A Phase I Study of the Chinese Herbal Medicine PHY906 as a Modulator of Irinotecan-based Chemotherapy in Patients with Advanced Colorectal Cancer

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Abstract

PHY906 is a novel Chinese herbal preparation that has been used in the Orient for over 1800 years to treat a wide range of gastrointestinal side effects including diarrhea, abdominal cramps, vomiting, fever, and headache. Preclinical and clinical studies were conducted to further investigate the biologic and clinical activities of this herbal medicine. To ensure standardization and maintain interbatch reliability of PHY906, high performance liquid chromatography (HPLC) was used to establish a "chemical fingerprint" of PHY906. In vivo preclinical studies using the murine Colon 39 tumor model showed that PHY906 protected against the weight loss associated with irinotecan treatment. In the presence of PHY906, mice were able to tolerate otherwise lethal doses of irinotecan. Significantly improved antitumor activity and overall survival were observed in animals treated with the combination of irinotecan and PHY906 versus irinotecan alone. The combination of PHY906 with irinotecan, 5-fluorouracil (5-FU), and leucovorin (LV) also resulted in at least additive antitumor activity with no increased host toxicity. Based on these in vivo studies, a phase I multicenter, double-blind, randomized, placebo-controlled, dose escalation, cross-over study of PHY906 as a modulator of the weekly, bolus regimen of irinotecan, 5-FU, and LV (IFL) in the first-line treatment of patients with advanced colorectal cancer (CRC) was conducted. The specific objectives of this clinical trial were to determine the safety and tolerability of PHY906 when administered concomitantly with the bolus, weekly IFL regimen. Treatment with PHY906 did not alter the pharmacokinetics of 5-FU, irinotecan, or the irinotecan metabolite SN-38.

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Introduction

Herbal medicines have been used for the treatment of cancer since ancient times. In the first century AD, Dioscorides compiled a compendium of medicinal herbs and botanicals that were used for over 15 centuries for the treatment of cancer.[1] Herbal medicines continue to be widely used in the United States and throughout the world, and they are now considered under the umbrella of complementary and alternative medicine (CAM). Such an integrative medicine approach encompasses several different modalities as an adjunct to conventional medicine. In general terms, CAM has been functionally defined as 'interventions neither taught widely in medical schools nor generally available in US hospitals.[2] The broad category of CAM has now been subdivided into 5 distinct domains, and they include alternative medical systems, mind-body interventions, biologic-based therapies (herbs), manipulative and body-based systems (eg, massages), and energy therapies (eg, magnet therapy).[3]

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Vitamins and herbs represent one of the most frequently used forms of complementary medicine.[4] Since the enactment of the Dietary Supplement Health and Education Act of 1994, dietary supplements have not been required to undergo rigorous clinical testing to characterize their biologic properties, individual constituents, drug-drug interactions, and/or optimal method of administration.[5,6] As a result, the formal development of scientific and clinical studies to document the benefit of dietary supplements and herbals has been challenging. In 2006, the Food & Drug Administration (FDA) approved the first botanical product, Veregen ointment, for the topical treatment of external genital and perianal warts in immunocompetent patients 18 years and older. The active ingredient of this topical ointment is sinecathecins, which is a partially pure fraction of the water extract of green tea leaves.

Traditional Chinese medicine (TCM) is based on the interaction of multiple components acting in a synergistic manner. In contrast to the reductionist approach taken by Western medicine, the incorporation of several key components within a Chinese herbal formulation is at the core of Chinese herbal medicine, and this approach may serve to enhance oral absorption, improve clinical efficacy, and reduce toxicity.[7,8] Chinese herbal formulas have been derived from empirical observations as well as from complex TCM theory, and they have been used in humans for over 2000 years. As such, herbals have been generally presumed to be safe and associated with relatively few side effects.

Presently, up to 30% to 40% of all anticancer agents currently being used to treat patients with cancer are derived from natural products. Given this history, there has been significant interest in developing herbal medicines for patients with cancer.[9] The potential role of herbal medicines in cancer chemotherapy include their use as anticancer agents, as cytoprotective agents to prevent and/or reduce toxicity from anticancer agents, and as chemoprevention agents to prevent and/or reduce the risk of development of cancer. One of the earliest herbal preparations evaluated as a cytoprotective agent for chemotherapy associated diarrhea was Kampo (Hangeshasin-to), an herbal medicine initially identified and used by the Chinese and further developed by the Japanese.[10] This medicine contains 6 main components, one of which is baicalin, a β-glucuronidase inhibitor that was shown in in vivo animal model systems to protect against irinotecan-associated diarrhea. Mori et al[11] reported the results of a randomized clinical trial administering Kampo along with irinotecan-based chemotherapy in patients with nonsmall lung cancer. Although treatment with Kampo did not result in a difference in the overall frequency or duration of diarrhea, a significant improvement in the incidence of grade 3/4 diarrhea was observed. Based on this study, it was felt that Kampo medicine could be used as an effective treatment to control irinotecan-associated diarrhea.

PHY906 is a novel Chinese herbal preparation that is composed of 4 main herbs, *Scutelleria baicalensis Georgi, Paeonia lactiflora Pall., Glycyrrhiza uralensis Fisch.,* and *Ziziphus jujuba Mill*, and it has been described in the Chinese pharmacopia for over 1800 years.[12] This herbal medicine has a wide range of pharmacological activities including antiviral, immunologic, analgesic, vasodilatory, hepatoprotective, antioxidant, and appetite stimulatory effects. In particular, it has been widely used to treat a variety of gastrointestinal side effects including diarrhea, abdominal cramps, vomiting, as well as fever and headache. To date, no serious side effects have been observed with this herbal treatment except for constipation that is readily reversible. Based on this extensive experience, we initiated further studies to investigate the biologic and clinical activity of this herbal medicine. As diarrhea is one of the most common dose-limiting side effects of the main anticancer agents used to treat colorectal cancer (CRC), including irinotecan and the fluoropyrimidine 5-fluorouracil (5-FU), we decided to investigate the potential cytoprotective effect of PHY906 in this setting.

Preliminary in vivo preclinical studies of PHY906 were conducted in mice inoculated with murine Colon 38 tumor cells and treated with irinotecan.[12] These studies showed a lower degree of weight loss when mice were treated with the combination of irinotecan plus PHY906. To ensure standardization and maintain interbatch reliability of PHY906, high performance liquid chromatography (HPLC) was used to establish a "chemical fingerprint" of PHY906, and this was standardized with marker substance of each herb presented in the formulation for each batch preparation.[13]

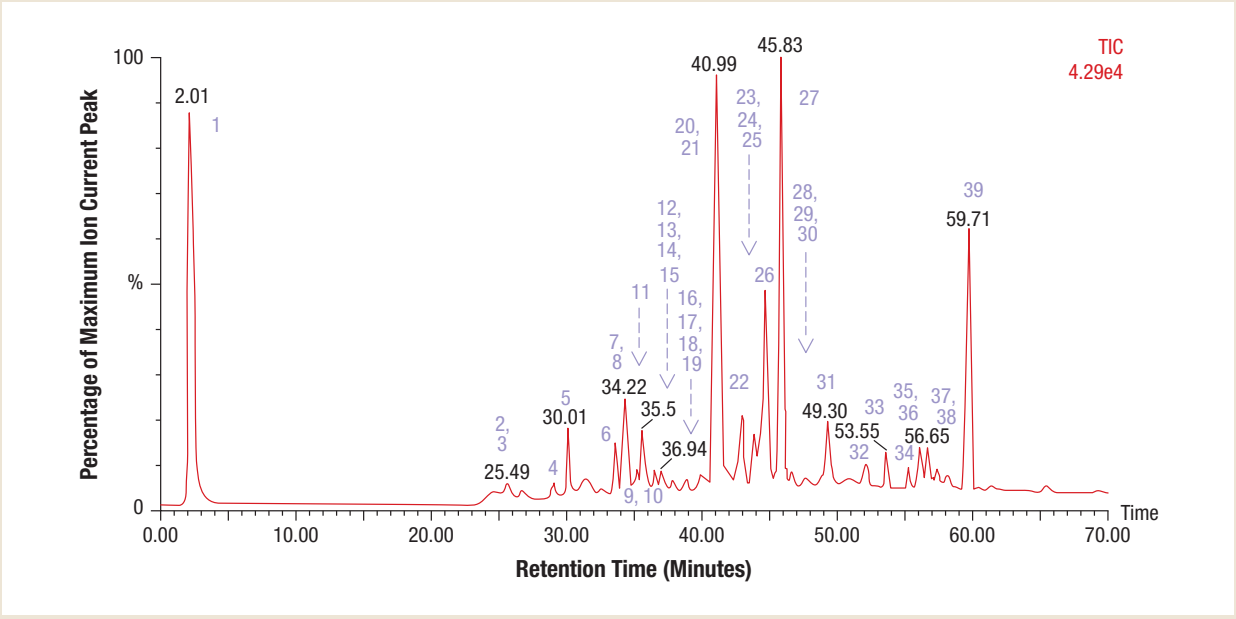
Based on the in vivo preclinical data, a clinical trial of PHY906 in combination with CPT-11 and 5-FU/LV (IFL) chemotherapy was conducted to assess the protective effect of PHY906 on irinotecan-associated diarrhea. The rationale for testing PHY906 with irinotecan-based chemotherapy was the approval of irinotecan in the United States in both first-line and second-line treatment of advanced metastatic CRC.[14-17] At the time this study was initially designed, the bolus, weekly IFL regimen was considered standard treatment in the first-line setting of metastatic CRC in the United States. Of note, the main side effects observed with this regimen were diarrhea, abdominal cramps, and dehydration. In some cases, these toxicities resulted in patient deaths. With this in mind, the goal of our study was to investigate the potential role of PHY906 in reducing the gastrointestinal toxicity associated with irinotecan-based chemotherapy. A phase I multicenter, double-blind, randomized, placebo-controlled, dose escalation, cross-over study of PHY906 as a modulator of irinotecan chemotherapy in patients with advanced CRC was initiated. The specific objectives of this clinical trial were to determine the safety and tolerability of PHY906 when administered concomitantly with the bolus, weekly schedule of IFL as first-line treatment for patients with advanced CRC. In addition, the effect of PHY906 on the pharmacokinetics of 5-FU and irinotecan was investigated to ensure that this herbal medicine did not alter their metabolism.

Methods

Quality Control Analysis of PHY906

PHY906 is comprised of a hot water extract of 4 commonly used herbs, *Scutelleria baicalensis Georgi* (S), *Paeonia lactiflora Pall.* (P), *Glycyrrhiza uralensis Fisch.* (G), and *Ziziphus jujuba Mill.* (Z), in a ratio of 3:2:2:2, respectively. This extract consists of a complex mixture of multiple phytochemicals with multiple biologic and pharmacological properties. The raw ingredients of PHY906 were preselected to meet rigid specifications set forth by the pharmaceutical sponsor of PHY906, PhytoCeutica, and by the herbal manufacturer, Sun Ten Pharmaceuticals in Taiwan. PHY906 is comprised of greater than 75% low molecular weight phytochemical compounds

Figure 1 LC/MS Chromatogram of PHY906



less than 1000 atomic mass units (amus), 10% macromolecular components including protein, nucleic acid, complex carbohydrates, and 5% water. In addition, 10% by weight of excipient insoluble cellulose is added during the spray dry step in manufacturing. Heavy metals (lead, mercury, cadmium, arseinc) are each less than 0.5 ppm, with mercury and cadmium less than 0.03 ppm, as detected by atomic absorption measurements. Pesticides levels (BHCs, DDTs, PCNB) are less than 0.2 ppm by liquid chromatography mass spectrometry (LC-MS) or gas chromatography MS. Total bacteria counts are 260 cfu/g whereas *E. coli* and *Salmonella* species are not detected. Over 90% by weight of PHY906, excluding water content (5%) and insoluble starch excipient (10%) can be re-extracted. The final PHY906 liquid extract (100 mg/mL) is stable for 18 hours at room temperature, and the stored bulk dry extract was stable for more than 3 years.

The chemical fingerprint analysis was conducted by capillary LC-MS using a reverse phase C-18 column and a QTOF-II MS instrument as described previously (Figure 1).[13] The fingerprint separates in the first dimension by chemical property, in the second dimension by exact mass, and in the third dimension by the intensity of each ion with mass between 50 and 1500 amu.

The extraction of PHY906 was carried by first weighing out 100 mg PHY906 powder in an Eppendorf tube. One mL of 80°C water was added to the PHY906, the mixture was vortexed for 1 minute, and placed in an 80°C water bath for 30 minutes, vortexing for 1 minute every 10 minutes in the water bath. The tube was then placed in a beaker of room temperature water for 10 minutes to allow it to cool. The sample was centrifuged for 10 minutes at 12,000 rpm and filtered through a 0.2 μM filter. A 20-μL aliquot was diluted with 980 μL of water for LC-MS analysis.

High-performance liquid chromatography (HPLC) was carried out using a Waters CapLC XE Pump equipped with a CapLC Autosampler and a CapLC 2996 Photodiode Array Detector. The eluents were A: 100% water with 0.1% formic acid and B: 100% acetonitrile with 0.1% formic acid. The column used was a Waters Atlantis dC18 3-μM 300 μM $\times$ 150 mm NanoEase column. The column was heated to 40°C and was preceded by a 0.5 μM precolumn frit. Gradient elution from 0% to 50% B over 70 minutes at 8 μL/minute was used with an initial hold of 5 minutes. The column was then ramped to 95% B over 4 minutes, held in place for 2 minutes, and brought back to initial conditions over 2 minutes. The total run time was 120 minutes.

Electrospray ionizations were performed on a Micromass Q-Tof-II mass spectrometer. Samples were introduced without any splitting into the electrospray interface through a 60-μM stainless steel capillary tube. A positive capillary voltage of 3.25 kV was used. The electrospray source was heated to 80°C and the desolvation gas (N2) was heated to 150°C at 400 L/hour. The Q-Tof was scanned from 50 to 2000 amu over 1 second.

Chemicals and Animals

Irinotecan (CPT-11) was obtained from Pfizer. 5-FU and LV were purchased from Sigma-Aldrich.

Female BDF-1 mice (4-6 weeks old) were purchased from Charles River Laboratories. Mice weighing 16 to 20 g were used in the in vivo animal studies.

Maintenance of Cell Lines In Vitro

The murine Colon 38 cell line was purchased from the American Type Culture Collection. Colon 38 cells were grown in RPMI 1640 medium (JRH Biosciences) supplemented with 10% fetal bovine serum and 100 μg/mL kanamycin. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2:95% air.

Effects of PHY906 On Antitumor Efficacy and Toxicity

Studies were conducted using murine Colon 38 cells (1-2 × 10^6 cells in 0.1 mL phosphate-buffered saline [PBS]) transplanted subcutaneously into 4 to 6-week-old female BDF1 mice (Colon 38/BDF-1 model). Tumor size, as determined by length and width, was measured with sliding calipers and was estimated according to the following formula: tumor size (mm^3) = (length in mm) × (width in mm^2).

After 10 to 14 days, mice with tumor sizes of 150 to 200 mm^3 were selected. Unless otherwise indicated, treatment groups consisted of 5 mice each. Tumor size, body weight, and mortality of the mice were monitored daily. Mice were euthanized when tumor size reached 10% of their body weight. The 5-FU, LV, and irinotecan were administered intraperitoneally (i.p.) whereas PHY906 was given orally. PHY906 (500 mg/kg) was given twice per day at approximately 10 am and 4 pm, and it was given 30 minutes before administration of the anticancer agents. For the control group, mice were administered a vehicle, either PBS for i.p. administration or water for oral administration.

The 4 treatment groups were (1) Control; (2) PHY906 (days 1-4) alone; (3) CPT-11/5-FU/LV only - LV (50 mg/kg, i.p.) 1 hour before administration of CPT-11 (300 mg/kg, i.p.), then immediately followed by LV (50 mg/kg, i.p.) 5-FU (100 mg/kg, i.p.); and (4) PHY906 plus CPT-11/5-FU/LV - first dose of LV (50 mg/kg, i.p.) 30 minutes before PHY906 (days 1-4), then 30 minutes later CPT-11 (300 mg/kg, i.p. on day 1 only), immediately followed by LV (50 mg/kg, i.p.) and 5-FU (100 mg/kg, i.p. on day 1 only).

Patient Eligibility

Patients between the ages of 18 and 75 years, with histologically advanced colorectal cancer previously untreated, were eligible. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate organ function, defined as absolute neutrophil count ≥ 1.5 × 10^9/L, platelets ≥ 100 × 10^9/L, hemoglobin ≥ 9.0 g/dL, creatinine ≤ 3.0 mg/dL, serum bilirubin ≤ 1.5 mg/dL, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤ 5 × institution's upper limit of normal.

All patients were required to have bidimensionally measurable disease. Exclusion criterion included patients with known hypersensitivity to the study medication, its excipients, its analogs, or any of the individual components contained within the herbal formulation; patients with active infections; active/progressive central nervous system (CNS) metastases; familial, nonhemolytic, acholuric jaundice (Gilbert syndrome); patients who received an investigational agent within 4 weeks prior to study entry; pregnant and/or lactating women; and patients with a permanent colostomy in whom evaluation of diarrhea would be difficult. The study was approved to proceed by the US FDA following submission of an investigational new drug (IND) application. The study was approved by the institutional review boards of the respective participating medical centers. All patients signed an informed consent voluntary participation prior to starting therapy.

Screening studies were performed within 2 weeks before starting treatment. All patients had a complete history, physical examination, complete blood count (CBC), serum chemistries, coagulation parameters (PT/PTT/INR), and electrocardiogram at baseline. Urinalysis including urine protein and microscopic examination was obtained. A serum pregnancy test was performed in women of childbearing potential. A computed tomography (CT) scan of chest, abdomen, and pelvis was performed within 4 weeks before starting treatment on study. During treatment, the following parameters were performed on the first day of each week during the two 6-week treatment courses (days 1, 8, 15, 22, 29, and 36 of each course), and at the end of the second treatment course (day 84). Physical examination, height and weight, and body surface area were recalculated if the weight changed by ± 5%. Assessment of performance status, CBC with differential, toxicity evaluation, adverse event (AE) evaluation, and concomitant medication monitoring were performed each week. Serum chemistries (including AST, ALT, ALP, albumin, prothrombin time, total bilirubin, serum creatinine, BUN, and electrolytes) were performed on days 1 and 22 of each course.

Pharmacokinetic analysis of irinotecan and 5-FU: blood samples were collected on day 8 or day 22 of each course and were obtained immediately before administration of irinotecan, immediately before administration of 5-FU, and postdose (ie, after completion of the 5-FU infusion) at 5 minutes and 0.25, 0.5, 0.75, 1, 2, 3, 5, 7, 24, and 48 hours. All collection times were relative to the start of the 5-FU bolus infusion (time point 0). The 5-FU bolus was administered as an intravenous "push" at a rate of approximately 2 mL per second. The total 5-FU infusion time did not exceed 10 seconds.

Patients were carefully educated by the medical and nursing staff as to how to manage and treat AEs, and they were monitored by telephone on the fourth day of each week of treatment with respect to development of new side effects.

Response Evaluation

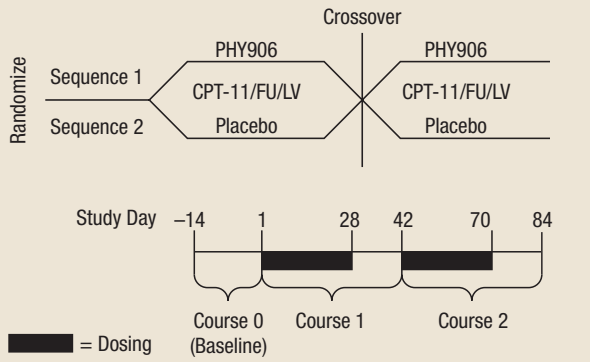
Clinical response (complete response, partial response, progressive disease, or stable disease) was assessed on the final study day (day 84) by CT scans using World Health Organization criteria.

Treatment Plan

Patients received IFL combination chemotherapy as first-line treatment of advanced CRC. Patients receiving the IFL regimen were administered irinotecan at a weekly dose of 125 mg/m^2, followed by leucovorin at 20 mg/m^2 and 5-FU at a dose of 500 mg/m^2. Chemotherapy was administered weekly for 4 weeks, followed by a 2-week rest period. Cycles were repeated every 6 weeks. Patients were randomized to 1 of 2 sequences, with half the patients receiving PHY906 during the first cycle of chemotherapy and placebo with the second cycle. The other cohort of patients received placebo with the first cycle and PHY906 with the second cycle (Figure 2). PHY906 was administered orally, and dose escalation was performed using the Fibonacci scheme at successive dose levels of 1.2, 2.4, and 3.6 g, respectively. No intrapatient dose escalation was allowed. No patients were enrolled into the subsequent cohort until at least 1 patient in the previous cohort had completed both 6-week courses and all 10 patients had been enrolled into the previous cohort.

All patients were required to maintain a medication administration diary, which included a daily patient record of the dose of study medication received. The diary also contained a daily record of diarrhea and associated symptoms. Patients were asked to record stool

Figure 2 Schematic Design of Clinical Trial



quality (formed, semisolid, and liquid) and frequency of bowel movements. Patient compliance with PHY906 was evaluated because of unused returned medication, and this was checked against each patient's medication administration diary.

Dose Modification

Patients were closely monitored for toxicity, and the following criterion were used for further treatment decisions: if grade 2 or higher toxicity (NCI-CTC version 2.0) occurred during the first course of treatment, the dose of chemotherapy was modified. The dose of study medication (PHY906 or placebo) was not reduced due to chemotherapy toxicity. If constipation occurred, study medication was to be temporarily discontinued for any remaining days of the 4 days of dosing during the week that the constipation occurred. The study medication was to be restarted for the entire 4-day dosing period during the following week, with one exception: study medication was not to be omitted on the day pharmacokinetic blood samples were to be drawn.

Doses of irinotecan and 5-FU were modified according to standard treatment guidelines. Loperamide is a standard antidiarrheal medication and was used for patients experiencing any grade of diarrhea. All patients received a 5-HT3 antagonist (granisteron, odansetron, or dolansetron) as premedication for chemotherapy. Additional antiemetic medications such as serotonin antagonists, prochlorperazine, or dexamethasone were used as needed during the course of treatment.

Results

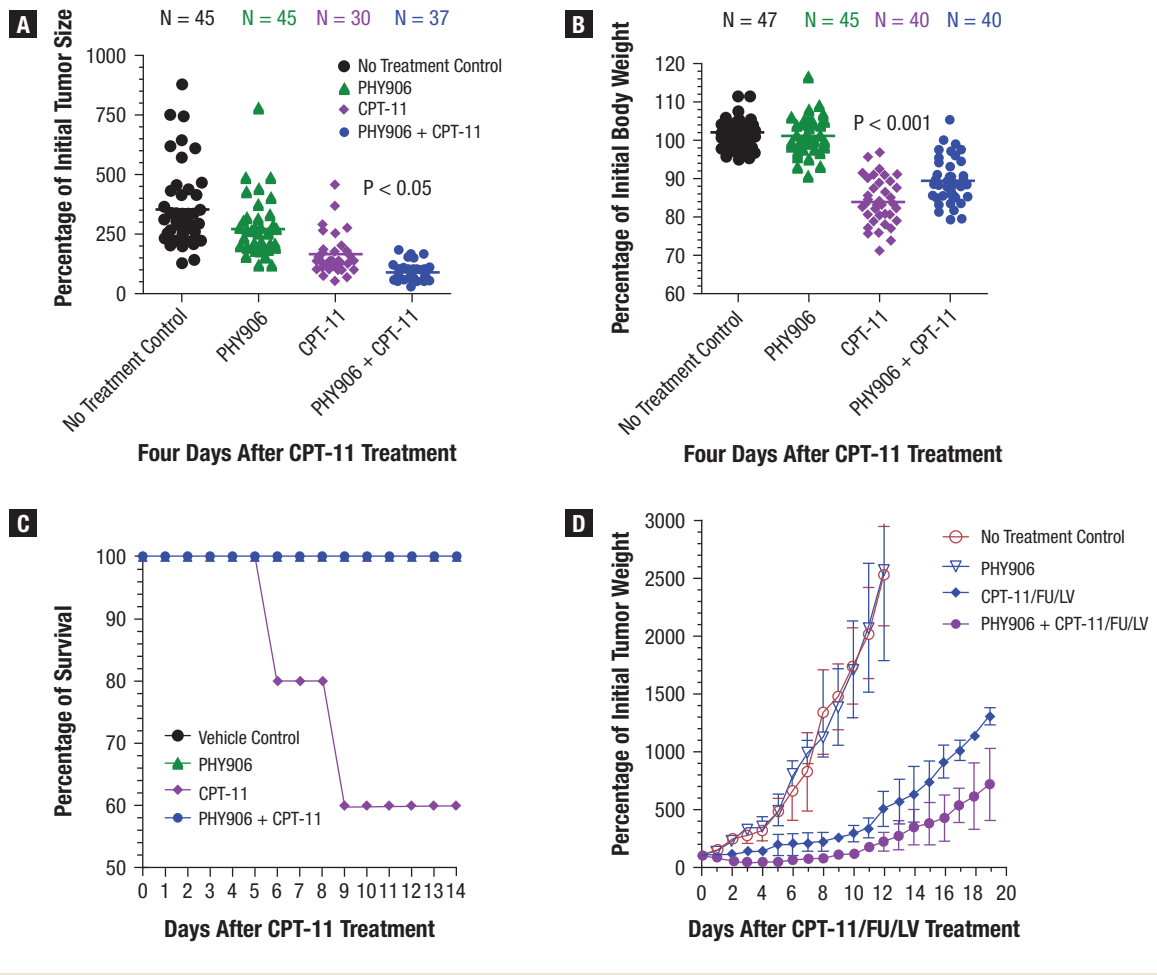
Quality Control Analysis of PHY906

The spectral profile of PHY906 is presented in Figure 1, and the tabulated peaks in the chemical fingerprint are presented in Table 1. Thirty-nine individual phytochemical peaks with intensities greater than 1% of the largest peak were used to define the overall chemical fingerprint pattern. These 39 phytochemical peaks make up more than 85% of the total ion current and 25 of the compounds are identified by the use of marker compounds, exact mass comparison and ms/ms fragmentation. Most of the molecular ions in the fingerprint arise from S (63%) followed by G (26%), P (8%), and Z (3%). The 39 peaks are uniquely identified to 1 of the 4 main

Table 1 Chemical Fingerprint Pattern and Identification

ID	Retention Time	Mass	Herb	Confirmed Chemical Identification	mg of Compound / Gram Dry Weight PHY906
1	2.3	159.1	Z		
2	26.57	304	S		
3	26.57	432.1	G		
4	28.93	480.2	P	Albiflorin	
5	30.02	178.1	P	Paeoniflorin	16.3 ± 0.4
6	33.5	256.1	G	Liquiritin	
7	34.11	256.1	G		
8	34.27	548.1	S		
9	34.9	548.1	S		
10	35.17	324.1	S		
11	35.56	548.1	S		
12	35.85	462.1	S	Scutellarin	1.3 ± 0.3
13	36.44	548.1	S		
14	36.96	480.2	P		
15	37.76	460.1	S	Wogonoside isomer	
16	38.75	346.1	S		
17	39.86	476.1	S		
18	40.14	430.1	G	Ononin	
19	40.36	256.1	G	Liquiritigenin	
20	41.02	446.1	S	Baicalin	46.4 ± 2.2
21	41.02	462.1	S		
22	42.91	446.1	S		
23	43.82	446.1	S		
24	43.82	476.1	S		
25	44.25	430.1	S		
26	44.63	460.1	S		
27	45.81	460.1	S		
28	46.67	490.1	S		
29	47.58	270	S	Apigenin	
30	48.76	300.1	S		
31	49.28	270	S	Baicalein	14.0 ± 1.0
32	52.12	984.5	G	Licorice Saponin A3	
33	53.57	880.4	G		
34	55.22	820.4	G		
35	56.1	284.1	S	Wogonin	7.1 ± 0.2
36	56.65	838.4	G	Licorice Saponin G2	
37	57.41	374.1	S		
38	58.12	284.1	S		
39	59.67	822.4	G	Glycyrrhizin	14.2 ± 0.5

Figure 3 BDF-1 Mice Bearing Muring Colon 30 Tumors were Treated with the Combination of PHY906 and Irinotecan in (A-C) as Outlined in the Methods Section. (A) Reflects the Effect of PHY906 on Tumor Growth. (B) Reflects the Effect of PHY906 on Body Weight. (C) Reflects the Effect of PHY906 on Survival. (D) Shows the Effect of PHY906 on the Antitumor Activity of CPT-11, 5-FU and LV, and the Combination of CPT-11, 5-FU, and LV



herbs, thereby resulting in a signature of both the composition, extraction method, and the herbal ratios. In addition to the chemical fingerprint, up to 4 marker compounds for each herbal ingredient are used for absolute quantitation; baicalin (S), baicalein (S), scutellarin (S), wogonin (S), glycyrrhizin (G), liquirtin (G), paeoniflorin (P), albiflorin (P). Using commercial marker standards for 6 of these compounds, absolute quantitation can be used to determine the amount of phytochemical in mg per gram of dry PHY906 powdered extract. The combination of chemical fingerprint that provides a global view of the phytochemicals and absolute quantitation of a subset of phytochemical markers provides a comprehensive chemical analysis of PHY906.

In Vivo Animal Studies

The effect of PHY906 on the antitumor efficacy and toxicity of irinotecan (CPT-11) monotherapy and the combination therapy of 5-FU, leucovorin, and irinotecan were evaluated using the murine Colon 38 xenograft model. Animal weight loss and mortality were monitored as a gross marker of toxicity secondary to irinotecan treatment. The maximum tolerable dose of CPT-11 in this model was 360 mg/kg (data not shown). Mice were treated with CPT-11 (360 mg/kg, i.p. day 1 only) in the absence or presence of PHY906, which was given orally twice a day at a dose of 500 mg/kg for 4 consecutive days. The profiles of antitumor activity as well as toxicity are presented in Figure 3A-D. As seen, PHY906 not only did not impair the antitumor efficacy of CPT-11, indeed, PHY906 significantly enhanced the antitumor activity of CPT-11 (Figure 3A; $P < .05$). In addition, PHY906 also reduced the toxicity of CPT-11-induced toxicity by reducing the body weight loss (Figure 3B) and mortality (Figure 3C).

For studies with the triple combination, PHY906 alone had no antitumor activity when compared with control mice (Figure 3D). Treatment with the combination of 5-FU, LV, and irinotecan yielded marked antitumor activity, which appeared enhanced upon addition of PHY906. This enhanced tumor inhibition with chemo-

Table 2 Patient Characteristics

Demographic	Cohort I PHY906 1.2 g Day/ Irinotecan/5-FU/LV (N = 13)	Cohort II PHY906 2.4 g Day/ Irinotecan/5-FU/LV (N = 4)
Age years		
Median	61	60
Range	44-74	46-64
Sex		
Male	12	1
Female	1	3
ECOG PS		
0	6	3
1	7	1

Table 3 Patient Outcomes

	Cohort I (N = 13)	Cohort II (N = 4)	Total (N = 17)
Evaluable-for-safety population	13	4	17
Completed study	9 (69.2%)	3 (75%)	12 (70.6%)
Discontinued early	4 (30.8%)	1 (25%)	5 (29.4%)
Disease progression	1 (7.7%)	0	1 (5.9%)
Adverse events	1 (7.7%)	0	1 (5.9%)
Patient withdrew consent	1 (7.7%)	0	1 (5.9%)
Intercurrent illness	0	0	0
Patient noncompliance	1 (7.7%)	1 (25%)	2 (11.8%)
Protocol violation	0	0	0
Lost to follow-up	0	0	0

therapy plus PHY906 approached significance by day 15 ($P = .05$), and was significant at day 20 ($P = .014$), when compared with mice treated with the same chemotherapy regimen plus placebo.

Clinical Trial

Patient Characteristics. Seventeen patients with advanced, metastatic CRC were enrolled on this clinical trial. The specific study sites included VACT Cancer Center/Yale Cancer Center, West Haven, CT; Weill Cornell Medical Center, New York, NY; Cancer Centers of the Carolinas, Greenville, SC; and Saint Francis Medical Center, Grand Island, NE. The median age of patients was 60 years, with 13 male and 4 female patients (Table 2). Five of 17 patients had received previous adjuvant 5-FU-based chemotherapy more than 1 year before enrollment into this study. One patient had received 5-FU-based chemotherapy for treatment of metastatic CRC, whereas 12 patients were previously untreated and had not received previous chemotherapy. Thirteen patients were enrolled into cohort I, and 4 patients into cohort II (Table 2).

All patients in cohort I received chemotherapy with the bolus, weekly IFL regimen, with either 1.2 g of PHY906 or placebo. Patients treated in Cohort II received 2.4 g of PHY906 or placebo.

Nine of 13 patients in cohort I (69.2%), and 3 of 4 patients in cohort II (60.0%) completed the study. Four of 13 patients enrolled in cohort I were discontinued from treatment, 1 due to toxicity, 1 due to disease progression, 1 withdrew consent, and 1 was noncompliant. In cohort II, 1 patient discontinued treatment because of noncompliance (Table 3). With respect to cohort I, the median total dose of PHY906 was 19.2 g (range, 5.2-19.2 g), and the median total dose of placebo was 19.2 g (range, 6.4-19.2 g). The median total number of days that the study drug was received was 16.0 days for PHY906 (range, 4.3-16.0 days) and 16.0 days for placebo (range, 5.3-16.0 days).

The median total dose of PHY906 administered in cohort II was 30.2 g (range, 8.0-38.4 g), and the median total dose of placebo was 38.4 g (range, 2.4-39.2 g). The median total number of days that the study drug was given on cohort II was 15.5 days for PHY906 (range, 3.3-16 days) and 16 days for placebo (range, 1.0-16.3 days).

Toxicity/Adverse Events

Treatment-related AEs were determined by the investigators to be related to treatment with study medication (PHY906 or placebo) or related to treatment with chemotherapy, and these results are presented in Tables 4A and 4B. Eleven patients in cohort I and 2 patients in cohort II experienced treatment-related side effects during treatment with PHY906. Eleven patients in cohort I and 2 patients in cohort II experienced treatment-related AEs during placebo treatment. No patients experienced treatment-related, life-threatening (grade 4) toxicity during treatment with PHY906 plus chemotherapy. In contrast, 2 of 16 patients (6.3%) experienced treatment-related, life-threatening (grade 4) AEs (neutropenia and GI hemorrhage) during treatment with placebo plus chemotherapy.

The most frequently experienced treatment-related, mild (grade 1) AEs during treatment with PHY906 included nausea, vomiting, and fatigue, whereas the main side effects experienced during placebo treatment were fatigue, nausea, diarrhea, abdominal pain, and anorexia. In cohort I, 4 of 11 patients in the PHY906 treatment group, and 6 of 12 patients in the placebo group developed diarrhea (all grades). Two patients in the group treated with PHY906 developed grade 2 diarrhea, and no patient developed grade 3. In the placebo group, 3 patients developed grade 2 diarrhea and no patient developed grade 3. In cohort II, for the PHY906 arm, 2 patients developed grade 2 diarrhea, whereas no patient developed grade 3. In patients treated on the placebo arm, no patients developed grade 2 diarrhea, whereas 2 patients developed grade 3 diarrhea.

As seen in Table 5, the use of the antidiarrheal loperamide or lomotil in cohort I, was markedly reduced during PHY906 treatment (3 of 11 patients, 27.3%) when compared with placebo treatment (7 of 12 patients, 58.3%). Although the patient numbers in cohort II are small, a similarly lower frequency of patients received loperamide while on PHY906 treatment (2 of 3 patients, 66.7%) than in those patients receiving placebo (3 of 3 patients, 100%).

The most frequently experienced treatment-related, moderate (grade 2) AE during placebo treatment (1.2 or 2.4 g daily of placebo plus chemotherapy) was vomiting, and this was observed in 5 of 16 patients (31.3%). One of 16 patients on placebo (cohort II) developed grade 3 vomiting. In the active treatment group, only 1 of 15

Table 4A Adverse Events (NCI-CTC Version 2.0 Grade 1 and 2)

Adverse Event	Toxicity Grade	Cohort I		Cohort II	
		PHY906 IFL (N = 11)*	Placebo IFL (N = 12)*	PHY906 IFL (N = 3)**	Placebo IFL (N = 3)**
Diarrhea	1	2	3	0	1
	2	2	3	2	0
Vomiting	1	1	1	1	0
	2	1	2	0	3
Nausea	1	5	8	1	0
	2	1	0	0	2
Neutropenia	1	1	2	0	0
	2	2	1	0	0
Anemia	1	1	0	0	0
	2	1	3	1	0
Thrombocytopenia	1	0	1	0	0
	2	0	1	0	0
DVT	1	0	0	0	0
	2	0	1	0	0
Anorexia	1	1	3	0	1
	2	1	0	0	1
Dehydration	1	0	0	0	0
	2	0	0	0	1
Fatigue	1	3	7	1	0

Abbreviation: DVT = deep vein thrombosis.
* Of 13 patients enrolled into cohort 1, 2 patients randomized to receive PHY906 were not evaluable for toxicity, while 1 patient on the placebo arm was not evaluable for toxicity.
** Of 4 patients enrolled into cohort 2, 1 patient was noncompliant and was not evaluable for toxicity.

Table 4B Adverse Events (NCI-CTC version 2.0 grade 3 and 4)

Adverse Event	Toxicity Grade	Cohort I		Cohort II	
		PHY906 IFL (N = 11)*	Placebo IFL (N = 12)*	PHY906 IFL (N = 3)**	Placebo IFL (N = 3)**
Diarrhea	3	0	0	0	2
Vomiting	3	0	0	0	1
Nausea	3	0	0	1	0
Neutropenia	3	3	2	1	0
Anemia	3	1	0	0	1
Thrombocytopenia	3	1	1	0	2
	4	0	1	0	0
Hypokalemia	3	0	0	1	1
	4	0	0	1	0
DVT	3	1	1	0	1
Anorexia	3	0	0	1	0
Dehydration	3	0	0	0	1
Sepsis	3	0	0	0	1
	4	0	0	1	0

Abbreviation: DVT = deep vein thrombosis.
* Of 13 patients enrolled into cohort 1, 2 patients randomized to receive PHY906 were not evaluable for toxicity, while 1 patient on the placebo arm was not evaluable for toxicity.
** Of 4 patients enrolled into cohort 2, 1 patient was noncompliant and was not evaluable for toxicity.

Table 5 Use of Anti-Diarrheal and Anti-Emetic Medications

Medication	Cohort I PHY906 IFL (N = 11)	Cohort I Placebo IFL (N = 12)	Cohort II PHY906 IFL (N = 3)	Cohort II Placebo IFL (N = 3)
Loperamide	3 (27.3%)	7 (58.3%)	2 (66.7%)	3 (100%)
Antiemetics	2 (18.2%)	2 (18.2%)	0	1 (33.3%)

patients developed grade 2 vomiting during treatment. No patient developed grade 3 vomiting while receiving PHY906.

Grade 3 neutropenia was observed in 5 of 15 patients (33.3%) while receiving PHY906 and in 3 of 16 patients (18.8%) during placebo treatment. Treatment-related, hematologic grade 4 AEs were experienced by no patients during treatment with PHY906 plus chemotherapy and by 1 of 16 patients (6.25%) during treatment with placebo plus chemotherapy.

Four patients in cohort I developed grade 2 anemia, 1 patient during treatment with PHY906, and 3 patients while receiving placebo. In cohort II, 1 patient on PHY906 developed grade 2 anemia, and 1 patient on placebo developed grade 3 anemia.

Of note, no patients died during the study or within 30 days of the last dose of study medication. A total of 5 patients experienced serious adverse events (SAEs) during the course of the study (Table 4B). One patient experienced serious toxicity during both cycles of treatment (with and without PHY906 plus chemotherapy). SAEs were experienced in 2 of 15 patients (13.3%) randomized to receive PHY906, with both of these patients receiving treatment in cohort II. However, these AEs were considered unrelated to the study drug as reviewed by the investigator. SAEs were experienced in 4 of 16 patients (25%) during placebo treatment, 3 in cohort I, and 1 in cohort II.

One serious AE involving a patient treated in cohort 1, was considered to be possibly related to chemotherapy and/or treatment with PHY906 or placebo. This event was rectal hemorrhage, which required hospitalization and admission into the intensive care unit and it involved a patient in cohort I. Given the severity of this event, the study blind was broken, and it was determined that the patient had been randomized to the placebo arm.

Pharmacokinetics

Maximum plasma concentration (C_{max}), time to maximal concentration (t_{max}), area under the time-concentration curve (AUC) from 0 to 24 hours ($AUC_{0\text{-}24}$) and from 0 hours to infinity ($AUC_{0\text{-}\mu}$), and elimination half-life ($t_{1/2}$) were calculated for day 8 using noncompartmental methods (Table 6).

Of the patients who completed both treatments, 10 patients were evaluable for pharmacokinetic and statistical analysis of the 5-FU concentration-time data. For irinotecan, 15 patients were evaluable for pharmacokinetics analysis and 12 patients were evaluable for statistical analysis. With respect to SN-38 metabolism, 15 patients were evaluable for pharmacokinetics and 11 patients for statistical analysis.

The half-life values for 5-FU were short, typically less than 1 hour, and in 7 of 10 patients, the estimated half-life was less than 20 minutes. The 5-FU t_{max} occurred at the end of the bolus dose. The C_{max} values were extremely variable, ranging from 2910 to 314,000 ng/mL. AUC values ranged from 1912 to 89,918 hours·ng/mL, and they were also highly variable, showing significant variation both within and between patients. Clearance values were high, ranging from 5.56-261.4 L/hour/m^2 and variable. Volume of distribution values were characteristic of 5-FU, ranging from 1.22 to 329.5 L/m^2.

With respect to irinotecan, half-life values ranged from 4.0 to 11.3 hours. Peak plasma concentrations ranged from 476 to 2390 ng/mL and the time of the peak concentration (t_{max}) typically occurred at the end, or shortly after the end, of the 90-minute infusion. Irinotecan $AUC_{0\text{-}\mu}$ values ranged from 2574 to 11,228 hours·ng/mL. CL and Vz estimates were consistent with those of a lipid soluble molecule with a relatively high extraction ratio. CL ranged from 11.1 to 48.6 L/hour/m^2 and Vz values ranged from 103.0 to 516.5 L/m^2. SN-38 half-life values were substantially longer than those observed with irinotecan, ranging from 4.5 to 105.4 hours. Peak plasma concentrations of the SN-38 metabolite were considerably lower than those of parent irinotecan, ranging from 3.4-37.1 ng/mL, and the time of the peak concentration (t_{max}) was typically after that of irinotecan, occurring slightly after the irinotecan t_{max}, with the exception of 1 patient, who was an outlier with a t_{max} value of 25.5 hours. SN-38 $AUC_{0\text{-}\mu}$ values were lower than those of irinotecan, ranging from 47.3-452.8 hours·ng/mL.

The mean and median values for the key pharmacokinetic parameters for 5-FU, irinotecan, and SN-38 were similar regardless of whether the patients received PHY906 or placebo. As seen in Table 6, the results of the paired *t* tests did not reveal any statistically significant differences.

Clinical Activity

Because the phase II part of the study was never completed due to the early termination of this study, the effect of PHY906 on the clinical activity of irinotecan-based chemotherapy could not be adequately investigated. Nevertheless, preliminary observations can still be made based on the phase I component. In cohort I, 4 patients experienced a partial response after 2 cycles of chemotherapy, 7 had stable disease, and 2 progressed on treatment. In cohort II, 3 patients were evaluable for response. Of this group, one had a partial response after 2 cycles of chemotherapy, and 2 patients had stable disease. In sum, 14 of 16 patients treated in this phase I part of the study experienced either a partial response or stable disease to give a tumor control rate of 87%.

Discussion

Botanicals and natural products have been a rich source for developing anticancer agents for nearly 40 years. In general, botanicals have been screened for initial antitumor activity and the active moiety is then isolated and further characterized. However, in traditional

Table 6 Effect of PHY906 on Pharmacokinetics of 5-FU, CPT-11 (Irinotecan), and SN-38

Analyte	Statistic	T_{max} μz (hr)		C_{max}(ng/mL)		$AUC_{0-\mu}$ (hr*ng/mL)		CL(mL/hr/m^2)	
		PHY906	Placebo	PHY906	Placebo	PHY906	Placebo	PHY906	Placebo
5-FU	Mean	0.23	0.20	51941	60765	16415	18520	86843	59364
	SD	0.10	0.12	88714	72610	25116	19485	73194	69664
	Min	0.08	0.11	4220	2910	2086	1911	5561	7039
	Median	0.21	0.18	32200	38300	7731	11761	64676	42515
	Max	0.39	0.54	314000	264000	89918	71035	239647	261590
	CV%	42	58	171	119	153	105	84	117
	P value	.4434		.4825		.6026		.3893	
CPT-11	Mean	8.46	8.28	899	1012	6275	5686	22159	25301
	SD	1.48	2.25	260	526	1996	2099	8206	10566
	Min	5.63	3.95	476	434	3169	2574	12702	13157
	Median	8.82	8.61	927	906	5992	5486	20870	22787
	Max	10.39	11.28	1480	2390	9841	9500	39450	48565
	CV%	17	27	29	52	32	37	37	42
	P value	.5739		.5024		.3807		.4622	
SN-38	Mean	20.41	25.02	13.5	15.3	161.3	194.9	NC	NC
	SD	8.32	27.48	7.9	11.5	86.6	117.7	NC	NC
	Min	11.79	6.48	4.2	3.4	47.3	48.9	NC	NC
	Median	16.97	17.65	12.0	13.0	127.1	196.9	NC	NC
	Max	40.25	105.36	25.3	37.1	321.7	436.8	NC	NC
	CV%	41	110	58	75	54	60	NC	NC
	P value	.6148		.3758		.4259		NC	

Abbreviations: T_{max} = time to maximal concentration; C_{max} = maximum plasma concentration; AUC = area under time-concentration curve; CL = clearance; NC = no change.

herbal medicine, multiple herbs are administered together as a mixture containing several active compounds. These individual components presumably act in a synergistic manner, and they are felt to enhance the overall efficacy of the formulation and/or to reduce the side effects of one or more components. The scientific and clinical evaluation of herbal preparations has been limited by several key issues, which include (1) quality control and standardization of batch-to-batch preparation of the active constituents contained in each herbal formulation, (2) the lack of well-defined and well-established parameters to document clinical efficacy, and (3) the relatively limited information on the drug interaction profile of commonly used herbal preparations. With respect to this latter issue, there is now an increasing appreciation for the potential for herb-drug interactions.[18] St. John's wort is one of the most commonly used herbs to treat depression, and several studies have shown that it can alter the metabolism of prescription and nonprescription drugs by altering the cytochrome P450 pathway.[19,20] With respect to the treatment of CRC, St. John's wort has been shown to impair the metabolic activation of irinotecan to SN-38, thereby reducing its clinical efficacy.[21] Moreover, an increasing number of herbal medicines, including kava, ginseng, garlic, and echinacea have been shown to have interactions with various anticancer drugs through induction of drug metabolizing enzymes, ATP-binding cassette drug transporters, and other key metabolic processes.[18]

Based on the growing use of herbal medicines by patients with cancer and the strong desire to incorporate them into clinical practice, a more rigorous set of regulatory, scientific, and clinical guidelines are required. Before the initiation of clinical trials, quality control and standardization must be ensured. Rapidly progressive renal failure and the development of urothelial carcinoma were reported in patients taking weight-reducing pills containing Chinese herbs.[22] Further evaluation revealed that a costly error had occurred in which one of the component herbs called *Stephania tetrandra* was replaced by a nephrotoxic and carcinogenic herb, *Aristolochia fangchi*.[23] PC-SPES is a combination of 8 different herbs, and when initially tested, this formulation showed promising clinical activity in the treatment of prostate cancer.[24] However, over a period of 5 years, several batches were found to have contaminants, including indomethacin, phytoestrogens, and warfarin.[25,26] These various agents were directly responsible for the increased risk of hemorrhagic as well as thromboembolic complications associated with PC-SPES. These findings led the FDA and the manufacturer to recall the medication and to terminate further clinical development of this herbal medicine.

Rigorous methods are clearly required to ensure similar composition of various constituents. The chemical composition of herbs is affected by several factors, including differences in soil,

temperature, and the time of harvesting. With this in mind, we believe that the LC-MS method that was developed for this study to establish a "chemical fingerprint" is a reliable and consistent methodology that can be used to easily compare the consistency and quality of different herbal batches. The 39 individual phytochemical peaks have been uniquely identified to 1 of the 4 main herbs contained within PHY906, and they form a signature that takes into account the composition, extraction method, and ratios of the individual components. Studies are ongoing to develop a biologic fingerprint analysis using a bioresponse gene expression profile that can provide a second, orthogonal method for characterizing PHY906. An informatics program is also being developed to integrate the chemical fingerprint and biologic fingerprint analyses to come up with what has been termed a phytomics similarity index. Such an integrated approach should be especially useful in ensuring quality control of herbal preparations and in identifying those herbal medicines that can be safely brought into early-phase clinical development.

PHY906 has been in human use in the Orient for nearly 2000 years without significant side effects other than reversible constipation. Its main use has been to treat multiple medical problems including GI complaints, specifically diarrhea. As diarrhea is one of the most significant dose-limiting toxicities of chemotherapy drugs used for the treatment of CRC, we decided to investigate the ability of PHY906 to reduce the GI toxicities associated with irinotecan-based chemotherapy. Although the overall number of patients enrolled on to this trial are relatively small, our study showed that there was indeed a reduction in the overall incidence of grade 3/4 diarrhea in patients treated with PHY906, which resulted in a lower use of antidiarrheals. In addition, there was also a trend toward lower frequency and severity of vomiting for cycles in which patients received PHY906 as opposed to placebo. Interestingly, during the conduct of this study, it was readily apparent to the patients, their family members, and the staff supporting this clinical trial that there was a difference in the overall qualitative function and quality of life between treatment with PHY906 and placebo. However, only at the time of unblinding was it clear which treatment patients had been randomized to receive. One of the clear advantages of this study was that it included a placebo arm, which helped to control for any placebo effect that might be present. Moreover, this study was double-blinded with neither the patients nor the investigators and support staff knowing which treatment the patient had been randomized to receive. This type of rigorous clinical design would seem to be particularly important for investigating herbal medicines, as this would provide an important safeguard mechanism against investigator bias and the potential for placebo effect.

Another potential concern associated with development of an herbal preparation in combination with cytotoxic chemotherapy relates to the potential effect of PHY906 on the pharmacokinetics of the individual chemotherapeutic agents and the potential for compromising their efficacy and/or safety profile. Our findings suggest that coadministration of PHY906 along with 5-FU or irinotecan did not alter the pharmacokinetic parameters of 5-FU or irinotecan nor did it affect the metabolism of irinotecan to the active metabolite SN-38.

As the study was closed early because of slow accrual resulting from changes in the standard care of treatment of patients with advanced CRC, it is difficult to make firm conclusions regarding the potential effect of PHY906 on the clinical efficacy of the IFL regimen. However, although the patient numbers enrolled on this study are small, our preliminary results suggest that PHY906 may not compromise the clinical activity of irinotecan-based chemotherapy as 14 of 16 patients showed either a partial response or stable disease when evaluated after 2 cycles of therapy. A formal randomized trial is needed to further evaluate the effect of PHY906 on the clinical activity and safety profile of irinotecan-based chemotherapy, whether it is irinotecan in combination with the infusional 5FULV2 regimen, with irinotecan in combination with the oral fluoropyrimidine capecitabine, or with irinotecan monotherapy.

The overall goal of this clinical trial was to begin to develop guidelines to investigate the role of Chinese herbal medicines in the treatment of cancer. By using the rigorous standards of evidence-based medicine, CAM therapies can be made available and used in conjunction with traditional Western medicine. Whereas further clinical trials exploring the role of herbal preparations in combination with standard cytotoxic chemotherapy are required, this study provides the rational framework for the development of such studies.

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