DCP Protocol #: NWU2014-03-01

Local Protocol #: NCI 2014-03-01

Phase I trial of Berberine in subjects with ulcerative colitis

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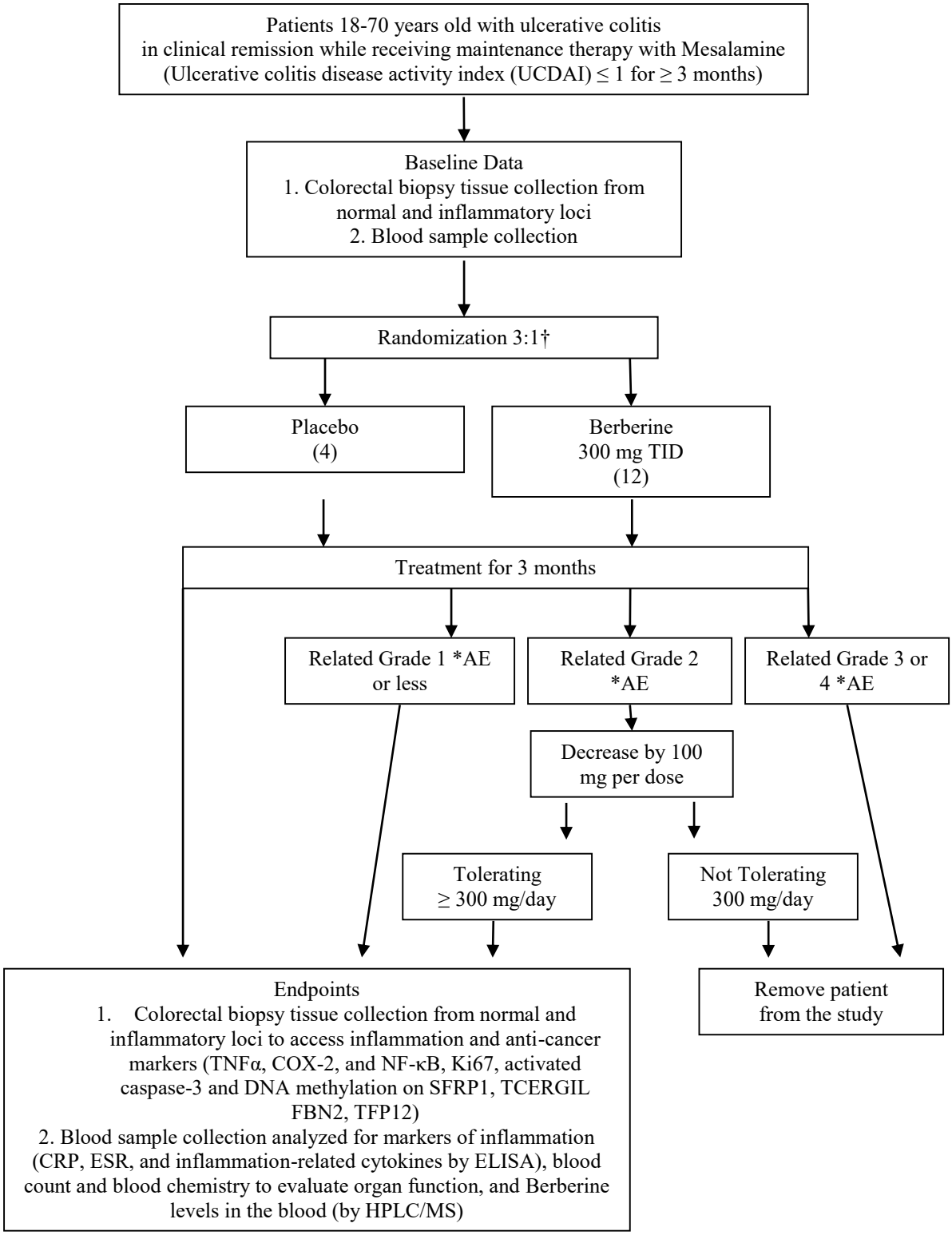
Agent(s)/Supplier:	Berberine/Shanghai Sine Tianping Pharmaceuticals
NCI Contract #	HHSN261201200035I
Protocol Version Date:	November 29, 2016
Protocol Revision or Amendment #	Version 5.7

SCHEMA

Phase I trial of Berberine in subjects with ulcerative colitis



†Sixteen subjects will be randomized. Two additional subjects are anticipated to be accrued (for a total of 18 subjects) to account for anticipated dropouts. Dropouts in either treatment group will be replaced by subjects assigned to that same treatment and gender group.

* = Adverse Event

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1. OBJECTIVES

1.1 Primary Objective

- To determine the safety of Berberine administered to participants with UC in clinical remission while receiving maintenance therapy with Mesalamine.

1.2 Secondary Objectives

- Determine the molecular efficacy of Berberine by examining the following biomarkers:
  - Plasma-based measures of inflammation, including the blood C-reaction protein (CRP) level, erythrocyte sedimentation rate (ESR), and cytokines such as TNFα, IL-4, IL-6, IL-8 and IL-10 measured by enzyme-linked immunosorbent assay (ELISA).
  - Tissue-based measures of inflammation, including TNFα, COX-2, and NF-κB by immunohistochemistry (IHC), and anti-cancer action, including Ki67 and activated caspase-3 by IHC, and DNA methylation on SFRP1, TCERGIL FBN2, TFP12 using the methylation-specific polymerase chain reaction (qMSP) strategy.
- Clinical Efficacy: UC related symptoms will be measured using the Ulcerative Colitis Disease Activity Index (i.e. the Mayo score) [UCDAI][13, 75].
- Histological analysis for inflammation: severity of histologic inflammation will be evaluated using the Geboes grading system[25, 73].
- Determine plasma concentration of Berberine.

2. BACKGROUND

2.1 Study Disease

Colorectal cancer (CRC) ranks high in cancer incidence and mortality worldwide, including in the US and China[74]. Specifically, CRC is the third most common type of cancer in both US and China, and accounts annually for ~11% and ~14% of all cancer deaths, respectively[9, 10, 18, 78]. Thus, identifying strategies to reduce its incidence is critically important.

Ulcerative colitis (UC) is a chronic intestinal inflammatory process that is clinically characterized by bloody diarrhea, fever, weight loss, and anemia[1]. Current global incidence and prevalence rates of UC are 8-14 per 100,000 persons and 120-200 per 100,000 persons, respectively[14], with the highest annual incidence of UC at 19.2 per 100,000 person-years in North America; it is 6.3 per 100,000 person-years in Asia[63, 67]. In a case-control study, it was reported that although the incidence of UC in the Chinese population is relatively lower compared to Caucasian populations, the risk factors associated with UC patients in both populations were similar[41]. In addition, there was no difference in clinical manifestations between Asian and Western countries[87]. Finally, it is believed that UC incidence in China is falsely low due to under diagnosis related to limited health care access, and that concurrent with economic development, access is increasing and incidence also appears to be increasing[96].

Individuals with UC are at high risk for CRC[3, 62, 82, 83]. Globally, the incidence of CRC in patients with UC is estimated to be 2 to 5 times higher than that in the general population of the same age group[28]. It was reported that the incidence of CRC in UC patients in US from 1998 to 2010 was 76.0 per 100,000 person-years[30], while in China from 1990 to 2003 it was 30.8[102]. The vast majority of UC patients are maintained on therapy based upon 5-aminosalicylic acid (5-ASA)[20, 21]. However, although Mesalamine, the 2nd generation of 5-ASA, effectively reduces periods of active disease and extends remission time[4, 59], UC often becomes refractory, and with prolonged UC, there is an increased risk of CRC (2% at 10 yr., 8% by 20 yr., and 18% by 30 yr.)[40]. Other available medical treatments, such as biological agents or immunosuppressants, are not only expensive, but are associated with potential adverse effects like infection and increased risk of malignancy[16]. Moreover, once CRC develops, treatment remains suboptimal and its prognosis is poorer for patients with UC compared to those without[38]. Therefore, the development of new therapies that combine high treatment efficacy, convenient dosing, low side effects, and, more importantly, with CRC chemopreventive ability, is an important goal for UC management and its associated CRC incidence reduction.

2.2 Study Agent

Berberine is a main constituent of Coptidis rhizome. A large body of evidence demonstrates that it inhibits colorectal carcinogenesis in humans, mainly through its anti-inflammation effects. Berberine is widely used to treat gastroenteritis and diarrhea in China[17, 19, 55, 80]. In two clinical trials in adult patients with acute diarrhea, Rabbani reported the efficacy and safety of Berberine[70, 71]. In another clinical trial on acute watery diarrhea, Berberine was found to be as effective as tetracycline[47]. In chloroquine resistant malaria, the combination of pyrimethamine and Berberine has been shown to optimally clear the parasite and reduce gastroenteritis symptoms[77]. Across different animal models of intestinal inflammation, Berberine is efficacious[99, 101, 104]. Of high importance, Berberine inhibits colon carcinogenesis in preclinical models. It has high activity in dextran sulphate sodium (DSS) and in trinitrobenzene sulfonic acid (TNBS) induced colitis in mice and rats[31, 57, 94, 103]. Furthermore, it prevents azoxymethane (AOM)-induced colon carcinogenesis in rats[81] with such effects being largely attributed to the anti-inflammatory properties of Berberine. Although not entirely clear, several lines of evidence support the notion that a primary effect of Berberine on the intestine relates to anti-inflammatory activity. In China, Berberine is widely used clinically to treat a range of inflammatory ailments, while specific anti-inflammatory activity has been demonstrated across of variety of models. Specifically, Berberine is efficacious in adjuvant-induced murine models of arthritis and in experimental models of osteoarthritis in the rat[33, 34, 48, 52, 96]. It suppresses autoimmune nephritis in BALB/c mice[64] and attenuates lipopolysaccharide-induced extracellular matrix accumulation and inflammation in rat mesangial cells[42]. In addition to these rigorously conducted and physiologically relevant studies, Berberine's basic anti-inflammatory action has been loosely linked to a number of downstream processes, including anti-oxidatic, anti-apoptotic, and anti-tumor activities in a wide array of reports (reviewed in[37, 84]). However, the design of such studies (typically involving very high concentrations of agent in vitro), places severe limitations on the meaning of resultant findings.

In considering the focus of this proposal on a Chinese population, it is important to consider that extensive safety information is available about the clinical use of Berberine in this cohort and is lacking in a US-based cohort. This is critical information in that drug safety is a central tenant of chemoprevention. Because genetic factors are major determinants of drug metabolism, thus underlying the field of pharmacogenomics, toxicity information cannot readily be extrapolated across populations. In addition to Berberine's wide spread clinical use in China, multiple clinical trials carried out in Asian populations indicate that Berberine (1.0 – 1.5 g daily for 3 months) is safe in this cohort[51, 87, 97, 100]. A separate revealing trial focused on patients with radiation-induced acute intestinal injury (RIAII); it randomized patients to 300mg Berberine orally three times daily versus control for three weeks[58]. The incidence, severity, and time to occurrence of RIAII were all significantly reduced by Berberine. Further, toxicities were lower with Berberine. The study indicated that oral Berberine was feasible, well tolerated, and effective[58]. Importantly, as RIAII is caused not only by death of the endothelial cells, but also by leukocyte recruitment and inflammatory response, RIAII mimics the active process of UC. As a traditional drug widely used for thousands of years in China, this CFDA (China Food and Drug Administration)-approved drug has been manufactured into an over-the-counter drug in China, formulated as tablets or capsules. The therapeutic dosage for most clinical situations is 300 mg orally two to four times daily[61]. Presently, Berberine is available as a dietary supplement in the form of 100-200 mg capsule dosage per day in the US, but it is not officially approved by the Food and Drug Administration (FDA) in the US. Based upon above findings, we hypothesize that Berberine will be safe and beneficial in UC patients, and that its administration to a Chinese cohort will be safe.

Some human studies have examined blood Berberine concentrations. In one study, 400 mg of oral Berberine gave a mean maximum plasma concentration (Cmax) of 0.4 ng/ml[35]. In another, 300 mg oral Berberine per day for 7 days gave a mean steady-state plasma concentration of 0.3 ng/ml[98]. Of high potential importance, current findings indicate that oral Berberine will provide for efficient delivery of agent to the target organ (i.e., the colorectum) with low exposure to systemic organs. Selective delivery to target organs uses pharmacology to optimize efficacy and minimize systemic toxicity, and is a highly desirable strategy for chemopreventive agents. Specifically, murine-based studies demonstrate that the oral bioavailability of Berberine is below 0.000%[46]. Also, a plateau in blood concentration was seen with increased oral doses. Of notable interest, intraperitoneal injection led to blood concentrations that were 20% of those of IV injection. These findings suggest that Berberine is absorbed through the intestinal lining (and is thus delivered to colonic epithelium and the supporting organ), but undergoes rapid first pass metabolism in the liver. Together, these findings further support our hypothesis that Berberine will be safe and beneficial in UC patients.

The ideal chemopreventive agent, in addition to being efficacious in the prevention of cancer, must be easily administered, affordable, safe, and well tolerated, with minimal side effects. Thus, Berberine holds great promise as an

efficacious chemopreventive agent for patients with UC who are at high risk for CRC. Because essentially all of those diagnosed with UC are maintained on Mesalamine, the first step in exploring Berberine's chemopreventive efficacy is to formally assess the safety of Berberine when administered to patients maintained on Mesalamine. There are no recognized contraindications for administering Berberine to UC patients maintained on Mesalamine and Chinese physicians do so under clinical circumstances. While it is reassuring that there are no reports of adverse interactions it needs to be underscored that the safety and efficacy of Berberine administered to a UC cohort maintained on Mesalamine has not yet been examined.

Therefore, we propose a randomized, placebo-controlled, phase I pilot study to investigate the safety of Berberine in a group of Chinese patients with UC in clinical remission while receiving maintenance therapy with Mesalamine. As such, this study will provide the necessary safety information to support future expanded investigation on Berberine as a CRC chemopreventive agent in UC patients. It will also generate pilot biomarker information that will directly support the design of future studies. Recognizing that an important parallel goal is to assess the feasibility of conducting early phase cancer chemoprevention trials in China, the proposed study is purposefully designed to be small and pilot in nature. The proposed study therefore represents an important first step in determining whether Berberine can provide protection for UC patients who are at risk for a common cancer and a common cause of death. It also represents an important first step in conducting studies in a country whose unique qualities and opportunities, including a well-established medical system for clinical trials, large populations, high density of patients in large hospitals (allowing for time- and cost-efficient recruitment), provide a highly leveraged opportunity to advance the field of chemoprevention in a manner that will directly improve the health of people who reside in the US.

2.3 Rationale

Berberine is efficacious against UC

The pathological features of UC are marked by inflammation, with infiltration of neutrophils and lymphocytes in the mucous membranes, which is believed to underlie the characteristic mucosal ulcerations[1]. Many different animal models of UC have been developed[90]. Both DSS- and TNBS- induced colitis are well-established rodent animal models of mucosal inflammation that have been used for over two decades to study UC pathogenesis and to support preclinical studies[69, 89]. Consistent with UC serving as a CRC risk factor, these same models are used to study colitis-associated carcinogenesis[12, 15, 44]. Moreover, changes in pro-(TNF-α, NF-κB, IL-6, IL-8) and anti-inflammatory (IL-10, IL-4) cytokine levels in these models emulate those observed in humans with UC[85]. Furthermore, therapeutic efficacy in these models, especially DSS-induced colitis, has been shown to accurately reflect efficacy in humans[65]. In both TNBS-induced colitis and DSS-induced colitis murine models, Berberine decreases colitis[31, 57, 103], and this is accompanied by induction of colonic macrophage apoptosis[94], and by inhibition of pro-inflammatory cytokine production and related signaling pathways in colonic macrophages and epithelial cells through suppressing the Nuclear factor-kappaB (NF-κB) pathway[8, 31, 57, 94, 103]. In addition, in macrophage-based experiments, Berberine was reported to inhibit inducible cyclooxygenase-2 (COX-2) expression, which in turn abolishes pro-inflammatory responses[39]. These findings demonstrate that Berberine inhibits pro-inflammatory processes and decreases colitis in established preclinical models of UC that have a track record of predicting efficacy in humans. Although it is unclear whether these molecules are primary or secondary factors involved in the regulation of the inflammation, they can be considered as biomarkers to test the drug efficacy and monitor inflammation severity. We hypothesize that Berberine will suppress these markers of inflammation in humans.

Berberine inhibits carcinogenesis

There is increasing evidence showing that Berberine inhibits inflammation-triggered carcinogenesis[24, 26]. In murine experiments, Berberine was found to significantly inhibit carcinogenesis induced by 20-methylcholanthrene or N-nitrosodiethylamine (NDEA) in a dose-dependent manner[2]. In addition, it was reported that Berberine prevents the appearance of malignant morphology and ultrastructural changes of AOM-induced rat colon cancer[81]. These studies demonstrate that Berberine protects against chemical carcinogenesis. Recent studies further demonstrate that Berberine-induced anticancer effects are strongly correlated with anti-inflammatory properties through cross talk between different targeted signaling pathways, such as the NF-κB pathway for anti-inflammatory effects and caspase-dependent pathways for apoptosis[54, 69]. Specifically, it is reported that Berberine suppresses the NF-κB pathway and inhibits COX-2 transcriptional activity effectively in a dose-dependent and time-dependent manner in colon cancer cells[23, 78, 92, 93]. By modulating the abnormal signaling pathways in cancer cells[11, 23, 60, 78, 79], Berberine blocked the proliferation of and induced death in multiple cancer cell types in vitro and in vivo[2, 7, 22, 27, 32, 36, 45, 91]. Chemoprevention of CRC is already possible with celecoxib, although it is still not the ultimate drug of choice, especially because of the cardiovascular risk

associated with COX-2 inhibitors[5]. With cardiovascular system protective effects[50, 56], we hypothesize that Berberine could be developed into an effective chemopreventive agent for CRC.

Primary Objective:

Objective: To determine the safety of Berberine administered to participants with UC in clinical remission while receiving maintenance therapy with Mesalamine.

Hypothesis: Berberine will be well tolerated by UC subjects in clinical remission.

Techniques for Endpoint Evaluation: Toxicity will be measured at study visits using the NCI criteria and recorded in case report forms. In addition to assessing clinical toxicity, we will collect tissue and blood samples from recruited participants at the beginning of the trial and at the end of Berberine administration (three months later) and evaluate organ toxicity by standard clinical chemistry tests. Finally, we will observe and record symptoms such as fever, fatigue, weight loss, appetite, stool frequency, bloody stool and other upper and lower gastrointestinal tract symptoms in participants.

Secondary Objectives:

Objectives:
- Determine clinical efficacy by measuring UC related symptoms.
- Determine the molecular efficacy of Berberine by examining blood- and tissue-based biomarkers of inflammation, apoptosis and DNA methylation.
- Examine the severity of inflammation using histologic analyses.
- Determine plasma concentration of Berberine.

Hypothesis: Berberine will inhibit inflammation pathways (as evidenced by the suppression of pro-inflammatory markers) and decrease ulcerative colitis, making it a potentially effective chemopreventive agent for CRC.

Techniques for Endpoint Evaluation:
- *Clinical efficacy*: Clinical efficacy will be measured using the UCDAI score[13, 74]. UCDAI assessment is a well-recognized standard assessment that can be reproducibly performed by endoscopists at Xijing Hospital.

- *Measures of inflammation:*
    - *Blood-based measures of inflammation:* Biomarker analysis will be conducted using commercially available ELISA kits for TNFα, IL-4, IL-6, IL-8 and IL-10 KIT (R&D Systems, Minneapolis, MN) according to the manufacturers' specifications. Other plasma-based measures of inflammation, including the blood CRP level and ESR will also be assessed.

    - *Tissue-based measures of inflammation and anti-cancer action*: Inflammation-related biomarkers (TNFα, COX-2, and NF-κB) and anti-cancer action biomarkers (Ki67 and activated capsase-3) in colorectal biopsy tissue will be evaluated by IHC. DNA methylation on SFRP1, TCERGIL FBN2, and TFP12 using qMSP strategy will also be performed.

        - *Histological analysis of inflammation*: Patients in clinical remission from UC still frequently have histologic features of inflammation, which correlate with endoscopic appearance[72]. We will utilize the same procedures for tissue acquisition, fixation and pathologic examination as described previously[72]. Six of the seven colon biopsies will be embedded for histology and immunohistochemistry. Histologic sections will be stained with H&E and the severity of histologic inflammation will be evaluated using the Geboes grading system[25, 72].

        - *Immunuhistochemical analysis of inflammation*: Six of the seven colon biopsies will be embedded for histology and IHC. IHC has the advantage of allowing identification of the cells being probed but has the disadvantage of being semi-quantitative with regards to intensity and expression. Colonic epithelial apoptosis and cell proliferation will be determined by cleaved caspase-3 and

Ki-67, respectively. Caspase-3 and Ki-67 will be assessed using IHC since the percentage of positive cells is the gold standard for reporting these results. Cleaved caspase-3 and Ki-67 are robust, well-validated measures of these parameters. TNFα, COX-2, and NF-κB will also be assessed by IHC. For this, we will use computer-aided quantitation to improve the robustness of expression analysis.

- *DNA methylation:* One of the seven colon biopsies will be used for DNA methylation analysis. DNA methylation has been reported to be correlated with the development of colitis-associated cancer. It has been reported that the methylation status of selective genes (SFRP1, TCERG1L, FBN2 and TFPI2) could be used as a risk marker for colon cancer in UC patients[49]. Methylation analysis will be performed using the MSP strategy, as previously described[29]. Changes will be compared with qMSP. The current study provides an opportunity to validate these associations, potentially leading to a means to identify high risk individuals with UC for progression towards colon cancer.

- *Blood Berberine concentration measurement*: Berberine concentrations with be measured using high-performance liquid chromatography /mass spectrometry (HPLC/MS).

- *Blood Count and Blood Chemistry:* Blood will also undergo standard clinical testing for complete blood count and blood chemistry to evaluate for end organ function.

Target Population

The goal of this Phase I trial is to determine the safety of Berberine, in subjects with UC in clinical remission and maintained with Mesalamine, therefore, the target population would be patients with UC in clinical remission, regardless how long they were diagnosed for UC. Entry criteria will consists of patients (age 18 – 70) with UC in clinical remission (UCDAI, Appendix A) ≤ 1 for ≥ 3 months) who are receiving maintenance therapy with Mesalamine. Participants must not have had any immunomodulatory treatment or any investigational agents within the past 3 month or take medicines that are inducers, inhibitors or substrates of select CYP isozymes (see Appendix B) within the past 3 months.

Why this trial is important

Prevention of CRC by administration of chemopreventive agents is one of the most promising options for decreasing CRC mortality, and especially so for high risk UC patients. Successful implementation of a chemopreventive strategy depends not only on accurate identification of high-risk populations, but also on the development of safe and effective drugs suitable for use in those specific populations. Despite this need, effective agents are lacking, and the field is searching for novel acting candidates. Berberine has a long history of use for anti-inflammatory ailments, lacks significant side effects, and has efficacy in relevant models of colitis and colorectal carcinogenesis, and as such it constitutes a novel agent with high potential as a chemoprevention drug for UC patients. Further, oral Berberine achieves high accumulated concentration to the target organ (i.e., the colonic lumen), while sparing systemic organs (and the associated toxicity), thus providing a highly desirable scenario for a chemopreventive agent. As such, Berberine also has potential use as a CRC chemopreventive agent in the general population. Although the body of preclinical in vitro and in vivo evidence is compelling for efficacy in UC, no controlled clinical trials have been carried out in human patients affected by UC. As the chemoprevention effects of Berberine currently remain preclinical, it is necessary to carry out such trial.

Why this study is being implemented in China

We have extensively discussed our plan to implement this study in China throughout the leadership of the Division Cancer Prevention (DCP), inclusive of its Director, Dr. Barry Kramer, and there is thematic support. Inclusion of China was highlighted by Dr. Kramer as important for the field of cancer prevention (AACR Frontiers of Cancer Prevention 2013 Special Lecture, "NCI Division of Cancer Prevention: Where we are and where we are headed"). Aside from thematic alignment with the stipulated goals of DCP, there are several reasons specific to this proposal. Berberine has been extensively used in China. Thus, Chinese physicians are intimately familiar with its use. A key related point is that its safety profile is well understood in the Chinese cohort. As the safety of chemopreventive agents is paramount, our proposed development plan provides an optimal pathway to evaluate agent efficacy, while optimizing patient safety. The scientific rational for this proposal was generated by the very people who will be conducting it. Alignment of those who generate an idea and preliminary supportive data with those who conduct a clinical trial has always been deemed optimal, and is universally recognized as such by review panels, and the general scientific community. Xijing Hospital of Digestive

Diseases in Xi'an constitutes an optimal clinical site. This hospital is completely dedicated to diseases of the gastrointestinal tract. In China, it has been continuously ranked as No 1 in the past 5 years in the gastrointestinal diseases field. It is world renowned for its expertise and scientific advances. Its Director, Dr. Daiming Fan is Vice President of the Chinese Academy of Engineering. The Principal Investigator of this study, Dr. Kaichun Wu is a physician (gastroenterologist) and scientist, whose research focuses upon UC. Further, he is Chairman of the Department of Gastroenterology, Xijing Hospital, and is fully committed to providing the resources necessary to successfully conduct this trial. There is a large and established cohort of UC patients within the Department of Gastroenterology, Xijing Hospital. Xijing Hospital is part of the Northwestern University Early Phase Chemoprevention Trials consortium, was part of the competitive renewal application, and was well received by the review panel. Dr. Raymond Bergan, former consortium PI, has extensive knowledge of Xijing Hospital, including its operations and capabilities. He is a Visiting Professor there, and co-operates a basic research laboratory there. Dr. Wu and Xijing Hospital's Department of Gastroenterology are committing high levels of resources to support this study. Thus, for a minimal cost to DCP, this study and its associated scientific advances can be realized. Further, the advances achieved in this study will serve to benefit people in the US. This is because findings from this study will serve to inform us about our ability to therapeutically manipulate colorectal carcinogenesis through a novel therapeutic approach. Positive findings can then be translated into the US population (a proportion of which are Chinese) from a scientifically informed standpoint.

There are cautionary aspects to implementing a study in China. We have extensively discussed these with DCP Officers. Following, we list cautionary aspects and how they will be mitigated:

- Sending U.S. NIH dollars to China under the current fiscal environment is not viewed as highly favorable. Xijing Hospital of Digestive Diseases is providing financial support for all China-based costs. No U.S. dollars will be sent to China.

- China is far away, making trial monitoring difficult, and this site has no experience in conducting chemoprevention trials. We are highly respectful of these facts and their implications. Therefore, the plan is to implement a very small pilot trial. A main goal is to demonstrate the feasibility of implementing a DCP study at this site. The Northwestern consortium and Dr. Bergan in particular, has an established track record of successfully working with sites and investigators who are new to the field of cancer chemoprevention. As such, Dr. Bergan has successfully brought new investigators into the field, which is widely deemed as critically important for the field. He now aims to do so for China, similarly deemed as important. Extensive monitoring of the site will be conducted. It will be monitored on site four times per year by Dr. Bergan (two times he will be accompanied by a Northwestern-based study monitor). Dr. Bergan has extensive interactions with China, and Dr. Lifang Hou (consortium co-PI) was trained as a physician in China, frequently travels there, has conducted several trials in China, and has successfully shipped clinical samples from China to the US. Finally, tissue endpoint biomarkers will be analyzed in China, and will then undergo complete re-analysis in the US by a GI pathologist, Dr. Guang-Yu Yang, thus providing rigorous quality control (QC). Dr. Yang is a US trained and practicing physician/scientist, whose research focuses upon colon carcinogenesis and its modulation by chemoprevention agents. Further, as a native born Chinese, he is well poised to ensure communication and QC in the realm of tissue processing.

- A minor issue was raised about the currently unpopular nature of investigating natural products for cancer prevention, and the fact that Berberine is a natural product. In this regard, it is important to consider that many non-natural products have also failed in cancer chemoprevention trials. Further, the majority of all FDA approved small molecule drugs are in fact natural products or directly inspired by them[105]. Specifically, for FDA approved small molecule drugs for cancer between 1/1981 and 12/2010, eighty percent were natural products or directly inspired by them.The fact is there is a large body of preliminary data related to Berberine to support its investigation for colorectal cancer, and there is a need for novel agents for exactly this purpose. Finally, a major asset of China is its rich source of bioactive agents. Such natural products have transformed the treatment of such unlikely diseases as acute promyelocytic anemia, where the Chinese natural product arsenic trioxide is now in widespread clinical use in the US[75].

3. SUMMARY OF STUDY PLAN

This is a single center, double-blind, randomized, placebo-controlled, pilot study. The goal of this Phase I trial is to determine the safety of Berberine in subjects with UC in clinical remission and maintained with Mesalamine. Pilot information about Berberine's ability to modulate relevant biomarkers at the dose being assessed will also be gathered.

Subjects will be randomized in a double-blind fashion to 300 mg TID Berberine (12 subjects) versus placebo (4 subjects), for 3 months. Two additional participants are anticipated to be accrued (for a total of 18 subjects) to account for an anticipated dropout rate of 10%. In order to keep the numbers in either treatment group as planned, dropouts in either treatment group will be replaced by subjects assigned to that same treatment and gender group. Assuming an accrual rate of approximately 1.5 per month, we expect the study to be completed in 12 months.

Subjects will be enrolled and followed up at Department of Gastroenterology, Xijing Hospital, Xi'an, China. For those subjects experiencing grade 2 toxicity felt to be due to Berberine, the dosage will be successively decreased by 100 mg decrements per dose until resolution (note, given TID dosing, this translates to 300 mg/day decrements). Those not tolerating 300 mg/day, will be removed from study, as will those experiencing grade 3 or 4 toxicity at any dose. Compliance will be assessed by pill counts and diary reviews at monthly clinical visits.and by measuring plasma Berberine concentrations at the end of the trial. Subjects whose compliance is below 80% will be removed from study.

Colorectal biopsies and blood samples will be taken at the initial visit (pre-treatment). At the end of the 3-month treatment, subjects will repeat earlier screening assessments to determine the outcomes of the primary and secondary objectives. One month post study, subjects will be contacted by clinical visit to assess symptoms.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Patients with ulcerative colitis in clinical remission (UCDAI, Appendix A) ≤ 1 for at least 3 months), regardless of how long ago they were diagnosed for UC.

4.1.2 Receiving maintenance therapy with Mesalamine for at least 3 months.

4.1.3 Age 18 - 70 years. Because no dosing or adverse event data are currently available on the use of Berberine in participants <18 years of age, children are excluded from this study but will be eligible for future pediatric trials, if applicable.

4.1.4 ECOG performance status ≤1 (Karnofsky ≥70%; see Appendix C).

4.1.5 Participants must have normal organ and marrow function as defined below:

Leukocytes	≥3,000/microliter
Absolute neutrophil count	≥1,500/microliter
Platelets	≥100,000/microliter
Total bilirubin	within normal institutional limits. Higher values (≤ 3 x institutional upper limit of normal (ULN)) are acceptable in participants with: 1. known or suspected cholangitis associated with Crohn's disease, or 2, known or suspected inborn errors of metabolism that lead to increased bilirubin.
AST (SGOT)/ALT (SGPT)	≤1.5 × institutional ULN
Creatinine	within normal institutional limits

4.1.6 Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation, due to unknown, but potential risk of Berberine causing uterine contractions and miscarriage[37]. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.1.7 Ability to read, understand, and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Participants who have had any immunomodulatory treatment in the past 3 months will be excluded.

4.2.2 Participants who have taken any medicines that are inducers, inhibitors or substrates of select CYP isozymes (see Appendix B) within the past 3 months will be excluded. Participants who have consumed either grapefruit juice or Seville orange juice in the past 7 days will be excluded.

4.2.3 Participants with Dysplasia-associated mass or lesion (DALM) due to longstanding idiopathic inflammatory bowel disease will be excluded.

4.2.4 Participants who are currently receiving any other investigational agents or have received investigational agents within the past 3 months will be excluded.

4.2.5 Participants with a history of allergic reactions attributed to compounds of similar chemical or biologic composition to Berberine will be excluded.

4.2.6 Patients with uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that in the opinion of investigators would jeopardize patient safety of data integrity are excluded. Individuals who are HIV positive will not necessarily be excluded, will be considered on a case-by-case basis, but will be required to meet criteria related to patient safety and data integrity, as assessed by investigators.

4.2.7 Pregnant women are excluded from this study because Berberine has a potential for causing uterine contractions and miscarriage[37] because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Berberine, breastfeeding should be discontinued if the mother is treated with Berberine. Women are considered to be of child-bearing potential if they are not surgically sterile or under the age 65 and have menstruated within the last two years.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.4 Recruitment and Retention Plan

Xijing Hospital of Digestive Diseases is solely dedicated to diseases of the gastrointestinal system. Annually, the hospital serves over 150,000 outpatients and over 6000 inpatients, performs over 20,000 endoscopic examinations with over 6000 being colonoscopies. Xijing Hospital of Digestive Diseases has established track records for Inflammatory Bowel Disease patients who are being followed-up. Thus potentially eligible subjects can be identified and contacted prior to the procedure. In addition, advertisements will be posted at Xijing Hospital of Digestive Diseases with study contact information. Adequate minority participation will be ensured with our patient population.

Once identified, eligible patients will be contacted by the study coordinator who will explain the details of the study and the requirements for participation. Patients who are interested in the trial will be given a copy of the IRB approved consent form. After the patient signs the consent form, the patient will then be monitored by the research coordinator. The coordinator will be the main contact for patients in accordance with the protocol to ensure pleasant clinic visits. Participant retention will be maximized by frequent patient contact. During each telephone contact and/or clinical visit, patients will be assessed for adverse events and counseled on AE management and the use of rescue medication. Compliance will be assessed by pill counts and diary reviews at monthly clinical visit, and by measuring plasma Berberine concentration at the end of the trial.

Northwestern University will work closely with Xijing Hospital of Digestive Diseases on an ongoing basis to monitor recruitment and adjust or employ new strategies as needed to maximize patient recruitment with an emphasis on ensuring a balanced representation of women and men. This assistance will include coordinating recruitment material design and placement.

Participants will not be reimbursed for participation, but all of their clinical test cost will be covered, including the Mesalamine that will be taken during the trial periods.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. The initial treatment will be 300 mg TID; if this is not tolerated, the dosage will be decreased by 100 mg/dose (300mg/day) until a tolerable dosage is reached. Reported adverse events (AEs) and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

16 participants will be randomized into one of two groups: 12 participants will receive 300 mg Berberine three times per day; 4 participants will receive placebo tablets three times per day. Two additional participants (for a total of 18 participants) are anticipated to be enrolled to account for an anticipated dropout rate of 10%. In order to keep the numbers in either treatment group as planned (3:1), dropouts in either treatment group will be replaced by subjects assigned to that same treatment and gender group. Dosing will extend for 90 days.

5.2 Berberine Administration

Participants will take 300 mg three times per day (3 x 100 mg tablets, three times a day) of Berberine for a period of 90 days. Participants should take tablets within one hour after a meal with water every 6-8 hours. Patients will be asked to return to the clinic within 6 days of the initial screening visit to pick up the study medication. If patients are unavailable to return to the clinic within this timeframe to pick up the study medication, the study medication may be shipped to the participant's home. Three (3) bottles (each containing 120 tablets) will be distributed initially and 3 bottles will be distributed at the Day 30 and Day 60 visits. An additional bottle may be dispensed if needed.

5.3 Run-in Procedures

N/A

5.4 Contraindications

None, other than pregnancy or unprotected intercourse for females of childbearing potential as Berberine has a potential for causing uterine contractions and miscarriage.

5.5 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included. Participants must not take any medicines that are inducers, inhibitors or substrates of select CYP isozymes (see Appendix B) within the past 3 months. Participants must not take over-the-counter berberine during the trial.

5.6 Dose Modification

The initial treatment will be 300 mg TID. Toxicity will be graded according to the NCI CTCAE version 4.0. For those subjects experiencing grade 2 toxicity felt to be due to Berberine, dosage will be successively decreased by 100 mg decrements per dose (i.e. take fewer pills) (note, given TID dosing, this translates to 300 mg/day decrements). If after one week the participant is still experiencing a grade 2 toxicity related to Berberine with the reduced dose, the dose will continue to be decreased (at one week intervals) until a tolerable dosage is reached (< grade 2 toxicity related to Berberine). Those not tolerating 300 mg/day, will be removed from study, as will those experiencing grade 3 or 4 toxicity (related to Berberine) at any dose. The dose will not be 'interrupted' other than stopping the drug to go off the study.

5.7 Adherence/Compliance

5.7.1 If less than 80% of the dose has been consumed during the study period, the subject will be considered non-compliant. All participants that receive a study agent for any period of time will be evaluable for efficacy and toxicity. Participants will not be replaced.

5.7.2 Clinical visits will be made each month to monitor compliance. Pill counts will be performed and patient diary will be reviewed at each clinical visit. Drug levels will be measured in blood on the last day of administration.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agent

Berberine ($C_{20}H_{18}NO_4$, molecular weight 353.36 Da), a natural plant alkaloid, is found in the roots, rhizomes, and stem bark of many plants and has been widely used to treat gastroenteritis and diarrhea in China [88, 97]. There is increasing evidence that it inhibits carcinogenesis primarily through anti-inflammatory action [53]. For this study, Berberine isolated from the *Coptidis* rhizome is supplied by Shanghai SINE TianPing Pharmaceutical Co. (Shanghai, China).

The investigational product to be used in the proposed clinical investigation is a film-coated tablet with a yellow core delivering 100 mg Berberine. Tablets allocated for this study met the requirements and standards from the Pharmacopoeia of the People's Republic of China (2010), are >98% pure, and have an expiration date of May 10, 2017 (Shanghai SINE TianPing Pharmaceutical Co. Ltd. Berberine Hydrochloride Testing Report, December 2, 2013). Additional supply with a later expiry date will be made available if the intervention phase of the study has not completed by that time. Matching placebo tablets will also be supplied by Shanghai SINE TianPing Pharmaceutical Co. Ltd.

6.2 Reported Adverse Events and Potential Risks

Berberine is available as a dietary supplement at doses of 100 to 200 mg/day in the US and has been approved by the Chinese Food and Drug Administration (CFDA) for diarrhea at a dose of 300 mg tid. Predominant clinical uses of Berberine include treatment of bacterial diarrhea, intestinal parasite infections, and ocular trachoma infections [68]. Additionally, Berberine has been investigated for treatment of type 2 diabetes, cardiovascular disease, hyperlipidemia, and cancer. In clinical studies for these indications, oral doses as high as 1500 mg Berberine/day for three months were administered[51, 87, 97, 100]. No severe adverse effects and no significant changes in plasma kidney and liver parameters were observed in any of the studies. Mild to moderate constipation was seen in 3−8% of subjects. In a study using 300 mg Berberine TID for 30 days in patients with acute coronary syndrome, three of the 61 patients discontinued treatment due to abdominal pain, rash, or constipation[66].

As most Berberine-containing plants are considered uterine stimulants, they should be used with care during pregnancy[6].

Berberine has not been shown to be genotoxic and does not induce significant cytotoxic, mutagenic, or recombinogenic effects[6]. The intravenous lethal dose for 50% (median) of test animals (LD_{50}) of Berberine sulfate in mice is approximately 25 mg/kg, while intravenous administration of Berberine to dogs at doses up to 45 mg/kg does not produce gross toxic effects.

6.3 Availability

Berberine and matching placebo will be manufactured and supplied by Shanghai SINE TianPing Pharmaceutical Co., Shanghai, China. Berberine and matching placebo will be packaged in bottles containing 120 tablets.

6.4 Agent Distribution

Shanghai SINE TianPing Pharmaceutical Co. will manufacture the Berberine tablets and matching placebo tables and supply them to the clinical site (Department of Gastroenterology, Xijing Hospital, Xi'an, China).

Xinyan Jiang, at Shanghai SINE TianPing Pharmaceutical Co, will be contacted for ordering (E-mail: cz_xn@aliyun.com, cell phone: +8618019168035).

6.5 Agent Accountability

The Protocol Lead Investigator, or a responsible party designated by the Protocol Lead Investigator, must maintain a careful record of the inventory and disposition of all agents received from Shanghai SINE TianPing Pharmaceutical Co. using the NCI Drug Accountability Record Form (DARF). The Protocol Lead Investigator is required to maintain adequate records of receipt, dispensing, and final disposition of study agent. This responsibility has been delegated to Department of Gastroenterology, Xijing Hospital. The research pharmacist at Xijing Hospital is Ms. Ying Song, who can be reached at 251653125@qq.com, phone: +86-15339072373.

Include on the receipt record from whom the study agent was received and to whom it was shipped, date, quantity, and batch or lot number. On the dispensing record, note quantities of and dates when study agent was dispensed to and returned by each participant.

6.6 Packaging and Labeling

Berberine and placebo tablets will be packaged, labeled and distributed by Shanghai SINE TianPing Pharmaceutical Co. Ltd, Shanghai, China. Tablets are packaged in 120-count bottles.

Each bottle will be labeled with a one-part label identifying study specific information, such as study title, DCP protocol number, dosing instructions, recommended storage conditions, the name and address of the distributor, randomization number, and a caution statement indicating that the agent is limited by China law to investigational use only and the agent should be kept out of reach of children.

6.7 Storage

The Berberine tablets that will be used in the study are stable when stored at room temperature (15 to 25 ºC) in an air-conditioned space for until dispensed to subjects. Once dispensed, the subjects will be instructed to store the drug in their homes protected from light, heat and moisture. If the results of stability tests at any time points are getting close to the limits of specification, we will contact Shanghai SINE TianPing Pharmaceutical Co. Ltd, to prepare a new batch for the clinical trial.

6.8 Registration/Randomization

A study coordinator must forward to Northwestern via the Northwestern Oncology Trials Information System (NOTIS), a signed and complete informed consent and a completed eligibility checklist for each participant identified as eligible to be entered into the study.

All participants must be registered with the Northwestern University Clinical Research Office within 24 hours of enrollment.

After registration, participants will be randomized by the Quality Assurance Monitors at Northwestern Lurie Cancer Center to either treatment with Berberine or placebo. The purpose of the placebo assignment is to allow evaluation of the perception of subtle, sub-clinical effects that might be associated with trial participation. Investigators and participants will be blinded as to the result of randomization. The following people will be un-blinded: The study statistician, the Lurie Cancer Center Quality Assurance Monitors, and the Investigational pharmacist at Xijing Hospital. The study statistician will set up randomization blocks, as previously described.

Full unblinded randomization information will be faxed to Xijing Hospital research pharmacist at: 86-298-253-9041. The clinical research coordinator will receive a blinded Participant ID code for the patient via fax. Participants must not start protocol treatment prior to randomization with Northwestern.

6.9 Blinding and Unblinding Methods

Study participants will receive a prescription, blinded, from the investigational pharmacy at Xijing Hospital. Unblinding will only occur when it is deemed medically necessary and will only take place after consultation with the NCI, DCP Medical Monitor:

DCP Medical /Scientific Monitor
Luz Maria Rodriguez, MD, FACS
NCI/Division of Cancer Prevention
9609 Medical Center Dr
Rm 5E-228
Telephone (240) 276-7039
Fax (240) 276-7848
Email: rodrigul@mail.nih.gov

If the medical monitor is unavailable or if after hours consultation is needed, please contact Dr. Seema Khan, at telephone: 312-503-4236, or cell: 312-307-3646.

6.10 Agent Destruction/Disposal

At the completion of the investigation, all returned or unused study agent will be disposed of according to institution-specific policy and procedures for handling investigational agents. Both Xijing hospital and Northwestern University will maintain a record of what is destroyed including name of agent, quantity, lot number and expiration date. All records will be maintained for 5 years from when the study is completed.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Evaluation/ Procedure	Screening	Randomization	Day 0*	Day 7 (+/- 3 days)	Day 30 (+/- 3 days)	Day 60 (+/- 7 days)	Day 90 (- 7 days)	Day 120 (+/- 7 days) or Early Termination
Informed Consent	X							
Assess Eligibility	X	X						
Medical History	X							
Physical Exam	X				X	X	X	X
Vital Signs, Height and Weight[1]	X				X	X	X	X
Baseline Symptoms	X							
UCDAI Questionnaire	X						X	
Laboratory Tests[2]	X						X	
Rectal Biopsy[3]	X						X	
Research Blood Collection[4]	X						X	
Concomitant Medications	X		X	X	X	X	X	X
Dispense Study Agent		X			X	X		
Pill Count				X	X	X	X	
Review Agent Diary/Record				X	X	X	X	
Adverse Events			X	X	X	X	X	X
Telephone Contact[5]			X	X				
Collect Study Agent							X	

1. Vital signs assessment is to include pulse, blood pressure, respiration rate, temperature and weight. Baseline assessment must also include height.
2. CBC, ESR, CRP, and Chemistry Panel (White blood count, RBC count, Hemoglobin, Hct, Platelets, Absolute Neutrophil Count, Glucose, Creatinine, Sodium, Potassium, Chloride, SGOT, SGPT, Total Bilirubin, INR). Baseline assessment must include a serum pregnancy test for all women of childbearing potential. Pregnancy test will only be performed at the screening visit.
3. Colonoscopy with 7 biopsies from inflammatory loci (or from regions close to the initial inflammatory area when the patient was first diagnosed). Biopsies taken on day 90 should be ≥5 cm distance from the original biopsy sites.
4. Research blood collection: 5ml into purple EDTA tube for plasma and 4ml into red top tube for cytokines.
5. For Day 0, either a telephone contact or an office visit will occur.

* Participant should begin taking the study medication within 7 days of the screening visit

7.2 Baseline Testing/Prestudy Evaluation

Screening

- Informed Consent – written informed consent must be obtained prior to any other study procedure. Participants' signature is required on the research informed consent (in addition to the procedure consent) prior to undergoing the screening colonoscopy.
- Participants will be told not to take Berberine for 3 days prior to the screening biopsy.
- Medical History and Baseline Symptom Review.
- Concomitant Medication Review – all medications that the participant has taken within the 3 months prior to consent are to be recorded.
- Physical Exam and Vital Signs assessment – vital signs assessment is to include pulse, blood pressure, respiration rate, temperature and weight. Baseline assessment must also include height.
- UCDAI questionnaire to help assess disease state.
- Inclusion / Exclusion Criteria Review.
- Clinical Lab Tests (27-31 ml blood) CBC, ESR, CRP and Chemistry Panel (White blood count, RBC count, Hemoglobin, Hct, Platelets, Absolute Neutrophil Count, Glucose, Creatinine, Sodium, Potassium, Chloride, SGOT, SGPT, Total Bilirubin). Baseline assessment must include an INR for all patients and a serum pregnancy test for all women of childbearing potential. Women of childbearing potential include those who have not been surgically sterilized and have had a period in the past year. Screening labs must be completed within two weeks of registration.
- Research blood will be collected: Peripheral blood for cytokines analysis (4ml in red top tube) and plasma for Berberine concentration analysis (5ml in purple EDTA tube).
- Colonoscopy with 7 biopsies from inflammatory loci (or from regions close to the initial inflammatory area when the patient was first diagnosed). Each biopsy site will be recorded by denoting the length in centimeters from the anal verge to the biopsy site as well as recording position of the participant during time of biopsy (e.g., lying on their left side).
- Register the patient with Northwestern University Clinical Research Office.
- Provide agent diary (Appendix D) and review it with participant.

Randomization

- Confirm that the patient meets all inclusion/exclusion criteria.
- Randomize the patient with Northwestern University Clinical Research Office.
- Participant will be asked to return to the clinic within 6 days of the screening visit to pick up the study medication. Three (3) bottles 100mg x 120# Berberine tablets/placebo for use as medication will be dispensed.
- If participant is unable to return to the clinic within 6 days, the study medication will be shipped to their home.

7.3 Evaluation During Study Intervention

Day 0

- Telephone contact or office visit with participant with instructions to begin study agent and diary completion.

- Participants should begin taking the study medication within 7 days of the screening visit.
- Assess Adverse Events
- Assess Concomitant Medications

Day 7 (+/- 3 days)

- Telephone contact with participant
- Assess study drug compliance
- Assess adverse events and discuss patient diary
- Assess Concomitant Medications

Day 30 (+/- 3 days)

- Physical Exam and Vital Signs assessment – vital signs assessment is to include pulse, blood pressure, respiration rate, temperature and weight.
- Assess study drug compliance
- Assess adverse events and discuss patient diary
- Assess Concomitant Medications
- Dispense 3 bottles 100mg x 120# Berberine tablets/placebo for use as medication.

Day 60 (+/- 7 days)

- Physical Exam and Vital Signs assessment – vital signs assessment is to include pulse, blood pressure, respiration rate, temperature and weight.
- Assess study drug compliance
- Assess adverse events and discuss patient diary
- Assess Concomitant Medications
- Dispense 3 bottles 100mg x 120# Berberine tablets/placebo for use as medication.

7.4 Evaluation at Completion of Study Intervention

Participants are to continue taking the study agent for the 90 day study period. All 90-day tests and procedures should take place as close to day 90 as possible, but may occur within 7 days of day 90 (from day 83-90) in order to accommodate participant conflicts and busy schedules.

Day 90 /-7 days)

- Concomitant Medication Review
- Physical Exam and Vital Signs assessment – vital signs assessment is to include pulse, blood pressure, respiration rate, temperature and weight.
- UCDAI questionnaire to help assess disease state.
- Clinical Lab Tests (12-16 ml blood) – CBC, ESR, CRP, and Chemistry Panel (White blood count, RBC count, Hemoglobin, Hct, Platelets, Absolute Neutrophil Count, Glucose, Creatinine, Sodium, Potassium, Chloride, SGOT, SGPT, Total Bilirubin).
- Research blood will be collected: Peripheral blood for cytokines analysis (4ml in red top tube) and plasma for Berberine concentration analysis (5ml in purple EDTA tube).
- Colonoscopy with 7 biopsies from same region as, but ⩾5 cm from, original biopsies taken at screening visit. If scars are visible from the site of screening biopies, then new biopsies will be taken >/= 5 cm from this site. If they are not visible, then biopsies will be taken >/= 5 cm from the prior recorded distance (with the participant in the same position as they were in for original biopsies).
- Assess adverse events and review patient diary
- Collect and count study medication from patient

7.5 Post-intervention Follow-up Period

Participants will be assessed 30 days after their last dose of the study agent for assessment of adverse events (participants who experienced adverse events will be followed for 30days after their last dose or until all side effects have been resolved, whichever occurs later). For participants who completed the study this visit would occur on day 120.

Day 120 (+/-7 days)

- Physical Exam and Vital Signs assessment – vital signs assessment is to include pulse, blood pressure, respiration rate, temperature and weight
- Assess adverse events and discuss patient diary
- Assess Concomitant Medications

7.6 Methods for Clinical Procedures

Colonoscopies: The initial colonoscopy will be standard procedure. The colonoscopy at the end of the trial will be research colonoscopy. At the scheduled day, the patients will come to the endoscopy unit fasting after midnight. On the day prior to the exam, the patient will begin a clear liquid diet starting at lunch. The patient will then undergo a standard colonoscopy preparation with PEG. This prep is indicated for standard "GI lavage" and contains PEG-4000, sodium chloride, sodium bicarbonate and potassium chloride for oral solution. After informed consent, the participants will be placed on their left side and a rectal exam will be performed. Under conscious sedation, an Olympus video colonoscope will be inserted into the rectum and with minimal insufflation of air advanced to the cecum. At the beginning of the trial, 7 biopsies from inflammatory loci will be collected, if applicable. If no such loci are found, 7 biopsies close to initial inflammatory area (when the patients were first diagnosed) will be collected. The area of biopsy collection will be recorded by denoting the length in centimeters from the anal verge to the biopsy site as well as recording position of the participant during time of biopsy. At the end of trial (3 months later), 7 biopsies close to, but ≥ 5 cm away from, this area will be collected. Six of these will be fixed in formalin and one will be flash frozen in liquid nitrogen. Specimen collection kits will be provided by the pathology department at Xijing Hospital. The study coordinator will arrange to have the biopsies processed for H& E sections by submitting to the study pathologists.

Research blood for drug concentration: 5ml blood will be collected (in purple-top EDTA tube) pre-dose (at the screening visit) and at 3 months after the start of dosing (at the Day 90 visit) for plasma drug concentration measurement. The clinical laboratory of Xijing hospital will provide specimen collection kits with all sample collection, labeling and packaging information. The research blood will be processed in the clinical laboratory of Xijing hospital. All samples will be logged in the lab logbook with the times of arrival to the lab. Plasma samples will be separated immediately by centrifugation at 4000 rpm for 3 min and stored at −80 ˚C until analysis.

Research blood for cytokine levels: 4 ml of research blood (in red-top tube) will be collected at the pre-study screening visit and at the 3 month visit for cytokines level measurement. The clinical laboratory of Xijing hospital will provide specimen collection kits with all sample collection, labeling and packaging information. The research blood will be processed in the clinical laboratory of Xijing hospital. All samples will be logged in the lab logbook with the times of arrival to the lab. The blood samples will be spun down at 2500RPM for 10 min immediately upon arrival. Serum will be aliquoted before further detection and stored at -20 ˚C until analysis.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

Safety will be measured at study visits using the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4 and recorded in case report forms. In addition to assessing clinical toxicity, we will collect tissue and blood samples from recruited participants at the beginning of the trial and at the end of Berberine administration (three months later) and evaluate organ toxicity by standard clinical chemistry tests. Finally, we will observe and record symptoms such as fever, fatigue, weight loss, appetite, stool frequency, bloody stool and other upper and lower gastrointestinal tract symptoms in participants.

8.2 Secondary Endpoints

Secondary endpoints will be evaluated in batch once the targeted number of study participants has completed the protocol.

- *Clinical efficacy*: Clinical efficacy will be measured using the UCDAI score[13, 74]. This will be done at the screening visit and at the end of the trial (90 day-7days) by an endoscopist.

- *Blood-based measures of inflammation:* Biomarker analysis will be conducted using commercially available enzyme-linked immunosorbent assay (ELISA) kits for TNFα, IL-4, IL-6, IL-8 and IL-10 KIT (R&D Systems, Minneapolis, MN) according to the manufacturers' specifications. Other plasma-based measures of inflammation, including the blood CRP level and ESR will also be assessed.

- *Tissue-based measures of inflammation and anti-cancer action*: Inflammation-related biomarkers (TNFα, COX-2, and NF-κB) and anti-cancer action biomarkers (Ki67 and activated capsase-3) in colorectal biopsy tissue will be evaluated by IHC and DNA methylation on SFRP1, TCERGIL FBN2, TFP12 using qMSP strategy.

- *Histological analysis of inflammation*: Histologic sections will be stained with H&E and the severity of histologic inflammation will be evaluated using the Geboes grading system[25, 72].

- *Blood Berberine concentration measurement*: Berberine concentrations with be measured using high-performance liquid chromatography /mass spectrometry (HPLC/MS).

- *Blood Count and Blood Chemistry:* Blood will also undergo standard clinical testing for complete blood count and blood chemistry to evaluate for end organ function.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, or medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. Subjects who stop treatment for toxicity reason will be followed until the issue is resolved or stabilized. If the drop-out rate is higher than expected, we will recruit more participants. Participants will not be replaced.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons:

- Adverse Event
- Death
- Disease Progression
- Lost to follow-up
- Other
- Participant Withdrawal
- Participant Refused Follow-up
- Physician Decision
- Protocol Defined Follow-up Completed
- Protocol Violation
- Study Complete
- Ineligible

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

A number of exploratory secondary endpoints will be assessed to guide future chemoprevention studies.
Clinical efficacy: Clinical efficacy will be measured using the UCDAI score[13, 74]. UCDAI assessment is a well-recognized standard assessment that can be reproducibly performed by endoscopists at Xijing Hospital.

Blood-based measures of inflammation: The conduct of early phase cancer trials provides the opportunity to evaluate systemic drug effect. Therapy-induced changes in systemic function will be expected to result from changes in cytokines expression. Pre- and post-treatment plasma will therefore be collected and stored for future batch analysis. Blood plasma cytokines changes will be measured using commercially available ELISA kits (for TNFα, IL-4, IL-6, IL-8 and IL-10), which is the most validated and widely used method for this purpose. Other plasma-based measures of inflammation, including the blood CRP level and ESR will also be assessed.

Histological analysis of inflammation: Patients in clinical remission from UC still frequently have histologic features of inflammation, which correlate with endoscopic appearance[72]. We will utilize the same procedures for tissue acquisition, fixation and pathologic examination as described previously[72]. Six of the seven colon biopsies will be embedded for histology and immunohistochemistry. Histologic sections will be stained with H&E and the severity of histologic inflammation will be evaluated using the Geboes grading system[25, 72].

Immunuhistochemical analysis of inflammation: Six of the seven colon biopsies will be embedded for histology and IHC. IHC has the advantage of allowing identification of the cells being probed but has the disadvantage of being semi-quantitative with regards to intensity and expression. Colonic epithelial apoptosis and cell proliferation will be determined by cleaved caspase-3 and Ki-67, respectively. Caspase-3 and Ki-67 will be assessed using IHC since the percentage of positive cells is the gold standard for reporting these results. Cleaved caspase-3 and Ki-67 are robust, well-validated measures of these parameters. TNFα, COX-2, and NF-κB will also be assessed by IHC. For this, we will use computer-aided quantitation to improve the robustness of expression analysis.

DNA methylation: One of the seven colon biopsies will be used for DNA methylation analysis. DNA methylation has been reported to be correlated with the development of colitis-associated cancer. It has been reported that the methylation status of selective genes (SFRP1, TCERG1L, FBN2 and TFPI2) could be used as a risk marker for colon cancer in UC patients[49]. Methylation analysis will be performed using the qMSP strategy, as previously described[29]. The current study provides an opportunity to validate these associations, potentially leading to a means to identify high risk individuals with UC for progression towards colon cancer.

Blood Berberine concentration measurement: Berberine concentrations in the research blood samples will be analyzed using high-performance liquid chromatography /mass spectrometry (HPLC/MS). The following factors should be used to assess assay performance in human plasma matrices: selectivity, linearity, precision, accuracy, recovery and stability, following the FDA's Guidance for Industry: Bioanalytical Method Validation [U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001.] The Phase I Clinical Trials Center of Xijing hospital has successfully utilized HPLC/MS method for plasma drug concentration in their previous trial.

Blood Count and Blood Chemistry: Blood will also undergo standard clinical testing for complete blood count and blood chemistry to evaluate for end organ function.

9.2 Comparable Methods

Proposed methods represent standard technology for chemoprevention trials in the colon.

10. SPECIMEN MANAGEMENT

10.1Laboratories

1. Dr. Aidong Wen's laboratory at Xijing Hospital will be responsible for measuring the plasma Berberine concentrations.

2. Clinical laboratory at Xijing Hospital will be responsible for the blood count and blood chemistry analyses as well as the ELISA assays.

3. Dr. Zengshan Li's laboratory will be responsible for the fixing, embedding, and sectioning of the biopsy samples and pathology review at Xijing Hospital. Tissue sections will be evaluated at both Pathologic Department of Xijing hospital and Pathology Core Facility (PCF) of Northwestern University.

4. Dr. Guang-Yu Yang's laboratory (PCF) will be responsible for the pathology review at Northwestern University. Dr. Li Xu will be responsible for shipping tissue sections to Dr. Yang at Pathology Core of Northwestern University, upon the approval of the Chinese government. Dr. Yang will be blinded to the nature of the sample and asked to review quantitation of expression by direct counts.

5. Dr. Kaichun Wu's laboratory will be responsible for the tissue sample DNA methylation analysis.

10.2 Collection and Handling Procedures

1. Blood for laboratory tests and research

a. ***Blood (for cytokine levels; 4 ml)***

i. Pre-visit – The night before the scheduled visit, the participant should fast after midnight.

ii. Collection - The clinical laboratory will provide specimen collection kits with all sample collection, labeling and packaging information. 4 ml of research blood will be collected in a red-top tubes for ELISA at the screening visit (before first dose) and at the 3 month visit. The participant should be fasting.

iii. Transportation - Blood samples will be transported to Xijing Hospital's Clinical Laboratory at room temperature. All samples will be logged in the lab logbook with the times of arrival to the lab.

iv. Labeling of specimen – Samples will be de-identified through established SOPs. All samples will be labeled with the patient's first initial of their last name followed by a number corresponding to their patient identification number assigned at the time of entry into the database (by research coordinator).

v. Tracking of specimens – The patient ID number will be used to link information on the database to clinical data.

vi. Processing - The blood samples will be spun down (2500 rpm for 10 min) and separated immediately upon arrival. Serum for cytokine detection will be stored at −20 ˚C until analysis.

vii. Archival - Unused serum samples will be stored at -20 ˚C for archival.

b. ***Blood (for Berberine Concentration; 5 ml)***

i. Pre-visit – The night before the scheduled visit, the participant should fast after midnight.

ii. Collection - The clinical laboratory will provide specimen collection kits with all sample collection, labeling and packaging information. 5 ml of research blood will be collected in a purple-top EDTA tube for drug concentration measurement at the screening visit (before first dose) and at the 3 month visit. The participant should be fasting.

iii. Labeling of specimen – Samples will be de-identified through established SOPs. All samples will be labeled with the patient's first initial of their last name followed by a number corresponding to

their patient identification number assigned at the time of entry into the database (by research coordinator).

iv. Tracking of specimens – The patient ID number will be used to link information on the database to clinical data.

v. Transportation - Blood samples will be transported to Dr. Zengshan Li's laboratory at room temperature.

vi. Processing - Plasma samples will be separated and spun down (4000 rpm for 3 min) immediately upon arrival. Samples will be aliquoted and stored at −80˚C until analysis.

vii. Archival – After analysis is complete, samples will be stored at −80˚C for archival.

2. Rectal Biopsies (7)

a. ***Colorectal Biopsy (for histology – IHC and H&E; 6 biopsies)***

i. Pre-visit – On the day prior to the exam, the patient will begin a clear liquid diet starting at lunch. On the scheduled day, the patients will come to the endoscopy unit fasting after midnight.

ii. Collection - The clinical laboratory will provide specimen collection kits with all sample collection, labeling and packaging information. 7 rectal biopsies will be collected. 6 of the biopsies will be placed in formalin immediately.

iii. Labeling of specimen – Samples will be de-identified through established SOPs. All samples will be labeled with the patient's first initial of their last name followed by a number corresponding to their patient identification number assigned at the time of entry into the database (by research coordinator).

iv. Tracking of specimens – The patient ID number will be used to link information on the database to clinical data. The number of biopsies for each container will be recorded for each procedure. The pathology readings and all research data regarding biopsies will be entered into this database.

v. Transportation - The biopsies in formalin will be transported (at room temperature) on the same day as the biopsy procedure to Dr. Zengshan Li's laboratory in the pathology department at Xijing Hospital for histology processing.

vi. Temperature storage requirements – Samples in formalin will be stored at room temperature until adequate fixation is achieved (i.e., overnight).

vii. Processing – After adequate fixation, tissues will be transferred through baths of progressively more concentrated ethanol to remove the water, and followed by a hydrophobic clearing agent (such as xylene) to remove the alcohol, and finally molten paraffin wax, the infiltration agent, which replaces the xylene. Tissues will then be embedded in paraffin wax. 5 um section cuts will be used for H&E and IHC.

viii. H&E Staining - For H&E staining, briefly, slides will be dried at 65℃ for 1-2 hours, pass through xylene to remove paraffin, and pass through baths of progressively less concentrated ethanol to remove xylene and rinsed in tap water to remove ethanol. Cellular nucleus will be stained with Hematoxylin for 10 minutes, rinsed through tap water to remove Hematoxylin, acided with 0.1% HCl, and stained with Eosin.

ix. IHC - Antigen retrieval of formalin fixed paraffin sections is performed using Target retrieval solution (Dako, Carpinteria, CA) with a decloaking chamber (Biocare medical, Concord CA). Sections are stained for Ki67 clone TEC-3 (1:400, Dako), active caspase 3 (San Diego, CA) or

anti- TNFα, anti-COX-2, and anti-NF-κB. After overnight incubation of primary antibody, sections were incubated with anti-rabbit or mouse HRP labeled polymer (Dako) or anti-rat VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA).

x. Archival – Tissues in paraffin blocks will be stored at room temperature until processed and then archived at room temperature for future studies. In some cases, it may be necessary to go back into a block to cut more slides. Thus, all blocks will be catalogued and preserved until they have been exhausted or until such time they are no longer useful.

xi. Shipping - For slides shipping to Northwestern, slides will be cut 1 day before shipping and shipped through FedEx at room temperature. Upon arrival, slides will be stored at room temperature and used for IHC and H&E staining.

b. ***Colorectal Biopsy (for DNA methylation; 1 biopsy)***

i. Pre-visit – On the day prior to the exam, the patient will begin a clear liquid diet starting at lunch. On the scheduled day, the patients will come to the endoscopy unit fasting after midnight.

ii. Collection - The clinical laboratory will provide specimen collection kits with all sample collection, labeling and packaging information. 7 rectal biopsies will be collected. 1 of the biopsies should be flash frozen in liquid nitrogen.

iii. Labeling of specimen – Samples will be de-identified through established SOPs. All samples will be labeled with the patient's first initial of their last name followed by a number corresponding to their patient identification number assigned at the time of entry into the database (by research coordinator).

iv. Tracking of specimens – The patient ID number will be used to link information on the database to clinical data. The number of biopsies for each container will be recorded for each procedure. The pathology readings and all research data regarding biopsies will be entered into this database.

v. Transportation - The biopsy that was flash frozen should be transported to Dr. Kaichun Wu's laboratory on dry ice.

vi. Temperature storage requirements – The frozen biopsy sample will be stored in a -80˚C freezer until processing.

vii. Processing – Methylation analysis will be performed using the qMSP strategy, as previously described[1,2]. DNA will be extracted following a standard phenol-chloroform extraction protocol. Bisulfite modification of DNA will be performed using the EZ DNA Methylation™ Kit (Zymo Research) according to the manufacturer's instructions. Methylation-specific PCR was carried out in a 25-µl reaction containing 10X MSP buffer, 10 mM dNTPs, 10 pmol of each of the methylated or unmethylated primers, 1 unit of JumpStart™ REDTaq® DNA polymerase (Sigma) and 4 µl of bisulfite-treated DNA. Amplification cycles were as follows: one cycle at 95°C for 5 min followed by 35 cycles of 95°C for 30 sec, annealing temp for 30 sec, 72°C for 30 sec and a final extension step at 72°C for 5 min.

viii. Archival – Samples will be stored until processed and then archived for future studies. Samples will be catalogued and preserved (-80˚C) until they have been exhausted or until such time they are no longer useful.

10.3 Shipping Instructions

Dr. Li Xu will be responsible for shipping tissue sections to Dr. Guang-Yu Yang at Pathology Core of Northwestern University for additional histological review upon the approval of the Chinese government.

Samples should be shipped at the end of the study.

Coordinate delivery with Dr. Guang-Yu Yang (g-yang@northwestern.edu) and send FedEx tracking number to ensure onsite tracking.

Note: Contact ncpc@northwestern.edu (312-695-1408) prior to sending samples to determine the best day to ship.

Northwestern will provide account number for samples shipments.

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

Samples should be shipped to the following address:

Dr. Guang-Yu Yang
Northwestern University
Department of Pathology
Ward 6-116
303 E. Chicago Avenue
Chicago, IL 60611
Phone: (312) 503-0645
Fax: (312) 503-0647
Email: g-yang@northwestern.edu

Upon arrival, samples should be stored at room temperature.

10.4 Tissue Banking

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in §6.2, Pharmaceutical Information, as well as the package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

- AE verbatim term
- System Organ Class (SOC)
- Common Terminology Criteria for Adverse Events v4.0 (CTCAE) AE term
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a serious adverse event (SAE)
- Whether or not the subject dropped due to the event
- Outcome of the event
- Report date
- Action

11.1.3 Severity of AEs

Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious Adverse Events

11.2.1 DEFINITION: The only clinical site is in China. The China Food and Drug Administration (formerly the State Food and Drug Administration) SFDA Order No. 3, Article 68, Sep. 1 2003 defines SAEs as those events which occur duirng a clinical trial and which include any of the following criteria:

- Inpatient hospitalization
- Prolongation of hospitalization
- Disability/incapacity
- Effect on work capability
- Life threatening
- Death
- Congenital anomaly

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE form found at http://prevention.cancer.gov/files/clinical-trials/SAE_form.doc.

11.2.2.2 Contact the DCP Medical Monitor by phone or email within 24 hours of knowledge of the event.

DCP Medical /Scientific Monitor
Luz Maria Rodriguez, MD, FACS
NCI/Division of Cancer Prevention
9609 Medical Center Dr
Rm 5E-228
Telephone (240) 276-7039
Fax (240) 276-7848
Email: rodrigul@mail.nih.gov
Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug and expectedness

11.2.2.3 The Lead Organization and all Participating Organizations will FAX or email written SAE reports to the DCP Medical Monitor within 48 hours of learning of the event using the paper SAE form. The written SAE reports will also be faxed (650-691-4410) or emailed (safety@ccsainc.com) to DCP's Regulatory Contractor, CCS Associates (phone: 650-691-4400). The written SAE reports will also be faxed (312-695-1352) or emailed (ncpc@northwestern.edu) to the Lead Organization Research Project Manager (phone: 312-695-0562) if sent by a Participating Organization. Because certified translations cannot be obtained in time, two copies of SAE reports will be completed: one in Chinese, and one in English. All SAE reports completed in Chinese will be translated into English then compared to the original English version to ensure accuracy.

11.2.2.4 The site-PI will determine which SAEs require submission to the China Food and Drug Administration.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC. The only clinical site for this study is in China**. SAE's will be reported to Northwestern University's IRB, Xijing Hospital's Ethics Committee, and to the CFDA in accordance with their respective reporting requirements.** A copy of all SAE reports sent to CFDA will be provided to DCP and translated into English.

11.2.3 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP and Lead Organization as soon as possible according to the DCP Serious Adverse Event Reporting Procedures and Guidelines. SAEs will be followed per protocol for 30 days after treatment completion or until all side effects have been resolved, whichever occurs later. As with the initial SAE report, follow-up SAE reports will be completed in Chinese and English. All follow-up SAE reports completed in Chinese will be translated into English then compared to the original English version to ensure accuracy.

12. STUDY MONITORING

12.1 Data Management

See Appendix E, Data Management Plan.

12.2 Case Report Forms

See Appendix E, Data Management Plan.

12.3 Source Documents

All source documents will be collected and stored at the study site. Any data recorded directly on CRFs that constitute no prior written or electronic record of data, will be specifically identified as source data.

12.4 Data and Safety Monitoring Plan

A comprehensive Data Safety and Monitoring Plan has been submitted by Northwestern University, approved by DCP, and is on file there.

The Participating Organization will be monitored by the Northwestern Cancer Prevention Consortium (NCPC) Research Project Manager. Given the difficulty of planning participant accrual and travel to China, the first monitoring visit will occur approximately after the fifth participant enrolls, with subsequent annual visits. The NCPC will employ an English/Chinese translator to assist with verifying Chinese source documents.

12.5 Sponsor or China Food and Drug Administration Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or CFDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB/Ethics Committee records and other regulatory documentation, will be retained by the Investigator in a secure storage facility in compliance with HIPAAOffice of Human Research Protections (OHRP), CFDA regulations and guidances, and NCI/DCP requirements,

unless the standard at the site is more stringent. The records for all studies performed in China will be retained for, at minimum, five years after the completion of the research. For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the China Food and Drug Administration. Because this study is done outside of the United States, applicable regulatory requirements for the China study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

All study agents are to be purchased commercially. No CRADA or CTA is applicable to this study.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is a Phase I, placebo controlled, safety and tolerability study. Randomization is double-blinded. The primary endpoint is clinical safety and tolerability. Secondary endpoints are plasma- and tissue-based measures of inflammation, clinical efficacy, and compliance (via blood drug concentrations).

13.2 Randomization/Stratification

Patients will be randomized by the study statistician. 12 subjects will receive treatment with Berberine and 4 subjects will receive the placebo, in a randomized, double-blinded manner and stratified by gender. Two additional participants are anticipated to be accrued (for a total of 18 subjects) to account for an anticipated dropout rate of 10%. In order to keep the numbers in either treatment group as planned, dropouts in either treatment group will be replaced by subjects assigned to that same treatment and gender group. The ratio of men to women in each treatment group (Berberine/placebo) will be 1:1. The randomization list will be provided by the study statistician to the Investigational pharmacist at Xijing Hospital. Only the Northwestern CRO Quality Assurance Monitors, statistician, and the research pharmacist at Xijing Hospital will know which patient is receiving which treatment.

13.3 Accrual and Feasibility

In a typical month, there are 3-4 subjects identified who would qualify for chemoprevention trials, and about 1/3 of these subjects are agreeable to consider entering trials. We plan to enroll a total of 16-18 subjects (1 treatment arm with 12 subjects and one placebo group with 4 subjects, with 2 additional subjects to account for anticipated attrition; dropouts in either group will be replaced by subjects assigned to that same treatment and gender group). We anticipate this taking about 6-12 months to complete. We also have subjects who participated in prior trials who have provided consent to be contacted for future studies.

13.4 Primary Objective, Endpoint(s), Analysis Plan

Primary Objective: To determine the safety of Berberine administered to patients with UC in clinical remission while receiving maintenance therapy with Mesalamine.

Endpoints: The primary objective will be evaluated by determining whether given subject experiences grade 2 systemic toxicity or not. Upon unblinding the placebo and treatment assignments, relevant counts and rates will be evaluated and reported. Specifically, in addition to assessing clinical toxicity, we will collect tissue and blood samples from recruited participants at the beginning of the trial and at the end of Berberine administration (three months later) and evaluate organ toxicity by standard clinical chemistry tests. Also, we will read tissue samples in China and the US. Finally, we will observe and record symptoms such as fever, fatigue, weight loss, appetite, stool frequency, bloody stool and other upper and lower gastrointestinal tract symptoms in participants.

Analysis: We plan to recruit 12 treatment and 4 placebo subjects for a total of 16 evaluable subjects. With potential dropout of 2 subjects we plan to recruit a total of 18 subjects. Dropouts in either treatment group will be replaced by subjects assigned to that same treatment and gender group. We do not expect to see any toxicity in either group of

patients; in fact we believe the underlying toxicity rate is the same in both groups. With no observed toxicities, we will be able to establish an approximate 95% upper bound on Prob(Tox) as the "Rule of Three": 3/(n+1) or 3/17 = 0.18 for both groups and 3/13 = 0.23 for the treatment group[43]. Other statistical analysis will consist of providing descriptive statistics (mean, SD, max, min) on patient characteristics and on toxicity rates; in case no events occur, appropriate methods will be used, such as the Rule of Three above for upper confidence bound, continuity correction or exact confidence bounds for two-sided intervals.

13.5 Secondary Objectives, Endpoints, Analysis Plans

Secondary Objectives: To determine the efficacy of Berberine and compliance of subjects.

Endpoints and Analyses:

- For plasma biomarkers, we will use descriptive statistics and graphical methods to present the evidence of possible correlations in outcomes of interest. Box plots and confidence intervals will be used to assign proper mass weight to groups of observations. "Before" and "after" intervention values and their difference will be compared with suitably chosen counterparts.

- Colorectal tissue IHC biomarkers and DNA methylation status for SFRP1, TCERGIL, FBN2 and TFP12 will be measured twice, before and after the treatment, and their difference will be studied in relative to values in their respective control groups. The Ki-67 and cleaved caspase 3 outcomes will be the number of cells positive over total number. The TNFα, COX-2, and NF-κB immunohistochemistry will be done by quantitation through chromovision and will be expressed as % of control (placebo, pre-treatment). The description and comparison of biomarkers and gene methylation status will be performed both with the view of Before – After possible change within each treatment group, as well as with the view of Treatment - Control groups for Before and for the After period. Descriptive statistics and graphical methods will be performed to present the evidence of possible correlations in outcomes of interest. Tissue biomarkers will be measured in China and separately in the US. Comparisons of before and after treatment will be performed separately as a function of country, and then differences between countries will be compared. This will be done with the purpose of gaining knowledge of the range of changes to plan future studies in these markers.

- Blood drug concentration will be measured before and after 3 months treatment. Comparisons of before and after treatment will be performed.

13.6 Reporting and Exclusions

Compliance will be formally assessed by clinical visit and pill counts each month, and by measuring blood drug concentrations at the end of the study. Patients will be considered compliant if they have taken 80% of their study agent doses as directed. During the first month of study, patients will be counseled regarding the importance of taking all doses, but will not be removed from the study for missing doses, unless 10 consecutive days are missed. Patients who are found to be non-compliant at the monthly assessments will be kept in the study; their information will be used in the final 'intent to treat' analysis, which will contain all randomized patients.

We expect that of 18 recruited participants, all will have complete data; it is possible that up to 10% of participants drop out. Participants will not be replaced. Analysis will be based upon complete data.

13.7 Evaluation of Toxicity

All participants will be evaluated for toxicity from the time of their first dose of Berberine through day 120 (corresponding to one month after completing the 90 day treatment period).

13.8 Evaluation of Response

All participants included in the study will be assessed for response to intervention, even if there are major protocol deviations or if they are ineligible. All of the participants who met the eligibility criteria (with the possible exception of those who did not receive study agent) will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants. Refer to section 13.5 for information on evaluation of response.

13.9 Interim Analysis

Toxicity will be evaluated continuously as outlined above.

13.10 Ancillary Studies

N/A

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Signed and dated current (within two years) CV or biosketch (translated into English, if necessary) for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable, and translated into English, if necessary) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP, and translated into English, if necessary) and lab normal ranges (translated into English, if necessary) for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in “Protection of Human Research Subjects” for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations will be obtained. For personnel in China, each certificate of human subjects' protection training will be obtained but will not be translated into English. Instead, we will obtain a letter, in English, on Xijing Hospital letter head and signed by the Ethics Committee, listing the names of the study personnel who have completed this training.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Package Insert acknowledgement form

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB/Ethics Committee. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB/Ethics Committee prior to implementation. Ethics Committee approvals and Informed Consent documents in Chinese will be translated into English as part of the site essential regulatory documents.

14.4 Informed Consent

All potential study participants will be given a copy of the IRB/Ethics Committee-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation. Ethics Committee approvals and Informed Consent documents in Chinese will be translated into English as part of the site essential regulatory documents.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to the DCP Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates
2001 Gateway PL, Suite 350W
San Jose, CA 95110

E-mail Submissions:
regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review at ncpc@northwestern.edu, which will then be electronically forwarded to the DCP Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

All research related costs associated with participating in this study will be paid for, and will not be the responsibility of

the participant.

Participants will not get monetary remuneration, but the cost of study-specific biopsies and exams, tests, and any other procedures will be paid for by the study. Special outpatient visiting time will be arranged for their visit.

REFERENCES

1 S. M. Adams, and P. H. Bornemann, 'Ulcerative Colitis', Am Fam Physician, 87 (2013), 699-705.
2 K. V. Anis, N. V. Rajeshkumar, and R. Kuttan, 'Inhibition of Chemical Carcinogenesis by Berberine in Rats and Mice', J Pharm Pharmacol, 53 (2001), 763-8.
3 L. Beaugerie, M. Svrcek, P. Seksik, A. M. Bouvier, T. Simon, M. Allez, H. Brixi, J. M. Gornet, R. Altwegg, P. Beau, B. Duclos, A. Bourreille, J. Faivre, L. Peyrin-Biroulet, J. F. Flejou, F. Carrat, and Cesame Study Group, 'Risk of Colorectal High-Grade Dysplasia and Cancer in a Prospective Observational Cohort of Patients with Inflammatory Bowel Disease', Gastroenterology, 145 (2013), 166-75 e8.
4 R. Bergman, and M. Parkes, 'Systematic Review: The Use of Mesalazine in Inflammatory Bowel Disease', Aliment Pharmacol Ther, 23 (2006), 841-55.
5 M. M. Bertagnolli, 'Chemoprevention of Colorectal Cancer with Cyclooxygenase-2 Inhibitors: Two Steps Forward, One Step Back', Lancet Oncol, 8 (2007), 439-43.
6 T.C. Birdsall, and G.S. Kelly, 'Berberine: Therapeutic Potential of an Alkaloid Found in Several Medicinal Plants', Altern. Med. Rev., 2 (1997), 94-103.
7 H. Cao, S. Song, H. Zhang, Y. Zhang, R. Qu, B. Yang, Y. Jing, T. Hu, F. Yan, and B. Wang, 'Chemopreventive Effects of Berberine on Intestinal Tumor Development in Apcmin/+ Mice', BMC Gastroenterol, 13 (2013), 163.
8 M. Cao, P. Wang, C. Sun, W. He, and F. Wang, 'Amelioration of Ifn-Gamma and Tnf-Alpha-Induced Intestinal Epithelial Barrier Dysfunction by Berberine Via Suppression of Mlck-Mlc Phosphorylation Signaling Pathway', PLoS One, 8 (2013), e61944.
9 W. Chen, R. Zheng, S. Zhang, P. Zhao, G. Li, L. Wu, and J. He, 'The Incidences and Mortalities of Major Cancers in China, 2009', Chin J Cancer, 32 (2013), 106-12.
10 W. Chen, R. Zheng, S. Zhang, P. Zhao, G. Li, L. Wu, J. He, 'Report of Incidence and Mortality in China Cancer Registries, 2009', Chin J Cancer Res, 25 (2013), 10-21.
11 K. N. Chidambara Murthy, G. K. Jayaprakasha, and B. S. Patil, 'The Natural Alkaloid Berberine Targets Multiple Pathways to Induce Cell Death in Cultured Human Colon Cancer Cells', Eur J Pharmacol, 688 (2012), 14-21.
12 M. L. Clapper, H. S. Cooper, and W. C. Chang, 'Dextran Sulfate Sodium-Induced Colitis-Associated Neoplasia: A Promising Model for the Development of Chemopreventive Interventions', Acta Pharmacol Sin, 28 (2007), 1450-9.
13 R. M. Cooney, B. F. Warren, D. G. Altman, M. T. Abreu, and S. P. Travis, 'Outcome Measurement in Clinical Trials for Ulcerative Colitis: Towards Standardisation', Trials, 8 (2007), 17.
14 J. Cosnes, C. Gower-Rousseau, P. Seksik, and A. Cortot, 'Epidemiology and Natural History of Inflammatory Bowel Diseases', Gastroenterology, 140 (2011), 1785-94.
15 M. De Robertis, E. Massi, M. L. Poeta, S. Carotti, S. Morini, L. Cecchetelli, E. Signori, and V. M. Fazio, 'The Aom/Dss Murine Model for the Study of Colon Carcinogenesis: From Pathways to Diagnosis and Therapy Studies', J Carcinog, 10 (2011), 9.
16 V. K. Denmark, and L. Mayer, 'Current Status of Monoclonal Antibody Therapy for the Treatment of Inflammatory Bowel Disease: An Update', Expert Rev Clin Immunol, 9 (2013), 77-92.
17 A. B. Desai, K. M. Shah, and D. M. Shah, 'Berberine in Treatment of Diarrhoea', Indian Pediatr, 8 (1971), 462-5.
18 B. K. Edwards, A. M. Noone, A. B. Mariotto, E. P. Simard, F. P. Boscoe, S. J. Henley, A. Jemal, H. Cho, R. N. Anderson, B. A. Kohler, C. R. Eheman, and E. M. Ward, 'Annual Report to the Nation on the Status of Cancer, 1975-2010, Featuring Prevalence of Comorbidity and Impact on Survival among Persons with Lung, Colorectal, Breast, or Prostate Cancer', Cancer (2013).
19 R. N. Fedorak, and M. Field, 'Antidiarrheal Therapy. Prospects for New Agents', Dig Dis Sci, 32 (1987), 195-205.
20 A. C. Ford, J. P. Achkar, K. J. Khan, S. V. Kane, N. J. Talley, J. K. Marshall, and P. Moayyedi, 'Efficacy of 5-Aminosalicylates in Ulcerative Colitis: Systematic Review and Meta-Analysis', Am J Gastroenterol, 106 (2011), 601-16.
21 A. C. Ford, K. J. Khan, J. P. Achkar, and P. Moayyedi, 'Efficacy of Oral Vs. Topical, or Combined Oral and Topical 5-Aminosalicylates, in Ulcerative Colitis: Systematic Review and Meta-Analysis', Am J Gastroenterol, 107 (2012), 167-76; author reply 77.
22 L. Fu, W. Chen, W. Guo, J. Wang, Y. Tian, D. Shi, X. Zhang, H. Qiu, X. Xiao, T. Kang, W. Huang, S. Wang, and W. Deng, 'Berberine Targets Ap-2/Htert, Nf-Kappab/Cox-2, Hif-1alpha/Vegf and Cytochrome-C/Caspase Signaling to Suppress Human Cancer Cell Growth', PLoS One, 8 (2013), e69240.
23 K. Fukuda, Y. Hibiya, M. Mutoh, M. Koshiji, S. Akao, and H. Fujiwara, 'Inhibition by Berberine of

Cyclooxygenase-2 Transcriptional Activity in Human Colon Cancer Cells', J Ethnopharmacol, 66 (1999), 227-33.
24 K. Fukuda, Y. Hibiya, M. Mutoh, M. Koshiji, S. Akao, H. Fujiwara, 'Inhibition of Activator Protein 1 Activity by Berberine in Human Hepatoma Cells', Planta Med, 65 (1999), 381-3.
25 K. Geboes, R. Riddell, A. Ost, B. Jensfelt, T. Persson, and R. Lofberg, 'A Reproducible Grading Scale for Histological Assessment of Inflammation in Ulcerative Colitis', Gut, 47 (2000), 404-9.
26 S. C. Gupta, J. H. Kim, S. Prasad, and B. B. Aggarwal, 'Regulation of Survival, Proliferation, Invasion, Angiogenesis, and Metastasis of Tumor Cells through Modulation of Inflammatory Pathways by Nutraceuticals', Cancer Metastasis Rev, 29 (2010), 405-34.
27 T. P. Hamsa, and G. Kuttan, 'Antiangiogenic Activity of Berberine Is Mediated through the Downregulation of Hypoxia-Inducible Factor-1, Vegf, and Proinflammatory Mediators', Drug Chem Toxicol, 35 (2012), 57-70.
28 K. Hemminki, X. Li, J. Sundquist, and K. Sundquist, 'Cancer Risks in Ulcerative Colitis Patients', Int J Cancer, 123 (2008), 1417-21.
29 J. G. Herman, J. R. Graff, S. Myohanen, B. D. Nelkin, and S. B. Baylin, 'Methylation-Specific Pcr: A Novel Pcr Assay for Methylation Status of Cpg Islands', Proc Natl Acad Sci U S A, 93 (1996), 9821-6.
30 L. J. Herrinton, L. Liu, T. R. Levin, J. E. Allison, J. D. Lewis, and F. Velayos, 'Incidence and Mortality of Colorectal Adenocarcinoma in Persons with Inflammatory Bowel Disease from 1998 to 2010', Gastroenterology, 143 (2012), 382-9.
31 T. Hong, Z. Yang, C. F. Lv, and Y. Zhang, 'Suppressive Effect of Berberine on Experimental Dextran Sulfate Sodium-Induced Colitis', Immunopharmacol Immunotoxicol, 34 (2012), 391-7.
32 W. H. Hsu, Y. S. Hsieh, H. C. Kuo, C. Y. Teng, H. I. Huang, C. J. Wang, S. F. Yang, Y. S. Liou, and W. H. Kuo, 'Berberine Induces Apoptosis in Sw620 Human Colonic Carcinoma Cells through Generation of Reactive Oxygen Species and Activation of Jnk/P38 Mapk and Fasl', Arch Toxicol, 81 (2007), 719-28.
33 P. F. Hu, W. P. Chen, J. L. Tang, J. P. Bao, and L. D. Wu, 'Protective Effects of Berberine in an Experimental Rat Osteoarthritis Model', Phytother Res, 25 (2011), 878-85.
34 Z. Hu, Q. Jiao, J. Ding, F. Liu, R. Liu, L. Shan, H. Zeng, J. Zhang, and W. Zhang, 'Berberine Induces Dendritic Cell Apoptosis and Has Therapeutic Potential for Rheumatoid Arthritis', Arthritis Rheum, 63 (2011), 949-59.
35 W. Hua, L. Ding, Y. Chen, B. Gong, J. He, and G. Xu, 'Determination of Berberine in Human Plasma by Liquid Chromatography-Electrospray Ionization-Mass Spectrometry', J Pharm Biomed Anal, 44 (2007), 931-7.
36 N. Iizuka, K. Miyamoto, K. Okita, A. Tangoku, H. Hayashi, S. Yosino, T. Abe, T. Morioka, S. Hazama, and M. Oka, 'Inhibitory Effect of Coptidis Rhizoma and Berberine on the Proliferation of Human Esophageal Cancer Cell Lines', Cancer Lett, 148 (2000), 19-25.
37 M. Imanshahidi, and H. Hosseinzadeh, 'Pharmacological and Therapeutic Effects of Berberis Vulgaris and Its Active Constituent, Berberine', Phytother Res, 22 (2008), 999-1012.
38 A. B. Jensen, M. Larsen, M. Gislum, M. V. Skriver, P. Jepsen, B. Norgaard, and H. T. Sorensen, 'Survival after Colorectal Cancer in Patients with Ulcerative Colitis: A Nationwide Population-Based Danish Study', Am J Gastroenterol, 101 (2006), 1283-7.
39 H. W. Jeong, K. C. Hsu, J. W. Lee, M. Ham, J. Y. Huh, H. J. Shin, W. S. Kim, and J. B. Kim, 'Berberine Suppresses Proinflammatory Responses through Ampk Activation in Macrophages', Am J Physiol Endocrinol Metab, 296 (2009), E955-64.
40 T. Jess, C. Rungoe, and L. Peyrin-Biroulet, 'Risk of Colorectal Cancer in Patients with Ulcerative Colitis: A Meta-Analysis of Population-Based Cohort Studies', Clin Gastroenterol Hepatol, 10 (2012), 639-45.
41 L. Jiang, B. Xia, J. Li, M. Ye, C. Deng, Y. Ding, H. Luo, H. Ren, X. Hou, H. Liu, H. Xu, H. Cheng, and H. Yang, 'Risk Factors for Ulcerative Colitis in a Chinese Population: An Age-Matched and Sex-Matched Case-Control Study', J Clin Gastroenterol, 41 (2007), 280-4.
42 Q. Jiang, P. Liu, X. Wu, W. Liu, X. Shen, T. Lan, S. Xu, J. Peng, X. Xie, and H. Huang, 'Berberine Attenuates Lipopolysaccharide-Induced Extracelluar Matrix Accumulation and Inflammation in Rat Mesangial Cells: Involvement of Nf-Kappab Signaling Pathway', Mol Cell Endocrinol, 331 (2011), 34-40.
43 B. Jovanovic, Rule of Three: Binomial Confidence Intervals When No Responses Are Observed. The Encyclopedia of Biostatistics. Armitage Pa and Colton T Eds. . ed. by Armitage PA and Colton T. Vol. 1 John Wiley and Sons Inc. Chichester, UK, (1998), pp. 358-9.
44 M. Kanneganti, M. Mino-Kenudson, and E. Mizoguchi, 'Animal Models of Colitis-Associated Carcinogenesis', J Biomed Biotechnol, (2011), 342637.
45 V. Kettmann, D. Kosfalova, S. Jantova, M. Cernakova, and J. Drimal, 'In Vitro Cytotoxicity of Berberine against Hela and L1210 Cancer Cell Lines', Pharmazie, 59 (2004), 548-51.
46 M. M. Kheir, Y. Wang, L. Hua, J. Hu, L. Li, F. Lei, and L. Du, 'Acute Toxicity of Berberine and Its Correlation

with the Blood Concentration in Mice', Food Chem Toxicol, 48 (2010), 1105-10.

47 U. Khin Maung, Khin Myo, Wai Nyunt Nyunt, Kyaw Aye, and U. Tin, 'Clinical Trial of Berberine in Acute Watery Diarrhoea', Br Med J (Clin Res Ed), 291 (1985), 1601-5.

48 B. H. Kim, M. Kim, C. H. Yin, J. G. Jee, C. Sandoval, H. Lee, E. A. Bach, D. H. Hahm, and G. H. Baeg, 'Inhibition of the Signalling Kinase Jak3 Alleviates Inflammation in Monoarthritic Rats', Br J Pharmacol, 164 (2011), 106-18.

49 T. O. Kim, J. Park, M. J. Kang, S. H. Lee, S. R. Jee, D. Y. Ryu, K. Yang, and J. M. Yi, 'DNA Hypermethylation of a Selective Gene Panel as a Risk Marker for Colon Cancer in Patients with Ulcerative Colitis', Int J Mol Med, 31 (2013), 1255-61.

50 W. H. Ko, X. Q. Yao, C. W. Lau, W. I. Law, Z. Y. Chen, W. Kwok, K. Ho, and Y. Huang, 'Vasorelaxant and Antiproliferative Effects of Berberine', Eur J Pharmacol, 399 (2000), 187-96.

51 W. Kong, J. Wei, P. Abidi, M. Lin, S. Inaba, C. Li, Y. Wang, Z. Wang, S. Si, H. Pan, S. Wang, J. Wu, Y. Wang, Z. Li, J. Liu, and J. D. Jiang, 'Berberine Is a Novel Cholesterol-Lowering Drug Working through a Unique Mechanism Distinct from Statins', Nat Med, 10 (2004), 1344-51.

52 M. Kubo, H. Matsuda, K. Tokuoka, S. Ma, and H. Shiomoto, 'Anti-Inflammatory Activities of Methanolic Extract and Alkaloidal Components from Corydalis Tuber', Biol Pharm Bull, 17 (1994), 262-5.

53 C.-L. Kuo, C.-W. Chi, and T.-Y. Liu, 'The Anti-Inflammatory Potential of Berberine {in Vitro} and {in Vivo}', Cancer Lett., 203 (2004), 127-37.

54 C. L. Kuo, C. W. Chi, and T. Y. Liu, 'The Anti-Inflammatory Potential of Berberine in Vitro and in Vivo', Cancer Lett, 203 (2004), 127-37.

55 S. C. Lahiri, and N. K. Dutta, 'Berberine and Chloramphenicol in the Treatment of Cholera and Severe Diarrhoea', J Indian Med Assoc, 48 (1967), 1-11.

56 C. W. Lau, X. Q. Yao, Z. Y. Chen, W. H. Ko, and Y. Huang, 'Cardiovascular Actions of Berberine', Cardiovasc Drug Rev, 19 (2001), 234-44.

57 I. A. Lee, Y. J. Hyun, and D. H. Kim, 'Berberine Ameliorates Tnbs-Induced Colitis by Inhibiting Lipid Peroxidation, Enterobacterial Growth and Nf-Kappab Activation', Eur J Pharmacol, 648 (2010), 162-70.

58 G. H. Li, D. L. Wang, Y. D. Hu, P. Pu, D. Z. Li, W. D. Wang, B. Zhu, P. Hao, J. Wang, X. Q. Xu, J. Q. Wan, Y. B. Zhou, and Z. T. Chen, 'Berberine Inhibits Acute Radiation Intestinal Syndrome in Human with Abdomen Radiotherapy', Med Oncol, 27 (2010), 919-25.

59 G. R. Lichtenstein, 'Current Research in Crohn's Disease and Ulcerative Colitis: Highlights from the 2010 Acg Meeting', Gastroenterol Hepatol (N Y), 6 (2010), 3-14.

60 H. L. Lin, T. Y. Liu, C. W. Wu, and C. W. Chi, 'Berberine Modulates Expression of Mdr1 Gene Product and the Responses of Digestive Track Cancer Cells to Paclitaxel', Br J Cancer, 81 (1999), 416-22.

61 No authors listed, 'Berberine', Altern Med Rev, 5 (2000), 175-7.

62 M. Lukas, 'Inflammatory Bowel Disease as a Risk Factor for Colorectal Cancer', Dig Dis, 28 (2010), 619-24.

63 Amosy E. M'Koma, 'Inflammatory Bowel Disease: An Expanding Global Health Problem', Clinical Medicine Insights: Gastroenterology, 6 (2013), 33-47.

64 E. K. Marinova, D. B. Nikolova, D. N. Popova, G. B. Gallacher, and N. D. Ivanovska, 'Suppression of Experimental Autoimmune Tubulointerstitial Nephritis in Balb/C Mice by Berberine', Immunopharmacology, 48 (2000), 9-16.

65 S. Melgar, L. Karlsson, E. Rehnstrom, A. Karlsson, H. Utkovic, L. Jansson, and E. Michaelsson, 'Validation of Murine Dextran Sulfate Sodium-Induced Colitis Using Four Therapeutic Agents for Human Inflammatory Bowel Disease', Int Immunopharmacol, 8 (2008), 836-44.

66 S. Meng, L.-S. Wang, Z.-Q. Huang, Q. Zhou, Y.-G. Sun, J.-T. Cao, Y.-G. Li, and C.-Q. Wang, 'Berberine Ameliorates Inflammation in Patients with Acute Coronary Syndrome Following Percutaneous Coronary Intervention', Clin. Exp. Pharmacol. Physiol., 39 (2012), 406-11.

67 N. A. Molodecky, I. S. Soon, D. M. Rabi, W. A. Ghali, M. Ferris, G. Chernoff, E. I. Benchimol, R. Panaccione, S. Ghosh, H. W. Barkema, and G. G. Kaplan, 'Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases with Time, Based on Systematic Review', Gastroenterology, 142 (2012), 46-54 e42; quiz e30.

68 M. Neurath, I. Fuss, and W. Strober, 'Tnbs-Colitis', Int Rev Immunol, 19 (2000), 51-62.

69 M. K. Pandey, B. Sung, A. B. Kunnumakkara, G. Sethi, M. M. Chaturvedi, and B. B. Aggarwal, 'Berberine Modifies Cysteine 179 of Ikappabalpha Kinase, Suppresses Nuclear Factor-Kappab-Regulated Antiapoptotic Gene Products, and Potentiates Apoptosis', Cancer Res, 68 (2008), 5370-9.

70 G. H. Rabbani, 'Mechanism and Treatment of Diarrhoea Due to Vibrio Cholerae and Escherichia Coli: Roles of Drugs and Prostaglandins', Dan Med Bull, 43 (1996), 173-85.

71 G. H. Rabbani, T. Butler, J. Knight, S. C. Sanyal, and K. Alam, 'Randomized Controlled Trial of Berberine Sulfate Therapy for Diarrhea Due to Enterotoxigenic Escherichia Coli and Vibrio Cholerae', J Infect Dis, 155 (1987), 979-84.

72 L. Rosenberg, K. S. Nanda, T. Zenlea, A. Gifford, G. O. Lawlor, K. R. Falchuk, J. L. Wolf, A. S. Cheifetz, J. D. Goldsmith, and A. C. Moss, 'Histologic Markers of Inflammation in Patients with Ulcerative Colitis in Clinical Remission', Clin Gastroenterol Hepatol, 11 (2013), 991-6.

73 K. Saika, and T. Sobue, '[Cancer Statistics in the World]', Gan To Kagaku Ryoho, 40 (2013), 2475-80.

74 K. W. Schroeder, W. J. Tremaine, and D. M. Ilstrup, 'Coated Oral 5-Aminosalicylic Acid Therapy for Mildly to Moderately Active Ulcerative Colitis. A Randomized Study', N Engl J Med, 317 (1987), 1625-9.

75 Z. X. Shen, G. Q. Chen, J. H. Ni, X. S. Li, S. M. Xiong, Q. Y. Qiu, J. Zhu, W. Tang, G. L. Sun, K. Q. Yang, Y. Chen, L. Zhou, Z. W. Fang, Y. T. Wang, J. Ma, P. Zhang, T. D. Zhang, S. J. Chen, Z. Chen, and Z. Y. Wang, 'Use of Arsenic Trioxide (As2o3) in the Treatment of Acute Promyelocytic Leukemia (Apl): Ii. Clinical Efficacy and Pharmacokinetics in Relapsed Patients', Blood, 89 (1997), 3354-60.

76 W. D. Sheng, M. S. Jiddawi, X. Q. Hong, and S. M. Abdulla, 'Treatment of Chloroquine-Resistant Malaria Using Pyrimethamine in Combination with Berberine, Tetracycline or Cotrimoxazole', East Afr Med J, 74 (1997), 283-4.

77 R. Siegel, D. Naishadham, and A. Jemal, 'Cancer Statistics, 2013', CA Cancer J Clin, 63 (2013), 11-30.

78 Y. Sun, K. Xun, Y. Wang, and X. Chen, 'A Systematic Review of the Anticancer Properties of Berberine, a Natural Product from Chinese Herbs', Anticancer Drugs, 20 (2009), 757-69.

79 J. Tang, Y. Feng, S. Tsao, N. Wang, R. Curtain, and Y. Wang, 'Berberine and Coptidis Rhizoma as Novel Antineoplastic Agents: A Review of Traditional Use and Biomedical Investigations', J Ethnopharmacol, 126 (2009), 5-17.

80 C. E. Taylor, and W. B. Greenough, 3rd, 'Control of Diarrheal Diseases', Annu Rev Public Health, 10 (1989), 221-44.

81 C. J. Thirupurasundari, R. Padmini, and S. N. Devaraj, 'Effect of Berberine on the Antioxidant Status, Ultrastructural Modifications and Protein Bound Carbohydrates in Azoxymethane-Induced Colon Cancer in Rats', Chem Biol Interact, 177 (2009), 190-5.

82 J. K. Triantafillidis, G. Nasioulas, and P. A. Kosmidis, 'Colorectal Cancer and Inflammatory Bowel Disease: Epidemiology, Risk Factors, Mechanisms of Carcinogenesis and Prevention Strategies', Anticancer Res, 29 (2009), 2727-37.

83 S. Viennot, A. Deleporte, D. Moussata, S. Nancey, B. Flourie, and J. M. Reimund, 'Colon Cancer in Inflammatory Bowel Disease: Recent Trends, Questions and Answers', Gastroenterol Clin Biol, 33 Suppl 3 (2009), S190-201.

84 P. R. Vuddanda, S. Chakraborty, and S. Singh, 'Berberine: A Potential Phytochemical with Multispectrum Therapeutic Activities', Expert Opin Investig Drugs, 19 (2010), 1297-307.

85 Y. M. Wan, Y. Q. Zhu, B. Xia, and J. Luo, 'Treating Tnbs-Induced Colitis in Rats with Probiotics', Turk J Gastroenterol, 22 (2011), 486-93.

86 Y. F. Wang, H. Zhang, and Q. Ouyang, 'Clinical Manifestations of Inflammatory Bowel Disease: East and West Differences', J Dig Dis, 8 (2007), 121-7.

87 W. Wei, H. Zhao, A. Wang, M. Sui, K. Liang, H. Deng, Y. Ma, Y. Zhang, H. Zhang, and Y. Guan, 'A Clinical Study on the Short-Term Effect of Berberine in Comparison to Metformin on the Metabolic Characteristics of Women with Polycystic Ovary Syndrome', Eur J Endocrinol, 166 (2012), 99-105.

88 W. Wei, H. Zhao, A. Wang, M.Sui, K. Liang, H. Deng, Y. Ma, Y. Zhang, H. Zhang, Y. Guan, 'A Clinical Study on the Short-Term Effect of Berberine in Comparison to Metformin on the Metabolic Characteristics of Women with Polycystic Ovary Syndrome', Eur. J. Endocrinol., 166 (2012), 99-105.

89 S. Wirtz, C. Neufert, B. Weigmann, and M. F. Neurath, 'Chemically Induced Mouse Models of Intestinal Inflammation', Nat Protoc, 2 (2007), 541-6.

90 S. Wirtz, and M. F. Neurath, 'Mouse Models of Inflammatory Bowel Disease', Adv Drug Deliv Rev, 59 (2007), 1073-83.

91 H. L. Wu, C. Y. Hsu, W. H. Liu, and B. Y. Yung, 'Berberine-Induced Apoptosis of Human Leukemia Hl-60 Cells Is Associated with Down-Regulation of Nucleophosmin/B23 and Telomerase Activity', Int J Cancer, 81 (1999), 923-9.

92 K. Wu, J. Yang, and Q. Zhou, '[Preventive Effects of Berberine on Experimental Colon Cancer and Relationship with Cyclooxygenase-2 Expression]', Zhongguo Zhong Yao Za Zhi, 35 (2010), 2768-73.

93 L. N. Xu, B. N. Lu, M. M. Hu, Y. W. Xu, X. Han, Y. Qi, and J. Y. Peng, 'Mechanisms Involved in the Cytotoxic Effects of Berberine on Human Colon Cancer Hct-8 Cells', Biocell, 36 (2012), 113-20.

94 F. Yan, L. Wang, Y. Shi, H. Cao, L. Liu, M. K. Washington, R. Chaturvedi, D. A. Israel, H. Cao, B. Wang, R. M. Peek, Jr., K. T. Wilson, and D. B. Polk, 'Berberine Promotes Recovery of Colitis and Inhibits Inflammatory Responses in Colonic Macrophages and Epithelial Cells in Dss-Treated Mice', Am J Physiol Gastrointest Liver Physiol, 302 (2012), G504-14.

95 L. Ye, Q. Cao, and J. Cheng, 'Review of Inflammatory Bowel Disease in China', ScientificWorldJournal, 2013 (2013), 296470.

96 E. Yesilada, and E. Kupeli, 'Berberis Crataegina Dc. Root Exhibits Potent Anti-Inflammatory, Analgesic and Febrifuge Effects in Mice and Rats', J Ethnopharmacol, 79 (2002), 237-48.

97 J. Yin, H. Xing, and J. Ye, 'Efficacy of Berberine in Patients with Type 2 Diabetes Mellitus', Metabolism, 57 (2008), 712-7.

98 Zhang H Yu CH, Pan JF, Hong YC, Ren JY, Zhu DY, and Xu XR, 'Determination and Preliminary Studies of Metabolism of Berberine in Human Urine after Oral Administration', Chin J Clin Pharmacol 16 (2000), 36-39.

99 M. F. Zhang, and Y. Q. Shen, '[Antidiarrheal and Anti-Inflammatory Effects of Berberine]', Zhongguo Yao Li Xue Bao, 10 (1989), 174-6.

100 Y. Zhang, X. Li, D. Zou, W. Liu, J. Yang, N. Zhu, L. Huo, M. Wang, J. Hong, P. Wu, G. Ren, and G. Ning, 'Treatment of Type 2 Diabetes and Dyslipidemia with the Natural Plant Alkaloid Berberine', J Clin Endocrinol Metab, 93 (2008), 2559-65.

101 Y. Zhang, X. Wang, S. Sha, S. Liang, L. Zhao, L. Liu, N. Chai, H. Wang, and K. Wu, 'Berberine Increases the Expression of Nhe3 and Aqp4 in Sennosidea-Induced Diarrhoea Model', Fitoterapia, 83 (2012), 1014-22.

102 J. J. Zheng, 'Clinical Aspects of Ulcerative Colitis in Mainland China', Chin J Dig Dis, 7 (2006), 71-5.

103 H. Zhou, and S. Mineshita, 'The Effect of Berberine Chloride on Experimental Colitis in Rats in Vivo and in Vitro', J Pharmacol Exp Ther, 294 (2000), 822-9.

104 B. Zhu, and F. A. Ahrens, 'Effect of Berberine on Intestinal Secretion Mediated by Escherichia Coli Heat-Stable Enterotoxin in Jejunum of Pigs', Am J Vet Res, 43 (1982), 1594-8.

105 D. J. Newman, G. M. Cragg, 'Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010', J Nat Prod, 75 (2012), 311-335.

INFORMED CONSENT

Northwestern University (NU)
The Robert H. Lurie Comprehensive Cancer Center
Xijing Hospital of the Fourth Military Medical University (FMMU)

CONSENT FORM FOR RESEARCH

Study Title for Study Participants: Testing Berberine for the Prevention of Colorectal Cancer in Patients with Ulcerative Colitis

Official Study Title for Internet Search on http://www.ClinicalTrials.gov: NWU2014-03-01, Phase I Trial of Berberine in Subjects with Ulcerative Colitis

Principal Investigator: Kaichun Wu, MD, PhD

Supported by: National Cancer Institute (NCI), Division of Cancer Prevention (DCP)

Introduction

You are being asked to take part in a research study. This document has important information about the reason for the study, what you will do if you choose to be in this research study, and the way we (i.e., Northwestern University (NU) in the United States of America, National Cancer Institute (NCI) Division of Cancer Prevention (DCP) in the United States of America, and Xijing Hospital) would like to use information about you and your health.

Dr. Wu, who is the person responsible for this research study, is interested in both your clinical care and the conduct of this research study. You may choose to seek the opinion of another doctor or someone not related to this research study before taking part in this research study. More importantly, you are not under any obligation to participate in any clinical research study offered by your doctor.

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part in the research. Please take your time to make your decision about volunteering. You may discuss your decision with your friends and family. You can also discuss this study with your health care team. If you have any questions, you can ask your study doctor for more of an explanation. **You should only agree to participate in this study if you are comfortable enough with the information so that you can make an informed decision about joining.**

What is the usual approach to my high risk condition for colorectal cancer?

You are being asked to take part in this study because you have ulcerative colitis and are at increased risk for colorectal cancer. The usual approach for someone at high risk for colorectal cancer includes periodic colonoscopies to see if there are any signs of colon cancer. People who are at increased risk and choose not to participate in a study are usually followed closely by their doctor to watch for the development of cancer. People who do participate in the study will also be followed closely by their doctor.

What are my other choices if I do not take part in this study?

If you decide not to take part in this study, you have other choices. For example:

- you may choose to have the usual approach described above,
- you may choose to take part in a different study, if one is available,
- or you may choose to do nothing.

Why is this study being done?

The purpose of this study is to test the safety of Berberine to find out what effects, if any, it has on people with ulcerative colitis in remission and their risk of colorectal cancer. We will test the effect Berberine has on inflammation (swelling) of the colon, and how much Berberine is found in your blood after taking it. Berberine is a plant-based substance that has been used in China as a medication you can get without a prescription from your physician (over-the-counter) for treatment for diarrhea. There will be 16-18 people taking part in this study.

What are the study groups?

This study has two study groups. Group 1 will receive the study drug Berberine and Group 2 will receive a placebo, a pill that looks like the study drug but contains no medication.

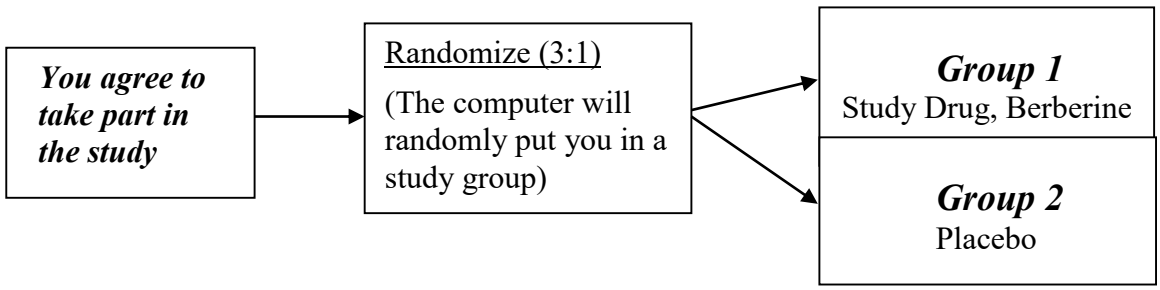
A computer will randomly put you in a study group—like a coin toss— to decide what group you get placed into. This is done because no one knows if one study group is better, the same, or worse than the other group.

The randomization assignment is 3:1 meaning that:

- The chances of receiving the study drug, Berberine, are 75%.
- The chances of receiving the placebo are 25%

For every group of 4 people to join the study, 3 people will receive the study drug Berberine and one person will receive a placebo.

Once you are put in a group, you cannot switch to the other group. Neither you nor your doctor will know if you are receiving the study drug or placebo. Your doctor cannot choose which group you will be in.



How long will I be in this study?

You will receive the study drugs for 3 months. Even if you do not finish the study, your doctor will continue to watch you for side effects and follow your condition for 30 days after you stopped taking the study medication, or until all side effects have been resolved, whichever happens later.

What extra tests and procedures will I have if I take part in this study?

Most of the exams, tests, and procedures you will have are part of the usual approach for your condition. However, there are some extra exams and tests that you will need to have if you take part in this study.

Before you begin the study:

Screening is a period during which tests and exams will be done to determine if you are eligible for

participation in the study. Some of the tests and exams may have been recently done by one of your doctors and might not need to be repeated. These tests and exams may be done on one day or two days. Most of these are part of regular care for conditions for your ulcerative colitis and would be done even if you do not join the study. Some blood tests will be done for research purposes only and will not change the way your disease is treated. Your study doctor will discuss this with you. You must sign the informed consent document before any screening tests or exams are done only for this study. Please do not take any medications that have not been prescribed by your physician (over the counter) in the 3 days prior to your screening. During this study, please do not take any berberine other than what is given to you for purposes of this study.

The following tests will be required to determine your eligibility before study treatment can begin:

- Review of your medical history (including a review of any medications you have taken in the past 3 months).

- Physical exam and Vital Signs Assessment (such as height, weight, heart rate, blood pressure, and temperature).

- Questionnaire about your bowel movements.

- Blood pregnancy test (for women who are able to have children). About ¾ of a teaspoon (3-4 milliliters) of blood will be collected for this test.

- Blood tests. About 2.5-3.5 teaspoons (12-18 milliliters) of blood will be collected for routine blood tests to determine your eligibility and to follow your safety, and an extra 2 teaspoons (9-10 milliliters) of blood for research. The total blood drawn will be 4.5-5.5 teaspoons (22-28 milliliters).

- The most common risks related to drawing blood from your arm are brief pain and possibly a bruise.

If the exams and tests show that you can take part in the study, and you choose to, then you will need the following extra procedures.

- Rectal biopsies (7 small pieces of colon or rectal tissue removed by surgery (biopsies) will be taken for the study before you begin study drug). This sample is required in order for you to take part in this study because the research on the sample is an important part of the study. This sample will be compared to the sample taken at the end of the study, to evaluate the effect of the drug.

    - The research biopsy is done in a similar way to biopsies done for diagnosis. This will be obtained during a standard colonoscopy procedure, which is a test that allows your doctor to look at the inner lining of your large intestine (rectum and colon). He or she uses a thin, flexible tube called a colonoscope to look at the colon. A colonoscopy helps find ulcers, colon polyps (abnormal tissue growths), tumors, and areas of inflammation or bleeding. Doctors will use this tissue to look for active signs of ulcerative colitis.

    - Common side effects of a biopsy are bloating, diarrhea, a small amount of bleeding at the time of the procedure, pain or discomfort at the biopsy site (which can be treated with regular pain medications), and bruising. Rarely, infection or a small hole might be made in the side of the rectum or colon that would require abdominal surgery.

    - You will sign a separate consent form before the biopsy is taken. This will be a standard surgical

consent form from the institution where the biopsy procedure takes place.

Following your colonoscopy, your study visit for screening will last approximately 60 to 90 minutes. Once you are enrolled in the study, you will receive instructions on when to take the drug and will be provided with a drug diary to document each dose of study medication that you take. You should mark any missed or skipped doses in this diary as well as any side effects that you experience.

You can pick up the study drug within 6 days of your initial visit (when the tests and procedures were done) at the clinic. If you are unable to return to the clinic to pick up the study medication, the medication will be shipped to your home via FEDEX. Three (3) bottles of pills (120-count each) will be provided to you at this time.

During the study:

Day 0
You will receive a phone call or come to the doctor's office to review any medications you are currently taking and any side effects that you have had since the previous visit.

You will also be given instructions to begin taking the study medication. You will be asked to take 3 pills three times per day (9 pills total each day). The pills should be taken after a meal with water every 6-8 hours. You will be asked to take the study medication for 90 days (3 months) and to keep a record of when you took the pills in a study diary (which was provided to you at the previous visit).

This phone call or office visit will take approximately 10-20 minutes.

Day 7 (+/- 3 days)
You will receive a telephone call to review any medications you are currently taking and any side effects that you have had since the previous visit. The study staff will also go over your diary with you and make sure you are taking the study medication as directed. You will continue to take the study medication as directed (3 pills, 3x each day). Participants should take tablets with water within one hour after a meal, every 6-8 hours. Please have your bottles of study medication available during the phone call.

This phone call will take approximately 10 minutes.

Day 30 (+/- 7 days)
You will go to the hospital for a clinical visit. The study staff will review any medications you are currently taking and any side effects that you have had since the previous visit. The study staff will also review your study diary with you and make sure you are taking the study medication as directed by counting how much medication you have left. You will continue to take the study medication as directed (3 pills, 3x each day). Participants should take tablets with water within one hour after a meal, every 6-8 hours. You may receive more study medication. You will also receive a physical exam. Please remember to bring your bottles of study medication with you to your visit.

This visit will take approximately 30 minutes.

Day 60 (+/- 7 days)
You will go to the hospital for a clinical visit. The study staff will review any medications you are currently taking and any side effects that you have had since the previous visit. The study staff will also review your study diary with you and make sure you are taking the study medication as directed by counting how much medication you have left. You will continue to take the study medication as directed (3 pills, 3x each day).

Participants should take tablets with water within one hour after a meal, every 6-8 hours. You may receive more study medication. You will also receive a physical exam. Please remember to bring your bottles of study medication with you to your visit.

This visit will take approximately 30 minutes.

After the study:

Day 90 (- 7 days)
You will go to the hospital for a clinical visit. The study staff will review any medications you are currently taking and any side effects that you have had since the previous visit. The study staff will also review your study diary with you and make sure you are taking the study medication as directed. You will also be asked to fill out a questionnaire about your bowel movements.

You will also receive a physical exam. Blood will be drawn (about 3-4 teaspoons or 15-20 mililiters) for laboratory tests and research. A colonoscopy will be done to collect rectal biopsies (7 small pieces of tissue) for research purposes. Please remember to bring all used and unused study medication bottles with you.

This visit will take approximately 60-90 minutes.

Post-study Follow-up:

Day 120 (+/- 7 days)
You will go to the hospital for a clinical visit. The study staff will review your current health status and any medications you have taken since the previous visit.

This final visit will take approximately 30 minutes.

What possible risks can I expect from taking part in this study?

If you choose to take part in this study, there is a risk that you may:

- Lose time at work or home and spend more time in the hospital or doctor's office than usual.

The drug used in this study may affect how different parts of your body work, such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

There is also a risk that you could have side effects.
Here are important points about side effects:

- The study doctors do not know who will or will not have side effects.
- Some side effects may go away soon, some may last a long time, or some may never go away.
- Some side effects may interfere with your ability to have children.
- Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:

- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
- The study doctor may be able to treat some side effects.
- The study doctor may adjust the study drugs to try to reduce side effects.

The tables below show the most common side effects that we know about Berberine, some of which may be serious. There might be other side effects that we do not yet know about. If important new side effects are found, the study doctor will discuss these with you.

Possible Side Effects of Berberine

COMMON, SOME MAY BE SERIOUS In 100 people receiving, more than 20 may have:
• Constipation (decreased number of or difficulty making bowel movements) • Abdominal pain (belly pain) • Rash

POSSIBLE, SOME MAY BE SERIOUS The frequency of some individual side effects has not yet been determined:
• Nausea • Vomiting • Drug fever • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Drug interactions with another medication that may change how your medications work or increase your risk for side effects • Increased risk for miscarriage (loss of an unborn baby from the uterus before it is able to survive outside the mother's body)

Reproductive risks: You should not get pregnant, breastfeed, or father a baby while in this study. Check with the study doctor about what types of birth control, or pregnancy prevention, to use while in this study.

Placebo risks: Those assigned to the placebo group will receive placebo (a pill which does not have any active ingredient) instead of active study medication (which is Berberine) for the duration of the study. The risks of receiving placebo are the same as not receiving any treatment for your condition. Any concerns you have about this should be discussed with the study doctor.

What possible benefits can I expect from taking part in this study? Participating in this study is unlikely to help your condition. This study may help us learn things that could help people in the future. The results from this study will provide information that will provide information that will help scientists to better understand how Berberine and similar drugs work, which would help other patients with ulcerative colitis in the future.

Can I stop taking part in this study? **Yes. You can decide to stop at any time. If you decide to stop for any reason, it is important to let the study doctor know as soon as possible so you can stop safely. If you stop, you can decide whether or not to let the study doctor continue to provide your medical information to the organization running the study.**

The study doctor will tell you about any new information or changes in the study that could affect your health or your willingness to continue in the study.

The study doctor may take you out of the study:

- If your health changes.
- If the study is no longer in your best interest.
- If new information becomes available.
- If you do not follow the study rules.
- If the study is stopped early for any reason by the sponsor, Institutional Review Board (IRB) or Ethics Committee, or CFDA.

What are my rights in this study?
Taking part in this study is your choice. No matter what decision you make, and even if your decision changes,

there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights while in this study, call Ms. Li Peng of the Xijing Hospital Ethics Committee at 86-29-84771794.

What are the costs of taking part in this study?

The study drug (or placebo) will be supplied at no charge while you take part in this study. The cost of study-specific biopsies and exams, tests, and any other procedures will be paid for by the study and will not be your responsibility.

Some costs associated with your care may be considered standard of care, and will be billed to you or your insurance company. You will have to pay for any costs (including deductibles and co-payments) not covered by your health insurer.

Before you decide to be in the study, you should check with your health plan or insurance company to find out exactly what they will pay for.

You will not be paid for taking part in this study.

What happens if I am injured or hurt because I took part in this study?

If you feel you have been injured or hurt as a result of taking part in the study, it is important that you tell the study doctor immediately. You will get medical treatment if you are injured or hurt as a result of taking part in this study.

The study sponsors will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance coverage, you would be responsible for any costs. Even though you are in a study, you keep all of your legal rights to receive payment for injury caused by medical errors.

Who will see my medical information?

Your privacy is very important to us and we will make every effort to protect it. Your information may be given out if required by law. For example, certain laws require doctors to report to health boards if they find a disease like tuberculosis. However, we will do our best to make sure that any information that is released will not be able to identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private. Some of these organizations are:

- The Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
- The Food and Drug Administration and the National Cancer Institute in the US, and similar organizations if other countries are involved in the study (such as the China Food and Drug Administration).
- The National Cancer Institute will obtain information for this clinical trial under data collection authority Title 42 U.S.C. 285.

Where can I get more information?

You may visit the NCI website at http://cancer.gov/ for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor, Dr. Li Xu, at 029-84771540.

This section is about optional studies you can choose to take part in.

Optional Sample Collections for Laboratory Studies and/or Bio-banking for Possible Future Studies

Researchers are trying to learn more about cancer, diabetes, and other health problems. Much of this research is done using samples from your biopsies, blood, urine, or other fluids. Through these studies, researchers hope to find new ways to prevent, detect, treat, or cure health problems.

Some of these studies may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment.

If you choose to take part, a sample of tissue from your previous biopsy will be collected. The researchers ask your permission to store and use your samples and health information for medical research. The research that may be done is unknown at this time. Storing samples for future studies is called "biobanking". The Biobank is being run by the Division of Cancer Prevention and supported by the National Cancer Institute.

WHAT IS INVOLVED?

1. If you agree to take part, your sample and some related information may be stored in the Biobank, along with samples and information from other people who take part. The samples will be kept until they are used up.
2. Qualified researchers can submit a request to use the materials stored in the Biobank. A research committee at the clinical trials organization, and/or the National Cancer Institute, will review each request. There will also be an ethics review to ensure that the request is necessary and proper. Researchers will not be given your name or any other information that could directly identify you.
3. Neither you nor your study doctor will be notified if/when research is conducted using your samples.
4. Some of your genetic and health information may be placed in central databases that may be public, along with information from many other people. Information that could directly identify you will not be included.

WHAT ARE THE POSSIBLE RISKS?

1. There is a risk that someone could get access to the personal information in your medical records or other information we have stored about you.
2. There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.
3. There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found

during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

HOW WILL INFORMATION ABOUT ME BE KEPT PRIVATE?

Your privacy is very important to the researchers and they will make every effort to protect it. Here are just a few of the steps they will take:

1. When your sample(s) is sent to the researchers, no information identifying you (such as your name or social security number) will be sent. Samples will be identified by a unique study code only.
2. The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and NCI staff with access to the list must sign an agreement to keep your identity confidential.
3. Researchers to whom the NCI sends your sample and information will not know who you are. They must also sign an agreement that they will not try to find out who you are.
4. Information that identifies you will not be given to anyone, unless required by law.
5. If research results are published, your name and other personal information will not be used.

WHAT ARE THE POSSIBLE BENEFITS?

You will not benefit from taking part. Your samples may be helpful to research whether you do or do not have cancer. The researchers, using the samples from you and others, might make discoveries that could help people in the future.

ARE THERE ANY COSTS OR PAYMENTS?

There are no costs to you or your insurance. You will not be paid for taking part. If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

WHAT IF I CHANGE MY MIND?

If you decide you no longer want your samples to be used, you can call the study doctor, Dr. Li Xu, at 029-84771540 who will let the researchers know. Then, any sample that remains in the bank will no longer be used. Samples or related information that have already been given to or used by researchers will not be returned.

WHAT IF I HAVE MORE QUESTIONS?

If you have questions about the use of your samples for research, contact the study doctor,
Dr. Li Xu, at 029-84771540.

Please circle your answer to show whether or not you would like to take part in each option (*include only applicable questions)*:

SAMPLES FOR THE LABORATORY STUDIES:

I agree to have my specimen collected and I agree that my specimen sample(s) and related information may be used for the laboratory study(ies) described above.

YES NO

I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to learn about results from this(ese) study(ies).

YES NO

SAMPLES FOR FUTURE RESEARCH STUDIES:

My samples and related information may be kept in a Biobank for use in future health research.

YES NO

I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to participate in other research in the future.

YES NO

This is the end of the section about optional studies within the consent template.

My Signature Agreeing to Take Part in the Main Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study.

Participant's signature _____

Date of signature_____

Signature of person(s) conducting the informed consent discussion _____

Date of signature_____

APPENDIX A
Ulcerative Colitis Disease Activity Index (UCDAI)

1. Stool Frequency*	Score
Normal number of stools/day for this patient	0
1–2 Stools/day>normal	1
3–4 Stools/day>normal	2
>4 Stools/day>normal	3
2. Rectal Bleeding†	Score
No blood seen	0
Streaks of blood with stool less than half of the time	1
Obvious blood with stool most of the time	2
Blood alone passed	3
3. Endoscopic Findings	Score
Normal or inactive disease	0
Mild disease (erythema, decreased vascular pattern, mild friability)	1
Moderate disease (marked erythema, absent vascular pattern, friability, erosions)	2
Severe disease (spontaneous bleeding, ulceration)	3
4. Physician's Global Assessment‡	Score
Normal	0
Mild disease	1
Moderate disease	2
Severe disease	3
UCDAI = total score	

* Each patient serves as his or her own control to establish the degree of abnormality of the stool frequency

† The daily bleeding score represents the most severe bleeding of the day

‡ The physician's global assessment acknowledges the 3 other criteria, as well as the patient's daily recall of abdominal discomfort, general sense of well-being, and other observations such as physical findings and the patient's performance status

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APPENDIX B

Select CYP450 Inducers, Inhibitors, and Substrates

	1A2	2C19	2D6	3A4
Inducers	ritonavir rifampin phenytoin omeprazole phenobarbital nicotine	rifampin carbamazepine ritonavir efavirenz	rifampin phenytoin phenobarbital carbamazepine	efavirenz, nevirapine, rifampin, phenytoin, phenobarbital, carbamazepine, glucocorticoids, St. John's Wort, ritonavir, etravirine
Inhibitors	fluoroquinolones cimetidine ticlopidine fluvoxamine amiodarone atazanavir	cimetidine ketoconazole omeprazole fluoxetine lansoprazole paroxetine etravirine	ritonavir paroxetine sertraline fluoxetine cimetidine celecoxib amiodarone quinidine methadone	ritonavir, indinavir, nelfinavir, amprenavir, atazanavir, saquinavir, delavirdine, fluconazole, ketaconazole, itraconazole, amiodarone, diltiazem, fluvoxamine, nefazodone, fluoxetine, clarithromycin, erythromycin, posaconazole, grapefruit juice, Seville orange juice
Substrates	haloperidol theophylline zileuton amitriptyline cyclobenzaprine olanzapine	nelfinavir lansoprazole omeprazole pantoprazole diazepam phenytoin voriconazole etravirine	metoprolol carvedilol codeine dextrometh-orphan tramadol venlafaxine	clarithromycin, cyclosporine, erythromycin, alprazolam, midazolam, triazolam, simvastatin, lovastatin, atorvastatin, nifedipine, nisoldipine, felodipine, PIs, nevirapine, efavirenz (2B6>3A4), delavirdine, sertraline, bepridil, propafenone, amiodarone, flecainide, irinotecan, pimozide, ergotamine, etravirine, maraviroc

*Table provided by http://www.hivguidelines.org/clinical-guidelines/adults/hiv-drug-drug-interactions/#APPENDIX%20A:%20ROUTES%20OF%20ELIMINATION%20OF%20HAART%20AND%20THE%20EFFECT%20ON%20CYP450

APPENDIX C
Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Participant Name_____

Participant ID_____

APPENDIX D
Patient Diary

DAY ___	DAY ___	DAY ___	DAY ___	DAY ___	DAY ___	DAY ___
DATE: ___/____/____	DATE: ___/____/____	DATE: ___/____/____	DATE: ___/____/____	DATE: ___/____/____	DATE: ___/____/____	DATE: ___/____/____
MORNING DOSE	MORNING DOSE	MORNING DOSE	MORNING DOSE	MORNING DOSE	MORNING DOSE	MORNING DOSE
Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____
☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken
MID-DAY DOSE	MID-DAY DOSE	MID-DAY DOSE	MID-DAY DOSE	MID-DAY DOSE	MID-DAY DOSE	MID-DAY DOSE
Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____
☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken
EVENING DOSE	EVENING DOSE	EVENING DOSE	EVENING DOSE	EVENING DOSE	EVENING DOSE	EVENING DOSE
Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____	Time ____:_____
☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken	☐ Not taken
Please list any new symptoms or reasons for missed doses:	Please list any new symptoms or reasons for missed doses:	Please list any new symptoms or reasons for missed doses:	Please list any new symptoms or reasons for missed doses:	Please list any new symptoms or reasons for missed doses:	Please list any new symptoms or reasons for missed doses:	Please list any new symptoms or reasons for missed doses:
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
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