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Original Article

Effect of Ling Zhi-8 supplementation on docetaxel-induced leukopenia and thrombocytopenia in patients with advanced non-small cell lung cancer

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Leukopenia and immunity impairment usually occur during cancer treatment, such as chemotherapy or radiotherapy. Ling zhi-8 (LZ-8), a water-soluble compound derived from the lingzhi mushroom, has demonstrated anticancer and immunomodulatory properties in an animal model. The objective of this Institutional Review Board-approved study was to evaluate the effect of LZ-8 on docetaxel-induced leukopenia and the anticancer activity of LZ-8 in patients with advanced non-small cell lung cancer. A total of 20 patients simultaneously received LZ-8 and weekly docetaxel and a total of 25 patients received weekly docetaxel alone. The quantities of immune cells in the blood of the participants were determined on days 1, 8, and 15 of chemotherapy and on day 22 when no chemotherapy was administered, either LZ-8-combined therapy or docetaxel alone. LZ-8-combined therapy significantly ameliorated the depletion of white blood cells, neutrophils, and platelets. In addition, an analysis of tumor biomarkers revealed that patients who received LZ-8 and docetaxel exhibited reduced levels of carcinoembryonic antigen and cancer antigen 125 when compared with those who received docetaxel alone. These findings indicated that LZ-8 treatment improves immune function in patients receiving chemotherapy, thereby enhancing their ability to fight cancer, as well as any secondary infections that could compromise their treatment or health.

Keywords: LZ-8, leukopenia, chemoprotective agents, lung cancer, chemotherapy

Introduction

The fruiting bodies of *Ganoderma* fungi, known as "lingzhi" in Chinese, have been widely used in traditional medicine in China and other Asian countries for more than 2000 years[1]. They have been reported to exert a range of beneficial effects, including immunomodulation, anti-tumor and antioxidant activities, and longevity enhancement[2,3]. The active constituents include proteins, peptides, polysaccharides, oxygenated triterpenoids, and lectins, which exhibit a broad range of biological activities and pharmacological functions[4-6].

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Immunomodulatory effects of an aqueous extract from the fruiting bodies of lingzhi fungi have been identified and attributed to an immunomodulatory protein, ling zhi-8 (LZ-8)[2,5,7,8]. LZ-8 has been isolated from G. *lucidum* mycelia[7] and found to belong to the fungal immunomodulatory protein (FIP) family together with FIP-gts (identical to LZ-8 but isolated from G. *tsugae* or G. *amboinense*), FIP-fve (from *Flammulina velutipes*), FIP-vvo (from *Volvariella volvacea*), and FIP-gsi (from G. *sinensis*)[9-12]. Studies have demonstrated the immunomodulatory effects of LZ-8 on autoimmunity and transplantation and the ability of LZ-8 to act as a mitogen in the activation of T cells[13]. The immunostimulatory effect of LZ-8 on human dendritic cells (DCs) has also been reported[14]. Taken together, these findings imply that LZ-8 should be considered an adjuvant candidate. Subsequent studies have shown that LZ-8 can be applied to vaccination or cancer therapy.

Docetaxel (taxotere) is a semi-synthetic anticancer agent in the taxane class of drugs that is widely used in the treatment of various malignancies, such as non-small-cell lung cancer (NSCLC), breast cancer, head and neck cancer, and prostate cancer. However, it may cause serious side effects such as myelotoxicity (neutropenia, anaemia, and thrombocytopenia), which limits its clinical use[15]. Chemotherapy-induced myelotoxicity is a common cause of blood cell injury, which can lead to low immune function and increased risk of infection[16,17]. Granulocyte colony-stimulating factor is the most effective cytokine for hematopoietic progenitor cells and is recommended for patients with chemotherapy-associated neutropenia in clinical practice. However, in such cases, patients are already in critical condition. Moreover, bone loss may be aggravated by chemotherapy drugs, such as methotrexate and ifosfamide, as well as the tumor itself, causing osteoporosis and fractures[18]. Therefore, developing an adjuvant or novel chemopreventive agent that can prevent these adverse effects is critical.

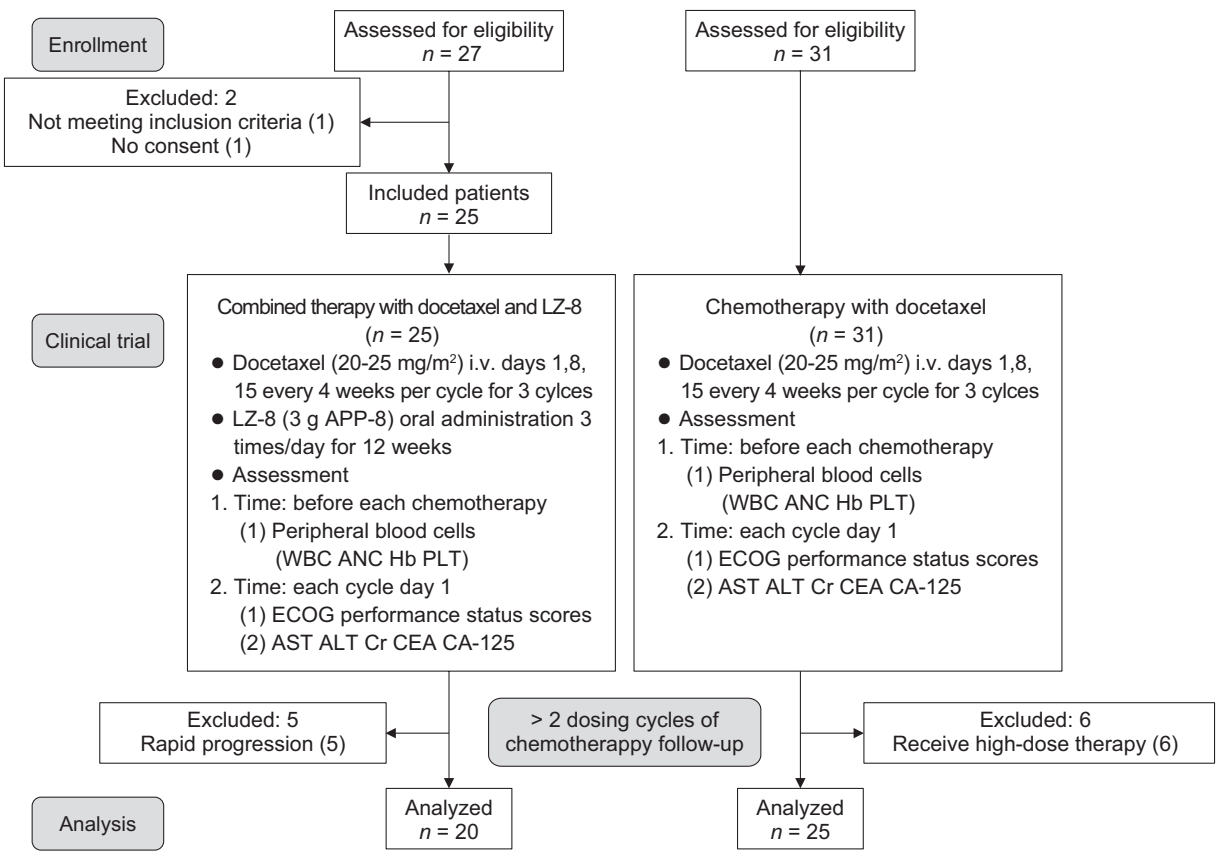

Figure 1. Flowchart of the study design.

In a previous study, we demonstrated that LZ-8 reverses docetaxel-induced reduction in white blood cells (WBCs) and platelets (PLTs) in mice[19]. Hematoxylin and eosin staining have revealed that LZ-8 reduces the number of docetaxel-induced empty vacuoles in the bone marrow (BM) and mitigates damage to the small intestinal mucosa[19]. Three-dimensional micro–computed tomography has revealed that LZ-8 significantly reverses docetaxel-induced reduction in percent bone volume, trabecular number, and bone surface density in mice[19]. Therefore, LZ-8 has the potential to serve as a novel therapeutic or protective agent in patients undergoing chemotherapy. Based on the findings of our previous *in vivo* study,

Table 1. Baseline characteristics of patients with lung cancer before clinical intervention

	Docetaxel alone				LZ-8-combined therapy				
Variable	N	%	Mean	SD	N	%	Mean	SD	*p* value
Gender									
Male	17	68			16	80			0.502
Female	8	32			4	20			
Tumor type									
Adenocarcinoma	16	64			13	65			0.597
Squamous	9	36			7	35			
Stage									
I	0	0			0	0			0.727
II	0	0			0	0			
III	6	24			6	30			
IV	19	76			14	70			
Metastasis									
-	6	24			6	30			0.741
+	19	76			14	70			
Age	25		63.44	12.11	20		65.45	10.9	0.486
WBC (/μl)	25		9668	9257	20		9175	4720	0.810
ANC (/μl)	25		5723	2852	20		7040	4879	0.749
Hb (g/dl)	24		11.69	1.53	19		12.09	1.92	0.714
PLT (x10^3/μl)	24		270	88	19		242	76	0.226
AST (IU/L)	10		25.4	17.59	8		27.13	13.18	0.398
ALT (IU/L)	12		21.75	13.88	8		23.38	9.59	0.332
Cr (mg/dl)	13		0.9	0.22	8		1.07	0.44	0.514
CEA (ng/ml)	8		259.33	701.84	12		77.00	130.9	0.487
CA-125 (U/ml)	9		214.11	337.10	10		96.87	118.33	0.624

Continuous data was compared using the Mann-Whitney U test, and categorical data was compared using Fisher's exact test.

Table 2. Decrease in blood cells counts in patients with lung cancer during clinical intervention

Variable	Docetaxel alone	LZ-8-combined therapy	F value	*p* value
	Decreased counts (Mean ± SD)	Decreased counts (Mean ± SD)		
WBC (/μl)	2568 ± 236	1396 ± 305	8.75	**0.004****
ANC (/μl)	2003 ± 178	1339 ± 236	4.71	0.033*
Hb (g/dl)	0.53 ± 0.09	0.64 ± 0.10	0.76	**0.384**
PLT (x10^3/μl)	40 ± 4	19 ± 7	7.92	**0.006****

Linear Mixed model; Adjustment for multiple variables including age, gender, tumor type, stage, and metastasis

we evaluated the protective effects of LZ-8 on the recovery of peripheral blood cells, performance status (PS), and tumor biomarkers in patients with lung cancer receiving docetaxel. The results revealed that LZ-8 can promote the recovery of peripheral blood cells, including WBCs, neutrophils, absolute neutrophil counts (ANCs), and PLTs, and reduce the level of tumor biomarkers in patients receiving docetaxel.

Results

No significant differences in biochemical variables at the start of intervention between the docetaxel alone and LZ-8-combined-therapy groups

Twenty-seven patients were enrolled in the LZ-8-combined-therapy group, of which 2 were ineligible. Of the 25 patients who received therapy, 5 were excluded from analysis because 1 refused continued treatment and 4 demonstrated rapid progression. Finally, 20 patients in the LZ-8-combined-therapy group were analyzed. In the docetaxel-therapy group, 6 patients were excluded because they received higher dose therapy. A total of 25 patients treated with docetaxel alone were analyzed in this study as the control group. The flowchart of the study design is shown in Figure 1.

Baseline clinicopathological data of the patients is presented in Table 1. No significant differences in age, sex, type of carcinoma, tumor stage, WBCs, ANCs, hemoglobin (Hb), PLTs, aspartate aminotransferase (AST), alanine transaminase (ALT), creatinine (Cr), carcinoembryonic antigen (CEA), cancer antigen 125 (CA-125), or Eastern Cooperative Oncology Group (ECOG) performance status (PS) score were observed between the docetaxel therapy alone and LZ-8-combined-therapy groups before clinical intervention. The clinical characteristics of these two groups were similar at the time the study began.

Table 3. Biochemical measurements in patients with lung cancer during clinical intervention

Variable	Docetaxel alone	LZ-8-combined therapy	F value	*p* value
	Concentration (Mean ± SD)	Concentration (Mean ± SD)		
AST (IU/L)	17.66 ± 1.09	28.46 ± 1.74	21.50	**< 0.001****
ALT (IU/L)	18.97 ± 2.06	26.21 ± 3.97	2.95	0.095
Cr (mg/dl)	0.90 ± 0.03	0.97 ± 0.05	1.12	0.295

Linear Mixed model; Adjustment for multiple variables including age, gender, tumor type, stage, and metastasis

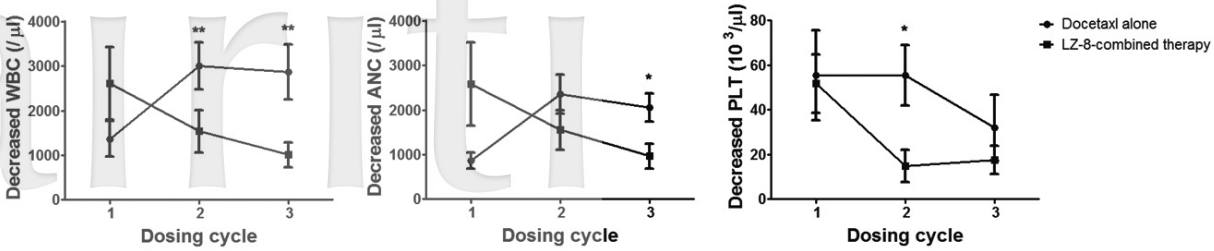

Figure 2. LZ-8-combined therapy suppresses the decrease in peripheral blood cells induced by docetaxel in patients with lung cancer.

LZ-8-combined therapy ameliorates the loss of peripheral blood cells in patients with lung cancer

The levels of peripheral blood cells in patients with lung cancer, during clinical intervention, are shown in Table 2 and Table 3. The reductions in blood cell counts caused by docetaxel therapy were reversed in the patients with lung cancer who received LZ-8-combined therapy. During the intervention period, WBC, ANC, and PLT levels were significantly restored in patients treated with LZ-8-combined therapy compared with those treated with docetaxel alone ($p = 0.004$, $p = 0.033$, $p < 0.001$, respectively). However, no significant differences in Hb level were observed between the two groups ($p = 0.384$). Furthermore, we evaluated the effects of LZ-8-combined therapy on restoration of these blood cell counts in patients with lung cancer who were treated with docetaxel at each cycle. As shown in Figure 2, WBC, ANC, and PLT levels were significantly restored in patients receiving LZ-8-combined therapy in the second or third cycle of docetaxel treatment, but not in the first cycle ($p < 0.05$).

Administration of LZ-8 does not induce liver or kidney damage

To determine whether LZ-8 supplementation induces liver and kidney damage in patients with lung cancer, we analyzed the serum levels of AST, ALT, and Cr during clinical intervention. Our data revealed that the serum levels of AST, ALT, and Cr are within normal range in patients with lung cancer receiving LZ-8-combined therapy or docetaxel alone (Table 3).

LZ-8-combined therapy reduces the levels of tumor biomarkers in patients with lung cancer

We determined the levels of tumor biomarkers CEA and CA-125 following clinical intervention. As shown in Table 4, serum levels of CEA and CA-125 were significantly lower in patients receiving LZ-8-combined therapy than in patients receiving docetaxel alone ($p < 0.044$, $p = 0.023$, respectively).

Discussion

Table 4. The levels of tumor markers in patients with lung cancer during clinical intervention

Variable	Docetaxel alone Concentration (Mean ± SD)	LZ-8-combined therapy Concentration (Mean ± SD)	F value	*p* value
CEA (ng/ml)	214.51 ± 29.94	38.07 ± 76.36	4.63	**< 0.044***
CA-125 (U/ml)	238.29 ± 27.72	93.99 ± 45.68	7.05	**< 0.023***

Linear Mixed model; Adjustment for multiple variables including age, gender, tumor type, stage, and metastasis

Fungal immunomodulatory proteins (FIPs) are known to promote immunomodulation and antitumor activity. However, evidence from clinical trials in humans is lacking, and data from *in vitro* and animal studies cannot always be extrapolated to clinical settings. The results of this study demonstrated the potential of LZ-8 as a chemoprotective agent in patients with lung cancer receiving chemotherapy. Patients with lung cancer treated with docetaxel exhibited decreases in WBCs, ANCs, Hb, and PLTs in the peripheral blood (Table 2). A 5-week LZ-8-combined therapy regimen alleviated the decreases in WBCs, ANCs, and PLTs in these patients (Figure 2). These results suggested that LZ-8-combined therapy ameliorates the loss of blood cells, including WBCs, ANCs, and PLTs, induced by docetaxel in patients with lung cancer, which supports our previous observation that LZ-8 alleviates docetaxel-induced myelotoxicity in an animal model[19]. LZ-8 stimulates human PBMCs to increase the expression of granulocyte-colony stimulating factor (G-CSF) *in vitro*. Then, G-CSF acts as a hematopoietic cytokine to promote granulocyte progenitor proliferation and differentiation. Therefore, by promoting activation of the hematopoietic system, long-term supplementation with LZ-8 may restore the levels of these blood cells in patients with lung cancer receiving docetaxel treatment. LZ-8 intervention may have the potential to reduce leukopenia, neutropenia, and thrombocytopenia through bone marrow (BM) regeneration. This hypothesis will be investigated in future studies.

The hematopoietic system continually renews itself; as billions of blood cells are produced daily in the BM through regulated proliferation and differentiation of hematopoietic stem cells (HSCs). Long-term BM (LT-BM) injury is a common late effect related to cancer treatment, which can result from ionizing-radiation- or chemotherapy-induced damage to HSCs[20]. Chemotherapy-induced BM nerve injury is a critical lesion that impairs hematopoietic regeneration and damages the host BM microenvironment[21]. In approximately 80% of patients undergoing treatment for cancer, decreases in WBCs and neutropenia are the most commonly reported complications[22-24]. Absolute neutrophil counts in the host play a critical role in the defense against infections. The exact incidence of neutropenia and the number of neutropenia-related cancer deaths are unclear, with mortality rates ranging from 5% to 30% among patients aged more than 70 years[25]. Many studies have identified natural plant products and extracts as potential chemoprotective agents. Polysaccharides, extracted from black soybeans, promote hematopoietic growth in animals with BM injury[26]. In addition, leukocyte and ANC counts are higher in patients receiving Chinese medicinal herb complex (CCMH)-combined therapy than in those receiving chemotherapy alone, demonstrating that CCMH supplementation may promote ANC and leukocyte recovery during chemotherapy or radiotherapy[23,24]. In an animal model, LZ-8 has been shown to reduce the myelotoxicity induced by chemotherapy by increasing the numbers of WBCs and PLTs[19,27]. In this study, we further demonstrated that LZ-8 supplementation promotes the recovery of ANCs, WBCs, and PLTs in patients with lung cancer receiving docetaxel treatment. In contrast, Hb was not drastically influenced by LZ-8 supplementation. Taken together, our results demonstrate the recovery of peripheral blood cells by LZ-8 in patients with lung cancer receiving docetaxel treatment.

In the present study, CA-125 and CEA levels decreased in patients with lung cancer receiving LZ-8-combined therapy compared with those receiving docetaxel alone. CA-125 is a glycoprotein produced in fetal tissue and mesothelial cells in adults. It has been extensively studied as a tumor marker in the screening and management of ovarian cancer[28,29]. CA-125 has also been reported as a tumor marker for lung cancer[30-32]. Serum CA-125 level is significantly higher in patients with bone metastasis than in those without bone metastasis[33]. Serum CA-125 level has been suggested as a prognostic factor in NSCLC[34-36]. Similarly, CEA is a glycoprotein tumor biomarker that was first identified in human colon adenocarcinoma[37]. CEA overexpression is observed in patients with a variety of carcinomas, including those of the colon, thyroid, lung, uterus, pancreas, and ovary, and serum CEA levels increase in certain cancerss[38].

CEA can be used as a prognostic marker for cancer after radiotherapy or chemotherapy[39,40]. Moreover, serum CEA level is an independent prognostic factor for overall survival and risk of recurrence in patients with NSCLC[41]. Consistent with these studies, our results suggested that LZ-8 supplementation-induced reductions in serum CA-125 and CEA are associated with prognostic outcome.

In conclusion, to the best of our knowledge, this is the first clinical trial to determine the efficacy of LZ-8 for treating docetaxel-induced leukopenia in patients with lung cancer. We assessed patients' dietary intakes during the study to minimize the effects of potential confounders such as leukocyte-protective nutrients, which is the strength of the present study. LZ-8 supplementation might not only suppress the reduction in peripheral blood cells induced by docetaxel, but also significantly reduce the levels of tumor biomarkers. Furthermore, the serum levels of AST, ALT, and Cr were normal in patients with lung cancer who received LZ-8-combined treatment during the clinical trial. The results suggested that administration of oral LZ-8 for 3 months is safe as it does not induce liver or kidney damage. We successfully determined the efficacy of LZ-8 in the protection of peripheral blood cells and enhancement of the sensitivity of cancer cells to docetaxel in patients with lung cancer. However, some limitations of the present study must be discussed. First, some of the control group data was obtained retrospectively, and some clinical parameters were not included in the analysis due to insufficient data, which may have affected the outcome of the study. Second, the sample size was relatively small, which may have hindered the interpretation of the data. Third, only patients from a single institution were included in this study. A randomized double-blind study is warranted to verify the results.

Methods

Study population

Consecutive patients diagnosed with lung cancer at Chung Shan Medical University Hospital (Taichung, Taiwan) between January 2013 and June 2017 were included in this study. All diagnoses were confirmed using histopathological examination of the samples harvested through biopsy or surgery. Criteria for exclusion from the study were terminal illness, renal function impairment, congestive heart failure, liver function impairment, secondary lung cancer, changes in drug regimen during the study, receiving radiotherapy, consumption of nutritional supplements within 3 months of the beginning of the study, pregnancy, and intolerance to or poor compliance with APP-8 (which contains the bioactive compound LZ-8). APP-8 was obtained from Yeastern Biotech Corp. (Taiwan). A total of 45 patients aged more than 18 years with stage III–IV lung cancer (according to the American Joint Committee on Cancer TNM staging system, 7th edition) were enrolled in this study. The treatment cycle was repeated every 4 weeks, and 2–6 cycles were administered. All patients with cancer undergoing chemotherapy (docetaxel) were assigned to receive either 9 g of APP-8 powder (N = 20) daily or chemotherapy alone (N = 25). A questionnaire provided to the patients at the beginning of the study was used to obtain information on concurrent diseases and medical history.

Ethical consideration

This trial was approved by the Institutional Review Board of Chung Shan Medical University Hospital, Taichung, Taiwan (IRB No.: CS14146). After the purpose of the study was explained to the participants, written informed consent was obtained from each participant prior to the study. All participants had the option of leaving the study at any time.

Preparation of APP-8

The investigational drug, APP-8 (Yeastern Biotech Corp., Taiwan), was extracted from antler-shaped fruiting bodies of G. *lucidum*. The major bioactive compound in APP-8 is LZ-8. The safety of APP-8 was confirmed by testing for heavy metals, pesticide residues, and microbial activity. APP-8 powder was stored in the clinical trial pharmacy department of Chung Shan Medical

University Hospital.

Study design

The patients who received LZ-8-combined therapy were solicited for the study as they were preparing to receive chemotherapy with weekly taxotere. The patients treated with weekly docetaxel alone were selected using a retrospective design, with individuals in each block matched by sex, age, stage, and docetaxel dosage for each cycle. Each participant completed a general questionnaire to provide demographic information and medical history.

Blood samples were collected before each docetaxel treatment in the hospital to measure serum WBC, ANC, Hb, PLT, AST, ALT, Cr, CEA, and CA-125 levels. In addition, PS was evaluated by clinicians based on the ECOG scale. Patients in the chemotherapy group (N = 25) received docetaxel (20–25 mg/m^2) on days 1, 8, and 15, every 4 weeks. Participants in the LZ-8-combined-therapy group (N = 20) received 3 g of APP-8 powder (Yeastern Biotech Corp.) three times daily from the first administration of docetaxel through the first three cycles of chemotherapy.

Biochemical measurements

Serum levels of WBCs, ANCs, Hb, PLTs, AST, ALT, CEA, and CA-125 were measured using the standard procedures of the Division of General Laboratory of Chung Shan Medical University Hospital.

Statistical analyses

Statistical analyses were performed using SPSS (version 16, SPSS Inc., Chicago, IL, USA). For all tests, $p < 0.05$ was considered significant, and all tested p values were two-sided. Mean values of biochemical measurements of the patients in the two groups at baseline were compared using the independent sample U test. Frequency values of biochemical measurements of the patients in the two groups at baseline were compared using Fisher's exact test. Within-group differences in serum WBC, ANC, Hb, PLT, AST, ALT, Cr, CEA, and CA-125 levels were evaluated at baseline and at the end of the trial using the linear mixed model. The log-rank test was used to analyze the Kaplan–Meier survival curves. For all analyses, the significance level was set at $p < 0.05$.

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Author contributions

Ming-Fang Wu, Jiunn-Liang Ko, and Dz-Chi Chen conducted the trial, participated in protocol design, and revised the manuscript. Chih-Hsien Wu participated in the protocol design. Chi-Wen Chen participated in statistical analyses. Yong-Shiang Lin performed the statistical analyses and wrote the manuscript.