A Phase II Study of Capecitabine, Oxaliplatin, Bevacizumab and Cetuximab in the Treatment of Metastatic Colorectal Cancer

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Abstract. Aim: This study was designed to determine the efficacy and tolerability of capecitabine, oxaliplatin and bevacizumab in combination with cetuximab as first-line therapy for advanced colorectal cancer. Patients and Methods: Patients with previously untreated advanced colorectal cancer received oxaliplatin 130 mg/m2 and bevacizumab 7.5 mg/kg every three weeks, capecitabine 850 mg/m2 twice daily on days 1-14, and cetuximab at 400 mg/m2 load and 250 mg/m2 weekly. KRAS, Braf and PI3K mutation status from paraffin-embedded tumor samples were assessed using real-time polymerase chain reaction. Results: Thirty patients were evaluable for safety and efficacy. One patient had a complete response and 12 patients had a partial response, giving an overall response rate of 43% (95% confidence interval (CI) 25%-63%). Fifteen patients had stable disease. The median time to progression was 10.3 months (95% CI, 6.8-16.3 months). The median overall survival was 18.8 months (95% CI, 14.2-23.7 months). Common grade ≥3 non-hematological toxicities were skin rash (37%), sensory neuropathy (27%) and diarrhea (17%). Grade ≥3 hematological toxicities were uncommon. Mutations in KRAS, Braf and PI3K occurred in 34.5%, 10.3% and 10.3% of patients respectively, but did not correlate with treatment outcome. Conclusion: The addition of cetuximab to capecitabine, oxaliplatin and bevacizumab did not improve the three-drug regimen activity compared to published data and was associated with significant toxicities requiring frequent dose modifications. KRAS, Braf, and PI3K mutation status were consistent with published literature, but did not affect outcome in this small study.

Treatment of colorectal cancer has evolved significantly over the past decade. In the first-line setting, current therapy usually consists of 5-fluorouracil (5-FU)-based combination chemotherapy plus a targeted antibody against either vascular endothelial cell growth factor (VEGF) or the epidermal growth factor receptor (EGFR) (1). Capecitabine is an oral pro-drug of 5-FU that allows greater patient convenience compared to infusional 5-FU. Randomized phase III trials have demonstrated non-inferiority of oral 5-FU in combination with oxaliplatin (XELOX) compared to infusional 5-FU and oxaliplatin (FOLFOX) in first- (2, 3) and second-line settings (4). Among biological agents added to chemotherapy, bevacizumab was the first to demonstrate clinical benefit in the first-line setting, initially with the irinotecan, 5-FU and leucovorin (IFL) regimen, and later with other regimens including 5-FU and leucovorin (5), capecitabine (6), and with FOLFOX and XELOX (7). The anti-EGFR monoclonal antibodies cetuximab and panitumumab have shown clinical benefit in patients with metastatic colorectal cancer whose tumors do not harbor a KRAS mutation. In the first-line setting, cetuximab has demonstrated a significant benefit in combination with infusional 5-FU plus irinotecan (FOLFIRI regimen) (8); however, the role for cetuximab combined with oxaliplatin-based regimens (FOLFOX or XELOX regimens) is controversial (9, 10).

At the time this study was initiated, a randomized study of 83 patients suggested a benefit from addition of bevacizumab to the regimen of cetuximab plus irinotecan in refractory colorectal cancer (11). Similarly, the combination
of capecitabine, oxaliplatin, and bevacizumab had just been shown to be tolerable and active (12, 13). Preclinical data had demonstrated greater antitumor activity when anti-VEGF and anti-EGFR agents were combined with chemotherapy compared to chemotherapy alone (14, 15) and when they were combined with each other compared to each agent alone (16-18). Therefore, this phase II study was conducted to evaluate the tolerability and activity of the XELOX-A regimen (capecitabine, oxaliplatin, bevacizumab) combined with cetuximab in first-line metastatic colorectal cancer. This trial was also designed to allow exploration of biomarkers of activity, including KRAS, BRAF, and PI3K, which were not yet well studied at the time of study initiation.

Patients and Methods

Patient selection. Patients with histologically confirmed colorectal adenocarcinoma and documented metastatic or incurable recurrent disease were eligible to participate in the trial. Patients were required to be age ≥18 years, with Eastern Cooperative Group (ECOG) performance status 0-2, normal organ and marrow function defined as absolute neutrophil count (ANC) >2,000/μl, platelets >100,000/μl, total bilirubin <2.0 × upper limit of normal (UNL), aspartate transaminase (AST) or alanine transaminase (ALT) <2.5 × UNL (<5 × UNL if liver metastasis present) and creatinine clearance >40 ml/min.

Exclusion criteria included having received prior chemotheray or biologic therapy for metastatic or recurrent disease (adjuvant or radiosensitizing fluoropyrimidines with or without leucovorin more than six months before study entry and adjuvant oxaliplatin more than 12 months before study entry were allowed), poorly controlled hypertension arterial thromboembolic events within six months of study entry, uncontrolled coagulopathy; major surgery within four weeks of initiation of study treatment, untreated leptomeningeal or brain metastases, grade ≥2 peripheral neuropathy, inability to tolerate oral medications, baseline urine protein:creatinine ratio >1.1, and pregnancy or lactation. Women of childbearing potential were required to have a negative pregnancy test within seven days prior to initiation of therapy. Effective contraception was required for sexually active female and participants male.

The study protocol (Clinicaltrials.gov NCT00290615) was approved by the Institutional Review Boards of each participating center and conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines. Written informed consent was obtained prior to any trial related procedures.

Study design and statistical methods. This was an open-label, multicenter, non-randomized phase II trial. The primary endpoint was overall response (complete and partial response), as defined by RECIST 1.0 criteria based on an intent-to-treat analysis. Secondary endpoints included time to progression, progression-free survival (PFS), overall survival, safety and tolerability, PFS was defined as the interval between start of treatment and the date of disease progression or death, censoring for loss to follow-up or start of new line of treatment (for patients who discontinued study treatment for reasons other than disease progression). A 2-stage modified Simon design was used to test the null hypothesis that the response rate was ≤30% versus an alternative favorable response ≥50%, with a significance level of 0.05 and power of 0.853. In the first stage, 15 patients were to be enrolled and the trial stopped if four or fewer patients showed response. If five or more patients responded in the first stage, the trial was to enroll an additional 30 patients in the second stage. If 18 or fewer patients out of 45 patients showed response, the treatment was to be considered to have a response rate of ≤30% and unworthy of further investigation. The null hypothesis was to be rejected if nineteen or more patients responded. The exact method was used to calculate 95% confidence interval of proportions. Survival duration was calculated using the Kaplan-Meier method and comparison between subgroups were performed using the log-rank test. Cox proportional hazard model was used to assess the effect of KRAS, BRAF and PI3K on PFS and overall survival.

Treatment schedule. Patients received treatment in 21-day cycles, comprising oral capecitabine 850 mg/m^2 every 12 hours on days 1-14, weekly cetuximab at an initial dose of 400 mg/m^2 intravenously over 120 minutes and subsequently 250 mg/m^2 over 60 minutes; on day one of each cycle, oxaliplatin 130 mg/m^2 was administered intravenously over two hours and bevacizumab 7.5 mg/kg was administered intravenously over 30-90 minutes. The use of growth factors was permitted. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events 3.0 (NCI CTCAE), Version 3.0. Neurosensory toxicity was graded according to the Neurologic Toxicity Scale for Oxaliplatin. Treatment on day one of each cycle was delayed until recovery of ANC >1,500/mm^3 and platelet count >75,000/mm^3 and recovery from any clinically significant treatment-related non-hematologic toxicity (except alopecia, anorexia or fatigue) to grade ≤1, or bilirubin and alanine transaminase to grade ≤1. Dose reduction due to adverse events was performed for each drug as specified in the study protocol, which included algorithms to manage oxaliplatin-related neuropathy, capecitabine-related diarrhea and hand-foot syndrome, cetuximab-related acne and infusion reactions and bevacizumab-related hypertension.

Patient evaluation. Vital signs, ECOG performance status, medical history, physical examination, neurosensory assessment, complete blood count (CBC), creatinine, AST, ALT, bilirubin, magnesium, urine protein to creatinine ratio, and toxicity assessments were performed at baseline and every three weeks prior to each treatment cycle. An electrocardiogram was performed at baseline and every three cycles. Formal toxicity assessments were performed weekly for the first three cycles, as well as weekly CBC for the first two cycles.

Tumor response was assessed every two cycles (nine weeks). Study specific assessment of tumor measurements were performed by a radiologist for all patients.

The primary study outcome was ‘on treatment’ PFS, defined from the start of study treatment to date of disease progression or death, whichever occurred earlier, with censoring of patients at the time of loss to follow-up or start of new line of treatment (for patients who discontinued study treatment for reasons other than disease progression). Responses were scored according to RECIST criteria version 1.0 (19).

Correlative studies. Formalin-fixed paraffin-embedded tumor tissue blocks were obtained for each patient. The tumor content was determined by a pathologist and paraffin blocks containing greater than 70% tumor were used for genomic DNA isolation. One 10 μm
cut was used to isolate the genomic DNA using an Ambion RecoverAll Total Nucleic Acid Isolation kit per manufacturer’s instructions (Foster City, CA, USA). **KRAS** mutation status was determined by real-time PCR using the DxS KRAS Mutation Test Kit from DxS Diagnostic Innovations (Manchester, UK), which is able to detect the seven common mutations of the **KRAS** gene at codons 12 and 13. **PI3K** mutation status was determined by real-time PCR using the DxS PI3K Mutation Test Kit from DxS Diagnostic Innovations, which is able to detect the following mutations in Exons 9 and 20 of the **PIK3CA** gene: H1047R, E542K, E545D, and E545K. **BRAF** mutational status was determined using a custom developed Taqman SNP assay from Applied Biosystems to detect the V600E mutation in the **BRAF** gene (Carlsbad, CA, USA).

**Results**

Thirty eligible patients were enrolled: 15 patients were treated in stage one and 15 were treated in stage two. Patient demographics are described in Table I. The median age of the patients was 57 years (range 33-77 years) and all had good baseline performance status ECOG 0-1. A minority (23%) had received prior adjuvant chemotherapy, including one patient who had received adjuvant oxaliplatin for rectal cancer. After the release of preliminary results from the CAIRO2 study (capecitabine, oxaliplatin, bevacizumab and cetuximab), which demonstrated a reduction in PFS with the addition of cetuximab to the combination of capecitabine, oxaliplatin and bevacizumab (20), a decision was made to close the present study before the completion of stage two.

Dose modifications for toxicity were required in most patients. Among the thirty patients, dose reductions of at least one agent by at least one dose level occurred in 21 patients. Seven patients required two or more reductions; eleven required discontinuation of one or more drugs.

The treatment-related adverse events are summarized in Table II. There were no unexpected adverse events encountered with the combination therapy. The most common non-hematological adverse event of any grade were skin rash (90%), sensory neuropathy (84%), diarrhea (70%), hypomagnesemia (56%), nausea (50%), hand-foot syndrome (23%), proteinuria (20%), fatigue (17%) and hypertension (10%). The most common grade three to five non-hematological toxicities were skin rashes (37%), sensory neuropathy (27%), diarrhea (17%) and paronychia (10%). The most common hematological adverse events of any grade included thrombocytopenia (50%), neutropenia (33%) and anemia (10%). Grade three to five anemia, thrombocytopenia and neutropenia occurred in 3%, 7% and 0%, respectively.

Twenty serious adverse events (excluding death) were documented among ten patients, including diarrhea (n=7), dehydration (n=1), dyspnea (n=1), elevated ALT/AST (n=1), sepsis (n=1), hyperbilirubinemia (n=1), urethral obstruction from disease progression (n=1), cerebrovascular accident...
(n=2), bowel obstruction (n=2), bowel perforation (n=1), pulmonary embolism (n=1) and deep venous thrombosis (n=1). There were two deaths unrelated to study drug; one occurred after elective resection of a liver metastasis and the other after evidence of disease progression.

Tumor tissue was available from 29 out of 30 patients for KRAS, BRAF and PI3K mutation status analysis. Ten patients had KRAS mutations (34.5%). BRAF mutations occurred in 3 out of 29 patients (10.3%) and were mutually exclusive of KRAS mutations. Hence, the incidence of BRAF mutations among the 19 patients with wild-type KRAS was 16%. PI3K mutations occurred in three patients (10.3%), of whom one also had a mutation in KRAS but not BRAF; one patient had no mutation in either KRAS or BRAF and another patient had a mutation in BRAF but not KRAS.

**Efficacy.** Among the entire cohort, the median PFS was 10.3 months (95% CI, 6.8 to 16.3 months) (Figure 1). Since there were no deaths prior to disease progression or censoring for progression, calculated time to progression yielded identical results to PFS. The median overall survival was 18.8 months (95% CI, 14.2 to 23.7 months) (Figure 2). The overall response rate was 43% (95% CI, 25% to 63%); one patient had a complete response, twelve patients had a partial response, and fifteen patients had stable disease as their best response.

An exploratory analysis of outcomes based upon tumor mutation status was also performed. The median PFS was 10.3 months for patients with wild-type KRAS tumors and 8.9 months for patients with mutant KRAS tumors. These differences were not statistically significant (log-rank test p=0.13) (Figure 3). Median overall survival was 18.0 months for patients with wild-type KRAS tumors and 21.1 months for patients with mutant KRAS tumors. These differences were also not statistically significant (log-rank test p=0.30) (Figure 4). The response rates for patients with wild-type KRAS tumors and mutant KRAS tumors were 37% and 60% respectively.

For patients with mutant versus wild-type BRAF tumors, median PFS and overall survival were significantly shorter (3.3 vs. 10.6 month; log-rank test p=<0.001 and 4.1 months vs. 19.2 months, log-rank test p=0.002, respectively). However, this analysis was limited by the small number of patients in the BRAF mutation subgroup (Figures 5 and 6). For the six patients with tumors that were wild-type for both KRAS and BRAF, the response rate was 38%.

There were no statistically significant differences in PFS or overall survival by PI3K mutation status (data not shown). For the 17 patients with tumors that were wild-type for both KRAS and PI3K, the response rate was 41%. For the two patients with tumors that were KRAS wild-type but PI3K mutant, the response rate was 0%. After adjusting for KRAS and PI3K mutation status in a Cox proportional hazard regression model, BRAF mutation remained a significant adverse predictor of PFS (hazard ratio 16.7, 95% CI, 2.6 to 109.4, p=0.003) and overall survival (hazard ratio 8.3, 95% CI, 1.9 to 37.4, p=0.006).

**Discussion**

In this phase II study for first-line metastatic colorectal cancer, the regimen of capecitabine, oxaliplatin, bevacizumab, and cetuximab had a median time to progression of 10.3 months and an objective response of 43%. In patients with wild-type KRAS tumors, PFS was 10.3 months and the response rate was 37%. These results are also consistent with results from two large phase III studies, CAIRO2 and PACCE.
The CAIRO2 study compared capecitabine, oxaliplatin, and bevacizumab with and without cetuximab (20); the PACCE study compared infusional 5-FU, oxaliplatin or irinotecan, and bevacizumab with and without panitumumab (21). In the CAIRO2 study, for patients treated with cetuximab, median PFS was 9.4 months for all patients and 10.5 months for patients with wild-type tumors. In the PACCE study, for patients treated with panitumumab, median PFS was 10.0 months for all patients and 9.8 months for patients with wild-type tumors. In both studies, the addition of an anti-EGFR antibody was associated with worse clinical outcome compared to the control treatment in unselected patients and in patients with ras mutant tumors.

The frequencies of KRAS, BRAF, and PI3K mutations in the present study are comparable to those found by other groups (8, 9, 22). Mutations in KRAS and BRAF have been associated with a lack of benefit from anti-EGFR monoclonal antibodies in metastatic colorectal cancer. Mutations in PI3K have been found in 10% of colorectal cancers (23-25), and have been associated with a lack of response to anti-EGFR directed therapy in some reports (26) but not in others (27). KRAS and PI3K mutation status was not associated with treatment outcome in the current study. This may be explained in part by the small sample sizes involved. Patients whose tumors were wild-type BRAF had better clinical outcomes compared to those whose tumors carried BRAF mutations, and this effect persisted after adjusting for KRAS and PI3K mutation status. These findings are consistent with the literature supporting the prognostic role of BRAF (28-30).
The regimen of capecitabine, oxaliplatin, bevacizumab and cetuximab was associated with significant toxicities. The rate of grade 3-4 toxicities in the current report are similar to those reported in CAIRO2 and PACCE. Importantly, in the current study, more than half of all patients required either multiple dose reductions or discontinuation of one or more study drugs for toxicity. Thus, poor long-term tolerability of the regimen may limit potential efficacy, particularly for progression time points beyond six months. The reasons for lack of benefit from the addition of an anti-EGFR therapy to first-line 5-FU, oxaliplatin, and bevacizumab-based treatment are not known. Combining anti-EGFR and anti-VEGF therapies has been useful as maintenance therapy for non-small cell lung cancer (31), but has had limited success in other settings (32, 33).

In conclusion, for patients with unknown KRAS mutation status and those with wild-type KRAS tumors, the combination of cetuximab with capecitabine, oxaliplatin and bevacizumab is only moderately well tolerated and offers no apparent clinical benefit over standard three drug regimens.

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References


