A phase II study of high-dose bevacizumab in combination with irinotecan, 5-fluorouracil, leucovorin, as initial therapy for advanced colorectal cancer: results from the eastern cooperative oncology group study E2200

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Aim: Patients with untreated advanced colorectal cancer were enrolled to this single arm phase II multi-center cooperative group trial of bevacizumab combined with IFL. The first 20 patients received irinotecan (125 mg/m²), 5-fluorouracil (500 mg/m²) and leucovorin (20 mg/m²) weekly for four of six weeks and high-dose bevacizumab (10 mg/kg) every other week. Following a toxicity review of other trials using IFL, subsequent patients were enrolled at reduced doses of irinotecan (100 mg/m²) and 5-fluorouracil (400 mg/m²).

Results: Of the 92 patients accrued to the study, toxicity data are available for 87 patients and efficacy data for 81 patients. At a median follow-up of 37.5 months, median overall survival is 26.3 months, median progression free survival is 10.7 months and 1-year survival is 85%. The overall response rate is 49.4% (6.2% complete responses). A reduction in the starting doses of irinotecan and 5-fluorouracil decreased the occurrence of vomiting, diarrhea and neutropenia related complications. Bleeding occurred in 37 patients; all events but two were grade 1 or grade 2. There were nine reports of grade 3 or grade 4 thrombo-embolic events. Hypertension of any grade occurred in 13% of patients and proteinuria was infrequent.

Conclusion: High-dose bevacizumab added to IFL is a well-tolerated and highly active regimen in patients with previously untreated metastatic colorectal cancer.

Key words: first-line therapy, high-dose bevacizumab, IFL, metastatic colorectal cancer

introduction

In the United States, colorectal cancer represents the second most common cause of cancer death [1]. Despite the improvements in response rates [2–5], progression-free survival (PFS) [2–5], and median survival [2, 4, 5] achieved by the addition of irinotecan and oxaliplatin to the 5-fluorouracil based treatments for advanced colorectal cancer, nearly all patients will succumb to their disease. Therapeutic agents that exploit the underlying molecular events involved in carcinogenesis, tumor growth and malignant dissemination provide the basis for new treatment strategies for this disease.

Angiogenesis, a complex physiologic process that plays a significant role in malignant diseases, is mediated by vascular endothelial growth factor (VEGF), a ligand for the VEGF family of transmembrane tyrosine kinases [6, 7]. Increased expression of VEGF has been found in most human cancers examined, including tumors of the lung, breast, gastrointestinal tract, kidney, bladder, ovary, and cervix [6]. In colon cancer, VEGF expression is greater in malignant colonic mucosa when compared to nearby normal tissue, and may have prognostic value [8, 9]. Inhibition of VEGF using an anti-VEGF monoclonal antibody blocks the growth of a number of human cancer cell lines in nude mice [6]. In addition, the combination of anti-VEGF antibody and chemotherapy in nude mice injected with human cancer xenografts results in an increased anti-tumor effect compared with antibody or chemotherapy alone [10, 11].

Bevacizumab (Avastin, Genentech BioOncology, South San Francisco, CA) is a recombinant humanized version of a murine anti-human VEGF monoclonal antibody (rhUAB-VEGF). With a high binding specificity for circulating VEGF, bevacizumab prevents its interaction with its receptors.
(specifically VEGFR-1 and VEGFR-2) on vascular endothelial cells, and abrogates their downstream biologic effects [12]. Phase I studies of bevacizumab demonstrated good tolerability when given as a single agent or in combination with three commonly used chemotherapy regimens [13, 14]. A phase II randomized trial of bevacizumab combined with 5-fluorouracil and leucovorin in colorectal cancer demonstrated improved response rates and time to progression [15]. Early clinical trials with bevacizumab identified bleeding, thrombosis, hypertension, and proteinuria as possible treatment-related side effects [15–17].

Building on the improvements in median progression free and overall survival for weekly irinotecan, 5-fluorouracil and leucovorin (IFL) [2], the Eastern Cooperative Oncology Group conducted a phase II clinical trial evaluating the addition of anti-angiogenesis therapy with high dose bevacizumab to IFL as first-line therapy for advanced colorectal cancer.

**materials and methods**

**eligibility criteria and patient evaluation**

This multi-institutional, phase II, NCI supported cooperative group study was open to patients with histologically confirmed colorectal cancer that was advanced or metastatic, measurable according the study. Any prior use of irinotecan or bevacizumab was not permitted. Provided that the last dose was more than 12 months prior to entering adjuvant therapy with a 5-fluorouracil based regimen were eligible for the study. All patients were followed until death. Each cycle of therapy was repeated every 42 days.

A total of 92 patients from 16 institutions in the United States were enrolled in the study between November 2000 and February 2002. Six who received treatment were determined to be ineligible, and five withdrew from the study prior to the start of treatment. Baseline patient characteristics are summarized in Table 1. The median age was 59 years, 60% were male, and 59% had an ECOG performance status of 0.

<table>
<thead>
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<th>Imitat</th>
<th>Neut / m</th>
<th>INFusion (90 min)</th>
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<tr>
<td>Irinotecan</td>
<td>125</td>
<td>mg/m</td>
<td>IV infusion (90 min)</td>
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<td>Leucovorin</td>
<td>20</td>
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<tr>
<td>Bevacizumab</td>
<td>10</td>
<td>mg/kg</td>
<td>IV infusion (90 min)</td>
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Each cycle of therapy was repeated every 42 days.

Accrual was suspended between April and August 2001 pending a review of toxic deaths that had been reported in several other studies using the IFL regimen, and adjustments to the starting doses of irinotecan and 5-fluorouracil were made. The subsequent 68 patients enrolled to the study were treated with the following doses:

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Following cycle 1 of therapy, a dose escalation to the original doses of IFL was permitted provided that no dose omissions occurred, and that diarrhea and neutropenia were never worse than grade 1.

**statistical considerations**

The primary objective of E2200 was the demonstration of an improvement in progression free survival for IFL and bevacizumab in previously untreated advanced colorectal carcinoma. Progression free survival was defined as the length of time from registration to the study to either disease progression or to death from disease or unknown causes. The study was also designed to evaluate the response rates, time to progression and toxicity for IFL and bevacizumab in this population.

Based upon a 7-month progression-free survival reported for IFL [2], the study was designed to have a greater than 90% power to detect a 70% PFS at 7 months (or a median PFS of 13.6 months) using a 0.05 level one-sided binomial test. In addition, there was a greater than 90% power to detect a 21% absolute increase in response rate from the null hypothesis of 40%. A 90% confidence interval for the true response rate would be no wider than 25%.

Patients who were eligible and who received treatment were included in the efficacy analysis. Patients who received any treatment were included in the toxicity analysis. The study was designed to have a >92% probability of observing toxicity with rates ≤5%. A 90% confidence interval for any true toxicity rate will be no wider than 25%.

**results**

**patient characteristics**

A total of 92 patients from 16 institutions in the United States were enrolled in the study between November 2000 and February 2002. Six who received treatment were determined to be ineligible, and five withdrew from the study prior to the start of treatment. Baseline patient characteristics are summarized in Table 1. The median age was 59 years, 60% were male, and 59% had an ECOG performance status of 0.
Metastatic sites included liver in 82% of patients and lung in 34% patients.

For all treated patients, the median number of cycles administered was six, with a maximum of 27. Of the patients registered to the trial following the modification of the starting doses of irinotecan and fluorouracil eleven (18%) underwent a dose escalation to the original starting doses of those agents at cycle 2.

**Toxicity**

Adverse events observed in this study are summarized in Table 2 for the 87 patients who received protocol therapy. Toxicity based on the starting doses of the chemotherapy agents is summarized in Table 3. Of the 87 patients with reported toxicity, 41 (47%) experienced grade 3 toxicity as their worst degree of toxicity and 25 (29%) had grade 4 toxicity as their worst grade. The most common grade 3 or higher toxicity was neutropenia (36%). There were two deaths on study. One patient died due to cerebrovascular ischemia, which was coded as possibly related to treatment. One patient died following surgery for bowel perforation and is described below. No deaths were reported within 60 days from the start of protocol therapy.

Forty-eight bleeding events occurred in 37 (43%) patients. All but two of these events were grade 1 or 2. The single most common event was epistaxis with 22 of the 23 events being grade 1. One individual with epistaxis required balloon tamponade to achieve hemostasis and was coded as grade 4. This individual also required a transfusion of packed red blood cells. Another individual experienced melena with an associated decline in hemoglobin and received a transfusion of packed red blood cells. Thrombosis occurred in 11 (13%) of patients, three of which were pulmonary embolism. Hypertension of any grade occurred in 11 (13%) of patients with two patients experiencing grade 3 hypertension. There were no reports of grade 3 or grade 4 proteinuria.

Two patients developed bowel perforation and one patient developed an enterocutaneous fistula while on study. One individual who experienced bowel perforation entered the study 3 weeks following the resection of the primary tumor. The perforation occurred 6 days after the first administration of therapy. At surgery, the perforation was identified at the colon–colon anastomotic site. Following recovery from surgical repair of the perforation, the individual continued on study without further complications. The second reported perforation occurred during the 10th cycle of therapy. The perforation was identified at a site of colonic recurrence and was associated...
with an abscess that extended into the right lower extremity. This patient died from sepsis despite surgical debridment of the affected tissues. The enterocutaneous fistula developed during the 6th cycle of therapy at a previous incision site and involved the transverse colon.

response
Of the 81 patients included in the efficacy evaluation, five complete responses (6.2%) and 35 partial responses were observed for an overall response rate of 49.4%. The median duration of response was 10.6 months.

progression free survival and overall survival
The median follow-up for all patients is 37.5 months. Of the 81 eligible and treated patients, 57 either relapsed or had progressive disease with a median progression free survival of 10.7 months (Figure 1). The seven-month progression free survival rate is 68% ± 5%, the one year overall survival rate is 85% ± 4%, and the median overall survival is 26.3 months (Figure 2).

Figure 1. Progression free survival curve for patients with metastatic colorectal cancer treated with bevacizumab (10 mg/kg), irinotecan, fluorouracil and leucovorin.

Figure 2. Overall survival curve for patients with metastatic colorectal cancer treated with bevacizumab (10 mg/kg), irinotecan, fluorouracil and leucovorin.

discussion
Vascular endothelial growth factor mediates tumor neo-vascularization via its interaction with the VEGF receptors found on vascular endothelial cells [19, 20]. Bevacizumab, a recombinant humanized monoclonal antibody, binds to all isoforms of VEGFα and the preclinical evaluation of the murine antibody from which bevacizumab is derived demonstrated dose-dependent anti-tumor activity in a VEGF expressing human colorectal cancer model [21]. Clinical studies of bevacizumab have shown improved efficacy when the drug is added to first-line fluorouracil based chemotherapy for advanced colorectal cancer [15, 22].

The combination of irinotecan, fluorouracil and leucovorin (IFL), all given by bolus intravenous injection, was established as a standard first-line regimen in the management of advanced colorectal cancer by a randomized Phase III trial that proved its superiority to 5-fluorouracil and leucovorin [2]. The present study demonstrates that the addition of bevacizumab, administered at a dose of 10 mg/kg, to IFL is well tolerated and may increase the proportion of patients achieving an objective response and progression free survival when compared with historical controls [2].

The dose of bevacizumab used in our study was chosen based on preclinical and clinical studies that support a dose–response effect for the agent [16, 23–26]. This finding, however, was challenged by a small phase II study of bevacizumab in combination with 5-fluorouracil and leucovorin for metastatic colorectal cancer that suggested bevacizumab administered at a dose of 5 mg/kg achieved higher response rates and a longer time to progression compared to 10 mg/kg [15]. However, the 95% confidence intervals for response rate and time to progression overlap for the two doses, and there were imbalances in the randomization that could have influenced the efficacy endpoints. We concluded from those data that 10 mg/kg is an active dose in advanced colorectal cancer that warranted further evaluation [15].

The addition of high-dose bevacizumab to IFL does not appear to exacerbate the described side effects for the regimen. As might be expected, a comparison of side effects based on the two sets of starting doses used in our study suggests a reduction in the incidence of grade 3 diarrhea and vomiting, and a reduction in the occurrence of complications from neutropenia for those patients treated with the lower doses of irinotecan and 5-fluorouracil. Grades 3 and 4 toxicity possibly attributed to bevacizumab consisted of bleeding, hypertension and thrombosis. In addition, bowel perforation in two patients may have been associated with the agent.

Our response rates and progression free survival are consistent with published results for the addition of bevacizumab to chemotherapy with IFL [27]. It is of particular interest that the response rate of 49.4%, mean PFS of 10.7 months for those treated in our study are similar to the 45% response rate, mean PFS of 10.3 months and overall survival of 19.5 months reported by Hurwitz and colleagues for patients who received IFL and 5 mg/kg of bevacizumab [27]. While the dose of bevacizumab in the current study was higher than that used by Hurwitz et al., it is important to
emphasize that the starting doses of irinotecan and 5-fluorouracil were reduced for 67 (83%) of the patients. Thus, despite the reduced chemotherapy doses, there was no apparent compromise in efficacy.

Our results demonstrate that high-dose bevacizumab when combined with IFL is well tolerated and active when compared to the published results for the chemotherapy regimen [2]. In addition, our results compare favorably to those for low dose bevacizumab (5 mg/kg) and full dose IFL [27] when used as first-line therapy for metastatic colorectal cancer.

**acknowledgements**

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**references**