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Effectiveness of a novel herbal agent MB-6 as a potential adjunct to 5-fluoracil–based chemotherapy in colorectal cancer☆,☆☆

William Tzu-Liang Chen[a,*], *Tsia-Sheng Yang*[b], *Hung-Chang Chen*[c], *Hong-Hwa Chen*[d], *Hua-Che Chiang*[a], *Tsang-Chi Lin*[c], *Chung-Hung Yeh*[e], *Tao-Wei Ke*[a], *Jen-Shi Chen*[b], *Koung-Hung Hsiao*[f], *Min-Liang Kuo*[g,h]

[a] *Division of Colorectal Surgery, Department of Surgery, China Medical University Hospital, Taichung, Taiwan*
[b] *Division of Medical Oncology in the Hematology–Oncology, Department of Internal Medicine, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan and Chang Gung University College of Medicine, Taoyuan, Taiwan*
[c] *Division of Colorectal Surgery, Department of Surgery, Changhua Christian Hospital, Changhua, Taiwan*
[d] *Division of Colon and Rectal Surgery, Department of Surgery, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan*
[e] *Division of Colon and Rectal Surgery, Jia-Yi St Martin de Porres Hospital, Chiayi, Taiwan*
[f] *Department of Colorectal Surgery, Taipei Buddhist Tzu Chi General Hospital, Taipei, Taiwan*
[g] *Institute of Biochemical Sciences, National Taiwan University College of Life Science and Institute of Toxicology of Medicine National Taiwan University, Taipei, Taiwan*
[h] *Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan*

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ABSTRACT

Natural products, such as fermented soybeans, have been used to treat various physical conditions, including cancer. MB-6 is a botanical preparation composed of fermented soybean extract, green tea extract, Antrodia camphorata mycelia, spirulina, grape seed extract, and curcumin extract. Based on this, we hypothesized that MB-6 would increase the effectiveness of chemotherapy in patients with colon cancer. In a rodent study, MB-6, in combination with leucovorin/5-fluorouracil chemotherapy, increased the survival rate and life span of colon cancer tumor-bearing BALB/c mice as compared with treatment with chemotherapy alone. In a proof-of-concept clinical study, 72 patients with metastatic colorectal cancer were randomized to receive leucovorin, 5-fluorouracil, and oxaliplatin in combination with either MB-6 or placebo for 16 weeks. The primary outcome was the best overall response, and secondary outcomes included progression-free survival, overall survival, and adverse effects. Up to 77 weeks after treatment, there was follow-up with the patients. No significant difference in the best overall response rate and overall survival was observed between the 2 groups. Patients in the MB-6 group had a significantly lower disease progression rate than patients in the placebo group, during the study period (0.0% vs 15.8%, $P = .026$). The placebo group had a significantly

Abbreviations: ACF, aberrant crypt foci; AE, adverse effects; CEA, carcinoembryonic antigen; CR, complete response; CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; ITT, intent to treat; LV, leucovorin; mCRC, metastatic colorectal cancer; NC, negative control; OS, overall survival; PFS, progression-free survival; PR, partial response; PSK, polysaccharide K.

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* *Corresponding author*. Department of Surgery and the Chief of Division of Colorectal Surgery, China Medical University Hospital, Taichung, Taiwan, 2 Yude Road, Taichung, 40447, Taiwan (ROC). Tel.: +886 4 22052121x1638; fax: +886 4 22053425.
E-mail address: wtchen@mail.cmuh.org.tw (W.T.-L. Chen).

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higher incidence of adverse events at least grade 4 compared with the MB-6 group (28.9% vs 2.9%, respectively, $P = .004$) and a significantly higher occurrence of increased serum creatinine compared with the MB-6 group (29% vs 5.9%, $P = .014$). MB-6 is a promising botanical supplement that may increase the effectiveness of chemotherapy in patients with metastatic colorectal cancer.

1. Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide and the second leading cause of cancer deaths in Western countries [1,2]. Patients with stage I, II, or III CRC, without serosal invasion, are treated by surgical resection with or without adjuvant chemotherapy, whereas stage IV disease with metastasis is treated with chemotherapy alone [3]. The European C95 and the US N9741 studies demonstrated the superiority of a combined regimen of leucovorin, 5-fluorouracil, and oxaliplatin (the FOLFOX4 regimen) over leucovorin/fluorouracil and leucovorin/fluorouracil bolus plus irinotecan, respectively [4,5]. These studies support FOLFOX4 as the standard first-line therapy for advanced disease. However, major side effects are associated with FOLFOX4, including neutropenia/granulocytopenia, febrile neutropenia, thromboembolic events, and neurosensory toxicity [6].

Current interest is focused upon the use of natural products, like fermented soybean, C-phycocyanin, PHY906, and curcumin, to treat a number of pathologic conditions that include immune suppression, liver disease, and various cancers [7-11]. MB-6 is a botanical product manufactured by Microbio Co, Ltd, a good manufacturing practice– and International Organization for Standardization 9001–certified pharmaceutical plant in Taipei, Taiwan. It is a formulation of fermented soybean extract (MicrSoy-20), green tea extract (*Camellia sinensis O*), Antrodia camphorata mycelia, spirulina (*Arthrospira platensis*), grape seed extract (*Vitis vinifera*), and curcumin extract (*Curcuma longa L*). Based on the properties of the ingredients [12-17], MB-6 is marketed as a dietary supplement to promote longevity. Furthermore, the active ingredients of MB-6 (fermented soybean extract, green tea extract, Antrodia camphorata mycelia, spirulina, grape seed extract, and curcumin) have been shown to have biological activity in a number of pathologic conditions [7,10,18-21]. Preliminary pharmacologic studies have suggested that MB-6 suppresses the growth of human colon adenocarcinoma grade II cell line human colon cancer cells and that green tea extract and curcumin play a major role in this tumor-suppressive activity (unpublished data).

Based on the aforementioned findings, we hypothesized that MB-6 would increase the effectiveness of chemotherapy in patients with colon cancer. To examine this hypothesis, we first performed a preclinical study to determine the effect of MB-6, in combination with chemotherapy leucovorin/5-fluorouracil (LV/5-FU), in colon cancer tumor-bearing BALB/c mice. This study showed that MB-6 increased the survival rate and life span of mice, compared with treatment with chemotherapy alone. Then, given the absence of major safety concerns with MB-6 from these preliminary data and the results of the preclinical study, we performed a proof-of-concept clinical study to evaluate the safety and efficacy of MB-6 in patients with metastatic colorectal cancer (mCRC).

2. Methods and materials

2.1. *Rat study-part I*

Animal studies were approved by the Institutional Animal Care and Use Committee of National Taiwan University, and all animals were treated according to the standard guidelines for the care and use of laboratory animals. Fischer 344 (F344) male rats, approximately 6-week old, were purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University. MB-6 was provided by Microbio Co Ltd and was aliquoted and stored in a desiccator until needed.

The male F344 rats were randomized into groups based on weight, with 8 rats per group. Beginning 1 week after adaptation, rats were fed the Harlan AIN-76 purified rodent diet (Harlan Laboratories, Indianapolis, IN, USA) with 17.7%, 64.9% carbohydrate, and 5.2% fat [22]. The rats were kept in stainless steel cages with access to food and distilled water ad libitum. Animal rooms were maintained in 23°C ± 1°C, 50% ± 10% humidity, and with a 12-hour light/dark cycle. Food intake and body weight were recorded weekly.

The rats were grouped as follows: C1, control group received 1,2-dimethylhydrazine (DMH) injection; C2, control group received saline injection; B1, experimental group received a low MB-6 dosage (17.3 mg/rat per day); B2, experimental group received a medium MB-6 dosage (34.6 mg/rat per day); and B3, experimental group received a high MB-6 dosage (69.2 mg/rat per day). The DMH was dissolved in saline (0.9% sodium chloride solution) to create a 2% solution, adjusted to pH 6.7, and then aliquoted and stored at –20°C until needed. Over a 4-week period, a weekly dosage of 20 mg/kg of DMH was administered by intraperitoneal (IP) injection (equivalent to injecting 1 mL of a 2% solution per kilogram body weight). The control group was given a saline injection (1 mL saline per kilogram body weight).

At week 15, the rats fasted for one night and then were euthanized with carbon dioxide inhalation the next day. Colons were then removed, the length and weight were measured, and the numbers of aberrant crypt foci (ACF) and the numbers of crypts composing each ACF were counted. In brief, the colon was sheered along the longitudinal axis, spread open with the mucosal lining facing up, and then cut into 3 segments (proximal, middle, and rear). Each segment was placed between 2 sheets of filter paper in a Petri dish that contained 10% buffered formalin and fixed for more than 24 hours. For ACF quantification, the fixed colon tissue was stained with 0.2% methylene blue (with Phosphate-Buffered Saline as solvent), and the number of ACF was assessed under light microscope at ×40 to ×100 magnification. To determine crypt multiplicity, the number of crypts per ACF were further categorized as 1 to 3 crypts, 4 to 6 crypts, and at least 7 crypts.

2.2. Mouse study—part II

Male BALB/c mice were purchased from the National Laboratory Animal Center in Taiwan and maintained in a 12-hour light/dark cycle at 21°C ± 2°C. The AIN-76 purified diet and distilled water were supplied ad libitum.

The mice were randomly divided into 7 groups, with 12 mice per group and 3 mice per cage. The Mouse Colon Carcinoma Cell Line (CT-26-VD) was cultured in complete Dulbecco's Modified Eagle Medium (GIBCO, Grand Island, NY, USA, cat no. 11995), which contained 10% fetal bovine serum at 37°C in an incubator containing 5% carbon dioxide. Establishment of a murine colon cancer model was previously described [23]. Briefly, the mice were weighed and anesthetized by sodium pentobarbital (10 μL/g, 6.5 mg/mL; Somnotol; MTC Pharmaceuticals, Cambridge, Canada), with the dosage based on body weight. On day 0, 100 μL of CT-26-VD cell suspension (2×10^5 cells/mL, 2×10^4 cells/mouse) was injected into the mouse spleen, and the animals were protected from hypothermia until recovery. The treated mice were administered 3 different doses of MB-6 (0.625 [MB-6-Low {MB-6-L}], 1.25 [MB-6-Medium dosage (MB-6-M)], and 2.5 [MB-6-High dosage (MB-6-H)] g/kg) by oral gavage. Mice were then treated with LV/5-FU on the third and sixth days after tumor cell injection. The negative control (NC) and positive groups of mice were administered sterile Mili-Q water (Taipei, Taiwan) and polysaccharide K (PSK) (0.325 g/kg), respectively, as a substitute for the test substance MB-6. A summary of the grouping is shown in Table 1.

The body weight of each mouse was monitored once a week. On day 15, the mice were injected with luciferin (150 mg/kg IP), anesthetized by inhalation of 3% isoflurane, and placed in the dark box with a noninvasive IVIS Imaging System 100 series (Xenogen, Alameda County, CA, USA). Bioluminescence was quantified using the system software for the determination of tumor growth. The survival mice were recorded up to day 55. After completion of the study, all animals were euthanized with carbon dioxide inhalation.

2.3. Clinical study

2.3.1. Patient eligibility

This study was a 2-arm, multicenter, parallel, double-blind, randomized, placebo-controlled clinical study that evaluated the efficacy and safety of MB-6 vs placebo, in combination with FOLFOX4, in patients with mCRC. Six hospitals in Taiwan were involved in the study (China Medical University Hospital, Changhua Christian Hospital, Buddhist Tzu Chi General Hospital, Chang Gang Memorial Hospital–Linkou Branch, Chang Gang Memorial Hospital–Kaohsiung Branch, and Chang Gang Memorial Hospital–Chiayi Branch). The clinical study was approved by the institutional review boards of the 6 hospitals participating in the study. All patients provided written informed consent.

Patients were eligible if they were aged 20 or more years; had histologically confirmed CRC and/or clinical evidence of metastasis; had at least one measurable lesion, either by computed tomography or magnetic resonance imaging; had an Eastern Cooperative Oncology Group performance status less than or equal to 2; possessed adequate bone marrow reserve (hemoglobin level at least 9 g/dL, absolute neutrophil count at least 1.5×10^9/L, and platelets at least 100×10^9/L); and hepatorenal function (total bilirubin less than or equal to 1.25×, creatinine less than or equal to 1.25, and alanine aminotransferase or aspartate aminotransferase less than 2.5× upper normal limits). Patients were excluded if they were pregnant or lactating, did not practice adequate contraceptive measures, showed evidence of central nervous system metastasis, presented with active infection that required systemic antimicrobial treatment, had current chronic diarrhea and/or other serious comorbidities (ie, angina, myocardial infarction, congestive heart failure, epilepsy, or other significant medical conditions as judged by the investigators), had a history of second primary malignancies (except for adequately treated basal cell skin carcinoma or cervical carcinoma in situ) or were undergoing concurrent treatment with any other anticancer therapy, or were treated with another investigational drug within the previous 4 weeks.

2.3.2. Treatment protocol

Patients were treated with MB-6 and concomitant FOLFOX4 [4,5]. The MB-6 dosage included 6 capsules of 320 mg each administered 3 times daily with meals. The placebo group received the same number of capsules containing only inactive ingredients and administered in the same manner as the treatment group. The FOLFOX4 chemotherapy regimen was given as a 2-hour infusion of leucovorin (LV) (200 mg/m^2) and oxaliplatin (85 mg/m^2), followed by a 22-hour infusion

Table 1 – Animal grouping in the preclinical study part II

Code	Group	Test article administered, route, and dosage	Animal model of injected substance (intraspleen/IP)
Sham	Sham	Sterile Mili-Q water, gavage, 10 mL/kg	Sterile normal saline/sterile normal saline
Tumor alone	Tumor alone	Sterile Mili-Q water, gavage, 10 mL/kg	CT-26-VD/sterile normal saline
NC	NC: tumor + chemotherapy	Sterile Mili-Q water, gavage, 10 mL/kg	CT-26-VD/LV/5-FU
PSK	Positive control: tumor + chemotherapy + PSK	PSK, gavage, 0.312 g/kg	CT-26-VD/LV/5-FU
MB-6-L	Low dosage: tumor + chemotherapy + MB-6-L	MB-6, gavage, 0.625 g/kg	CT-26-VD/LV/5-FU
MB-6-M	Medium dosage: tumor + chemotherapy + MB-6-M	MB-6, gavage, 1.25 g/kg	CT-26-VD/LV/5-FU
MB-6-H	High dosage: tumor + chemotherapy + MB-6-H	MB-6, gavage, 2.50 g/kg	CT-26-VD/LV/5-FU

MB-6-L, 0.625 g/kg MB-6; MB-6-M, 1.25 g/kg; and MB-6-H, 2.5 g/kg MB-6.

of 400 mg/m^2 5-fluorouracil bolus and 600 mg/m^2 on the first day. On the second day, a 2-hour infusion of LV 200 mg/m^2, followed by a 22-hour infusion of 400 mg/m^2 5-fluorouracil bolus and 600 mg/m^2 were provided. The FOLFOX4 regimen was repeated every 2 weeks for a total of 16 weeks.

2.3.3. *Follow-up assessment*

Vital signs and body weight were measured before randomization and every 2 weeks for the duration of the study. Serum carcinoembryonic antigen (CEA) levels and tests of immune function (interleukin 2, interferon γ, interleukin 10, and tumor necrosis factor α levels) and hematologic and biochemical parameters (red blood cell count, hematocrit, white blood cell, platelet count, absolute neutrophil count, differential count, total bilirubin, creatinine, alanine aminotransferase, and aspartate aminotransferase) were performed every 4 weeks. Females of child-bearing age were tested for pregnancy. Tumor assessments were performed every 8 weeks. Drug compliance was documented at each study visit. Total follow-up duration was up to 77 weeks.

2.3.4. *Primary end point*

The primary efficacy end point of this study was evaluated by calculating the best overall response (complete response [CR] + partial response [PR] by RECIST 1.1 (National Cancer Institute of Canada, ON, Canada) criteria) to MB-6, as previously described [24] and then comparing the results to that of patients who received the placebo.

2.3.5. *Secondary end points*

Progression-free survival (PFS) was assessed as the duration from the date of randomization to the date when disease progression was observed. Death from any cause was regarded as a progression event. Patients without documented disease progression or survival follow-up were censored on the date of the last objective tumor assessment. In the case of no postbaseline tumor assessment or survival follow-up, progression was censored on the day of study medication initiation. Overall survival (OS) was assessed from the date of initiation of study medication (MB-6 or placebo) until the date of patient death, due to any cause. Patients not known to have died were censored for survival as of the last date known alive.

Adverse effects (AE) that manifested at the time of or after initiation of study medication were classified according to System Organ Class and Preferred Term via the MedDRA (McLean, VA, USA) coding dictionary [25]. The duration, severity, relationship to study medication, action taken, and outcome of the AE were recorded. Whether the event was classified as a serious adverse event was also recorded.

2.4. *Statistical analyses*

In the preclinical study, continuous variables among the groups were compared by one-way analysis of variance. When a significant difference between groups was apparent, multiple comparisons of means were performed using the Bonferroni procedure. In the clinical study, comparison of demographic and baseline clinical characteristics between MB-6 and placebo groups was analyzed using independent 2-sample t test for continuous variables and Fisher exact test for categorical variables. Data of continuous variables were presented as means ± SD, whereas categorical variables were presented as counts (percentages). The efficacy analysis was performed on the *intent-to-treat* (*ITT*) population, which was defined as all patients who were randomized to the study treatment and took at least one dose of study medication. The best overall response rate and rates of tumor response were compared between the 2 groups, using Fisher exact test. Survival curves, including PFS and OS, were estimated using the Kaplan-Meier method, and the differences were examined by the log-rank test. Intergroup differences of body weight, CEA level, and immune function were assessed by the linear mixed model. The least square means from the linear mixed model were used to obtain the 2-sided 95% confidence intervals of difference between the 2 groups. The incidence of adverse events were displayed and tested for intergroup differences by using Fisher exact test.

For primary end point analysis, the incidence of best overall response and patients with no postbaseline tumor assessments were not classified in the efficacy analysis for the tested group. Missing data for laboratory tests were inserted using the last observation carried forward method. For all analyses, a 2-sided $P < .05$ was considered significant. Statistical analyses were performed using SAS 9.0 (SAS Institute Inc, Cary, NC, USA).

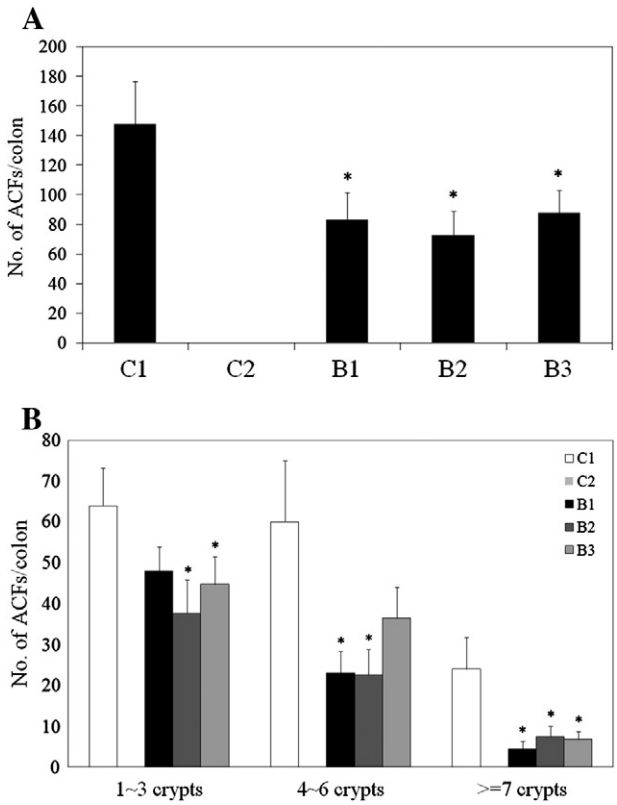

Fig. 1 – Aberrant crypt foci in rat colon. A, MB-6 (B1, 17.3 mg/rat per day; B2, 34.6 mg/rat per day; and B3, 69.2 mg/rat per day)-treated rats showed a significant decrease in the total number of ACF/colon (40.6%-51.0%, $^*P < .05$) compared with C1 (DMH control) rats. B, The number of crypts per ACF was classified into 1 to 3 crypts, 4 to 6 crypts, and at least 7 crypts. MB-6 significantly prevented the formation of chemical-induced ACF, and the highest inhibition rate was observed in the group with at least 7 crypts (81.7%, $^*P < .05$ vs group C1).

3. Results

3.1. Rat study—part I

A total of 50 F344 rats were randomly assigned to groups C1 (DMH control), C2 (normal control), B1 (MB-6-L), B2 (MB-6-M), or B3 (MB-6-H). There was no significant difference in the body weight among the 5 groups over time. The number of ACF per colon is shown in Fig. 1. MB-6–treated rats showed a significant decrease in the total number of ACF/colon (40.6%-51.0%, $P < .05$) compared with C1 (DMH control) rats. The number of crypts per ACF was classified into 1 to 3 crypts, 4 to 6 crypts, and at least 7 crypts. MB-6 significantly prevented the formation of chemical-induced ACF, and the highest inhibition rate was observed in the group with at least 7 crypts (81.7%, Fig. 1B).

3.2. Mouse study—part II

Compared with the LV/5-FU chemotherapy group, MB-6 did not affect feed intake and body weight in CT-26-VD–bearing BALB/c mice (Fig. 2). The result of bioluminescence quantification showed that the administration of 1.25 or 2.5 g/kg MB-6 significantly increased the efficacy of LV/5-FU chemotherapy treatment (Fig. 3). The median survival periods were 27.5 days (tumor alone), 30.5 days (NC), 34.5 days (PSK), 35 days (MB-6-L), 39 days (MB-6-M), and 39 days (MB-6-H) (Fig. 4). The administration of 0.625 to 2.5 g/kg of MB-6 significantly prolonged the life span of the tumor-bearing mice receiving chemotherapy, wherein MB-6-M prolonged the life span by more than 30% compared with the NC group (Fig. 5).

3.3. Clinical study

3.3.1. Patient demographics

A total of 72 patients with mCRC were recruited from 26 October 2009 to 2 May 2011. Study subjects were randomized in a double-blind fashion to receive either MB-6 or placebo at a 1:1 ratio, using random permuted block sizes. Thirty-four patients were randomized to the MB-6 group and 38 to the placebo group. A total of 60 patients (29 in the MB-6 group and 31 in the placebo group) completed the 16-week study (83.3%). The reasons for early termination are shown in the study flowchart (Fig. 6). All 72 patients were included in the ITT population. There were no differences in patient demographic and baseline characteristics between the 2 groups ($P > .05$) (Table 2).

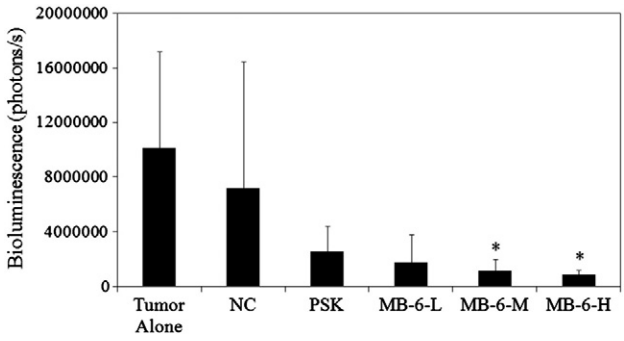

Fig. 3 – Effect of MB-6 on the bioluminescence of CT-26-VD colon tumor-bearing and LV/5-FU–treated BALB/c mice. Leucovorin/5-fluorouracil chemotherapy was administered twice by IP injection. The administration of 1.25 and 2.5 g/kg of MB-6 significantly increased the efficacy of LV/5-FU. Results represent means ± SD. *$P < .05$ vs NC group. MB-6-L, 0.625 g/kg MB-6; MB-6-M, 1.25 g/kg; and MB-6-H, 2.5 g/kg MB-6.

3.3.2. Primary outcome

The best overall response rates were 41.2% in the MB-6 group and 39.5% in the placebo group. The difference was not significant ($P = 1.000$, Table 3). The tumor response rate (CR +

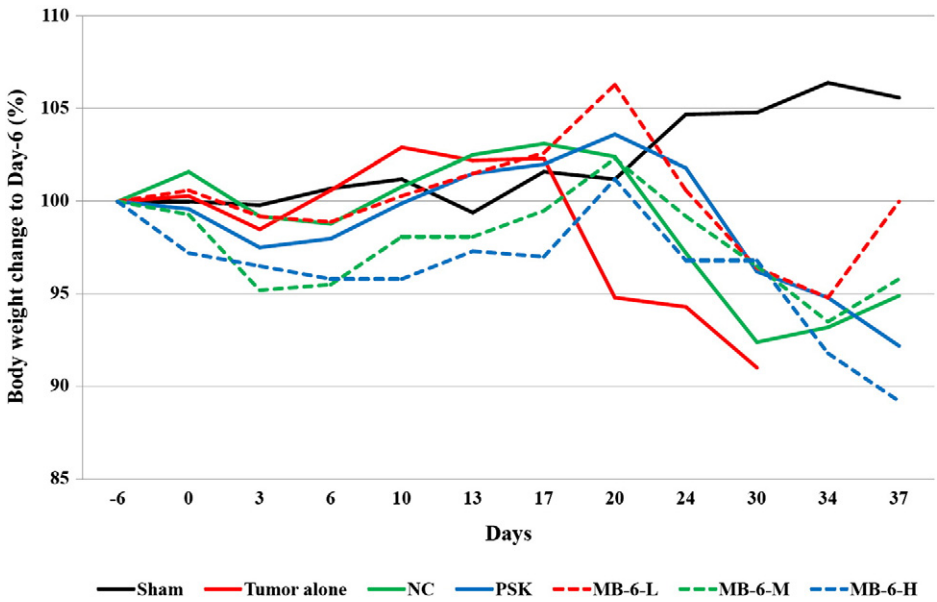

Fig. 2 – Effect of MB-6 on the body weight change of chemotherapy-treated colorectal tumor-bearing mice (n = 12). Leucovorin/5-fluorouracil chemotherapy was administered twice by IP injection. The effect of MB-6 was insignificant compared with the NC group of mice. MB-6-L, 0.625 g/kg MB-6; MB-6-M, 1.25 g/kg; and MB-6-H, 2.5 g/kg MB-6.

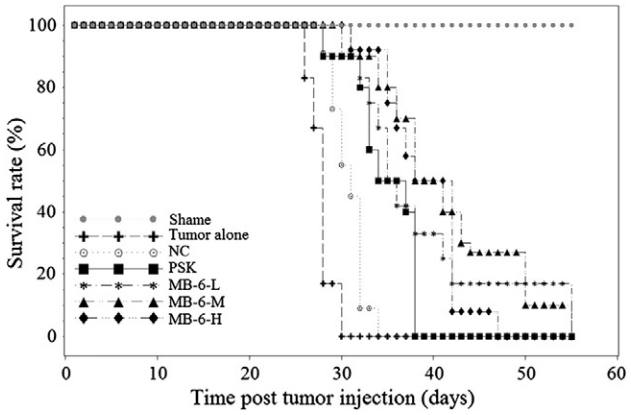

Fig. 4 – Survival of colon tumor CT-26-VD–bearing BALB/c mice treated with MB-6 and LV/5-FU chemotherapy. The median survival periods were 27.5 days (tumor alone), 30.5 days (NC), 34.5 days (PSK), 35 days (MB-6-L), 39 days (MB-6-M), and 39 days (MB-6-H). MB-6-L, 0.625 g/kg MB-6; MB-6-M, 1.25 g/kg; and MB-6-H, 2.5 g/kg MB-6.

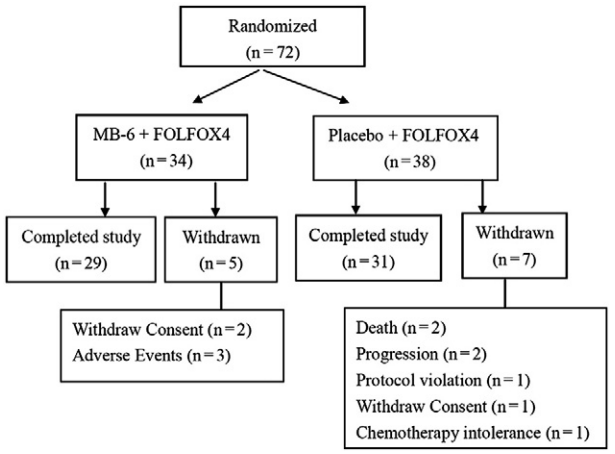

Fig. 6 – Schematic of patient disposition.

PR) at the last measurement per RECIST 1.1 criteria was 61.8% for the MB-6 group and 63.2% for the placebo group.

3.3.3. *Secondary outcomes*

None of the patients in the MB-6 group showed disease progression over the 16-week study period, whereas 6 patients in the placebo group did. Patients in the MB-6 group had a significantly lower disease progression rate than patients in the placebo group (0.0% vs 15.8%, $P = .026$). The PFS rates at 24, 36, and 48 weeks were 100%, 28.6%, and 28.6%, respectively, for the MB-6 group and were 55.3%, 41.5%, and 13.8%, respectively, for the placebo group. There was no significant difference in PFS between the 2 groups after the initial 16-week study period ($P = .249$, Fig. 7A). The median PFS for the MB-6 and placebo groups was 246 days and 236 days, respectively. There were no deaths in the MB-6 group, whereas 2 patients in the placebo group expired during the 16-week study period. There was no significant difference in the crude mortality rate between the 2 groups (0.0% vs 5.3%, $P = .495$) or OS between the 2 groups ($P = .347$, Fig. 7B).

There was no significant decrease in body weight from baseline in the MB-6 group, whereas the placebo group exhibited a significant decrease in body weight at weeks 4, 6, and 8 of the study ($P < .05$, Fig. 7C). However, the difference in body weight change between the 2 groups at the end of study was not significant ($P = .114$). As expected, there was a significant decrease in serum CEA level at week 8 with FOLFOX4 chemotherapy, and the reduction was similar in the presence of MB-6 (Fig. 7D). No significant changes in the levels of immune markers in the 2 groups were noted (data not shown). Thus, the addition of MB-6 to chemotherapy does not appear to compromise or interfere with the effect of chemotherapeutic treatment.

The compliance with MB-6 was 93.8% ± 11.6% and with placebo was 94.1% ± 11.4% ($P = .919$). Interestingly, the MB-6 group showed a significantly higher compliance to FOLFOX4 chemotherapy compared with the placebo group (94.4% ± 7.8% vs 89.0% ± 13.2%, respectively, $P = .037$).

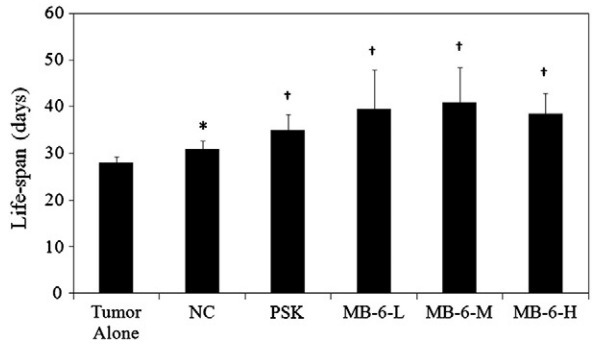

Fig. 5 – Effect of MB-6 combined with LV/5-FU chemotherapy on the life span of the CT-26-VD colon tumor-bearing BALB/c mice. Leucovorin/5-fluorouracil was administered twice by IP injection. The administration of 0.625 to 2.5 g/kg MB-6 significantly prolonged the life span of LV/5-FU–treated CT-26-VD–bearing mice. Results represent means ± SD. *P < .05 vs tumor alone: †P < 05 vs NC group. MB-6-L, 0.625 g/kg MB-6; MB-6-M, 1.25 g/kg; and MB-6-H, 2.5 g/kg MB-6.

3.3.4. *Safety*

The severity of AE was graded according to Common Terminology Criteria for Adverse Events 3.0, where 1, mild; 2, moderate; 3, severe; 4, life threatening; and 5, death. The overall incidence rates of AE over 10% are summarized in Supplemental Table. The placebo group had a significantly higher incidence of AE at least grade 4 compared with the MB-6 group (28.9% vs 2.9%, respectively, $P = .004$). The placebo group also had a significantly higher occurrence of increased serum creatinine compared with the MB-6 group (29% vs 5.9%, $P = .014$). A total of 19 patients (6 in the MB-6 group and 13 in the placebo group) reported 28 serious adverse events. No significant difference in liver function tests or neuronal toxicity between the MB-6 group and placebo group was noted.

4. Discussion

The results of this study confirm the hypothesis that MB-6 increases the effectiveness of chemotherapy in patients with

Table 2 – Patient demographic and baseline characteristics

	MB-6 + FOLFOX4 (n = 34)	Placebo + FOLFOX4 (n = 38)	P [a b]
Sex			.632
Male	22 (64.7)	22 (57.9)	
Female	12 (35.3)	16 (42.19)	
Age (y)	63.4 (13.66)	63.9 (13.25)	.893
BMI (kg/m^2)	22.3 (2.79)	23.0 (4.92)	.478
ECOG score			.666
0	6 (17.7)	7 (18.4)	
1	25 (73.5)	25 (65.8)	
2	3 (8.8)	6 (15.8)	
Current disease site			
Primary site	5 (14.7)	12 (31.6)	.105
Regional nodes	9 (26.5)	9 (23.7)	.793
Distant nodes	12 (35.3)	10 (26.3)	.451
Lung/pleura	13 (38.2)	11 (29.0)	.459
Liver	20 (58.8)	23 (60.53)	1.000
Bone	1 (2.9)	4 (10.53)	.361
Other abdominal regions	1 (2.9)	3(7.9)	.617
Other than above	4 (11.8)	12 (31.6)	.052

Abbreviations: *BMI*, body mass index; *ECOG*, Eastern Cooperative Oncology Group.
P values are from
[a] Fisher exact test and
[b] Independent 2-sample *t* test.
Data are displayed as number (percentage) and means ± SD.

colon cancer. MB-6 was able to prolong the life span of colon cancer-bearing BALB/c mice treated with LV/5-FU. The results of the clinical study showed that patients who received MB-6 had a significantly greater PFS compared with the placebo group during the 16-week study period. A similar reduction in serum CEA level at week 8 was seen in the MB-6 and placebo groups, indicating that the addition of MB-6 did not compromise the effect of FOLFOX4. Significantly greater compliance with FOLFOX4 in the MB-6 group was observed, indicating better tolerance. Furthermore, the MB-6 group had a significantly lower incidence of AE at least grade 4.

Herbal medicines have been used for thousands of years in Asian countries [26]. The cytoprotective activity of a number of herbal medicines has resulted in their use in conjunction with cancer chemotherapy to reduce toxicity, maintain optimal dosages of chemotherapeutic agents, and improve quality of life [9]. Our data are consistent with these reports and suggests that the addition of MB-6 to FOLFOX4 is associated with improved tolerance of FOLFOX4 and thus superior for therapeutic compliance and activity.

Patients in the MB-6 group had a significantly higher median PFS and slower disease progression, suggesting that MB-6 improved the overall quality of survival in patients with mCRC treated with FOLFOX4. These data are consistent with the preclinical studies showing that MB-6 prolonged the life span of chemotherapy-treated tumor-bearing mice by more than 30%. Active ingredients of MB-6 include fermented soybean extract, green tea extract, Antrodia camphorata mycelia, spirulina, grape seed extract, and curcumin, and they have been shown to have biological activity in a number

Table 3 – Best overall response and tumor response at end of treatment

Response profile	MB-6 + FOLFOX4 (n = 34)	Placebo + FOLFOX4 (n = 38)	P
Best overall response			.578
PR	14 (41.2%)	15 (39.5%)	
Stable disease	16 (47.1%)	19 (50.0%)	
Progression disease	0 (0.0%)	2 (5.3%)	
Not evaluable	4 (11.8%)	2 (5.3%)	
Best overall response	14 (41.2%)	15 (39.5%)	1.000
Tumor response			.136
CR	2 (5.9%)	0 (0.0%)	
PR	19 (55.9%)	24 (63.2%)	
Stable disease	9 (26.5%)	8 (21.1%)	
Progression disease	0 (0.0%)	4 (10.5%)	
Not evaluable	4 (11.8%)	2 (5.3%)	
Response rate (CR + PR)	21 (61.8%)	24 (63.2%)	1.000

P values are based on the Fisher exact test.

of pathologic conditions. Fermented soybean extract enhances natural killer activity and immune function under chemotherapy [7], in addition to possessing potent antioxidant activity [18]. Curcumin induces apoptosis of Human Colon Cancer Cells via an E2F4-dependent mechanism [10], and it inhibits the growth of FOLFOX-resistant colon cancer cells by suppressing Wnt signaling pathways and cell-cell adhesion [19,20]. A number of clinical trials are underway to evaluate the antitumor and antimetastatic role of curcumin [21]. Epigallocatechin-3-gallate, the active ingredient of green tea extract, inhibits cell proliferation via inhibition of the Extracellular Regulated Protein Kinases1/2 and Nuclear Factor Kappa-light-chain-enhancer of activated B cells pathways [27], and preliminary studies indicate that grape seed extract inhibits proliferation and induces apoptosis in colon cancer cell lines [28]. Thus, it is suggested that the mechanism of action for MB-6 is multifactorial and could be mediated by more than one of its active ingredients.

Although there was no significant difference in the best overall response rate (CR + PR) between the MB-6 group and the placebo group, our data were consistent with a previous study, which showed that the overall tumor response rate for FOLFOX4 was 58.5% [29]. This suggests that MB-6 did not diminish the effect of FOLFOX4 in patients with mCRC. Of note, there was also no significant difference in CEA levels and immune function between the 2 groups at the end of the study, thus confirming that MB-6 had no negative impact on the chemotherapy regimen. In other words, MB-6 did

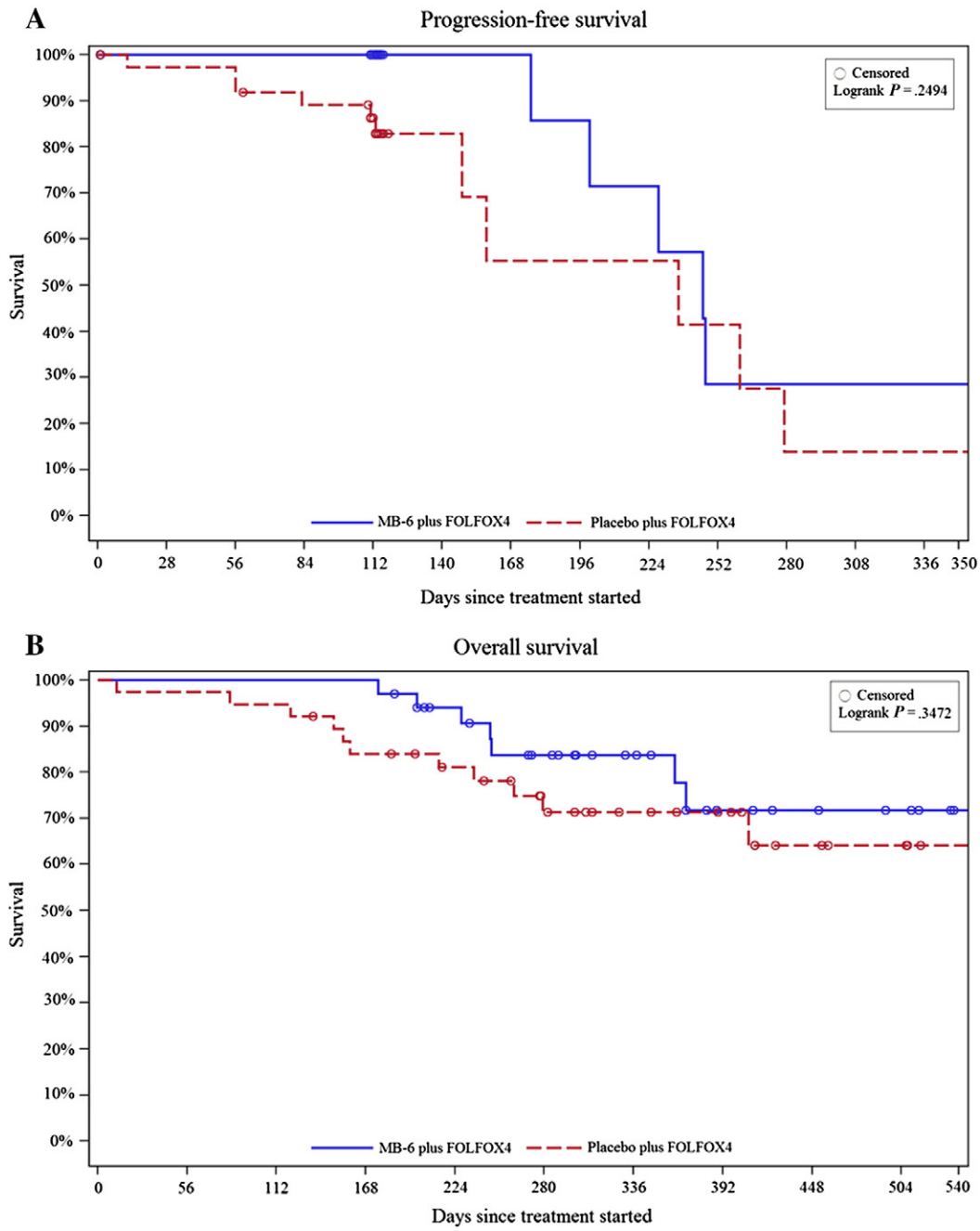

Fig. 7 – Secondary end points in the intention-to-treat population. Progression-free survival (A), OS (B), body weight (C), and CEA level (D).

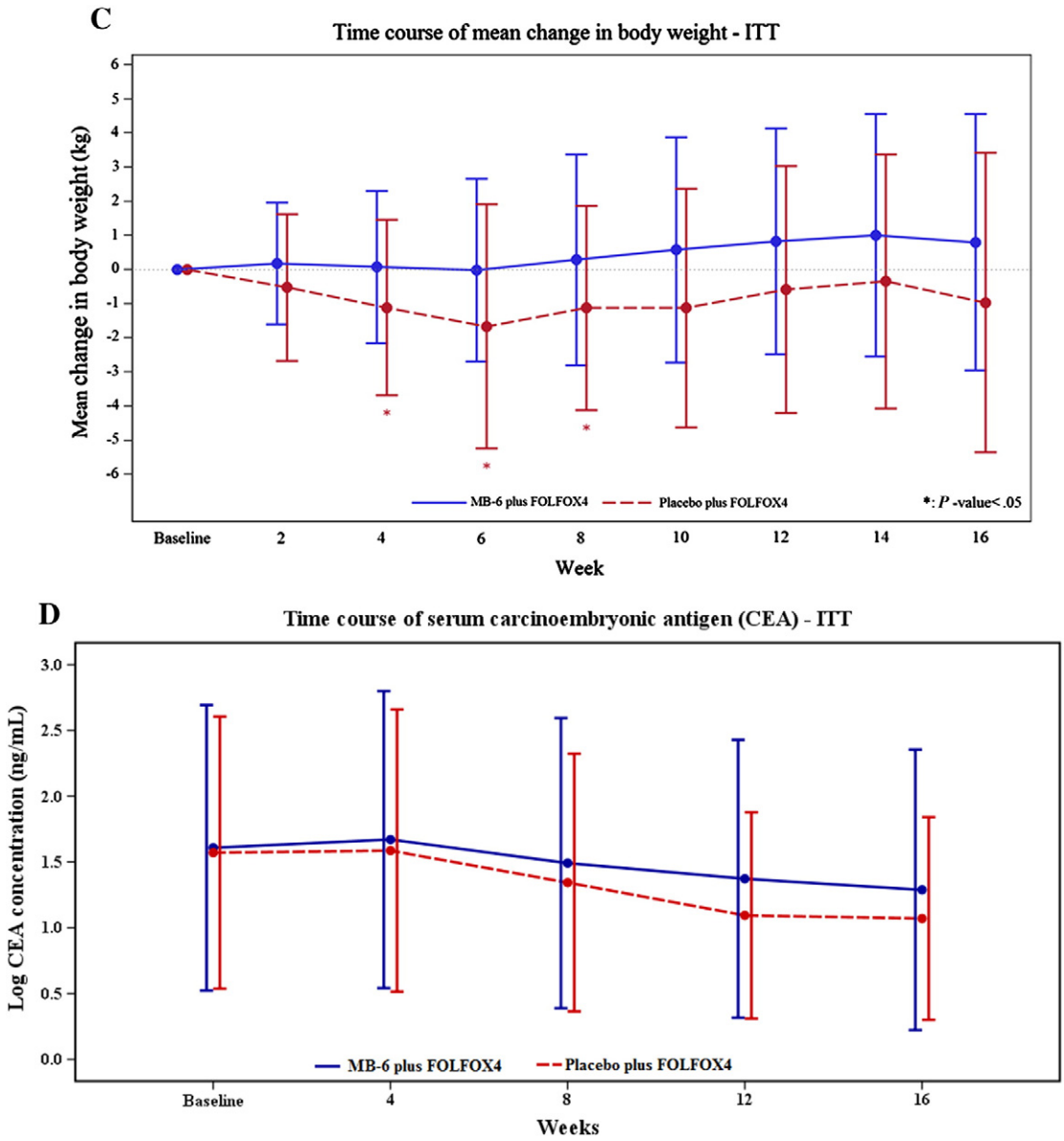

Fig. 7 (*continued*).

not interfere with the cytotoxic effect of FOLFOX4 in these mCRC patients.

There are a number of limitations of this study that should be considered. This current study was designed as a proof-of-concept investigation, and as such, the sample size was relatively small. A power analysis was not performed, and MB-6 was used as an adjuvant treatment with FOLFOX4 over a relatively short 16-week period. In addition, the optimal dose of MB-6 for humans was not investigated, and the follow-up period was not long enough to evaluate clinical benefits in terms of OS. Lastly, MB-6 is a complex mixture of a number of ingredients, the active ingredients are not known, and the mechanisms of action at the molecular level were not investigated.

In summary, animal studies showed that MB-6 was able to prolong the life span of colon cancer-bearing BALB/c mice treated with LV/5-FU. The results of the clinical study showed that patients who received MB-6 had a significantly greater PFS compared with the placebo group during the 16-week study period, and they had a significantly lower incidence of AE at least grade 4. Additional clinical studies are warranted to confirm these findings and pursue further understanding of the potential mechanisms of action for MB-6.

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