USC Norris Comprehensive Cancer Center
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Protocol Title: Phase I/II Study of Celebrex and EPO906 in Patients with Metastatic Colorectal Cancer (CEPO906AUS10)

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**UNIVERSITY OF SOUTHERN CALIFORNIA/KENNETH NORRIS, JR.
COMPREHENSIVE CANCER CENTER AND HOSPITAL
CLINICAL INVESTIGATIONS SUPPORT OFFICE
1441 Eastlake Avenue, Room 7408 MS-74
Los Angeles, California 90089-9177
Telephone (323) 865-0450**

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CO-INVESTIGATORS:	Syma Iqbal, MD Anthony El-Khoueiry, MD
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Schema for the Phase I part of the study:

Rules for Dose Escalation (See Section 6.0 for Details)

1.0 OBJECTIVES

1.1 Primary Objective

1.1.1. To determine the maximum tolerated dose (MTD) of Celebrex in combination with EPO906 in patients with metastatic colorectal cancer.

1.1.2. Once MTD is established we propose to review the data and expand a dose level felt to be appropriate for a Phase II trial (which will not be above the MTD reached in the Phase I portion of the study) to evaluate response.

1.2 Secondary Objectives

1.2.1 To estimate the time to progression, survival and incidence of grade 3-4 diarrhea in patients with metastatic colorectal cancer who failed 5-FU/LV, CPT-11 and/or oxaliplatin based chemotherapy and receive Celebrex in combination with EPO906.

1.2.2 To further assess toxicity of this regimen.

1.2.3 To investigate whether the molecular biomarkers including protein expression changes from plasma, the expression levels of VEGF, E-cadherin, TP, COX-2, and β-tubulin in tumor tissue associated with clinical outcome for this regimen.

2.0 BACKGROUND AND HYPOTHESES

2.1 Incidence of Colorectal Cancer

In 1999, an estimated 139,400 new cases of colorectal cancer will be diagnosed in the United States and 56,600 deaths from this disease will occur.[1] Colorectal cancer is the third most common cause of cancer-related death in men and women. Current concepts and clinical practice regarding the prognosis and the therapy for patients with colon cancer rests on clinical/pathological staging maintained for over 60 years. Identification of molecular determinants of chemotherapy efficacy and toxicity is of critical importance for the development of more efficient and less toxic treatment strategies for patients with colon cancer.

2.2 Current Strategies for Metastatic Colorectal Cancer

Most recently CPT-11, a potent topoisomerase inhibitor in combination with 5-FU and leucovorin has established a role in the treatment of colorectal cancer. Several phase II/III trials in previously untreated patients with metastatic colorectal cancer have reported significant activity in patients with metastatic colorectal cancer. At ASCO 2000, the Saltz regimen demonstrated superior response, time to progression and overall survival when compared to 5-FU-containing regimens, which made it the new standard of therapy for metastatic colorectal cancer.[2] There were concerns regarding the potential side effects with this combination therapy. Serious adverse events included grade 3 or 4 diarrhea, leukopenia/neutropenia. [2] NCCTG reported a trial in previously untreated metastatic colorectal cancer patients with a 29% partial response rate and a median duration of response of 4.4 months. The median survival for all

patients was 11.7 months. This also demonstrated late diarrhea and myelosuppression, that is grade 3 and 4 toxicities at 28 % (24).[3] Because of significant toxicities and early death in the adjuvant trial of the Intergroup, this study was held for further evaluation of the toxicities of this regimen. An expert panel concluded that there is not an increased risk of mortality with this regimen if precautions and early intervention of side effects are followed (JCO paper).

Recently, during the ASCO 2002 meeting, data from the Intergroup study 9781 were reported. This study was prematurely terminated because it demonstrated superior clinical benefit for one of the treatment arms reaching the stopping rule. Patients treated with 5-FU/Oxaliplatin (FOLFOX) showed higher response rates, longer time to progression and longer overall survival and significant less toxicities except neurotoxicity.[4] Based on these and other data, oxaliplatin has been approved in the second-line therapy of metastatic colorectal cancer.

With the development of new effective anticancer drugs such as topoisomerase I inhibitors, oxaliplatin, oral fluoropyrimidines and other agents such as Epothilone B with novel mechanisms, it is of clinical significance to better understand the metabolism and the mechanism of resistance of these drugs. It is essential to understand why some patients develop life-threatening toxicity and why some tumors are resistant to these drugs.

EPO906 (epothilone B) is an investigational agent which, like the taxanes paclitaxel (Taxol®)[1] and docetaxel (Taxotere®), induces polymerization of tubulin dimers into stable microtubules, eventually leading to arrest of cell proliferation and apoptosis. *In vitro*, EPO906 is a potent inhibitor of cell growth in a variety of human cancer cell lines. In contrast to paclitaxel, EPO906 is equally cytotoxic to Taxol®-sensitive and Taxol®-resistant cells that display a multidrug-resistant phenotype due to overexpression of the P-glycoprotein (P-gp) efflux pump. *In vivo*, EPO906 is a potent inhibitor of tumor growth in P-gp-mediated multidrug-resistant human tumor models. Thus, regressions were observed in two models of tumors that are either poorly or completely non-responsive to treatment with Taxol.® Regressions were also seen in a colon model that is resistant to 5-fluorouracil. Potent tumor growth inhibition was also observed in a Taxol®-resistant model of human lung carcinoma A549, where resistance most likely is not mediated by P-gp overexpression. Phase II studies in colon cancer are ongoing.

2.3 Study Rationale

Cyclooxygenase-II (COX-II) is an attractive target for inhibition in patients with advanced large bowel malignancies. Epidemiological, clinical, animal and laboratory studies combine to present convincing evidence that traditional Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin and sulindac can protect against the development of CRC. The epidemiological studies indicate an approximate 50% reduction in the incidence of, or death from, CRC among regular users of aspirin and other NSAIDs.[5,6] Traditional NSAIDs inhibit both isoforms of cyclo-oxygenase (COX), the rate-limiting enzyme in prostaglandin biosynthesis. COX-I is constitutively expressed in most tissues and has a role in normal gastrointestinal tissue homeostasis. COX-II is induced by a variety of cytokines and mitogens and cannot be detected in

[1]The term "Taxol®" is used when referring to the formulated drug, as it was used in all *in vivo* experiments. The term "paclitaxel" refers to the non-formulated active drug substance of Taxol®.

most normal tissues. Direct genetic evidence that COX-II has a role in intestinal tumorigenesis has been obtained using mouse models of familial adenomatous polyposis (FAP).

Both COX-II protein and mRNA are overexpressed in most colorectal cancers relative to normal large bowel mucosa, and expression is correlated with larger tumor size, deeper invasion, and higher rates of lymph node and distant metastases.[7] In potentially curatively resected colorectal cancers, high levels of COX-II expression in the primary cancer correlate with statistically worse recurrence-free survival for the patients.[8] There exist a number of mechanisms by which COX-II expression is linked to the initiation or promotion of invasive cancer, with pre-clinical support for each. These include enhancement of tumor-induced angiogenesis; causing increases in tumor cell proliferation, motility, and general invasiveness; enhancing resistance to normal apoptosis; and decreasing tumor host immune surveillance by increasing interleukin 10 and decreasing IL-12 production.

Selective COX-II inhibitors are not as toxic as the traditional NSAIDs. The COX-II selective drugs retain the anti-neoplastic effects of traditional NSAIDs and appear, like aspirin and sulindac, to be effective intestinal neoplasia chemopreventive agents in a number of murine models. In fact, when evaluated using similar protocols, they are more effective than the NSAIDs aspirin, ibuprofen, sulindac and piroxicam. COX-II selective NSAIDs inhibit angiogenesis factors and endothelial tube formation, and they induce cell cycle arrest and apoptosis in CRC cells. Selective COX-2 inhibitors can also be used to treat established tumors. Celecoxib, one such drug, has been shown to significantly diminish the polyp burden of familial adenomatous polyposis patients when given at a dose of 400 milligrams po BID.[9] Additionally, in laboratory models of CRC, celecoxib decreases primary tumor growth and inhibits lung metastases. These effects are additive or synergistic with the chemotherapeutic drugs CPT-11 and 5FU.[10]

Celecoxib has been combined safely with chemotherapy in humans. A Phase II trial testing 400 mg PO BID with the standard Saltz CPT-11-based regimen has accrued 21 evaluable previously untreated advanced CRC patients. The objective response rate is 29% and median survival is 11 months. Interestingly, that trial reports grade 3/4 neutropenia rates of 27%, about half that expected from the same chemotherapy alone. The Hoosier Oncology Group also reported a decrease in IFL-induced severe or greater neutropenia (31%) in CRC patients with the addition of celecoxib, though that trial also added glutamine to the Saltz regimen. Investigators at USC have retrospectively reviewed their data on CRC patients treated on a phase II trial of oxaliplatin with 5-FU (oxaliplatin 130 mg/m2 IV over 2 hours every 3 weeks; 5-FU 200 mg/m2 CI weekly x 10) with or without celecoxib (200-400 mg daily). The rate of serious neuropathy (> grade 1) was 14% for the 175 patients treated without celecoxib and 4% for the 75 patients given the COX-II inhibitor. No notable increase in other toxic events was seen in those given celecoxib versus the patients treated with chemotherapy alone. Finally, M. D. Anderson investigators retrospectively analyzed 67 colorectal cancer patients taking capecitabine alone, or capecitabine with celecoxib (200 mg po BID) in both first and second-line settings. The addition of the COX-II inhibitor significantly reduced rates of hand-foot syndrome (> grade 1) and diarrhea (> grade 2).

COX-2 selective inhibitors, which have far fewer adverse side effects than conventional NSAIDs, are effective chemopreventive agents and potential chemotherapeutic agents, and there is rationale for combining them with oxaliplatin-

containing regimens. Celecoxib specifically has important antineoplastic properties, making it an excellent candidate to be used in treatment of established disease. It demonstrates additive or synergistic effects when combined with fluoropyrimidines pre-clinically. In phase II studies celecoxib potentially decreases the major toxicities of drugs commonly used to treat CRC, including 5-FU, oxaliplatin, and capecitabine.

The combination of Celebrex with EPO906 may show additive or synergistic effect in colon cancer. In addition, Celebrex may be able to modulate the diarrhea caused by EPO906 and allow dosing at a higher MTD. The DLT in both of the Phase I dose escalation trials the DLT was diarrhea. In the Phase I trial using the q 3 week schedule used in this study, three complete responses were observed in colorectal patients, two at the 8.0 mg/m2 level and one at the 7.0 mg/m2 dose level. However, this Phase I trial determined the MTD to be 6.0 mg/m2, with the DLT being diarrhea. Hopefully by adding Celebrex to the EPO906 and implementing strict guidelines for management of diarrhea a higher, more efficacious MTD of EPO906 can be reached in patients with advanced colorectal cancer. Based on preclinical and clinical data demonstrating a synergistic effect between chemotherapeutic agents and Celebrex in patients with metastatic lung and colon cancer, the combination of Celebrex and EPO906 should be explored since EPO906 has shown activity not only in breast but also in colon cancers. The basis for a potential synergistic effect is the inhibition of COX-2 allowing direct impact on apoptosis, angiogenesis and DNA repair.

Preclinical data in a rat model of diarrhea indicate that Celebrex was effective in decreasing the incidence and severity of diarrhea caused by EPO906. In addition, preclinical toxicology data looking at the possible mechanisms of diarrhea caused by EPO906 in beagle dogs noted an increase in COX-2 expression in the small bowel tissues of these animals at seven days post-dosing with EPO906 (data on file at Novartis Pharmaceuticals). The primary dose limiting toxicity and the most commonly reported adverse event with EPO906 treatment is diarrhea. Diarrhea of any grade was reported by 81% of patients from 8 Phase II studies in a variety of indications. Grade 3 diarrhea was reported by 16% of patients and grade 4 diarrhea by 1.4%. Two Phase II studies done in second and third line colorectal cancer revealed that diarrhea was more severe in this population with 26% of patients reporting grade 3 diarrhea.

2. 4 Molecular Markers

The molecular targets of EPO906 are the microtubulins and genes involved in DNA repair, apoptosis, cell cycle control, transcriptional regulation based on the mechanisms of EPO906. The molecular targets of Celebrex include COX-2 and genes from angiogenesis, adhesion, cytokines and apoptosis. The biological activity will be monitored by markers of cell cycle, apoptosis and target genes.

1. Target Genes of Celebrex: COX-2, IL-10, VEGF, PDGF-R
2. Target Genes of EPO906: βtubulin, Cox-2, ERCC-1, XPD
3. Apoptosis: Induces of apoptosis, bcl-2, bax
4. Cell Cycle: Stabilizes cell cycle regulatory proteins such as p21, p53 and p27, blocking the G2-M phase.

2.4.1 Target Genes of Celebrex

Cyclooxygenase (COX) is the key enzyme in the metabolism of arachidonic acid. It catalyzes the biosynthesis of prostaglandin H_2 - the precursor of other prostaglandins and prostanoids. There are two known isoenzymes of COX. COX-1 which is

constitutively expressed in most tissues and involved in various physiologic functions and COX-2 which is induced by several stimuli and associated with tissue inflammation, cell activation and proliferation.[7] COX-2 isoform has been found to be overexpressed in a variety of malignant tumor cells and in angiogenic endothelial cells.[8] There is compelling evidence indicating that it plays a role in pathogenesis of tumor growth, tumor angiogenesis and metastasis. The precise molecular mechanism by which overexpression of COX-2 enhances malignant transformation and invasiveness is unknown, but it appears to inhibit apoptosis and increase ability to degrade extracellular matrix proteins by activation of the matrix metalloproteinase MMP-2.[9,10]

Arachidonic Acid is oxidized by 3 different oxygenases, including cyclooxygenase (COX), and its metabolites include PGs, thromboxanes, and leukotrienes. AA mobilization is linked to a wide variety of biologic signal transduction pathways, and this process is tightly regulated in the GI tract. Prostanoid synthesis is enhanced by a variety of growth factors, and PGH 2 synthase (another name for COX), is homologous to the product of a proliferation –associated gene. Tumor cells produce larger amounts of certain PGE2 than surrounding mucosa. It has been shown that patients with metastatic colorectal cancer have statistically significantly elevated levels of peripheral blood PGE2 versus those with localized cancers. Other mechanisms for PG-induced tumor initiation and promotion exist: Colony-stimulating factors released by tumors can cause mononuclear cells to secrete PGE2, which enhances IL-10 production and influences activity of T-cells and natural killer cells, altering immune surveillance. PGs regulate platelet function, and tumor-platelet aggregates are proposed to activate the cancer cells' vascular attachment, promoting metastases. E-series PGs are angiogenic and tumor-induced angiogenesis is strongly tied to growth and metastasis.

Utilizing immunohistochemistry, COX-2 expression was determined in patients with squamous cell carcinoma (SCC) and adenocarcinoma (ADC) of the esophagus. It was determined that COX-2 was expressed in 91% of the SCCs and 78% of the ADCs. COX expression was also evaluated in two esophageal cancer cell lines, OSC-1 and OSC-2. OSC-2 cells expressed COX-2 but not COX-1 and OSC-2 cells expressed high levels of COX-1 but showed only a very weak COX-2 expression. PGE2 synthesis was 600 times higher in the OSC-2 cells as compared with the OSC-1 cells. Treatment of the OSC-2 cells with selective COX-2 inhibitors suppressed PGE2 synthesis and proliferation and also induced apoptosis, no effect was seen on the OSC-1 cells.[18] The expression in squamous cell carcinoma of the esophagus has been confirmed as detection of COX-2 was strongly positive in well-differentiated regions of the esophageal tumor, whereas normal squamous epithelium stained only weakly positive. In this study, poorly differentiated areas of the esophageal tumor were negative.[19] COX-2 expression in SCC correlated with proliferation activity assessed by the proliferating cell nuclear antigen index in dysplastic lesions, but not in SCCs. COX-2 expression was found to be a marker for high grade dysplasia and suggests that COX-2 may be involved in early stages of squamous carcinogenesis of the esophagus.[20] COX-2 mRNA levels in Barrett's mucosa were also found to be increased when evaluated by immunohistochemical staining and Western blot.[21]

2.4.1.1 Molecular determinants of angiogenesis, adhesion and invasion

The majority of malignant solid tumors overexpressed at least one of the angiogenic growth factors, such as acidic fibroblast growth factor (FGF-1), basic fibroblast growth factor (FGF-2), vascular endothelial growth factor (VEGF), platelet-derived endothelial cell growth factor/thymidine phosphorylase (PDEGF/TP) and others. Increased

expression of growth factors and their receptors have been demonstrated in variety of malignancies.[22,23] Expression of thymidine phosphorylase is associated with increased microvessel density, local tumor invasion and decreased survival in colorectal cancer.[24,25] VEGF has been found to be closely associated with microvessel density formation in various cancers.[26,27] A recent study showed that TP and VEGF expressions in colon cancer appeared to be inversely correlated, that is in tumors with a high vessel density, TP expression was high when VEGF was low and vice versa.[24] Vessel count and expression of VEGF have been shown to predict distant recurrence in patients with node-negative colon cancer.[28,29] A number of cell adhesion molecules are expressed on endothelial cells and tumor cells and play a critical role in angiogenesis by modulating cell-cell and cell-extracellular matrix interactions.[30-33] One of these molecules is integrin alpha v beta 3, which binds to the arginine-glycine-aspartic acid (RGD) sequence of vitronectin, fibronectin, fibrinogen and other subendothelial proteins of the extracellular matrix.[34,35]

2.4.1.2 Metalloproteinases (MMPs)

The matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are essential for tumor invasion and metastasis. Matrix metalloproteinases, a family of zinc-dependent proteases, participate in several steps in tumor progression, including invasion, metastasis and angiogenesis and each of these enzymes is secreted as a proenzyme that requires activation.[36] A positive correlation between tumor aggressiveness, metastatic potential and prognosis and levels of MMP and TIMP has been documented.[37]

The levels of MMP-2 and MMP-9 mRNA were significantly higher in primary colorectal cancers than in their adjacent normal tissue and were significantly higher in liver metastases than in the primary colorectal cancer.[38] Increased expression of MMP-2 is associated with decreased survival in patients with breast cancer.[38] Downregulation of MMP-9 results in reduction of tumor cell invasion and metastatic potential. Elevation of serum levels of MMP-2 was shown to be a predictor of recurrence and disease free survival in patients with urothelial carcinomas.[38]

Patterns of expression of described molecular determinants of angiogenesis, adhesion and invasion before therapy, during treatment with Celebrex and at the time of progression, may provide important insights into its mechanism of anti-tumor activity.

2.4.1.3 COX-2, VEGF, E-Cadherin

Several studies suggest that cell-substrate adhesion may play an important role in colon tumorigenicity since cells that have been transformed by a virus or with chemical carcinogens demonstrate altered adhesion to ECM compared with non-transformed cells.[11,12] In fact, changes in the expression and function of adhesion molecules are important characteristics in the development of gastrointestinal malignancies and might be used in the future as prognostic factors or as new targets in diagnosis and therapy. Recent data suggest that cells overexpressing COX-2 can attach to basement membrane and survive on it, whereas cells with low COX-2 expression are unable to do so, indicating that the extracellular adhesion pathway was altered by COX-2 overexpression, and suggesting that cell survival was affected as well.[13] COX-2 expression increased the ability of rat intestinal cells to attach to laminin and matrigel, but it is of interest that each group of cells bound to the fibronectin coated plates equally well suggesting that not all components of the extracellular adhesion pathway were affected.[14]

Very recently, COX-2 overexpression was found to decrease E-cadherin expression in epithelial cells.[14] E-cadherin, an epithelial adhesion molecule, is critical for the maintenance of cell polarity and differentiation. Down-regulation of E-cadherin has been reported to occur in a variety of solid tumors and is closely correlated to tumor invasion.[13,14] For tumor formation, local invasion is thought to be one of the most important factors, and E-cadherin may act as an invasion suppressor gene. Therefore, the effect of COX-2 expression on E-cadherin expression levels could play a significant role in tumorigenesis. Decreased cell-cell adhesion and increased motility on laminin have been correlated with more poorly differentiated and aggressive carcinomas. A defect in the cadherin cell-cell adhesion complex, often found in poorly differentiated and highly invasive tumors, facilitates increased cell-cell adhesion and retards tumor cell migration on basement membrane and stromal proteins.[15] E-cadherin negative tumor cells are far more likely than E-cadherin positive cells to detach from a tumor mass, pointing to a novel mechanism, whereby defects in cell-cell adhesion could lead to carcinoma cell dissemination.[16] E-cadherin has been shown to be an independent prognostic factor in colon cancer to identify patients with poor prognosis.[17-19] More than 80% of poorly differentiated tumors lack expression of E-cadherin suggesting that the presence of E-cadherin is important in cell differentiation and that down-regulation of E-cadherin is associated with local invasion of tumor cells. It is unknown in what adhesion pathways COX-2 may be involved. There are preliminary data suggesting that COX-2 overexpression increased adhesion to the extracellular matrix. One of the potential molecular effects of COX-2 inhibition is on the integrin family particular $\alpha v\beta 1$ and $\beta 3$ based on the findings that COX-2 expression has been associated with increased attachment to laminin and matrigel.

Two angiogenic factors, TP and VEGF, have been shown to play an important prognostic role in colorectal cancer, and very recently, thymidine phosphorylase has been shown to increase COX activity and COX-2 protein expression in several cell types.[20] Thymidine phosphorylase is associated with micro-vessel density, local tumor invasion, and decreased survival in colorectal cancer. TP was recently discovered to be identical to the angiogenic factor platelet-derived endothelial cell growth factor (PD-ECGF).[21] VEGF has been found to be closely associated with micro-vessel density formation in various cancers.[22,23] A recent study showed that TP and VEGF expressions in colon cancer appeared to be anti-coordinated, that is, in tumors with a high vessel density, TP expression was high when VEGF was low, and vice versa.[24] Vessel count and expression of VEGF have been show to predict distant recurrence in patients with node-negative colon cancer.[25] Thus, if VEGF is also associated with clinical outcome, the use of both TP and VEGF expressions simultaneously may identify patients who benefit from COX-2 inhibitors. **In this study we will evaluate whether the expression levels of VEGF, E-cadherin, TP, and COX-2 will predict for tumor response determined by pathological evaluation.**

2.4.2 Targets of EPO906

The targets of EPO906 are microtubulins. We have established a method to measure gene expression levels of β-tubulin as wells as the β tubulin mutations in paraffin embedded tissue which may predict response and outcome in patients treated with EPO906.

2.4.2.1 Apoptosis

Apoptosis is an active, energy-dependent process, which depends on the expression of certain genes such as p53, p21, bcl-2 and bax. The tumor suppressor gene p53 is a

critical mediator of cellular responses to DNA damage in mammalian cells. Both G1 cell cycle arrest and apoptotic cell death following chemotherapy have been shown to be dependent on normal p53 function.[26-28] P53-induced apoptosis, however, can be blocked by gene transfer-mediated elevations in the levels of the bcl-2 protein, suggesting that p53 and bcl-2 may participate in a common pathway for regulation of cell life and death.[12] Furthermore it has been demonstrated that p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo.[12]

It has been shown that high expression of bcl-2 inhibits apoptosis, and that increased bcl-2 expression enhances resistance of several lymphoid cell lines to chemotherapeutic drugs.[29] It is possible that bcl-2 expression might cause even tumors with wt p53 to be resistant. Regulating the activity of bcl-2 in cells is the protein product of the bax gene, which forms an inhibitory heterodimer with bcl-2. Thus, the activity of bcl-2 in tumors may actually depend on the ratio of bcl-2/bax.[12]

Recent data showed that EPO906 can induce apoptosis, indicating a direct interaction with the apoptotic pathway. We will determine whether bcl-2, bax, p53 and p21 protein and gene expression levels will predict response to EPO906. Furthermore, we will determine the percentage of apoptotic cells by using a terminal deoxynucleotidyl transferase (TdT) assay that labels the 3'-OH ends of DNA fragments produced in apoptotic cells (ApopTag Detection Kit, Oncor). We will be able to address these questions by obtaining not only a biopsy prior to treatment but also a second biopsy directly after chemotherapy.

2.4.2.2 Cell Cycle Modulation/Apoptosis.

p27/KIP1 encodes a Cdk inhibitor that associates with cyclin/cdk complexes inhibiting their cell cycling activity.[29] p27 is a downstream component of the *TGF-B1* growth inhibitory signal pathway and is most often associated with Cyclin E/Cdk2 complexes, but is also active against complexes containing Cyclin A or Cyclin D. Although the N-terminus of p27 is homologous to the Cdk inhibitor p21, it contains a carboxy-terminal sequence that is unique and of unknown function. The mechanism of p27 downregulation has been shown to be through the ubiquitin-proteasome degradation pathway.[30]

Recently, loss of p27 staining by immunohistochemistry was shown to provide prognostic information in two studies of NSCLC (n=108, p=0.0012 and n=149, p=0.03).[31,32] In the former study, it was shown that *p27* mRNA was present in all tumors examined and that the levels of p27 protein correlated inversely with ubiquitin-mediated degradation activity. Additionally, the total loss of *p27/KIP1* protein was observed in 68% of prostate carcinomas (CaPs). Although not addressed by the authors, close inspection of the CaP staining patterns revealed strong cytoplasmic staining. This is consistent with an unusual mechanism of sequestering p27 recently identified in anchorage-independent transformed cells: cytoplasmic presence of Cyclin E/Cdk2 complexes that bind p27.[33] Whether this mechanism occurs in human tumors has yet to be studied. A more recent study found that 78% of metastatic CaP lesions had low or undetectable nuclear expression of p27 in the presence of abundant p27 mRNA, suggesting a post-transcriptional mechanism of gene expression downregulation.[34] Thus, while abnormalities of *p27* expression are associated with CaP progression, the unique mechanism of inactivation of this tumor suppressor offers the potential to be exploited for therapeutic advantage in many advanced tumors of different types. This would involve either the induction of protein levels to exceed the

levels of degradation or stabilization of the protein by inhibiting degradation to result in levels sufficient to induce apoptosis.

2.5 Biomarker Development

In addition to testing the safety and effectiveness of the experimental drug EPO906 in this Study, the Lenz laboratory will develop blood test(s) that will identify patients most likely to be helped by EPO906.

Development of Potential Biomarkers

Biomarkers are objectively measured and evaluated indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (see footnote 1 below for reference). In oncology, there is particular interest in the molecular changes underlying the oncogenic processes that may assess amount of tumor growth or predict disease progression, metastasis and responses/toxicities to EPO906. Therefore biomarker analyses have been incorporated into this clinical study. The purpose of biomarker investigations is to identify alterations in protein expression in the blood that may be useful in developing markers to guide therapy and/or predict toxicity. More specifically, these biomarker studies are restricted in scope to proteomics studies in plasma to look for markers that correlate with toxicity and EPO906 effect.

3.0 DRUG INFORMATION

3.1 CELEBREX

3.1.1 Description

Celecoxib is chemically designated as 4-(5-(4-methylphenyl)-3(trifluoromethyl))-1H-pyrazol-1-yl)) benzylsulfonamide. It is a diarylsubstituted pyrazole which is a reversible inhibitor of the COX enzyme. Antiinflammatory activity has been demonstrated for this compound in animal models.

3.1.2 Drug Administration

Celecoxib is commercially available in hard gelatin capsules containing 200 mg of the drug as well as hydrous lactose. The drug is rapidly absorbed following oral administration, reaching peak plasma concentrations approximately 3 hours after oral dosing. Patients are instructed to take capsules with food.

3.1.3 Storage and Stability

The drug should be stored at 59-77° F(15-25° C)

3.1.4 Toxicity

The following toxicities occurred in ≥2% of patients given celecoxib for arthritis: gastrointestinal (abdominal pain, diarrhea, dyspepsia, flatulence, nausea); nervous system (dizziness, headache, insomnia), respiratory (pharyngitis, rhinitis, sinusitis), integument (rash), other (back pain, peripheral edema). As with non-steroidal anti-

inflammatory drugs, patients should be monitored closely for liver function abnormalities, renal injury, anemia, aspirin-sensitive asthma, and gastrointestinal bleeding. Co-administration of celecoxib with drugs known to interact with cytochrome P450 2C9 (e.g., HMG-CoA Reductase inhibitors, tolbutamide) should be performed cautiously. There is potential for interaction with drugs metabolized with p450 2D6 (e.g., morphine. Please see investigator brochure for an up to date list of these drugs. A list of the currently known drugs metabolized with p450 2D6 is in Appendix I).

3.1.5 Source of Drug

Celebrex is manufactured by Pfizer; the study sponsor, Novartis, will provide Celebrex for this trial.

3.2 EPO906

3.2.1 Description

EPO906 (epothilone B) is an investigational agent which, like the taxanes paclitaxel (Taxol®)[2] and docetaxel (Taxotere®), induces polymerization of tubulin dimers into stable microtubules, eventually leading to arrest of cell proliferation and apoptosis. *In vitro*, EPO906 is a potent inhibitor of cell growth in a variety of human cancer cell lines. In contrast to paclitaxel, EPO906 is equally cytotoxic to Taxol®-sensitive and Taxol®-resistant cells that display a multidrug-resistant phenotype due to overexpression of the P-glycoprotein (P-gp) efflux pump. *In vivo*, EPO906 is a potent inhibitor of tumor growth in P-gp-mediated multidrug-resistant human tumor models. Thus, regressions were observed in two models of tumors that are either poorly or completely non-responsive to treatment with Taxol.® Regressions were also seen in a colon model that is resistant to 5-fluorouracil. Potent tumor growth inhibition was also observed in a Taxol®-resistant model of human lung carcinoma A549, where resistance most likely is not mediated by P-gp overexpression. Although EPO906 can be associated with body weight loss and mortality in animals at high doses, efficacy was achieved at dose levels that were reasonably well tolerated by the animals, suggesting that EPO906 could be an appropriate treatment for multidrug-resistant tumors in humans.

3.2.2 Clinical Experience with EPO906

EPO906 was tested in humans in two-phase 1 studies. In study 101, EPO906 was administered as a five-minute bolus infusion every three weeks. Dosing started at 0.3 mg/m^2, and reached doses of 8.0 mg/m^2. Doses up to 5.4 mg/m^2 were well tolerated, but patients receiving the 8.0 mg/m^2 dose frequently experienced episodes of grade 3 diarrhea, and that dose was considered to be above the maximum tolerated dose (MTD). The dose was reduced in the next cohort to 7.0 mg/m^2, but this dose also proved to be above the MTD due to diarrhea. Twelve patients were then enrolled at 6.0 mg/m^2 with only one DLT, a grade 3 diarrhea. Based on these findings, 6.0 mg/m^2 was determined to be the recommended dose for this schedule. Other frequently reported adverse events have included fatigue, nausea and sensory neuropathies. One partial response (breast cancer) was observed at the 0.3 mg/m^2 dose level, two (colon,

[2]The term "Taxol®" is used when referring to the formulated drug, as it was used in all *in vivo* experiments. The term "paclitaxel" refers to the non-formulated active drug substance of Taxol®.

unknown primary) were observed at the 8.0 mg/m^2 dose level, and two (colon, ovarian) at the 7.0 mg/m^2 dose level. No responses were seen at 6.0 mg/m^2.

In study 102, EPO906 was initially administered as a five-minute bolus infusion every week for six weeks, followed by three weeks off (each cycle equals nine weeks). Dosing started at 0.3 mg/m^2, and reached levels of 3.6 mg/m^2. Doses up to 1.85 mg/m^2 were well tolerated, but patients receiving 3.6 mg/m^2 frequently experienced episodes of grade 3 diarrhea, and that dose was considered to be above the MTD. The dose was reduced in the next cohort to 3.0 mg/m^2, but this dose also proved to be above the MTD. Twelve patients were treated at 2.5 mg/m^2 with no major problems, and this dose was determined to be the recommended dose for this schedule. To try to further reduce the incidence of grade 1 and 2 diarrhea in these patients, an additional cohort of patients was initiated at 2.5 mg/m^2 given for three weeks followed by one week off. These patients did not experience any diarrhea, and an additional cohort at 3.0 mg/m^2 was enrolled on this new schedule. Two of the six patients in this cohort experienced grade 3 diarrhea, and enrollment was terminated. Twenty additional patients were then enrolled into the 2.5 mg/m^2 cohort, with no additional DLTs reported.The most common adverse events other than diarrhea recorded to date have included fatigue, nausea and sensory neuropathies. One partial response (breast cancer) was observed at the 1.85 mg/m^2 dose level, and three (ovarian, carcinoid, endometrial) at the 2.5 mg/m^2 dose level. One durable (2 years and counting) minimal response (NSCLC) was seen at the 3.0 mg/m^2 dose level.

Five Phase1b studies are currently ongoing. In all studies except for one, EPO906 is being dosed at the three weeks on/one week off (qW) schedule. There have been no unexpected toxicities reported in these studies. The most common dose limiting toxicity has been diarrhea.

1. In study 2103 EPO906 is currently being administered at 2.0 mg/m^2 in combination with standard Xeloda therapy. One serious adverse event (grade 3 diarrhea, dehydration and mucositis resulting in death) has been observed, leading to the enrollment of four additional patients in that cohort.
2. Study 2104 completed with the recommended Phase 2 dose for EPO906 at 2.5 mg/m^2 in combination with weekly carboplatin. Two disease-related DLTs were seen in the first cohort (0.5mg/m2) that were not attributed to the EPO906 component, and the cohort was expanded to five patients with no further problems.
3. Study 2108 is currently dosing at the fourth cohort at 2.0 mg/m^2 in combination with estramune. Four patients have been enrolled and there have been no DLTs seen to date.
4. Study 2110 is currently dosing at 2.0 mg/m^2 in combination with gemcitabine. Twelve patients have been enrolled and no DLTs have been observed to date.
5. Study 2113 has completed the second cohort at 4.8 mg/m^2 in combination with carboplatin with both drugs being dosed on a q3 weekly schedule. There have been no DLTs seen to date. The study will be amended to increase the dose of carboplatin to AUC=56 before further escalating the EPO906 dose.

Nine Phase 2 studies are also currently ongoing. The most common adverse events occurring in these studies include: diarrhea, nausea, fatigue, vomiting, abdominal pain, and anorexia. Confirmed partial responses were observed in most indications with the exception of melanoma. Patients also exhibited prolonged stable disease during treatment with durations of between 4 and 18 months. One patient expired due to

cardiac failure where drug related diarrhea leading to dehydration was a contributory factor to fatal outcome according to the reporter. Other deaths in EPO906 studies have been related to underlying disease.

Results from two Phase I/II studies of EP0906 were recently presented at ASCO 2004 and these results have been updated by Novartis.[35-37] In both studies a diarrhea management algorithm was implemented, and this, along with patient education have enabled the management of the dose limiting toxicity of diarrhea, which has resulted in an escalation of MTD.

One trial consists of patients diagnosed with advanced ovarian, primary fallopian or primary peritoneal cancer who failed to respond or relapsed within six months of first-line taxane plus platinum therapy. The other trial consists of patients diagnosed with stage IIIB/IV NSCLC that relapsed after platinum based therapy. Both trials utilize the q 3 week regimen. As can be seen from the table below, an MTD has not been reached yet in these pre-treated populations, with dosing currently at 13 mg/m2.

In the refractory ovarian trial (n=30) an objective response rate of 23% was observed (1 complete response, 6 partial responses), with an approximately 6 month median rate of response. Nine additional patients experienced stable disease lasting approximately 6 months.[37] In the second line NSCLC trial (n=39) an objective response rate of 10% was observed (4 partial responses) with approximately 4 ½ months median duration of response. Thirteen patients had stable disease with a median duration of 4.2 months. In patients pre-treated with paclitaxel (13 of 39), 2 had partial responses and 2 had stable disease.[37] A summary table of common adverse events as of March 2005 is also provided below.

Cycle 1 DLTs by dose

Dose (mg/m2)	Total DLTs (# pts in cohort)	Ovarian	NSCLC
6.5	0 (6)	0	0
7.0	0 (6)	0	0
7.5	1 (9)	0	Gr3 asthenia
8.0	2 (12)	Gr 3 Fatigue	Gr3 asthenia
8.5	2(12)	Gr 3 Fatigue	Gr 3 asthenia
9.0	1 (9)	Gr 4 uric acid	0
9.5	0 (6)	0	0
10.0	0 (6)	0	0
10.5	0 (6)	0	0
11.0	0 (9)	Gr 3 Diarrhea	0
11.5	0 (3)		0
12.0	0 (3)		0
13.0	1 (3)		Gr3 diarrhea

Common AEs

Adverse event	N = 95 (as %)	N = 351 (as %)
	6.5-11.0mg/m2	2.5mg/m2/week x3
	q3Weekly	q4Weekly
Diarrhea	58 (61)	270 (77)
Nausea	26 (27)	189 (54)

Fatigue	40 (42)	177 (50)
Peripheral neuropathy	22 (23)	100 (28)
Vomiting NOS	24 (25)	116 (33)

3.2.3 Pharmacokinetics of EPO906

Pharmacokinetics of EPO906 has been evaluated in two Phase 1 dose-escalation clinical trials, studies 101 and 102. The starting dose was 0.3 mg/m^2 for both studies. In study 101, EPO906 was administered intravenously once every three weeks. In study 102, EPO906 was administered weekly for 6 weeks followed by a 3-week drug holiday. Later, the schedule was changed to weekly for 3 weeks followed by one-week drug holiday to improve the tolerability.

Table 3.2.3 Summary of studies 101 and 102

Study No.	Patient population	Dosing regimen (short IV infusion)	Patients with PK sampling N	Dose range studied (mg/m^2)	DLT	MTD mg/m2
101	Solid tumors	once every 3 weeks	50	0.3-8	Diarrhea	6
102	Solid tumors	Weekly*6+3-week off	53	0.3-3.6	Diarrhea	
		weekly*3+1-week off	26	2.5-3.0		2.5

In study 101, pharmacokinetics of EPO906 was evaluated by both non-compartmental and compartmental methods. After a short infusion, blood EPO906 disposition was multiphasic. EPO906 concentrations declined rapidly followed by a prolonged terminal phase with t½ of 4.5 days. The total body clearance of EPO906 was approximately 8.98 L/h (150 ml/min). The estimated large volume of distribution (>1000 L) was consistent with extensive tissue uptake of EPO906 reported in preclinical studies. Renal excretion of unchanged EPO906 in 48 h was 0.05% of total dose infused, indicating that the major elimination route was nonrenal.

In study 102, pharmacokinetics of EPO906 were evaluated in patients by a non-compartmental method. In general, blood and urine pharmacokinetic profiles were similar to that obtained from study 101. The lower $AUC_{0\text{-}inf}$ and shorter terminal $t_{½}$ compared to those obtained from study 101 may be due to a short sampling collection interval with a weekly dosing schedule, which may result in underestimation of AUC0-inf and t½ and overestimation of total body clearance. Renal excretion of unchanged drug in 48 h was <0.1% of total dose infused. No statistical analysis was performed to compare the peak drug concentrations (Cmax) between 2 infusion schedules because of inconsistent sampling time with respect to the end of infusion.

Following intravenous administration of EPO906, blood disposition of EPO906 was multiphasic. EPO906 concentrations declined rapidly with an initial distribution t½ of 7

min followed by a prolonged terminal phase with t½ of 4-5 days. Renal excretion of unchanged drug was negligible (<0.1% of the total drug infused), suggesting that the clearance of EPO906 was nonrenal. The estimated large volume of distribution (>1000 L) was consistent with extensive tissue uptake of EPO906 reported in preclinical studies. Systemic drug exposure was close to being dose proportional over a dose range of 0.3-8 mg/m2. Drug accumulation was not evident with a once every 3-week dosing schedule, and there was < 2-fold increase in drug exposure after 6 doses with a 3-week on one-week off schedule. Protein binding was 95% and blood cell uptake may be saturable. No statistically significant dependence was found for the blood clearance rate (log-transformed) of EPO906 as a function of age, gender, body weight, and BSA. There was no statistically significant difference in EPO906 blood clearance rate (log-transformed) between patients with and without liver metastases. In patients receiving EPO906 once every 3 weeks, the occurrence/severity of diarrhea was associated with dose, systemic exposure, Cmax or duration of time that blood concentrations remained above a "threshold" concentration, 1.2 nM or 0.6 ng/mL. The occurrence/severity of diarrhea was also found to be associated with dose or systemic exposure in patients receiving EPO906 with a weekly dosing schedule.

The capability of EPO906 to inhibit P450 enzymes in human liver microsomes was investigated in two independent studies. Although being a substrate for CYP enzymes, in vitro experiments using human liver microsomes suggest that the oxidative pathway plays only a minor role for the metabolic clearance of EPO906. These studies indicate that EPO906 is a competitive inhibitor of CYP2C19 and CYP3A4/5 (Ki: 3.5 and 3.1 µmol/L, respectively) and a metabolism-dependent inhibitor of CYP3A4/5 (Ki: 55 µmol/L). Based on in vitro data, metabolic clearance of 5-Fluorouracil, warfarin and Gleevec (STI-571) appear not to be significantly affected at clinically relevant doses of EPO906.

3.2.4 Preparation and administration precautions:

EPO906 is available at two dosage strengths, 5mg and 10mg. EPO906 LIVI 5mg/2mL in 6ml glass vials and EPO906 LIVI 10mg/4ml in 10ml glass vials are delivered as a concentrate for solution for injection and requires two dilution steps prior to administration. Please follow the preparation instruction provided below.

Important notes:

- **The vials contain an overfill**
- **Each vial is for a single dose and may not be used for multiple doses.**
- **Do not directly inject the concentrate for solution for infusion.**

The drug is formulated in polyethylene glycol 300 (PEG 300) and must be pre-diluted in 0.9% Sodium Chloride Solution to obtain a concentration of 1mg/ml. All preparation steps have to be performed under aseptic conditions

Preparation of the Initial Diluted Solution

EPO906 5mg/2mL LIVI :Dilute the vial contents with 3.4 mL of physiological saline resulting in 5 mL (usable volume) of a 1.0 mg/mL solution of EPO906. Shake the vial gently and allow it to stand for at least 5 min to allow air bubbles formed during the dilution to separate. The pre-diluted product must be inspected visually for particulate matter and discoloration, if either is present, a new vial must be used.

EPO906 10mg/4mL LIVI: Dilute the vial contents with 6.5 mL of physiological saline resulting in 10 mL (usable volume) of a 1.0 mg/mL solution of EPO906. Shake the vial gently and allow it to stand for at least 5 min to allow air bubbles formed during the dilution to separate. The pre-diluted product must be inspected visually for particulate matter and discoloration, if either is present, a new vial must be used.

Preparation the Final Dilution for Injection

Aseptically withdraw the required volume of the initial diluted EPO906 solution from the vial using a graduated disposable syringe.

Transfer under aseptic conditions the required content from the syringe into infusion bottles/infusion bags typically containing 50ml physiological saline (0.9% NaCl) to produce a final concentration of 0.05mg/ml to 0.2mg/ml EPO906. Thoroughly mix the infusion by manual rotation.

Note: Materials tested and compatible in the final concentration range of 0.05mg/ml to 0.2mg/ml EPO906 are glass, Polyethylene and Polypropylene.

3.2.5 Administration:

Prior to administration check visually for particulate matter or discoloration. If the final solution is not clear, free of particles or discolored, the solution should be discarded.

EPO906 should be administered intravenously over a period of 5 to 10 minutes using the best available in-dwelling line. The line should be thoroughly flushed with 4-5 ml saline immediately following the administration of EPO906. If an in-dwelling line is not available, a butterfly infusion line should be inserted and utilized, and flushed as previously described immediately following completion of the administration of EPO906.

3.2.6. Storage/Stability:

The unopened EPO906 concentrate in vial is stable until the expiration date on the package when stored refrigerated (2°C to 8°C) and protected from bright light.

The final dilution for injection should be administered preferably within 1 hour after the start of the preparation. If not possible the solution should be kept at 2-8°C and allowed to come to room temperature before administration. The overall standing time of the solution between first dilution and final application should not exceed 24hours when stored for 22hours at 2-8°C, followed by equilibration to room temperature. After that time the solution should be discarded and a new preparation has to be made.

3.2.7 Availability: EPO906 will be provided by Novartis Pharmaceuticals.

3.3 Concomitant therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed, provided their use is documented

in the patient records and on the appropriate case report form. Patients should be maintained on the same medications throughout the study period, if medically appropriate. All medications taken within 30 days of the screening visit should be recorded on the CRF.

In the phase 1 studies, diarrhea was determined to be the dose-limiting toxicity for EPO906. Therefore, the anti-diarrhea medication algorithm contained in Appendix 2 or a treatment algorithm in accordance with local guidelines are recommended at the first sign of abdominal cramping, loose stools or overt diarrhea.

The administration of other anticancer agents including chemotherapy, investigational agents, and biologic agents is NOT permitted while patients are enrolled in this study. The need for other anti-cancer therapy will be attributed to disease progression and will require the patient to withdraw from the study. Radiotherapy for local peripheral metastases not being used as marker lesions is permitted, but the need for such therapy may be an indication of disease progression and should be discussed prior to continued administration of the study drug. Radiotherapy for central metastases (e.g., vertebral, mediastinal) is not permitted. Patients requiring such therapy prior to completion of the study should be considered as having progression of disease and discontinued from the study. All cancer medications/therapies given to the patient within 28 days after the last dose of study drug must be recorded on the data forms.

Hematopoietic growth factors may not be administered prophylactically in cycle 1, but may be administered therapeutically if indicated at any time. Prophylactic use in subsequent cycles is allowed if, in the opinion of the investigator, failure to do so could compromise a patient's ability to remain in the study. All use of growth factors must be in accordance with the guidelines established by the American Society of Clinical Oncology. The guidelines can be found on the Internet at the following location: http://www.asco.org/prof/pp/html/guide/csfg/m_csfg.htm (ASCO members; or http://www.asco.org> Practice Guidelines > Clinical Practice Guidelines > 2000 Update of Recommendations for the use of Hematopoietic Colony Stimulating Factors)

EPO906 has been found to cause mild to moderate emetogenicity. Patients may receive prophylactic antiemetics as per institutional guidelines or at the discretion of the treating physician.

Baseline analgesics for tumor-related pain should be maintained during the study. However, an increase in analgesic use or a step up on the WHO analgesic ladder for control of tumor-related pain may indicate disease progression. If an increase in analgesic medication from baseline is required during the study, the patient should be evaluated for progression of disease.

Although the overwhelming body of preclinical data suggests that EPO906 does not act biologically as a Pgp substrate, one *in vitro* assay performed using Caco-2 cells suggests otherwise. Therefore, patients receiving any concomitant medications known to inhibit Pgp function (e.g., verapamil, diltiazem, nicardipine, cyclosporine, quinine) will not be excluded from the protocol, but must be observed closely for toxic effects of EPO906 (e.g. myelosuppression and neurotoxicity).

In preclinical studies, cytochrome P450 enzymes did not appear to play a major role in EPO906 metabolism, and the drug did not appear to inhibit the metabolism of any other agent. Therefore, patients receiving concomitant medications known to be metabolized by the cytochrome P450 enzymes 2C19, 3A4/5, 2C9 and 2D6 will not be excluded from the study, but should be carefully monitored for potentiation of toxicity due to the individual concomitant medication. Should an event occur, whenever

feasible a sample should be obtained for measurement of the blood level of the concomitant medication as soon as possible. A list of drugs that are substrates of human liver microsomal enzymes is included in Appendix 1.

Four patients receiving Coumadin® in the phase 1 studies had elevations of their coagulation parameters requiring reductions in their Coumadin® dose. None of these patients was symptomatic. Preclinical studies revealed no interaction between these agents, and no correlation between EPO906 blood levels and increased Coumadin® potency could be established. Twenty-eight other patients receiving Coumadin® did not experience coagulation irregularities. However, in order to ensure patient safety, administration of Coumadin® and any agents containing warfarin are not permitted while on study. Patients receiving any warfarin-based anti-coagulant should be switched to heparin prior to study initiation; if this is not possible, they should not be enrolled. Exceptions may be made for patients receiving 1.0 mg or less of Coumadin® daily prophylaxis for maintenance of in-dwelling venous or arterial catheters, but these patients should be followed closely for any evidence of bleeding disorders.

No data exists regarding the interaction of EPO906 with commonly used herbal or non-traditional medications. Patients should be instructed not to use such medications while receiving EPO906 therapy.

Mukherjee et al. has reported an increased risk of cardiovascular events associated with selective COX-2 inhibitors.[38] In this article previous trials were examined, including two randomized trials, the Vioxx Gastrointestinal Outcomes Research Study (VIGOR) and the Celecoxib Long-term Arthritis Safety Study (CLASS), as well as 2 smaller trials. The VIGOR results showed that the RR risk of developing a confirmed adjudicated thrombotic cardiovascular event (myocardial infarction, unstable angina, cardiac thrombus, resuscitated cardiac arrest, sudden or unexplained death, ischemic stroke, and transient ischemic attacks) with rofecoxib treatment vs. naproxen was 2.38 (95% confidence interval, 1.39-4.00; *P* =.002). There was no statistically significant difference in cardiovascular event (myocardial infarction, stroke, and death) rates between celecoxib and nonsteroidal anti-inflammatory agents in CLASS. The annualized myocardial infarction rates for COX-2 inhibitors in both VIGOR and CLASS were statistically significantly higher than that in the placebo group of a recent meta-analysis of 23,407 patients in primary prevention trials (0.52%): 0.74% with rofecoxib (*P* =.04 compared with the placebo group of the meta-analysis) and 0.80% with celecoxib (*P* =.02 compared with the placebo group of the meta-analysis).

Atherosclerosis is a process with inflammatory features and selective cyclooxygenase 2 (COX-2) inhibitors may potentially have antiatherogenic effects by virtue of inhibiting inflammation. However, by decreasing vasodilatory and antiaggregatory prostacyclin production, COX-2 antagonists may lead to increased prothrombotic activity. The report by Mukherjee et al. raised a cautionary flag about the risk of cardiovascular events with COX-2 inhibitors.[38] Because of this, all patients that fall into at least one of the following categories will be required to take low-dose aspirin throughout the study starting on the first day of study drug:

A) Age $\geq$ 65 years
B) Hypertension (SBP $\geq$ 160 or DBP $\geq$ 95 on separate occasions)
C) Hypercholesterolemia (total blood cholesterol $\geq$ 240 mg/dL)
D) History of any of the following: Myocardial infarction, Unstable angina, CVA/TIA, Peripheral vascular disease, Smoking, Strong family history (primary relative with MI under age 55)

4.0 STAGING CRITERIA

This is a phase I/II study open for patients with metastatic colon cancer. Patients should be staged for their disease by the appropriate, standard TNM staging system of the American Joint Committee on Cancer.

5.0 ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

5.1.1 Histologically confirmed metastatic adenocarcinoma of the colon or rectum for which no further standard chemotherapy is considered to be effective. Patients must have failed (progressed on or were intolerant of) 5-FU, CPT-11 and/or oxaliplatin based chemotherapy. All patients will have malignancy confirmed by review of their biopsy specimens by the Division of Pathology of the University of Southern California/LA County/Norris Comprehensive Cancer Center.

5.1.2 SWOG performance status 0-1

5.1.3 ANC>1000, platelets >100,000.

5.1.4 Total bilirubin $\leq$ 2 x upper limit of normal. Transaminase (AST and/or ALT) $\leq$ 2 x upper limit of normal or $\leq$ 5 x upper limit of normal in patients with liver metastasis.

5.1.5 Serum creatinine $\leq$ 1 institutional upper limit of normal.

5.1.6 Female patients of child-bearing potential must have negative pregnancy test within 7 days before initiation of study drug dosing. Post menopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential. Male and female patients of reproductive potential must agree to employ an effective barrier method of birth control throughout the study and for up to 3 months following discontinuation of study drug.

5.1.7 All patients who are enrolled in the Phase II portion of this study must have measurable disease.

5.1.8 Signed informed consent.

5.1.9 Patients who fulfill at least one of the following criteria will be required to take low-dose aspirin throughout the study starting on the first day of study drug:

Age $\geq$ 65 years
Hypertension (SBP $\geq$ 160 or DBP $\geq$ 95 on separate occasions)
Hypercholesterolemia (total blood cholesterol $\geq$ 240 mg/dL)
History of any of the following:
- Myocardial infarction
- Unstable angina
- CVA/TIA

Peripheral vascular disease
Smoking
Strong family history (primary relative with MI under age 55)

5.1.10 Patients in the Phase II portion of the study must agree to blood draws for pharmacogenomics/biomarker development at baseline, with each cycle and off study.

5.2 Exclusion Criteria

5.2.1 Patient has received any other investigational agent within 28 days of first day of study drug dosing.

5.2.2 History of another malignancy within 3 years prior to study entry, except curatively treated non-melanoma skin cancer, prostate cancer, or cervical cancer in situ.

5.2.3 Patient has another severe and/or life-threatening medical disease.

5.2.4 Patient has an acute or known chronic liver or kidney disease (e.g., chronic active hepatitis, cirrhosis, chronic renal insufficiency).

5.2.5 Patient has a known diagnosis of human immunodeficiency virus (HIV) infection.

5.2.6 Patient has received chemotherapy within 4 weeks (6 weeks for nitrosourea, mitomycin-C or any antibody therapy)

5.2.7 Patients with symptomatic brain metastasis.

5.2.8 Patient with Grade III/IV cardiac problems as defined by the New York Heart Association Criteria. (e.g. congestive heart failure, myocardial infarction within 6 months of study)

5.2.9 Medical, social or psychological factors interfering with compliance.

5.2.10 Patients who have undergone major surgery for any cause less than 4 weeks prior to study entry.

5.2.11 Patients taking Coumadin® or other agents containing warfarin, with the exception of low dose Coumadin® (1 mg or less) administered prophylactically for maintenance of in-dwelling lines or ports.

5.2.12 Any peripheral neuropathy > Grade 1.

5.2.13 Patients with unresolved diarrhea > Grade 1.

5.2.14 Patients may not have a history of an allergy to sulfonamide drugs.

5.2.15 Patients may not have active peptic ulcer disease or other contraindications to chronic NSAID use or aspirin use.

5.2.16 Patients with lactose intolerance.

5.2.17 Patients taking full-dose NSAIDs, including aspirin, regularly for any reason (e.g., arthritis,history of TIA or myocardial infarction). Patients taking cardiac preventive dose ASA (<81mg daily) are eligible. Patients should stop taking any other NSAIDs 14 days prior to receiving first dose of Celecoxib.

5.2.18 Patients with hypersensitivity to COX-2 inhibitors, NSAIDS or salycilate.

5.2.19 Patients taking fluconazole or lithium.

6.0 STUDY DESIGN

This is a phase I/II study to be conducted at USC. This study is expected to accrue a minimum of 6+11 evaluable patients and a maximum of 18+62. It should take approximately 24 months to complete this trial.

6.1 Definition of Dose Limiting Toxicity and Maximum Tolerated Dose

This study will use the NCI CTCAE version 3.0 for toxicity and Adverse Event Reporting. A copy of the latest version of the CTC can be downloaded from the CTEP home page (***http://ctep.info.nih.gov***).

6.1.1 Dose Limiting Toxicity (DLT)

The term DLT will refer to unacceptable toxicity which in a patient during the first course of treatment (3 weeks). DLT is defined as:

A: Any Grade 3 non-hematologic toxicity not reversible to Grade 2 or less within 96 hours with the following exceptions:

- Nausea or Vomiting (≥ Grade 3) that occurs without maximal anti-emetic therapy
- Diarrhea(≥ Grade 3) that occurs following patient noncompliance with loperamide therapy
- Fatigue (Grade 3)

B: Any Grade 4 toxicity with the exception of Grade 4 Fatigue

C: Any toxicity that results in treatment delays of > 4 weeks.

To be evaluable for toxicity, a patient must complete one course of treatment (3 weeks) and be observed during that time or have experienced DLT attributable to EPO906 and Celebrex. A DLT attributable to Celebrex or EPO906 is a toxicity with an attribution of “definite” or “probably” or “possible”.

All patients who are not evaluable for toxicity will be replaced. All side effects observed, for all patients who received any of the study drugs, will be reported.

6.1.2 Maximum tolerated dose (MTD).

The MTD is the highest dose tested in which none or only one patient experienced DLT attributable to Celebrex or EPO906, when at least six patients are treated at that dose and are

evaluable for toxicity. The MTD is one dose level below the highest dose tested in which 2 or more patients experience DLT attributable to Celebrex or EPO906.

6.2 Dose Escalation Schedule and Rules

6.2.1 Dose Escalation Schedule

EPO906 will be administered as a single intravenous infusion over five to ten minutes every three weeks. This dosing schedule will be repeated until either progression of disease or unacceptable toxicities occur. Where appropriate, patients may receive treatment as outpatients.

In this study, the dose of Celebrex will be fixed at 400 mg po twice daily within patients with modification for toxicity only (see Section 8.5). Patients will begin receiving Celebrex one week before their first infusion of EP0906 and will not take the Celebrex on days they receive EP0906. Based on previous Phase I trials of EPO906 monotherapy, the initial dose of EPO906 will be 7.0 mg/m^2 IV every 3 weeks, with dose escalation in increments of 1.0 mg/m^2. We do not anticipate major toxicity with the regimen, but will not, for practical reason escalate beyond the dose level of 13.0 mg/m^2. If no DLT attributable to the study drugs is observed prior to this highest dose level, then we will treat 6 patients at this dose level. If DLT attributable to EPO906 or Celebrex is observed in two or more patients at Dose Level I, then Dose Level -I (6.0 mg/m^2) will be tested. Dose Level –II of EPO906 will not be tested, it will be used for deescalation within patients for observed toxicity only.

Dose Level	**Dose of Celebrex (to be deescalated within patients for observed toxicity)**
I	400mg po bid
-I	300 mg po bid
-II	200 mg po bid
-III	100 mg po bid.

Dose Level	**Dose of EPO906 mg/m^2**
-II*	5.0 mg
-I	6.0 mg
I	7.0 mg
II	8.0 mg
III	9.0 mg
IV	10.0 mg
V	11.0 mg
VI	12.0 mg
VII	13.0 mg

* Dose Level –II will not be tested, it will be used for deescalation within patients for observed toxicity only.

6.2.2 Rules for Dose Escalation

For this trial, we will use the standard NCI 3+3 rules. Because Celebrex is an agent that is known to be effective, we will not incorporate dose escalation of Celebrex in this design. Initially, three patients will be treated at each new dose level. If no patients (0/3) experience DLT attributable to Celebrex or EPO906 during the first course at that dose level, the dose level will be escalated in the next three patients. If DLT attributable to either Celebrex or EPO906 is observed in one patient (1/3) exactly, 3 more patients (for a total of 6) will be treated at that dose level. If no additional DLT is observed at the expanded dose level (i.e., 1/6 with DLT), the dose will be escalated in the next three patients. As soon as DLT attributable to Celebrex or EPO906 is

observed in two or more patients at a dose level, escalation will stop. If, at the next lower dose level only 3 patients have been treated, additional 3 patients will be treated at that dose level.

The Phase I trial will be closed when either (a) 6 patients have been treated at the next lower dose level, and at most 1/6 patients experience DLT attributable to Celebrex or EPO906 (if more than 1/6 patients experience DLT, the next lower dose will be expanded), or (b) 6 patients are treated at the highest dose level of EPO906 of 8.0 mg/m^2, and none or only one experiences DLT.

Treatment will continue in an individual patient at the same dose level for the duration of therapy if no DLT is observed and if benefit is observed (see Section 8.4); patients will go off therapy if excessive toxicity is experienced. Before escalating to the next dose level in a cohort, all patients at the current dose level must have completed the first course of therapy and be observed for a minimum of 3 weeks after starting the first course – or have experienced DLT attributable to the study drugs.

There will be no dose escalation of EPO906 within a patient.

6.3 Expansion of the MTD / Phase II Trial

Once the MTD is established, the Phase II portion of this trial will begin. The patients used to establish the MTD will not be included in the Phase II portion of this study. A modified Simon 2-stage phase II trial will be performed to assess activity of EPO906 in combination with Celebrex. a.[39-41] In this trial, tumor response will be the primary endpoint used to determine the required sample size. The patients enrolled in this trial have previously received 5-FU, CPT-11, and/or Oxaliplatin based therapy and therefore if Celebrex and EPO906 together does not produce responses (CR's or PR's) (the response rate is 2% or less), then there will be little interest in studying the regimen further. If the response rate is statistically significantly greater than 2%, the recommendation of further studying this regimen will be supported. Otherwise, this regimen is not promising for further study. Tumor response will be assessed using the RECIST criteria version 1.1 after 6 weeks of Celebrex and EPO906. Patients who receive less than 6 weeks of treatment due to unacceptable toxicity or progressive disease will still be evaluable for response. 18 patients will be accrued, treated, and evaluated for tumor response during stage I. If none of 18 evaluable patients have complete (CR) or partial response (PR), we will stop accrual. If 1 or more patients have CR or PR, we will continue to enroll until a total of 37 patients have been treated and evaluated for tumor response. If 2 or fewer out of 37 evaluable patients have CR or PR, further study of this regimen will not be recommended in this setting. If 3 or more patients have CR or PR, and toxicity is acceptable, further investigation of EPO906 and Celebrex is warranted.
With this design, the probability of recommending this regimen will be 0.033 (alpha) and 0.901 (power) if the true response rate is 2% and 15%, respectively. If the regimen is not promising (the response rate is 2%), the probability of ending the trial during stage I is 0.695.

The overall response rate will be calculated as the percent of evaluable patients whose best response is a CR or PR in the end of the Phase II trial. With 37 patients, the response rate will be estimated with a half width of 0.161 or less.

The time to progression, survival and incidence of grade 3-4 diarrhea in patients with metastatic colorectal cancer treated with EPO906 and Celebrex will be secondary endpoints. A diary of diarrhea (see Appendix 3 for details) and CTCAE version 3.0 will be used to determine the course of treatment and the grade of diarrhea. Patients will fill out the diarrhea diary for a week before the start of treatment to obtain the baseline diarrhea measure. The diary includes

items regarding number of bowel movements, number of times of abdominal cramping, and number of times incontinent during the day (morning to bedtime) and night (bed time to morning). The diary also queries whether diarrhea interferes with daily activities and what medications for diarrhea are taken. Patients who complete two courses of treatment (6 weeks) and are observed during that time, or have experienced Grade 3-4 diarrhea will be evaluable for diarrhea.

After 12 patients were accrued to the Phase II portion of the study at the 11 mg/m2 dose, accrual was halted to the study for a safety review. As per Sections 8.4 and 12.4 of the study too many patients had experienced unacceptable toxicity. Their Grade 3/4/5 toxicities are as follows: Three patients died (2 due to disease progression), all of whom experienced Grade 3 diarrhea, Grade 3 nausea and Grade 3 vomiting. Of the other 9 patients 7 experienced Grade 3 toxicity, which included Grade 3 dehydration (n=1), Grade 3 fatigue (n=1), Grade 3 diarrhea (n=4), Grade 3 anorexia (n=1), Grade 3 nausea (n=2), Grade 3 vomiting (n=2) and Grade 3 joint pain (n=1).

The MTD reached in the Phase I portion of the trial was 12 mg/m2. As the toxicity experienced by patients on the 12 mg/m2 seemed too high in courses subsequent to course one to be well tolerated as a Phase II dose, the 11 mg/m2 dose was used as a Phase II dose. Due to the excessive toxicity experienced by our patients at this dose to date, we now think this dose is too high. The dose for the Phase II portion of the trial will now be 9.0 mg/m2, which is below the 10 mg/m2 dose used in a large (n=829) randomized trial of EPO906 or Doxil in up to fourth line patients with ovarian, fallopian or peritoneal cancer, which was recently completed. We have treated 6 patients at the 9 mg/m2 dose level on our trial to date, who received 2-8 cycles of therapy with acceptable toxicity.

7.0 TREATMENT PLAN

Phase I

Patients will receive increasing doses of EPO906 which will be started at 7.0 mg/m^2 iv, on Day 1 of each cycle. Celebrex will be given at 400 mg po bid, beginning one week prior to the first EP906 infusion (days -7 through -1 of study). Celebrex will be held on the days EPO906 is infused, and will be given at 400 mg po bid for the rest of the cycle (days 2-21 of each cycle). The first treatment cycle, therefore, will be four weeks, all cycles thereafter will be 3 weeks.

Phase II

All patients will receive the 9.0 mg/m2. Celebrex will be given at 400 mg po bid, beginning one week prior to the first EP906 infusion (days -7 through -1 of study). Celebrex will be held on the days EPO906 is infused and will be given at 400 mg po bid for the rest of the cycle (days 2-21 of each cycle). The first treatment cycle, therefore, will be four weeks, all cycles thereafter will be 3 weeks.

7.1 Duration of Therapy

In the absence of treatment delays due to toxicity, treatment continues until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable toxicity
- Patient decides to withdraw from the study, or

- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigatorTwo grade 3-4 diarrhea episodes despite dose reduction

8.0 TOXICITIES TO BE MONITORED AND DOSAGE MODIFICATIONS

8.1 General Approach to Patient Toxicity

8.1.1 All toxicities will be graded according to NCI Common Toxicity Criteria (CTCAE version 3.0). If multiple toxicities occur, the next dose administered should be based on the most severe toxicity experienced.

Adverse events (see Section 6) will use the descriptions and grading scales found in the revised CTCAE version 3.0 for toxicity and Adverse Drug Experience reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov/CTC3/ctc_ind_term.htm). All appropriate treatment areas will have access to a copy of the CTCAE version 3.0.

8.2 Dosing Delays and Modifications of Celebrex

8.2.1 Hematologic Toxicity

- Doses of celecoxib will not in general be modified for hematologic toxicity.
- Each treatment course will begin when hematologic parameters recover to neutrophils $\geq$1,000/uL and platelets $\geq$100,000/uL.

8.2.2 Gastrointestinal Toxicity

- GI toxicity will be based on the worst toxicity observed during the previous course.
- Doses of celecoxib will not be modified for the individual patient *unless* a grade 3 or 4 GI toxicity persists greater than 10 days from the last dose of EPO906, at which time the celecoxib dose can be decreased to 200 mg, po, bid. If the toxicity does not decrease to grade 0 or 1 within 21 days from the last dose of chemotherapy, the patient must be taken off celecoxib. Patients requiring celecoxib cessation may continue on EPO906 at the discretion of the investigator.
- Prior to the start of the next course of Celebrex, the patient should have complete resolution of stomatitis/pharyngitis and diarrhea.

8.2.3 Renal Toxicity and Duodenal Ulceration

	During a course of therapy	At the start of subsequent courses of therapy
Renal Toxicity Grade 2 Creatinine	Hold Celebrex.	If improved to less or equal grade 1, decrease celecoxib to 200 mg, po, bid.**
Duodenal ulceration, gastric ulceration, gastritis, gastrointestinal bleeding (endoscopically documented)		
1. grade 1 or 2	Continue celecoxib at same dose, institute anti-ulcer therapy.	Continue celecoxib at same dose, institute anti-ulcer therapy.
2. grade 3 or 4	Discontinue celecoxib.***	Discontinue celecoxib***

** If toxicity does not resolve to grade 1 and patient is already receiving celecoxib 200 mg, po, bid, then discontinue celecoxib permanently - patients may continue on EPO906 at the discretion of the investigator.
*** Patients may continue on EPO906 at the discretion of the investigator.

8.2.4 Fluconazole

If a patient requires short-term fluconazole therapy while on study, the celecoxib dose will be held and resumed 10 days after the completion of fluconazole therapy. If doses of celecoxib are missed or held for any reason it will be recorded in the CRF.

8.3 Dose modification for EPO906

8.3.1 Dose Interruption or Reduction

Patients who experience any of the following toxicities during a treatment course shall have the next scheduled dose of EPO906 withheld until recovery to at least grade 1 (grade 2 for nausea, vomiting, myalgias, arthralgias or mucositis; full recovery for diarrhea):

- ≥ CTC grade 3 non-hematological toxicity (excluding nausea and vomiting controlled by antiemetics)
- ≥ CTC grade 3 hematological toxicity
- ≥ CTC grade 3 neurotoxicity
- ≥ CTC grade 2 cardiac toxicity
- Serum creatinine ≥ 2.0 x ULN

Following recovery from the above toxicities to at least Grade 1 (grade 2 for nausea, vomiting, myalgias, arthralgias or mucositis; full recovery for diarrhea), patients may continue to receive EPO906 at the next lower dose level. If the toxicity reoccurs at the same grade following a one dose level reduction, the dose should be further reduced one dose level. If the toxicity continues EPO906 should be discontinued, unless in the opinion of the investigator, the patient is benefiting from EPO906 treatment. Further dose reductions or continued dosing

despite these adverse events should be discussed on a case by case basis with Novartis. Any interruption or change in the dose must be captured on the Dose Administration Record CRF.

If a patient is discontinued due to an adverse event(s), the end of study evaluations should be performed and the patient should be followed weekly until the toxicity causing the discontinuation resolves or stabilizes.

The above guidelines for dose reduction or discontinuation are to be interpreted considering the overall condition of the patient, the patient's overall tolerance of treatment, and any indication of patient benefit. Dose interruptions or reductions generally should be applied whenever this appears to be in the best interest of the patient. All dose interruptions and modifications guidelines apply only to adverse events that are thought to be at least possibly related to the study drug. If an adverse event is not thought to be related to study drug, the patient may restart treatment at the current dose level as per the treating physician's discretion.

8.3.2 See appendix 2 for full diarrhea management instructions, note the following in that appendix .

If a patient experiences grade 2 or higher diarrhea they will be seen by the treating physician weekly until it resolves to at least grade 1.

All patients experiencing grade 3 or higher diarrhea should be placed on a quinolone antibiotic until the diarrhea resolves to grade 1. If a grade 3 or higher diarrhea occurs patients should receive a quinolone antibiotic with subsequent cycles of EPO906 for 5 to 7 days prophylactically.

8.4 Monitoring for Excessive Toxicity in Phase II portion of trial

Although we expect this regimen to be well tolerated, the guidelines listed below will be used to raise a flag if the number of patients who experience unacceptable toxicity is large enough to strongly suggest that the true probability of unacceptable toxicity is > 20%. Once the MTD is established, the guideline will be only applied to the Phase II portion of this study. Unacceptable toxicity will be defined as:

(a) any Grade 3 non-hematologic toxicity not reversible to Grade 1 or less within 96 hours with the exception of Grade 3 diarrhea that occurs following patient noncompliance with loperamide therapy or
(b) any Grade 4 non-hematologic toxicity or
(c) any Grade 4 hematologic toxicity not resolving to Grade 1 or less within 5 days, despite supportive care or
(d) Grade 4 neutropenia associated with fever or
(e) Grade 4 thrombocytopenia.

An unacceptable toxicity, regardless of attribution, observed during any course, will be used in the decision to suspend accrual.

8.5 Serious and Unexpected Adverse Events

A serious adverse event will refer to any adverse event that:

a) is fatal (i.e., results in death from any cause at any time);
b) is life threatening (i.e., the patient was, in the view of the Investigator, at immediate risk of death from the reaction as it occurred);

c) results in persistent or significant disability / incapacity;
d) requires, or prolongs, hospitalization;
e) is a congenital anomaly / birth defect;
f) is medically significant, may jeopardize the subject's prognosis, and may require medical or surgical intervention to prevent one of the outcomes listed above..

An unexpected event is any adverse event that is not identified in nature, severity or frequency in the Clinical Investigator Brochure.

8.5.1 Reporting responsibility

Each serious adverse event (but not pregnancies) must be reported by the investigator to Novartis within 24 hours of learning of its occurrence, even if it is not felt to be treatment-related. Follow-up information about a previously reported serious adverse event must also be reported to Novartis within 24 hours of receiving it. If the serious adverse event has not been previously documented (new occurrence) and it is thought to be related to study drug (or therapy), the Medical Safety Expert of the Clinical Safety & Epidemiology (CS&E) Department may contact the investigator to obtain further information. If warranted, an investigator alert may be issued, to inform all investigators involved in any study with the same drug (or therapy) that this serious adverse event has been reported. Any serious adverse events that occur should also be reported to the USC IRB via the online iStar system located at:: https://istar-chla.usc.edu/istar/. Follow-up reports on SAE's should also be submitted to the USC IRB via the iStar system.

8.5.2 Reporting procedures

For all serious adverse events, the investigator must complete the Medwatch Report Form in English and SAE fax cover sheet, assess the relationship to study treatment, and send both of the completed forms, by fax, to:

- Novartis Clinical Safety & Epidemiology (CS&E) Department **1.888.299.4565**
- Novartis Medical Affairs Department **1.973.781.7453**

The Investigator must also ensure that the forms are accurately and fully completed. The original and the duplicate copies of the Medwatch Form, SAE cover sheet, and the fax confirmation sheet must be kept with the case report forms at the study site.

Follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or discontinued study participation. The form and fax confirmation sheet must be retained. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, and the presence or absence of any congenital abnormalities or birth defects.

8.5.3 Reporting Guidelines

Actions towards FDA:

For Investigator Sponsored IND Studies, some additional reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR § 600.80.

Events meeting the following criteria need to be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

***7 Calendar-Day Telephone or Fax Report*:**
The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of EPO906. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be telephoned or faxed to the FDA and Novartis within 7 calendar days of first learning of the event. Each telephone call or fax transmission (see fax number below) should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research.

***15 Calendar-Day Written Report*:**

The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of EPO906. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted the FDA, Novartis, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500 Form but alternative formats are acceptable (e.g. summary letter).

FDA fax number for IND Safety Reports:

1 (800) FDA 1078 Include IND # 69,334 on the report

All written IND Safety Reports submitted to the FDA by the Sponsor-Investigator must also be submitted to the USC IRB via istar and to Novartis at the FAX numbers listed above.

Additional information may be added to a previously submitted report by any of the following methods:

Adding to the original MedWatch 3500 report and submitting it as follow-up
Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500 form
Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

8.5.4 IND Annual Report

The principal investigator is responsible for preparing and submitting the IND annual reports to the FDA (CFR312.33) within 60 days of the anniversary date that the IND went into effect. All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Novartis. This report will include all adverse events in all patients.

9.0 STUDY CALENDAR

Celebrex will be taken p.o. b.i.d. daily, starting one week prior to the first infusion of EPO906 (cycle one). Celebrex will be taken p.o., bid, daily on all days but Day 1 of each cycle (Celebrex will held on the days EPO906 is infused for all cycles). EPO906 will be administered intravenously on Day 1 of each cycle. One cycle is considered 3 weeks, however the first cycle will be four weeks due to the one week pre-loading of Celebrex.

	Each Cycle						
Parameter	**Pre-Treatment[7]**	**Day -7**	**Day 1**	**Day 8**	**Day 15**	**End of Study**	**Every 2 Cycles**
History & Physical Exam	X		X	X[13]		X	
Toxicity Assessment	X		X	X[13]	X[13]	X	
Weight, Performance Status	X		X			X	
WBC(differential), Hgb, Platelets[6]	X		X	X		X	
Electrolytes, MG, BUN, Cr	X		X	X		X	
LFT (SGOT and Total Bili)	X		X			X	
Cholesterol	X						
Diarrhea Assessment[9]	X		X	X	X	X	
CEA and/or other markers	X		X			X	
EKG	X					X[4]	
CXR	X[12]						
Serum Pregnancy Test	X[11]						
Tumor Biopsy	X[5]					X[10]	
Radiology or Scans for Tumor Assessment[2]	X						X[2]
Pharmacogenomics/ Biomarker [3]	X		X[8]			X	
Celebrex Administration*		Celebrex administration should start one week prior to Cycle 1 (given Day -7 through Day -1). Celebrex should not be given on Day 1 of each cycle (should not be given on days of EPO906 infusion), thus it should be given days 2-21 of each cycle.					
EPO906 Administration**			X				

1 If used to follow measurable disease.
2 If measurable disease is present.
3 Two purple top tube blood samples , for Lenz laboratory. At baseline two additional tubes should be drawn – one red top and one purple top. This is mandatory for Phase II patients.
4 As clinically indicated.
5 If paraffin embedded tissue is not available, if feasible (ct guided or colonoscopy) and patient consents.
6 Increase to 3 times weekly if Grade 4 neutropenia occurs on day 7 or 14.
7 Pretreatment lab work should be done within 72 hours and no labs are necessary on day 1.
8 All Cycles but Cycle 1
9. Diarrhea will be assessed weekly through a patient diary attached as Appendix 3. Assessment should begin 7 days prior to Day 1 of Cycle 1.
10. If patient consents and the biopsy is feasible (ct guided or colonoscopy).
11. For women of childbearing potential.
12. If other imagining of the chest is done (CT/MRI), CXR is not necessary
13 Cycle 1 only
* Celebrex administered orally twice per day starting one week prior to Cycle 1, held on Days EP0906 is given (Day 1 of every cycle) and orally twice a day thereafter. .
** EPO906 administered IV on Day 1 of each 21-day cycle

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

All patients who are registered will be accounted for in the report of the results. All patients who begin the first course of treatment will be included in the analysis of survival and time-to-failure.

The outcome status (in terms of toxicity, response, reason for withdrawal, progression, and survival) of all eligible patients will be reported. The Response Evaluation Criteria In Solid Tumors (RECIST) (version 1.1) and CTCAE version 3.0 toxicity criteria will be used.

10.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 2 cycles. In addition to a baseline scan, confirmatory scans should also be obtained ≤ 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

10.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with celebrex.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

10.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area will not be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

10.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination

unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in

trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

10.1.4 **Response Criteria**

10.1.4.1 **Evaluation of Target Lesions**

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD):	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

10.1.4.2 **Evaluation of Non-Target Lesions**

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

10.1.4.3 **Evaluation of Best Overall Response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

	Non-Target Lesions	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

10.1.5 **Duration of Response**

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

10.1.6 **Progression-Free Survival**

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

10.2 Other Endpoint Definitions

10.4.1 Overall Survival. Defined as the time from first day of treatment to time of death due to any cause. If a patient is still alive, survival time is censored at the time of last follow-up.

10.4.2 Time to progression. Defined as the time from first day of treatment to the first observation of disease progression or death due to disease. If failure has not occurred, failure time is censored at the time of last follow-up.

11.0 SPECIAL INSTRUCTIONS

We will request tumor samples which have been collected at the time of diagnosis or prior to entry into this protocol. We will ask for 10 unstained slides (10um thick) from the paraffin-embedded tumor sections for RNA and DNA analyses. We will also collect a purple and red

top blood sample prior to start of chemotherapy. Questions regarding tissue or blood collection should be referred to Dana Agafitei at 323 865 0467.

11.1 Handling of blood samples for USC Laboratory

2 purple top Blood samples should be sent within 2 hours to Dr. Lenz Laboratory at room temperature. In addition, at baseline only, two additional tubes should be drawn – one red top and one purple top, which should also be sent within 2 hours to the Lenz Lab at room temperature.: Questions should be referred to Dana Agafitei or Dr. Zhang.

Dr. Wu Zhang Norris 5th Floor: 5410, Tel 323 865 0572.

11.2 Handling of the tumor tissues

Tumor samples should be sent to Dana Agafitei:
Unstained Paraffin embedded tumor section 10x 10um thick containing tumor and normal tissue should be send by room temperature to Dana Agafitei. A tumor block (paraffin embedded tumor tissues) can be used instead of sending the unstained slides. Questions should be referred to Dana Agafitei.

Dana Agafitei
USC/Norris Comprehensive Cancer Center
CISO office 7th floor
1441 Eastlake Ave
Los Angeles, CA 90033
Tel: 323 865 0467

11.3 Experimental Methods and Procedures

11.3.1 Polymorphism: We will isolate genomic DNA from whole blood samples. An approximate 200bp region of the COX gene containing the polymorphic site will be PCR amplified using a P-33 end-labeled forward primer and an unlabeled reverse primer. PCR products will be separated on sequencing gels and autoradiographed, and products will be sized by comparison to a known genotypes. We will also collect serum to allow tumor DNA analysis for gene discussed in this proposal.

11.3.2 Quantitation of mRNA levels: The cDNA library created from each tumor as part of this technology (RT-PCR) contains a quantitative and qualitative record of all of the tumor's expressed genes, and mRNA quantitation as well as mutations screening of 30-40 expressed genes is possible with material obtained from an average tumor biopsy. We have successfully quantitated gene expression in specimens of less than 1 mg and fine needle biopsies only by changing the PCR conditions. Quantitative RT-PCR is carried out essentially as previously described in with some modifications (29-35). The choice of the internal standard is critical for obtaining meaningful results with PCR quantitation. This gene should be one that is expressed constitutively with a level of per cell expression that is constant among different tissues or at least in similar tissues from different individuals.

12.0 STATISTICAL CONSIDERATIONS

This is a Phase I/II study to determine MTD and pharmacokinetics of EPO906 in combination with Celebrex in patients with metastatic colorectal cancer who have failed 5-FU, CPT-11 and/or Oxaliplatin based chemotherapy and to assess the response rate to this regimen, time to progression, and survival in those patients. This study will also evaluate whether Celebrex can reduce the incidence of diarrhea associated with EPO906 administration. In addition, this study is to obtain preliminary data on molecular correlates to determine clinical efficacy and toxicity with this regimen.

12.1 Phase I trial

The standard NCI 3+3 design is used in the Phase I portion of this study. DLT and MTD are defined in the section 6.1. Dose escalation schedule and rules are defined in the section 6.2.

12.2 Phase II trial

A two-stage Phase II portion of this trial will use 9.0 mg/m2 as the dose of EPO906. This will assess activity of the regimen and initial estimates of the tumor response rate. With 37 evaluable patients the response rate can be estimated with a standard error of no more than +/- 8.2%. A detailed explanation of the two-stage design can be found in section 6.3.

12.3 Accrual

This study is expected to accrue a minimum of 6+11 evaluable patients and a maximum of 18+37. It should take approximately 24 months to complete this trial.

12.4 Monitoring for Excessive Toxicity in the Phase II portion of this study

Although we expect this regimen to be well tolerated, the guidelines listed below will be used to raise a flag if the number of patients who experience unacceptable toxicity is large enough to strongly suggest that the true probability of unacceptable toxicity is > 20%. The rules given below will trigger such a review and are based on the sequential probability ratio test with theoretical parameters set (α=0.10, β=0.10, p_o=0.20, p_a=0.40). Once the MTD is established, the guideline will be only applied to the Phase II portion of this study. Unacceptable toxicity will be defined as in section 8.4.

To be evaluable for excessive toxicity a patient must complete a minimum of 2 courses of treatment or have experienced unacceptable toxicity. Patients who do not complete 2 courses and who do not experience any unacceptable toxicity will not be used in the decision to continue or suspend accrual to the trials, for reasons for excessive toxicity. Every time unacceptable toxicity is observed, the number of patients (X) who have experienced unacceptable toxicity will be compared to the number of patients (N) who are evaluable for excessive toxicity. If the number of patients, N, is greater than N_X, the number given in column 2 of the Table 1, below, then accrual will not be suspended. If N is less than or equal to N_X, then accrual will be suspended for review of the data.

Table 1: Criteria for Continuing Accrual

X = Total Number of Patients with Unacceptable Toxicity	N_X: Suspend the Trial if Number of Evaluable Patients Is Less Than or Equal to:

4	≤ 6
5	≤ 9
6	≤ 12
7	≤ 16
8	≤ 19
9	≤ 23
10	≤ 26
11	≤ 29
12	≤ 33
13	≤ 36
14	suspend

Using this rule with 37 patients, the probability of correctly suspending this regimen for review toxicities is 0.82 if the true chance of unacceptable toxicity is 40% or greater. The probability of falsely suspending this regimen for review toxicities is 0.069, if the true chance of unacceptable toxicity is ≤ 20%. Estimations of the probabilities of suspending this regimen are based on 10,000 simulations.

Table 2: Probability of Suspending Accrual Because of Excessive Unacceptable Toxicities					
True Chance of Unacceptable Toxicity	**15%**	**20%**	**35%**	**40%**	**45%**
Probability of Suspending Accrual to Review Toxicities	0.018	0.069	0.64	0.82	0.93

12.5 Clinical Endpoints**.**

The outcome status (in terms of toxicity, response, reason off study, time to progression, and survival) of all eligible patients who are registered, will be reported. Patients who complete 3 weeks of therapy or who experience any Grade 2 or greater toxicity will be included in the analysis of toxicity. Patients with measurable tumor who complete 6 weeks of therapy, or who terminate treatment for reasons of toxicity or progression, will be included in analysis of tumor response. All eligible patients who begin treatment will be included in the analysis of survival and progression-free survival.

12.5.1 **Toxicities** observed at each dose level will be summarized in terms of type (organ affected, laboratory determination), severity (by CTC and nadir or maximum values for the laboratory measures), time of onset, duration, and reversibility or outcome. Tables will be created to summarize these toxicities and side effects by dose and by month of therapy. Baseline information (e.g. the extent of prior therapy, extent of disease) and demographic information will be presented to describe the patients treated in this study.

12.5.2 **Diarrhea** observed during Phase II portion of this study will be summarized in terms of grade (all grades, and Grade 3-4), course of treatment, duration, and reversibility based on CTCAE version 3.0 and a diary of diarrhea (see Appendix 3 for details). Tables will be constructed to evaluate whether the incidence of diarrhea is less than the previously reported incidence. Exact binomial probabilities will be calculated to examine whether the incidence of diarrhea is lower than previously reported rates (80% in all grades) during the 1st two courses of treatment. Time to occurrence of the first Grade 2+ diarrhea will be summarized with Kaplan-Meier plots to describe patterns of Grade 2+ diarrhea onset and the effect of medications for diarrhea taken before the start of treatment among patients treated on this regimen.

12.5.3 **Tumor response** will be assessed using the RECIST criteria after 6 weeks of Celebrex and EPO906. Patients who receive less than 6 weeks of treatment due to unacceptable toxicity or progressive disease will still be evaluable for response. All responses will be reported. Response rates will be calculated as the percent of evaluable patients whose best response is a CR or PR, and exact 95% confidence intervals will be calculated for this estimate. Exact binomial probability will be calculated to determine whether the response rate in this regimen is statistically significantly greater than 5% in traditional chemotherapy.

12.5.4 Overall **survival and time-to-progression** will be summarized with Kaplan-Meier plots to describe the outcome of patients treated on this protocol.

12.6 Molecular Studies

12.6.1 Analysis of Molecular Markers (gene expression and genomic polymorphisms): **Baseline** gene expression levels of (1) the target genes (ERCC-1, COX-2, Beta tubulins, for EPO906 and COX-2, IL10 for Celebrex), (2) genes associated with induction of apoptosis (bcl-2, bax), and (3) cell cycle regulatory genes (p53, p21, p27) – in the tumor and blood cells and plasma– will be summarized overall and according to dose, response and toxicity (if numbers permit), using medians, quartiles and ranges – or if a transformation is found to render the data compatible with the normal assumptions, with means, standard deviations, and confidence intervals. The relationship between dose and gene expression levels will be assessed using plots and ANOVA or a Kruskal-Wallis test (if the normal assumptions are violated). The association with progression-free survival or overall survival will be assessed by dichotomizing the measures of gene expression at the median (or by previously established cut-points) and constructing Kaplan-Meier plots.

Association of types of polymorphisms for genes involved in the metabolic pathways (beta tubulins, COX-2) will be summarized overall and by response and presence of toxicity, using contingency tables and percentages. The association between the gene expression and the polymorphism will be summarized using medians, quartiles and ranges – or if a transformation is found to render the data compatible with the normal assumptions, with means, standard deviations, and confidence intervals. The association with progression-free survival or overall survival will be assessed by constructing the Kaplan-Meier curves according to the polymorphisms observed.

These analyses of the molecular biomarkers and scans will be descriptive and exploratory, in order to identify questions and patterns for future study; the numbers of patients expected (nearly 100% for the specimens obtained at baseline and once after start of treatment, and probably about 12-18 at the time of progression) will not permit formal comparisons of patients according to toxicity and response, but will allow us to estimate the patient-to-patient variability in this well-defined group of patients, as well as the change at the time of progression. These analyses will be descriptive in nature.

Where appropriate, data from the above studies will be submitted to the Cancer Center Statistical Center for determination of any significance. Correlation between outcome and these molecular markers are purely exploratory but may

serve to provide initial impetus to further investigations on the possible prognostic role for these markers in tumor response to chemotherapy.

12.6.2 **Data Analysis of Novartis biomarker exploratory development**

A mass spectrometry (MS) method such as SELDI will be utilized for these biomarker discovery studies. The purpose is to find correlations between differentially expressed peptides/proteins and EPO906 laboratory and clinical effects. If the specific proteins can be identified following protein purification, we would then use an ELISA assay or related method to confirm if the proteins are differentially expressed. Since we have only recently initiated proteomics studies in EPO906 single agent studies and because this is the first clinical study of this drug combination, no specific proteins for statistical analysis can be listed at this time. Therefore, the analyses of the biomarkers will be descriptive and exploratory in order to identify putative markers and MS patterns for future study. As with the RNA expression studies described above, the numbers of patients expected (nearly 100% for the specimens obtained at baseline and once after start of treatment, and probably about 12-18 at the time of progression) will not permit formal comparisons of patients according to toxicity and response

12.7 Justification of Study Design in Phase II Portion

Using two-stage design with 18+19 (a total of 37) patients, we will have 90% power to recommend this regimen for further study if the true response rate is 15%, and have 3.3% chance (Type I error) to recommend this regimen for further study if the true response rate is 2%. The probability of ending the trial during stage I and rejecting the null during stage II by the true response rate is listed in Table 3. Calculations of power and the probability of Type I error and early stopping are based on exact binomial probabilities.

Table 3 the probability of ending the trial during stage I and rejecting the null during stage II using the two-stage design of the phase II portion by the true response rate (RR)

RR	Probability of early stopping at stage I	Probability of rejecting the null at stage II
3%	0.578	0.088
4%	0.480	0.165
5%	0.397	0.255
10%	0.150	0.685
11%	0.123	0.746
12%	0.100	0.797
13%	0.082	0.839
20%	0.018	0.973

The chance of stopping accrual at stage I is high and rejecting the null at stage II is low when the response rate is 3% or lower, and the chance of early stopping is low and rejecting the null at stage II is high when response rate is 12% or higher using the current design.

Assessment of incidence of diarrhea of Grade 1-4 will be performed in the end of this trial. We will have 77% power to detect a reduction in the incidence of Grade 1-4 diarrhea compared to the historical rate (≥ 80%) if the true incidence of Grade 1-4 diarrhea is 60% using a one-sided 0.05-level exact test.

13. PROTOCOL AMENDMENTS

Any change or addition (excluding administrative) to this protocol requires a written protocol amendment that must be approved by Novartis and each investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study require additional approval by the IRB at each center. A copy of the written approval of the IRB must be provided to Novartis. Examples of amendments requiring such approval are:

- increases in drug dose or duration of exposure of subjects
- significant changes in the study design (e.g. addition or deletion of a control group),
- increases in the number of invasive procedures
- addition or deletions of a test procedure required for monitoring of safety.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by Novartis in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons Novartis must be notified and the IRB at the center must be informed immediately. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB must be kept informed of such administrative changes.

14.0 REGISTRATION GUIDELINES

This study will be conducted at the hospitals affiliated with the USC/Norris Comprehensive Cancer Center. All patients will be registered with the USC/Norris Comprehensive Cancer Center Clinical Investigations Support Office (CISO).

To register a patient, the research nurse or data manager must complete the eligibility form and the registration form and give (or FAX) copies to the Data Manager. After verifying the eligibility, the Data Manager will assign a study number, assign a dose, and register the patient onto the study.

At the time of registration, three copies of the signed and dated patient Informed Consent form with the Human Rights must be available (an original for the patient's medical chart, one copy for the patient, and one copy for Clinical Investigations Support Office.

15.0 RECORDS TO BE KEPT AND DATA SUBMISSION SCHEDULE

15.1 Confidentiality of Records: the original data collection forms will be stored in secure file cabinets in the CISO.

15.2 Patient Consent Form: At the time of registration, three signed and dated copies of the patient Informed Consent form with the Human Rights must be available (for patient, chart, and Clinical Investigations Support Office.

15.3 Registration Eligibility Worksheet: At the time of registration, the information requested on the On-Study/Eligibility Form will be submitted along with the Informed Consent to the Quality Assurance Monitor at CISO.

16.0 MINORITIES AND WOMEN STATEMENT

Patients of both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria outlined in Section 5.0. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to accrue a representative sample. However, since this is a Phase I trial, considerations for patient safety and a reluctance to expose patients either to a potentially toxic and/or ineffective treatment, will limit the total number of patients entered. If differences in outcome appear to be associated with gender or ethnic identity, then a follow-up study will be designed to investigate those differences more fully.

ETHNIC AND GENDER DISTRIBUTION OF NEWLY DIAGNOSED CANCER PATIENTS(1) IN LOS ANGELES COUNTY IN 1999(2)

Primary Site of Tumor	Total # of Patients	Males %	Females %	White %	Black %	Hispanic %	Asian/Other %
ALL INVASIVE TUMORS	**33,194**	**49**	**51**	**61**	**12**	**18**	**10**
ORAL CAVITY/PHARYNX	**749**	**66**	**34**	**64**	**13**	**12**	**11**
DIGESTIVE SYSTEM	**6,727**	**52**	**48**	**56**	**12**	**18**	**13**
Esophagus	294	72	28	61	13	17	10
Stomach	823	60	40	41	12	27	21
Colon	2,677	49	51	62	14	14	11
Rectum/Anus	547	49	51	63	10	17	10
Liver	479	66	34	34	10	29	27
Pancreas	718	50	50	63	11	18	9
RESPIRATORY SYSTEM	**4,341**	**56**	**44**	**64**	**15**	**11**	**10**
Lung and Bronchus	3,949	54	46	65	15	11	10
BONES AND JOINTS	**72**	**58**	**42**	**43**	**11**	**33**	**13**
SOFT TISSUE INCL. HEART	**223**	**54**	**46**	**49**	**14**	**27**	**9**
MELANOMAS OF THE SKIN	**1,101**	**56**	**44**	**91**	**1**	**7**	**1**
BREAST	**5,489**	**1**	**99**	**63**	**11**	**16**	**11**
FEMALE GENITAL SYSTEM	**2,334**	**0**	**100**	**54**	**9**	**26**	**11**
Cervix Uteri	573	0	100	35	10	45	10
Corpus Uteri	954	0	100	63	10	18	9
Ovary	684	0	100	56	8	23	13
MALE GENITAL SYSTEM	**5,404**	**100**	**0**	**59**	**16**	**18**	**7**
Testis	195	100	0	54	2	41	4
Prostate	5,176	100	0	59	17	17	7
URINARY SYSTEM	**1,549**	**65**	**35**	**65**	**11**	**18**	**7**
Invasive Bladder	656	73	27	73	8	12	8
Renal	830	59	41	58	13	23	7
EYE AND ORBIT	**59**	**63**	**37**	**66**	**7**	**24**	**3**
BRAIN /NERVOUS SYSTEM	**481**	**55**	**45**	**61**	**8**	**24**	**6**
ENDOCRINE/THYROID	**656**	**26**	**74**	**53**	**6**	**26**	**15**
HODGKIN'S DISEASE	**245**	**49**	**51**	**53**	**10**	**33**	**4**
NON-HODGKIN'S LYMPHOMA	**1,430**	**55**	**45**	**62**	**8**	**20**	**10**
MULTIPLE MYELOMA	**408**	**57**	**43**	**49**	**23**	**20**	**8**
LEUKEMIA	**841**	**61**	**39**	**56**	**8**	**26**	**9**
Lymphocytic Leukemia	330	60	40	55	10	31	5
Non-Lymphocytic Leukemia	511	61	39	57	8	23	12
***IN SITU* DISEASE**							
in situ Breast	1,063	1	99	67	10	12	11
in situ Bladder	648	77	23	78	6	8	8
in situ Melanoma	658	54	46	92	1	6	1

(1) Invasive cancer with the inclusion of specified *in situ* breast, melanoma and bladder cancer cases.

(2) Data provided by Los Angeles County Cancer Surveillance Program; Department of Preventive Medicine; University of Southern California; 1540 Alcazar St.; LA CA 90033.

17.0 ETHICAL AND REGULATORY CONSIDERATIONS

17.1 All patients will have signed an informed consent for participation in research activities in accord with all institutional, NCI and Federal regulations, and will have been given a copy of the Experimental Subject's Bill of Rights.

18.0 PATHOLOGY REVIEW

All patients will have advanced malignancy confirmed by review of their biopsy specimens by the Division of Pathology of the University of Southern California/LA County or Norris Comprehensive Cancer Center.

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Appendix 1: Drugs known to be metabolized by CYP450 isoenzymes 2D6 and 3A4

CYP2D6	
Substrates	
Amitriptyline (hydroxylation)	Methamphetamine
Amphetamine	Metoclopramide
Betaxolol	Metoprolol
Bisoprolol	Mexitetine
Brofaromine	Mianserin
Buturolol	Meperidine
Bupropion	Methadone Mirtazapine (hydroxylation)
Captopril	Molindone
Carvedilol	Morphine
Cevimeline	Nortriptyline (hydroxylation)
Chlorpheniramine	Olanzapine (minor, hydroxymethylation)
Chlorpromazine	Ondansetron
Cinnarizine	Orphenadrine
Clomipramine (hydroxylation)	Oxycodone
Clozapine (minor pathway)	Papaverine
Codeine (hydroxylation, o-demelhylation)	Paroxetine (minor pathway)
Cyclobenzaprine (hydroxylation)	Penbutolol
Cyclophosphamide	Pentazocine
Debrisoquin	Perhexiline
Delavirdine	Perphenazine
Desipramine	Phenformin
Dexfenfluramine	Pindolol
Dextromethorphan (o-demethylation)	Promethazine
Dihydrocodeine	Propafenone
Diphenhydramine	Propranolol
Dolasetron	Quetiapine
Donepezil	Remoxipride
Doxepin	Risperidone
Encainide	Ritonavir (minor)
Fenlluramine	Ropivacaine
Flecainide	Selegiline
Fluosetine (minor pathway)	Sertindole
Fluphenazine	Sertratine (minor pathway)
Haiofantrine	Sparteine
Haioperidol (minor pathway)	Tamoxifen
Hydrocodone	Thioridazine
Hydrocortisone	Tiagabine

CYP2D6	
Hydroxyamphetamine	Timolol
Imipramine (hydroxylation)	Tolterodine
Labetalol	Tramadol
Loratadine	Trazodone
Maprotiline	Trimipramine
m-Chlorophenylpiperazine (m-CPP)	Tropisetron
	Venlafaxine (o-desmethylation)
	Yohimbine

Inhibitors	
Amiodarone	Methadone
Celecoxib	Mibefradil
Chloroquine	Moclobemide
Chlorpromazine	Nortluoxeline
Cimelidine	Paroxetine
Citalopram	Perphenazine
Clomipramine	Propafenone
Codeine	Quinacrine
Deiavirdine	Quinidine
Desipramine	Ranitidine
Dextropropoxyphene	Risperidone (weak)
Diltiazem	Ritonavir
Doxorubicin	Sertindole
Entacapone (high dose)	Sertraline (weak)
Fluoxetine	Thioridazine
Fluphenazine	Vaiprolc acid
Fluvoxamine	Venlafaxine (weak)
Haloperidol	Vinblastine
Labetalol	Vincristine
Lobeline	Vinorelbine
Lomustine	Yohimbine

CYP3A3/4	
Substrates	
Acetaminophen	Chlorpromazine
Alfentanil	Cimetidine
Alosetron	Cisapride
Alprazolam	Citalopram
Amiodarone	Clarithromycin
Amitriptyline (minor)	Clindamycin
Amlodipine	Clomipramine
Anastrozole	Clonazepam
Androsterone	Clozapine
Antipyrine	Cocaine
Astemizole	Codeine (demethylation)
Atorvastatin	Cortisol
Benzphetamine	Cortisone
Bepridil	Cyclobenzaprine (demethylation)
Bexarotene	Cyclophosphamide
Bromazepam	Cyclosporine

CYP3A3/4

Bromocriptine
Budesonide
Bupropion (minor)
Buspirone
Busulfan
Caffeine
Cannabinoids
Carbamazepine
Cevimeline
Cerivastatin
Digitoxin
Diltiazem
Disopyramide
Docetaxel
Dolasetron
Donepezil
Doxorubicin
Doxycycline
Dronabinol
Enalapril
Erythromycin
Estradiol
Ethinyl estradiol
Ethosuximide
Etoposide
Exemestane
Dofetilide (minor)
Felodipine
Fentanyl
Fexofenadine
Finasteride
Fluoxetine
Flutamide
Glyburide
Granisetron
Halofantrine
Hydrocortisone
Hydroxyarginine
Ifosfamide
Imipramine
Indinavir
Isradipine
Dapsone
Dihydroepiandrostendione
Delavirdine
Des-methyldiazepam
Dexamethasone
Dextromethorphan (minor, N-demethylation)
Diazepam (minor; hydroxylation, N-demethylation)

Nefazodone
Nelfinavir
Nevirapine
Nicardipine
Nifedipine
Niludipine
Nimodipine
Nisoldipine
Nitrendipine
Omeprazole (sulfonation)
Ondansetron
Oral contraceptives
Orphenadrine
Paclitaxel
Pantoprazole
Pimozide
Pioglitazone
Pravastatin
Prednisone
Progesterone
Proguanil
Propafenone
Quercetin
Quetiapine
Quinidine
Quinine
Repaglinide
Retinoic acid
Rifampin
Risperidone
Ritonavir
Salmeterol

CYP3A3/4	
Itraconazole	Saquinavir
Ketoconazole	Sertindole
Lansoprazole (minor)	Sertraline
Letrozole	Sibutramine
Levobupivacaine	Sildenafil citrate
Lidocaine	Simvastatin
Loratadine	Sirolimus
Losartan	Sufentanil
Lovastatin	Tacrolimus
Methadone	Tamoxifen
Mibefradil	Temazepam
Miconazole	Teniposide
Midazolam	Terfenadine
Mifepristone	Testosterone
Mirtazapine (N-demethylation)	Tetrahydrocannabinol
Montelukast	Theophylline
Navelbine	Tiagabine
	Tolterodine
Toremifene	Vincristine
Trazodone	Warfarin (R-warfarin)
Tretinoin	Yohimbine
Triazolam	Zaleplon (minor pathway)
Troglitazone	Zatosetron
Troleandomycin	Zileuton
Venlafaxine (N-demethylation)	Ziprasidone
Verapamil	Zolpidem
Vinblastine	Zonisamide
Inducers	
Carbamazepine	Phenytoin
Dexamethasone	Primidone
Ethosuximide	Progesterone
Glucocorticoids	Rifabutin
Griseofulvin	Rifampin
Nafcillin	Rofecoxib (mild)
Nelfinavir	St John's wort
Nevirapine	Sulfadimidine
Oxcarbazepine	Sulfinpyrazone
Phenobarbital	Troglitazone
Phenylbutazone	
Inhibitors	
Amiodarone	Ketoconazole

CYP3A3/4	
Anastrozole	Metronidazole
Azithromycin	Mibefradil
Cannabinoids	Miconazole (moderate)
Cimetidine	Nefazodone
Clarithromycin	Nelfinavir
Clotrimazole	Nevirapine
Cyclosporine	Norfloxacin
Danazol	Norfluoxetine
Delavirdine	Omeprazole (weak)
Dexamethasone	Oxiconazole
Diethyldithiocarbamate	Paroxetine (weak)
Diltiazem	Propoxyphene
Dirithromycin	Quinidine
Disulfiram	Quinine
Entacapone (high dose)	Quinupristin and dalfopristin
Erythromycin	Ranitidine
Ethinyl estradiol	Ritonavir
Fluconazole (weak)	Saquinavir
Fluoxetine	Sertindole
Fluvoxamine	Sertraline
Gestodene	Troglitazone
Grapefruit juice	Troleandomycin
indinavir	Valproic acid (weak)
isoniazid	Verapamil
itraconazole	Zafirlukast
	Zileuton

Adapted from Cytochrome P-450 Enzymes and Drug metabolism. In : Lacy CF, Armstrong LL, Goldman MP, Lance LL eds. Drug Information Handbook 8th ed. Hudson, OH; LexiComp Inc. 2000: 1364-1371

Appendix 2 EPO906 Diarrhea Management AlgorithmEPO906 Diarrhea Management Algorithm

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Diarrhea management[1,2]

NCI CTC grading of diarrhea for patients with and without colostomy

DIARRHEA GRADING according to NCI CTC version 2.					
Toxicity	**0**	**1**	**2**	**3**	**4**
Diarrhea Patients without colostomy:	None	increase of < 4 stools/day over pre-treatment	increase of 4-6 stools/day, or nocturnal stools	increase of ≥ 7 stools/day or incontinence; or need for parenteral support for dehydration	physiologic consequences requiring intensive care; or hemodynamic collapse
Patients with a colostomy:	None	mild increase in loose, watery colostomy output compared with pre-treatment	moderate increase in loose, watery colostomy output compared with pre-treatment, but not interfering with normal activity	severe increase in loose, watery colostomy output compared with pretreatment, interfering with normal activity	physiologic consequences, requiring intensive care; or hemodynamic collapse

History of diarrhea

- Review previous medical history of diarrhea within the last 12 months (use of laxatives, CID agents like 5-FU, CPT-11 etc, colon surgery, abdominal and pelvic irradiation, nocturnal diarrhea, pain)
- Patient suffering from diarrhea at study screening are usually excluded (consult protocol)
- Stop all diarrheogenic agents at screening if possible, otherwise exclude from trial
- Instruct patients regarding risk of developing diarrhea
- Perform baseline clinical / laboratory studies according to the trial protocol (e.g. one could rule out carrier state of Salmonella spp., Clostridium difficile, Campylobacter spp., Giardia, Entamoeba, Cryptosporidium which can lead to opportunistic infections in immunosuppressed patients)
- Explain the frequency of diarrhea and its relationship to CTC grading

Proactively look for occurrence of diarrhea

- Call patients at home 3 times a week after start of EPO906 treatment to detect diarrhea early
- Check the appropriate website 3 times a week when electronic diaries are used in the trial (according to the particular trial protocol)

First report of diarrhea

- Obtain history of onset and duration of diarrhea

- Description of number of stools and stool composition (eg. watery, blood, mucus in stool)
- Assess patient for fever, abdominal pain, cramps, distension, bloating, nausea, vomiting, dizziness, weakness (ie, rule out risk for sepsis, bowel obstruction, dehydration)
- Medication profile (ie, to identify any diarrheogenic agents)
- Dietary profile (ie, to identify diarrhea-enhancing foods)

Management

- Stop all lactose-containing products, alcohol
- Stop laxatives, bulk fiber (Metamucil, Procter & Gamble), and stool softeners(docusate sodium; Colace, Roberts)
- Drink 8 to 10 large glasses of clear liquids per day (water, Pedialyte [Ross],Gatorade [Quaker], broth)
- Eat frequent small meals (bananas, rice, applesauce, Ensure, toast)
- Stop high-osmolar food supplements such as Ensure Plus and Jevity Plus (with fiber)

Treatment of diarrhea grade 1 or 2

- Diarrhea grade 1 or 2 will be treated with **standard loperamide** (initial at first administration 4 mg, then 2mg every 4 hours or after each unformed stool). 12-24 hours later: If a patient experiences Grade 2 or higher diarrhea they will be seen by the treating physician weekly until it resolves to at least Grade 1.

Diarrhea resolved

- **Continue instructions for dietary modification**
- Gradually add solid foods to diet
- Discontinue loperamide after 12-hours diarrhea-free interval

Diarrhea unresolved

- Persisting diarrhea grade 1 or 2 will be treated with **high dose loperamide** (initial 4 mg, then 2mg every 2 hours) with monitoring of patients condition to rule out dehydration, sepsis, ileus) medical check and selected workup if patient does not need hospitalization (see section Diarrhea workup and additional test in the particular trial protocol). Observe patient for response to antidiarrheal treatment.
- Persisting diarrhea grade 3 or 4 will be treated with **hospitalization**, **high dose loperamide** (initial 4 mg, then 2mg every 2 hours) and addition of **opium tincture or dihydrocodeine tartrate tablets / injections**, start of i.v. fluids and antibiotics as needed with monitoring of patients condition (to rule out dehydration, sepsis, ileus) medical check and workup (see section Diarrhea workup and additional test in the particular trial protocol). Observe patient for response.
- After 12-24 hours:

Diarrhea resolved

- Continue instructions for dietary modification
- Gradually add solid foods to diet
- Discontinue loperamide and / or other treatment after 12-hours diarrhea-free interval

Diarrhea unresolved

- If diarrhea still persisting (NCI CTC grades 1 and 2), after 2x 24 hours with low and high dose loperamide then admit to hospital and employ measures as for grade 3 and 4 until diarrhea resolved
- If diarrhea still persisting and progressed to NCI grades 3 and 4, employ measures described below.

Treatment of diarrhea grade 3 or 4

- Severe diarrhea grade 3 or 4 will be treated with **hospitalization**, **high dose loperamide** (initial 4 mg, then 2mg every 2 hours and addition of **opium tincture or dihydrocodeine tartrate tablets / injections**, start of i.v. fluids and antibiotics as needed with monitoring of patients condition (to rule out dehydration, sepsis, ileus) medical check and workup (see section Diarrhea workup and additional test in the particular trial protocol). Observe patient for response.

After 12-24 hours:

- If diarrhea persisting administer s.c. **Sandostatin / octreotide** (100-500 µg tid)
- Continue i.v. fluids and antibiotics as needed
- If diarrhea grade 3 or 4 still persists patients should receive opium tincture or dihydrocodeine tartrate injections s.c. or i.m.
- If diarrhea grade 3 or 4 is still persisting s.c. **Sandostatin / octreotide** (500-1000 µg tid) should be administered.
- All patients experiencing grade 3 or 4 diarrhea should be placed on a quinolone antibiotic until the diarrhea resolves to grade 1. If a grade 3 or higher diarrhea occurs patients should receive a quinolone antibiotic with subsequent cycles of EPO906 for 5 to 7 days prophylactically. Patients will be seen by the treating physician weekly until the diarrhea resolves to at least Grade 1.
- To control and/or resolve diarrhea, next cycle of treatment should be delayed by 1 or 2 weeks. Treatment should be continued only when diarrhea resolved.

Diarrhea workup[3]

Additional assessments to be considered for hospitalized patients (*i.e., optional*), selected tests can be done in outpatients if deemed useful.

Spot stool analysis

- Collect stool separating it from urine (special containers, analysis immediately, exceptionally freeze samples)
- Blood
- pH (fresh stool)
- Osmolarity (fresh stool)
- Stool creatinine (rules out contamination by urine)
- Fecal leukocytes (Wright's staining and microscopy) or
- Fecal calprotectin - marker of intestinal inflammation[4]
- C. difficile toxin

- Fecal cultures including the tests mentioned in section **History of diarrhea** plus Shigella and pathogenic E. coli - enterotoxigenic, enterohemorrhagic etc., possibly Aeromonas, Plesiomonas (if suspected exposure to contaminated water)

Quantitative stool analysis

- Collect a 24 hour stool specimen and weigh, analyze immediately.
- Measure electrolytes and calculate osmotic gap[3] in stool water from the specimen (unabsorbed Na^+ and K^+ in the intestinal lumen cause secretory diarrhea), formula: 290-2([Na^+]+ [K^+]), suspected secretory diarrhea when osmotic gap <50mOsm/kg, suspected osmotic diarrhea when osmotic gap >125mOsm/kg

Bacterial overgrowth

- Culture of jejunal liquid
- Excretion of hydrogen in breath test in 15-30 minutes intervals after ingestion of 50-100 g glucose, 12-20ppm positive for bacterial overgrowth of small bowel and glucose fermentation

Endoscopic examinations

- Gastroscopy to obtain jejunal fluid - re. bacterial overgrowth for cultures and biopsy of proximal jejunum to assess extent of inflammatory jejunitis
- Sigmoidoscopy - re. assessment of colitis

References:

1. Adapted from Wadler S, Benson A, Engelking C, et al. Recommended guidelines for the treatment of chemotherapy induced diarrhea. J Clin Oncol 1998; 16: 3169-3178
2. Adapted from Kornblau S et al. Management of Cancer Treatment–Related Diarrhea: Issues and Therapeutic Strategies. Journal of Pain and Symptom Management 2000; 19: 118-129
3. Adapted from AGA Technical Review on the Evaluation and Management of chronic Dairrhea. Gastroenterol 1999; 116: 1464-1486
4. Adapted from Arnott DR et al. Review article: is clinical remission the optimum therapeutic goal in the treatment of Crohn's disease? Aliment Pharmacol Ther 2002; 16: 857-867

Appendix 3: Patient Diaries – Diarrhea Assessment

Patient Diary for Patients without ostomies

Patient Diary for Patients with ostomies

Date	Morning to Bedtime			Bedtime to Morning			Did Diarrhea interfere with your activities today?	Medications For Diarrhea* and Number of Times Taken per Day
	Number of Bowel Movements	Number of Times Abdominal Cramping	Number of Times Could not reach toilet before Bowel Movement	Number of Bowel Movements	Number of Times Abdominal Cramping	Number of Times Could not reach toilet before Bowel Movement		
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	

*Medications Include Loperamide (Imodium), Sandostatin (octreotide acetate) and opium tincture

Date	Morning to Bedtime			Bedtime to Morning			Did Diarrhea interfere with your activities today?	Medications For Diarrhea* and Number of Times Taken per Day
	Number of Times Emptied Ostomy Bag	Number of Times Abdominal Cramping	Number of Times Ostomy Bag Overflowed	Number of Times Emptied Ostomy Bag	Number of Times Abdominal Cramping	Number of Times Ostomy Bag Overflowed		
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	
							Yes ___ No ___	

*Medications Include Loperamide (Imodium), Sandostatin (octreotide) and opium tincture