aventis and Merck Serono; Dr. van Krieken, grant support from the Dutch Cancer Foundation and Roche; Dr. van Krieken, grant support from the Dutch Cancer Foundation; and Dr. Punt, grant support from the Dutch Cancer Foundation and Roche and consulting fees from Roche and Merck Serono. No other potential conflict of interest relevant to this article was reported.

We thank Marjolijn J.L. Litgenberg, Ph.D., for the interpretation of the KRAS data and Annelies Klaasen, B.Sc., for laboratory assistance.

**APPENDIX**

REFERENCES


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