

## Association Between COX-2 Expression and Effectiveness of COX-2 Inhibitors in a Phase II Trial in Patients with Metastatic Colorectal Adenocarcinoma

KHALDOUN ALMHANNA<sup>1</sup>, BASSEL EL-RAYES<sup>2</sup>, SEEMA SETHI<sup>3</sup>, GREGORY DYSON<sup>3</sup>, LANCE HEILBRUN<sup>3</sup>, PHILIP A PHILIP<sup>3</sup> and FAZLUL SARKAR<sup>4</sup>

<sup>1</sup>Department of Gastrointestinal Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, U.S.A.;

<sup>2</sup>Department of Hematology and Oncology, Winship Cancer Institute, Atlanta, GA, U.S.A.;

<sup>3</sup>Department of Hematology and Oncology, Karmanos Cancer Institute, Wayne State University, Detroit, MI, U.S.A.

**Abstract.** *Aim: The role for the cyclooxygenase-2 (COX-2) pathway in colorectal carcinogenesis has been suggested in pre-clinical models. In a previously reported phase II trial, the addition of COX-2 inhibitor celecoxib to irinotecan and capecitabine did not appear to significantly increase the activity of chemotherapy in patients with metastatic colorectal carcinoma (mCRC). We evaluated the COX-2 expression in the available tumors from enrolled patients by immunohistochemistry, as well as its correlation with clinical outcome. Patients and Methods: Fifty-one patients with mCRC were enrolled in the phase II study between June 2002 and November 2005. Patients received a combination of irinotecan 70 mg/m<sup>2</sup> over 30 min i.v. on days 1 and 8, capecitabine 1,000 mg/m<sup>2</sup> twice per day orally on days 1-14 and the COX-2 inhibitor celecoxib at a daily dose of 800 mg continuously. Cycles were repeated every 21 days. Formalin-fixed paraffin-embedded tumor tissue samples were available for 17 patients enrolled on this study. COX-2 expression was evaluated by immunohistochemistry and was correlated with clinical outcome. Results: In the phase II study, the objective response rate was 41%. The median time to progression was 7.7 months and median survival time was 21.2 months. Tumor COX-2 expression, by immunohistochemistry, was assessed for 17 patients enrolled in the same phase II study. While not statistically significant, the response rate was better for patients in the low COX-2 expression group, while time to progression and overall survival was longer in patients in the high COX-2 expression group. This discrepancy can be partially attributed to the small sample*

*size. Conclusion: In the previously published phase II study, the addition of celecoxib to irinotecan and capecitabine did not appear to significantly increase the activity of chemotherapy. COX-2 expression by immunohistochemistry was neither prognostic nor predictive for response.*

Colorectal cancer (CRC) is the second most common cause of cancer death in the United States resulting in 55,000 deaths per year (1). Cytotoxic and targeted agents have significantly increased survival in patients with metastatic colorectal cancer (mCRC) (2). Despite the advances in the treatment of mCRC in the last 10 years, fewer than 50% of patients remain alive two years following diagnosis. The improvement in outcome in mCRC is dependent on developing more effective therapies with novel mechanisms of action.

The cyclo-oxygenase (COX) enzymes catalyze the rate-limiting step in the conversion of arachidonate to prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), the immediate substrate for prostaglandin, and thromboxane synthesis (3). COX-2 plays an important role in colorectal carcinogenesis, angiogenesis, metastasis (4-6), and chemoresistance (7-9). Seventy percent of CRC cases express COX-2 (10, 11) as compared to adjacent normal epithelium, which makes it a very attractive target in the therapy of CRC. Inhibiting COX-2 with anti-inflammatory drugs was suggested to reduce polyps recurrence in multiple studies (12, 13).

Between June 2002 and November 2005, 51 patients with pathologically or cytologically confirmed diagnosis of metastatic adenocarcinoma of the colon or rectum were enrolled in a phase II trial where patients received combination of irinotecan 70 mg/m<sup>2</sup> over 30 min i.v. on days 1 and 8, capecitabine 1,000 mg/m<sup>2</sup> twice per day orally on days 1-14, and the COX-2 inhibitor celecoxib at a daily dose of 800 mg continuously. Cycles were repeated every 21 days. In that study, the objective response rate was 41%, with median time to progression (TTP) of 7.7 months (95%

Correspondence to: Khaldoun Almhanna, MD, MPH, 12902 Magnolia Drive, FOB-2, Tampa, FL 334612, U.S.A. Tel +1 8137457257 Fax: +1 8137457229, e-mail: Khaldoun.almhanna@moffitt.org

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Table I. Response rate in high- and low-cyclooxygenase-2 expression groups.

	CR/PR	SD/PD	Response rate (80% CI)	
Low-expression score (0-3)	4	5	44.4% (26-65%)	<i>p</i> =0.63
High-expression score (4-6)	2	5	28.6% (12-53%)	

CR: Complete response, PR: partial response, PD: progressive disease, SD: stable disease, CI: confidence interval.

confidence interval CI=6.2-8.6 months) (14). Antitumor activity of irinotecan and capecitabine did not significantly improve with concurrent administration of the COX-2 inhibitor. The lack of benefit could be related, at least in part, to the non-selective nature of the study.

In this study we examined the expression of COX-2 in available tumor tissues from patients enrolled in that same phase II trial to evaluate whether COX-2 expression correlates with response to COX-2 inhibitor.

### Patients and Methods

**Study cohort.** Fifty-one patients were enrolled in the phase II study. Cases were retrieved from the computerized database of the department of Pathology, Karmanos Cancer Institute/Wayne State University School of Medicine, Detroit, MI., USA. After obtaining approval from the Institutional Review Board, a retrospective chart review of each patient's demographic, clinical and pathological data was performed. In each case, histopathology slides were microscopically reviewed to select a representative tumor block. (n=17)

**Immunohistochemical analysis.** Four-micron tissue sections were cut from the selected tumor block on charged slides and stained for immunohistochemical analysis using specific antibodies for COX-2 (Zymed Laboratories Inc., San Francisco, CA., USA). Standard staining protocols according to the laboratory manual were used as previously described (15). The protocol was then optimized for antigen retrieval, antibody dilution and incubation conditions. A tissue known for COX-2 positivity was stained with each investigative case study.

Briefly, after de-paraffinizing and hydrating to phosphate-buffered saline buffer (pH 7.4), the sections were pre-treated with hydrogen peroxide (3%) for 10 min to remove endogenous peroxidase, followed by antigen retrieval *via* steam bath for 20 min in EDTA. The primary antibody was then applied, followed by washing and incubation with the biotinylated secondary antibody for 30 min at room temperature. Detection was performed with diaminobenzidine and counterstained with Mayer hematoxylin followed by dehydration and mounting.

**Assessment of COX-2 expression.** *A priori* hypothesis was generated that COX-2 expression would correlate with response to celecoxib. Immunohistochemical staining was performed for tumors of 23 patients on paraffin-embedded tumors. COX-2 immunostained slides were studied under a transmission light microscope to blindly score the expression levels based on staining intensity. COX-2 expression

was graded using a standardized grading system as absent (score=0) if COX-2 expression in the tumor had the same level of intensity as in the adjacent normal epithelium, weak staining (score=1), or strong staining (score=2); and using the percentage of positively stained cells (1=10%; 2=11-50%; 3≥50%). A final score was obtained by multiplying the two scores (0 to 6). Cases were classified as low (0-3), or high (4-6) expressers.

Among the 23 samples that were stained, six had to be excluded: one because it was a breast case; one because there was no tissue left in the block; one because there was no tumor; one because the sample could not be matched to a patient in the study; and two because they were duplicates. This resulted in 17 analyzable samples.

**Endpoints.** Three endpoints were examined in this study: response rate (complete response plus partial response), TTP (time from trial registration until disease progression or death) and overall survival (OS) (time from trial registration until death). Disease progression was evaluated every two cycles. OS was monitored until the termination of the study trial in November 2005.

**Statistical methods.** Fisher's exact test was used to determine if the response rate was different between individuals in high- and low-expression groups. Kaplan-Meier methods were used to estimate the survival curves for both endpoints discussed in this article (TTP and OS). The log-rank test was used to test the difference between survival curves by the derived expression category variable. The Cox proportional hazards model was used for both endpoints to estimate the hazard ratio of experiencing an event in the high-expression group as compared to the low-expression group, with *p*-values less than 0, using 0.20 as the significance level (appropriate for a small pilot study such as this one).

### Results

Fifty-one patients were enrolled onto the study between June 2002 and November 2005. A total of 354 cycles were administered with a median of eight cycles per patient. Two patients (4%) had CR. Nineteen (37%) patients had a PR and 19 patients had stable disease. The overall response rate (CR plus PR) based on an intent-to-treat analysis was 41% (95% CI=0.28-0.55). The median TTP was 7.7 months (95% CI=6.2-8.6 months) and the median survival time was 21.2 months. There were no cardiovascular complications potentially attributed to celecoxib, irinotecan, or capecitabine (14).

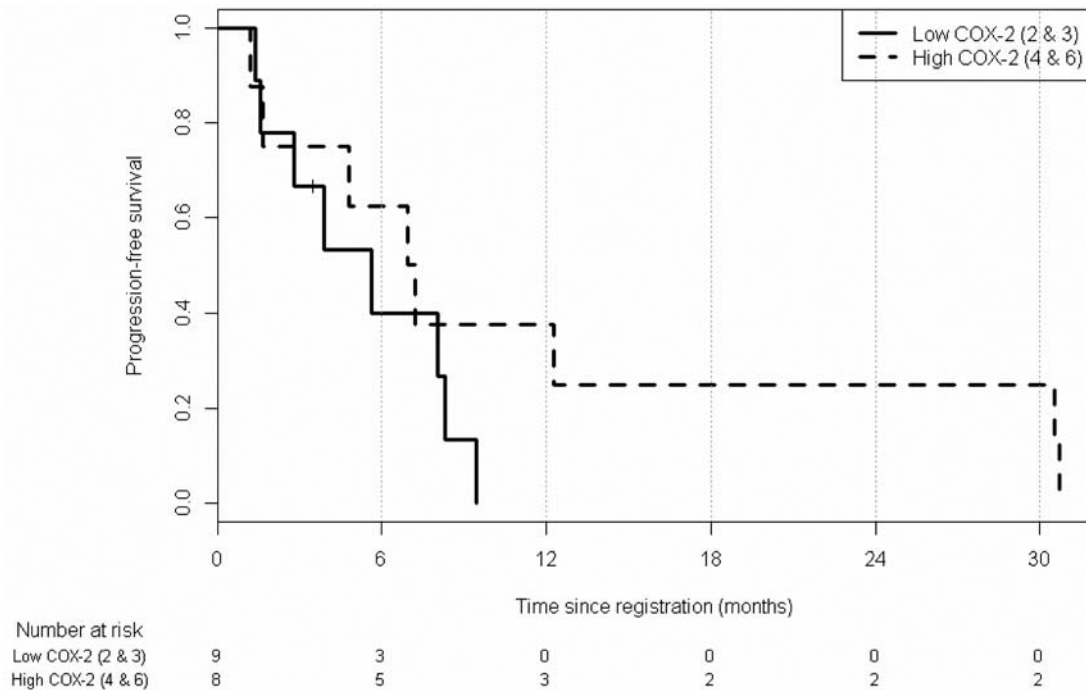


Figure 1. Kaplan-Meier curves of progression-free survival in high- and low-cyclooxygenase-2 (COX-2) expression groups.

**Association between COX-2 expression and response rate.** The median COX-2 expression score was 3. Among the nine patients with low-expression scores, two patients had score of 2 and seven patients a score of 3. The high-expressers consisted of eight patients with a score of 4 (n=1) or 6 (n=7). The association between scoring and response rate is summarized in Table I. The response rate was higher in the low-expression group, but not statistically different from that of high-expression group (Fisher's exact test,  $p$  0.63).

**Association between COX-2 expression and TTP.** Only one out of the 17 patients was censored for TTP. The median TTP (80% CI) of patients in the low- and high-expression groups was 5.62 (2.79-8.31) months and 7.06 (4.80-30.52) months respectively. The log-rank test  $p$ -value for comparison of these two groups was 0.24 (Figure 1).

The hazard ratio and 80% CI for progression among patients in the high COX-2 expression group *versus* the (reference) low COX-2-expression group was 0.51 (0.24-1.07) with a  $p$ -value of 0.25.

**Association between COX-2 expression and OS.** We performed an exploratory analysis using OS and COX-2 expression category. Nine out of the 17 patients were censored for OS. The median OS (80% CI) of patients in the low COX-2-expression group was 17.8 months (15.5 month- not reached).

The median OS of patients in the high COX-2-expression group was not reached at the time of the analysis (19.4 months, NR). The log-rank test  $p$ -value for comparison of these two groups was 0.09 (Figure 2).

The hazard ratio and 80% CI for progression among patients in the high COX-2-expression group *versus* the (reference) low COX-2-expression group was 0.25 (0.08-0.75). The small sample size, and the even smaller number of cases available for pathology review, limits the inference that can be drawn from this analysis, although the trend is consistent with longer OS in the high COX-2 expression group.

## Discussion

Our phase II study hypothesized that COX-2 inhibition by celecoxib would improve the outcome of patients with mCRC treated with combination chemotherapy. To indicate a successful study, a response rate of 55% or more was required. Results showed no overall benefit for celecoxib, with no significant improvements in response rate, progression-free survival (PFS) or OS. Potential reasons for the lack of benefit are that the drug is inactive; or the fact that the patients were not selected based on COX-2 expression and that a higher dose of COX-2 inhibitors was possibly needed. Previous data have suggested that expression of COX-2 is a poor prognostic feature in prostate (16), lung (17) and colon cancer (18). Regular use of aspirin (a COX-2 inhibitor) was found to

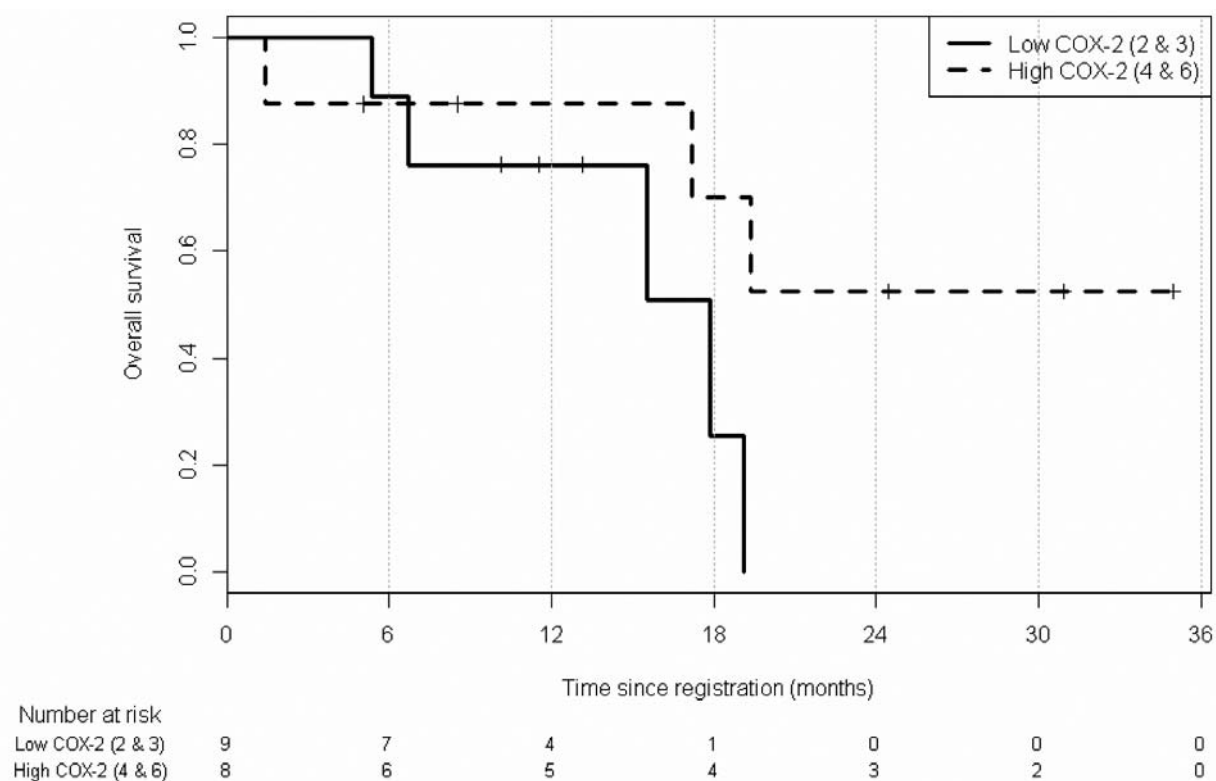


Figure 2. Kaplan-Meier curves of overall survival in high- and low-cyclooxygenase-2 (COX-2) expression groups.

reduce the risk of CRC overexpressing COX-2 but not the risk of CRC with weak or no expression of COX-2 (13). In a recent phase III randomized trial assessing the COX-2 inhibitor rofecoxib in the adjuvant setting of CRC (the VICTOR trial), rofecoxib did not improve OS or protect from recurrence in unselected patients. COX-2 expression did not correlate with prognosis or predict effectiveness of COX-2 inhibitors (19). Our analysis also failed to show any benefits in the metastatic setting, possibly secondary to the small number of samples analyzed as only 17 tumors were evaluated for COX-2 expression.

In our analysis, patients with low COX-2 expression had a higher response rate, but shorter TTP and OS. This could be related, at least in part, to the small sample size.

There is still significant interest in the potential role of COX-2 inhibition in CRC treatment and prevention. Further trials targeting patients who could benefit from inhibition of the COX-2 pathway based on predictive markers are needed. A phase III trial of 6 versus 12 treatments of adjuvant oxaliplatin, leucovorin calcium, and fluorouracil (FOLFOX) plus celecoxib or placebo for patients with resected stage III colon cancer is currently recruiting patients (ClinicalTrials.gov:NCT01150045) and the results will be awaited with interest.

**Disclosures**

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