

FOLFIRI Plus Bevacizumab 5 mg/kg Versus 10 mg/kg as Second-line Therapy in Patients with Metastatic Colorectal Cancer Who Have Failed First-line Bevacizumab Plus Oxaliplatin-based Therapy: A Randomized Phase III Study (EAGLE Study)

Hideyuki Mishima^{1,*}, Koji Oba², Junichi Sakamoto³, Kei Muro⁴, Takayuki Yoshino⁵, Ichinosuke Hyodo⁶ and Yoshihiko Maehara⁷

¹Department of Surgery, National Hospital Organization, Osaka National Hospital, Osaka, ²Translational Research and Clinical Trial Center, Hokkaido University Hospital, Hokkaido, ³Social Life Science Specified Non-profit Corporation Epidemiological Clinical Trial Research Support Organization, Nagoya University Graduate School of Medicine, Aichi, ⁴Department of Clinical Oncology, Aichi Cancer Center Hospital, Aichi, ⁵Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Chiba, ⁶Department of Gastroenterology, Institute of Clinical Medicine, University of Tsukuba Graduate School of Comprehensive Human Sciences, Ibaraki and ⁷Department of Surgery and Science, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan

*For reprints and all correspondence: Hideyuki Mishima, Department of Surgery, National Hospital Organization, Osaka National Hospital, 2-1-14, Hoenzaka, Chuo-ku, Osaka-shi, Osaka 540-0006, Japan. E-mail: hmishima@onh.go.jp

Received June 1, 2011; accepted November 15, 2011

We planned a multicenter randomized phase III study to evaluate the efficacy of appropriate dose of bevacizumab (5 or 10 mg/kg) with FOLFIRI in patients with advanced/metastatic colorectal cancer who have failed prior bevacizumab plus oxaliplatin-based therapy. The primary endpoint is progression-free survival. The secondary endpoints are the toxicity, response rate, time to treatment failure, overall survival, overall survival from the start of the first-line treatment and second progression-free survival (time duration from the initiation of the first-line treatment until progression after the protocol treatment). A total of 370 patients were considered to be appropriate for this trial.

Key words: bevacizumab – FOLFIRI – irinotecan – beyond progression – advanced/metastatic colorectal cancer

INTRODUCTION

Age-adjusted prevalence of colorectal cancer (CRC) is the second largest percentage after that of gastric cancer in males and breast cancer in females in Japan (1). According to the CONCORD study, it is reported that Japanese men attain the first place and Japanese women attain sixth for a 5-year survival rate with CRC in the world (2). Japanese patient's clinical registered data from 1991 to 1994 by the Japanese Society for Cancer of the Colon and Rectum is superior to the same period's data from Survival Epidemiology

and End Results and National Cancer Data Base for each of Stage I, II, III CRC, at most 20%.

It is estimated that the number of CRC patients will be 480 396 in 2015 and 512 225 in 2020 (1). It is also expected that the incidence of CRC will overtake that of breast cancer after 2010. Although CRC screening rates were improved, considerably large number of patients had a locally advanced or metastatic disease at the time of diagnosis. For patients with metastatic CRC, recommended first-line regimens by guidelines are FOLFOX or FOLFIRI (3,4) plus biological agents.

Bevacizumab (Avastin; Genentec, Inc., South San Francisco, CA), a recombinant, humanized monoclonal antibody that binds to and neutralizes vascular endothelial growth factor (VEGF) is one of the biological agents and was proved to improve overall survival (OS) and progression-free survival (PFS) in bevacizumab-naïve patients with metastatic CRC when administered to first- and second-line chemotherapy.

For patients with previously treated metastatic CRC, treatment results of FOLFIRI or FOLFOX as a second-line therapy were reported from the phase III study. PFS was 2.5 and 4.2 months, respectively (5). Treatment results of FOLFIRI plus bevacizumab at 5 mg/kg and FOLFOX plus bevacizumab at 5 mg/kg as a second-line treatment were reported from the phase II study. PFS was 7.8 and 5.3 months, respectively (6). In addition, the treatment result of FOLFOX4 plus bevacizumab at 10 mg/kg as a second-line therapy was reported from a randomized phase III study. OS as the primary objective was 12.9 months compared with 10.8 months of FOLFOX4 alone (HR, 0.66; $P < 0.0011$). PFS was 7.3 months, which is also significantly improved compared with 4.7 months of FOLFOX4 alone (HR, 0.61; $P < 0.0001$) (7). However, all of these treatments were examined for previously bevacizumab-naïve patients.

A key element of continuous administration of bevacizumab beyond progression is as shown below. In basic research, regrowth of tumor vessels are often observed soon after cessation of bevacizumab administration (8–10) and VEGF expression is identified across the board from the initial period of the tumor lifecycle (11). Several experimental studies have examined that the muMAb 4.6.1 antibody, mouse monoclonal precursor of VEGF inhibitors in CRC xenograft models prevents growth of tumor cells at metastatic sites dose dependently (12). In addition, the BRiTE study (13), one of the observational cohort studies in the USA provides supportive clinical data about the foregoing. Median OS were 12.6, 19.9 and 31.8 months in the no post-progressive disease (PD) treatment, chemotherapy without bevacizumab and chemotherapy with bevacizumab groups, respectively.

After adjustment for other prognostic factors, bevacizumab treatment beyond progression maintained a statistically significant effect on survival after PD, compared with no post-PD bevacizumab (HR, 0.49; 95% CI, 0.41–0.58; $P < 0.001$). In this study, the proportion of bevacizumab doses administered as the second-line therapy were 90.7% (5 mg/kg), 3.6% (7.5 mg/kg) and 2.3% (10 mg/kg). These results from the BRiTE study suggest that continuous VEGF inhibition with bevacizumab beyond initial PD could play an important role for prolonging survival of patients with metastatic CRC.

There are three major clinical questions to be solved about second-line biological agents in metastatic colorectal cancer. The first clinical question about the continuation of bevacizumab after exposure to bevacizumab treatment will be revealed from the results of the on-going trial 'AIO 0504'. The second clinical question about the drug selection between bevacizumab and anti-epidermal growth

factor receptor antibodies with KRAS wild type after a first-line bevacizumab-containing regimen will also be answered by the on-going trial 'SPIRITT'.

On the other hand, the third clinical question about the optimal doses of bevacizumab as second-line treatment followed by a bevacizumab-containing regimen is still remains unsolved. The verified data indicates the efficacy of bevacizumab at 5 mg/kg/weekly (=10 mg/kg/biweekly) in the second-line setting followed by bevacizumab-naïve treatment (7). The recommended dose of bevacizumab is 5 mg/kg/weekly (=10 mg/kg/biweekly) in non-small cell lung cancer, breast cancer, renal cell cancer and second-line colorectal cancer (14–19), but 2.5 mg/kg/weekly (=5 mg/kg/biweekly) in the first-line CRC treatment. The dose of bevacizumab 2.5 mg/kg/weekly (=5 mg/kg/biweekly) could be lower than the recommended dose in the second-line CRC treatment.

Thus, it is necessary for us to investigate the effectiveness of high-dose bevacizumab for metastatic CRC.

Accordingly, we have conducted a randomized phase III study of FOLFIRI plus bevacizumab 5 mg/kg versus 10 mg/kg as second-line therapy in patients with metastatic CRC who have failed first-line bevacizumab plus oxaliplatin-based therapy (EAGLE study).

The study protocol was approved by the institutional review boards of each participating institution. The study met the ethical guidelines for clinical studies of the Health, Labor and Welfare Ministry in Japan, and was conducted in compliance with the Declaration of Helsinki. All patients provided written informed consent.

PROTOCOL DESIGN FOR EAGLE STUDY

OBJECTIVE

A multicenter randomized phase III study of adding bevacizumab 5 or 10 mg/kg to FOLFIRI in advanced/metastatic CRC who have failed prior bevacizumab plus oxaliplatin-based first-line therapy.

ENDPOINT

The primary endpoint is PFS. The secondary endpoints are the toxicity, response rate, time to treatment failure, OS, OS from the start of the first-line treatment and second PFS (time duration from the initiation of the first-line treatment until progression after the protocol treatment). The progression will be evaluated on the basis of response evaluation criteria in solid tumors (RECIST) ver. 1.1.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA

- (i) PD after chemotherapy with bevacizumab plus oxaliplatin-based therapy as the first-line treatment

(with measurable lesions in the RECIST criteria) or difficult to continue the first-line therapy due to the other reasons.

- (ii) Oxaliplatin and bevacizumab were administered for more than four times in the first-line treatment.
- (iii) Cytologically and/or histologically proven CRC.
- (iv) Written informed consent.
- (v) Aged 20 years old and above.
- (vi) Eastern Cooperative Oncology Group performance status (ECOG PS) 0 or 1.
- (vii) Life expectancy estimated ≥ 3 months.
- (viii) Sufficient organ functions.

EXCLUSION CRITERIA

- (i) Previous irinotecan treatment.
- (ii) Administration of transfusion/hematopoietic factor or antithrombotic drug within 14 days.
- (iii) Serious renal dysfunction.
- (iv) Serious drug hypersensitivity or a history of drug allergy.
- (v) Active concomitant malignancy.
- (vi) Active infections.
- (vii) Symptomatic or asymptomatic heart disease that is being treated at the time of registration to the trial.
- (viii) History of thrombosis, interstitial pneumonia, pulmonary fibrosis or high-grade pulmonary emphysema.
- (ix) Fresh hemorrhage from the digestive tube, intestinal tube paralysis, intestinal obstruction and peptic ulcer.
- (x) Pleural effusion, peritoneal fluid and pericardial fluid.
- (xi) Symptomatic brain metastasis.
- (xii) History of mental disturbances or cerebrovascular accident.
- (xiii) High blood pressure and diabetes that cannot be controlled.
- (xiv) Uncontrolled diarrhea.
- (xv) Serious non-healing wound and/or major surgical procedure within 4 weeks prior to enrolling in this trial.
- (xvi) Traumatic fracture that has not been healed at the time of enrollment.
- (xvii) Bleeding tendency and anti-platelet therapy (including aspirin and non-steroidal anti-inflammatory drugs).
- (xviii) Pregnant women, possibly pregnant women, wishing to become pregnant and nursing mothers.
- (xix) Needing treatment with atazanavir sulfate.
- (xx) Paralyzed bowel.

REGISTRATION

Any medical institution that would like to participate could contact a secretariat at Epidemiological and Clinical Research Information Network (ECRIN) or publicly contact: Hideyuki Mishima at the Department of Surgery, National

Hospital Organization Osaka National Hospital, Osaka, Japan.

Registration forms are sent from the ECRIN to the medical institution for registration.

Registered patients are allocated randomly into the FOLFIRI + 5 mg of bevacizumab arm (arm A) or the FOLFIRI + 10 mg of bevacizumab arm (arm B) at the data-center. For randomization, a minimization method or dynamic randomization is used with five balancing factors: baseline ECOG PS, number of metastasis ($2 >$, $2 \leq$), reason for a change in therapy to second-line treatment (PD in first-line treatment/non-PD), early recurrence within 6 months (during/after adjuvant treatment) and institutions.

TREATMENT METHODS

FOLFIRI plus bevacizumab consists of bevacizumab at 5 mg/kg (or 10 mg/kg) as a 30-min infusion and *l*-leucovorin 200 mg/m² as a 2-h infusion, and concurrently irinotecan 150 mg/m² as an over 90-min infusion, followed by bolus fluorouracil (5-FU) 400 mg/m² within 15 min and 46-h infusion of 5-FU 2400 mg/m². Patients randomly assigned to arm A receive FOLFIRI plus bevacizumab 5 mg/kg. FOLFIRI plus bevacizumab 10 mg/kg is administered to patients randomly assigned to arm B. These treatments are repeated every 2 weeks until disease progression, unacceptable toxicity or patient choice.

FOLLOW-UP

Disease progression and occurrence of new diseases are monitored by using abdominal radiography, abdominal computed tomography (CT) or magnetic resonance imaging, and thoracic CT, and by measuring levels of the tumor markers CEA and CA19–9 at the baseline and every 8 weeks during the treatment period (tumor marker levels are measured every 4 weeks). Blood tests and symptom checks (collecting adverse events) will be carried out throughout the treatment period. In case of dyspnea, arterial blood gases will be tested and chest X-ray test will be carried out. In case of arrhythmia, a 12 lead electrocardiogram will be carried out. The follow-up period is 1 year after the registration of the last patient.

STUDY DESIGN AND STATISTICAL ANALYSIS

The primary objective of this trial is to evaluate whether arm B (FOLFIRI plus 10 mg/kg of bevacizumab therapy) significantly improves PFS compared with arm A (FOLFIRI plus 5 mg/kg of bevacizumab therapy). The null hypothesis, if the PFS of both arms is equal, is tested by the stratified log-rank test with the balancing variables (except for the institutions) as the stratification factor. If arm B showed a statistically significant prolonging effect on PFS compared with the other arm, it is concluded that arm B is more

beneficial therapy. The overall significance level of the trial is set as 5% for the two-sided test.

PFS curves are depicted by the Kaplan–Meier method. Median PFS and the annual PFS rates are also estimated using the Kaplan–Meier method with the two-sided 95% confidence interval using the Greenwood formula (20). The stratified Cox proportional hazards model is used to assess the hazard ratio with Wald-type 95% confidence intervals for the treatment effect between both arms.

Median PFS of arm A in this trial is assumed to be 5.0 months based on previous studies (6,7) and it is considered as a clinically relevant prolongation if the median PFS of arm B is 7.0 months (risk reduction 30%). At the start of this trial, the planned sample size was 280 patients to detect 30% risk reduction with 80% power for a log-rank test comparing two survival curves with a two-sided significance level of 0.05, assuming an accrual time of 2 years and a follow-up time of 1 year (21). This calculation was carried out by employing nQuery Advisor 7.0 software (Statistical Solutions, Saugus, MA, USA). On 8 April 2011, an independent data monitoring committee of the EAGLE trial recommended that the statistical power be amended from 80 to 90% with the consideration of the promising enrollment of patients. As a result, 358 patients (330 events) will be needed to detect 90% power under the same assumption. Taking some dropouts into account, the sample size to be accrued was set at 370 patients in total.

THE EAGLE TRIAL GROUP

Principal investigator: H. Mishima (Osaka National Hospital, Osaka, Japan).

Promotion committee chairman: Y. Maehara (Graduate School of Medical Science, Kyushu University, Fukuoka, Japan).

Data and safety monitoring board: I. Hyodo (University of Tsukuba Graduate School of Comprehensive Human Sciences, Ibaraki, Japan), K. Muro (Aichi Cancer Center Hospital, Aichi, Japan) and T. Yoshino (National Cancer Center Hospital East, Chiba, Japan).

Data center: J. Sakamoto (Nagoya University Graduate School of Medicine, Aichi, Japan) and C. Abe (ECRIN, Kyoto, Japan).

Statistical advisor: K. Oba (Hokkaido University, Hokkaido, Japan).

Participating institutions: Approximately 150 Japanese institutions and hospitals are participating in this trial.

Acknowledgement

This study is and will be supported in part by a non-profit organization ECRIN.

Funding

Research grant from a non-profit organization ECRIN.

Conflict of interest statement

None declared.

References

- Center for Cancer Control and Information Services, National Cancer Center, Japan. *Statistics*. <http://ganjoho.jp/public/statistics/index.html> (in Japanese).
- Coleman MP, Quaresma M, Berrino F, Lutz JM, De Angelis R, Capocaccia R, et al. Cancer survival in five continents: a worldwide population-based study (CONCORD). *Lancet Oncol* 2008;9:730–56.
- National Comprehensive Cancer Network. *NCCN Clinical Practice Guidelines in Oncology*. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp.
- Colorectal cancer treatment guideline for doctor ver, 2009. <http://www.jscrc.jp/guideline.html>.
- Tournigand C, Andre T, Achille E, Flesh M, Mery-Migmard D, Quinaux E, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004;22:229–37.
- Senellart H, Borg C, Delord JP, Hussein F, Trillet-Lenoir V, Faroux R, et al. Bevacizumab combined with chemotherapy in the second-line treatment of metastatic colorectal cancer (mCRC): 12-month results of a phase II study (bevacolor). In: 2008 Gastrointestinal Cancers Symposium (abstract 493).
- Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* 2007;25:1539–44.
- Vosseler S, Mirancea N, Bohlen P, Mueller MM, Fussenig NE. Angiogenesis inhibition by vascular endothelial growth factor receptor-2 blockade reduces stromal matrix metalloproteinase expression, normalizes stromal tissue, and reverts epithelial tumor phenotype in surface heterotransplants. *Cancer Res* 2005;65:1294–305.
- Kamba T, Tam B, Hashizume H, Haskell A, Sennino B, Mancuso MR, et al. VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. *Am J Physiol Heart Circ Physiol* 2006;290:H560–76.
- Inai T, Mancuso M, Hashizume H, Baffert F, Haskell A, Baluk P, et al. Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. *Am J Pathol* 2004;165:35–52.
- Folkman J. Angiogenesis: an organizing principle for drug discovery? *Nat Rev Drug Discov* 2007;6:273–86.
- Warren RS, Yuan H, Matli MR, Gillett NA, Ferrara N. Regulation by vascular endothelial growth factor of human colon cancer tumor genesis in a mouse model of experimental liver metastasis. *J Clin Invest* 1995;95:1789–97.
- Grothey A, Sugrue MM, Purdie DM, Dong W, Sargent D, Hedrick E, et al. Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: results from a large observational cohort study (BRI TE). *J Clin Oncol* 2008;26:5326–34.
- Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 2004;22:2184–91.
- Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel–carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006;355:2542–50.
- Manegold C, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, et al. BO17704 (AVAiL): a phase III randomized study of first line bevacizumab combined with cisplatin/gemcitabine (CG) in patients

- (PTS) with advanced or recurrent non-squamous, non-small cell lung cancer (NSCLC). *Ann Oncol* 2008;19 (late-breaking abstract 1).
17. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 2003;349:427–34.
 18. Miles D, Chan A, Romieu G, Dirix LY, Cortes J, Pivot X, et al. A randomized, double-blind study of bevacizumab in combination with docetaxel as first-line treatment of patients with HER2-negative locally recurrent or metastatic breast cancer: efficacy and safety: AVADO. *J Clin Oncol* 2008;26 (meeting abstracts): LBA1011.
 19. Horita Y, Hirashima Y, Yamada Y, Kato K, Nakamoto A, Nishitani H, et al. Effects of bevacizumab on plasma concentration of irinotecan and its metabolites in advanced colorectal cancer patients receiving FOLFIRI with bevacizumab as second-line chemotherapy. 2009 Gastrointestinal Cancers Symposium (abstract 380).
 20. Greenwood M. The Natural Duration of Cancer. Reports on Public Health and Medical Subjects No. 33. London: Her Majesty's Stationery Office, 1926;1–26.
 21. Lakatos E, Lan KK. A comparison of sample size methods for the logrank statistics. *Stat Med* 1992;11:179–91.