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CANCER AND LEUKEMIA GROUP B

CALGB/SWOG C80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (CALGB IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

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This study is supported by the NCI Cancer Trials Support Unit (CTSU).
Institutions not aligned with CALGB will participate through the CTSU mechanism as outlined below and detailed in the CTSU logistical appendix.

Investigators must have a current affiliation with CALGB or CTSU to receive investigational agent and/or an Investigator's Brochure for this protocol.

- The **study protocol and all related forms and documents** must be downloaded from the protocol-specific Web page of the CTSU Members' side of the website located at https://www.ctsu.org
- Send completed **site registration documents** to the CTSU Regulatory Office. Refer to the CTSU logistical appendix for specific instructions and documents to be submitted.
- **Patient enrollments** will be conducted by the CTSU. Refer to the CTSU logistical appendix for specific instructions and forms to be submitted.
- Data management will be performed by the CALGB. **Case report forms** (with the exception of patient enrollment forms)**, clinical reports, and transmittals** must be sent to CALGB unless otherwise directed by the protocol. Do not send study data or case report forms to the CTSU Data Operations.
- **Data query and delinquency reports** will be sent directly to the enrolling site by CALGB. (generally via email but may be sent via fax or postal mail). Please send query responses and delinquent data to CALGB and do not copy the CTSU Data Operations. Query responses should be sent to CALGB via postal mail or fax (no transmittal form needs to accompany response). Each site should have a designated CTSU Administrator and Data Administrator and must keep their CTEP IAM account contact information current. This will ensure timely communication between the clinical site and the CALGB Statistical Center.

Investigators from SWOG must enroll patients through the CTSU.

The pharmacogenomic component of this study is conducted as part of the NIH Pharmacogenomics Research Network, which is funded through a separate U01 mechanism (see http://www.nigms.nih.gov/pharmacogenomics/research_net.html) for details

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Patient Eligibility

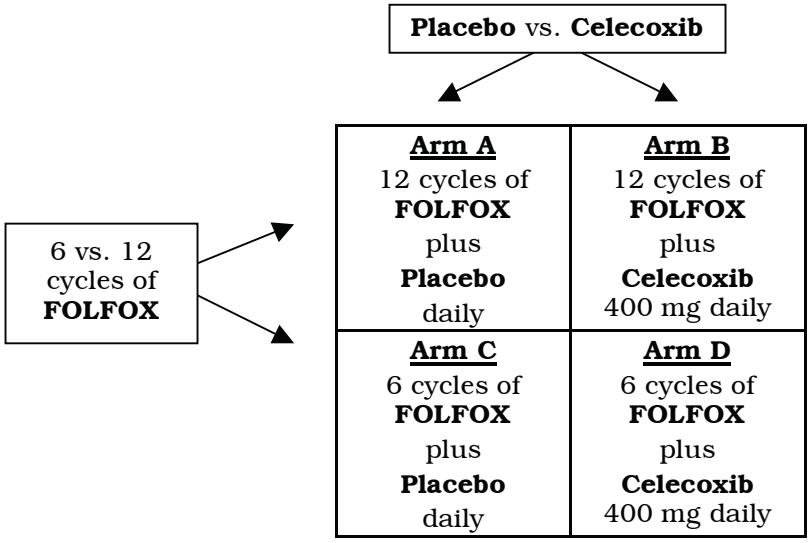
Histologically documented adenocarcinoma of the colon (see §4.1.1)
Completely resected tumors w/ R_o en bloc resection for tumors adherent to adjacent structures (see §4.1.2)
At least one pathologically confirmed positive lymph node (see §4.1.3)
No evidence of residual involved lymph node disease or metastatic disease at time of registration (see §4.1.4)
Patients with synchronous colon cancers are eligible but patients with synchronous colon and rectal primary tumors are not eligible (see §4.1.5)
Patients are ineligible if they use NSAIDs at any dose or aspirin at more than 325 mg at least three times per week, on average. Low-dose aspirin not exceeding 100 mg/day is permitted.
No previous or concurrent malignancy, except treated basal cell or squamous cell cancer of skin, treated in situ cervical cancer, treated lobular or ductal carcinoma in situ in one breast, or other cancer for which patient has been disease-free for ≥5 years (see §4.3)
No neurosensory or neuromotor toxicity ≥ grade 2
No known allergy to platinum compounds
No prior allergic reaction or hypersensitivity to sulfonamides, celecoxib or NSAIDs
No history of upper gastrointestinal ulceration, bleeding, or perforation within past 3 years
No symptomatic pulmonary fibrosis or interstitial pneumonitis
No cardiac risk factors including uncontrolled high BP (systolic > 150), unstable angina, history of documented MI or cerebrovascular accident, or NYHA Class III or IV heart failure
Non-pregnant and not nursing (see §4.10)
ECOG Performance Status: 0-2
Age ≥ 18 years

Required Initial Laboratory Values	
Granulocytes	≥ 1500/μL
Platelet Count	≥ 100,000/μL
Creatinine	≤ 1.5 x ULN
Bilirubin	≤ 1.5 x ULN

Schema

2 x 2 Factorial Randomization

1 Cycle = 14 Days



Celecoxib/placebo continued for a total of 3 years

Stratification for the duration of adjuvant chemotherapy randomization:
- Number of positive lymph nodes (1-3 vs. 4 or more)

Stratification for the celecoxib randomization:
- Number of positive lymph nodes (1-3 vs. 4 or more)
- Current regular low dose aspirin usage (Yes vs. No)

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1.0 INTRODUCTION

1.1 Colorectal cancer

In the United States, colorectal cancer is the fourth most common malignancy and the second most frequent cause of cancer-related death.[1] In 2008, an estimated 148,810 cases of colorectal cancer were diagnosed and 49,960 people died from this disease. Surgery is the primary modality of treatment for colorectal cancer, and a 'curative intent' resection occurs in 80-85% of patients with non-metastatic disease (stages I-III). Among patients with potentially curable colorectal cancer, pathologic stage (including depth of invasion in the bowel, involvement of regional lymph nodes and distant metastasis) is considered critical in determining prognosis and in whether treatment in addition to surgery is necessary. Overall, 35-40% of colorectal cancer patients have stage III disease at diagnosis (~55,000 people in the United States annually).[2] Of patients with stage III colon cancer, 40-70% will develop cancer recurrence (dependent on substage of stage III disease and other factors) despite curative-intent surgery and postoperative adjuvant chemotherapy.

1.2 Adjuvant therapy for colon cancer

Adjuvant therapy has evolved in the past two decades for stage III colon cancer. In 1990, a National Cancer Institute consensus conference recommended fluorouracil-based adjuvant therapy as standard of care for patients with resected stage III colon cancer based on trials demonstrating a statistically significant 40% improvement in disease-free survival and 33% improvement in overall survival.[3] A pooled analysis of stage III patients participating in seven adjuvant therapy clinical trials demonstrated that such chemotherapy increased the probability of remaining free of tumor recurrence after five years from 42 percent to 58 percent and the likelihood of five-year overall survival from 51 percent to 64 percent.[4]

1.3 Oxaliplatin

Oxaliplatin (trans-*l*-1,2-diaminocyclohexane oxalatoplatinum) is an antineoplastic platinum derivative with a 1,2-diaminocyclohexane [DACH] carrier ligand. Although the precise mechanism of action is unknown, platinum compounds are thought to exert their cytotoxic effects through the formation of DNA adducts that block both DNA replication and transcription, resulting in cell death in actively dividing cells as well as the induction of apoptosis. Like cisplatin, oxaliplatin reacts with DNA, forming mainly platinated intra-strand links with two adjacent guanines or a guanine adjacent to an adenine.[5-7] However, DACH-platinum adducts formed by oxaliplatin are apparently more effective at inhibiting DNA synthesis[7] and are more cytotoxic than cis-diamine-platinum adducts formed from cisplatin and carboplatin.[7,8]

1.4 FOLFOX for colon cancer

Oxaliplatin administration has been coupled with 5-fluorouracil infusion mainly in two ways. In the method developed by de Gramont, patients are given a loading dose of 400 mg/m^2 5-fluorouracil as a bolus injection administered after a two-hour leucovorin infusion at a dose of 400 mg/m^2. The loading dose is then followed by a 22-hour 5-fluorouracil infusion of 600 mg/m^2 via a pump programmed to provide a constant drug infusion rate.[9] This program is repeated on two consecutive days every two weeks. A version termed FOLFOX-4 utilizes 85 mg/m^2 of oxaliplatin. In order to ease the administration and reduce the number of clinic visits, a modified version, FOLFOX-6, has been routinely used in clinical practice and recent clinical trials. Patients receive a loading dose of 400 mg/m^2 5-fluorouracil as a bolus injection given after a two-hour leucovorin infusion at a dose of 400 mg/m^2. The loading dose is then followed by a 46-hour 5-fluorouracil infusion of 2,400 mg/m^2 via a pump programmed to provide a constant drug infusion rate.[10-12]

Randomized clinical trials have consistently shown FOLFOX to result in superior response rates and times to disease progression compared to fluorouracil and leucovorin alone when given as first[9,13,14] or second-line[15] treatment of advanced colorectal cancer. The NCCTG trial N9741 treated 265 patients with advanced colorectal cancer not previously treated with chemotherapy for advanced disease using the FOLFOX-4 regimen. In this trial, IFL was used as the comparator arm.[16] The FOLFOX regimen resulted in a statistically significant advantage in time to progression from a median of 6.9 to 8.8 months (p= .0009), a response rate advantage of 29% versus 38% (p= .03), and a median survival advantage of 14.1 versus 18.6 months (p= .002) over IFL. Toxicity also favored FOLFOX over IFL with significantly less grade 3 or greater nausea (6 versus 15%), vomiting (4 versus 12%), diarrhea (13 versus 33%) and 1.8 % versus 4.5% 60-day all cause mortality observed. More paresthesias were observed with FOLFOX than with IFL (18% versus 2%).

In 2003, the first analysis of the Multicenter International Study of Oxaliplatin/Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) study was presented and showed a statistically significant improvement in disease-free survival with the addition of oxaliplatin to 5-fluorouracil and leucovorin in stage III patients.[17] Soon after this presentation, FOLFOX became increasingly the standard of care for stage III colon cancer adjuvant therapy and the backbone of subsequent adjuvant therapy trials. However, the recent update of the MOSAIC trial demonstrated a 5-year disease-free survival of 66% with FOLFOX compared to 59% with 5-fluorouracil and leucovorin only in stage III patients.[18] Clearly, with 34% of stage III patients eventually manifesting recurrent disease, there is a need for further improvement in outcomes of stage III colon cancer patients. Further, the addition of oxaliplatin to fluoropyrimidine therapy increases the risk of toxicities, including bone marrow suppression and neurosensory symptoms. The latter is particularly problematic for some patients. At the completion of therapy in the MOSAIC trial, 12% of patients had grade 3 peripheral neuropathy and 92% had some level of neuropathy.[19] In a recent update, 15% of patients still had some level of residual neuropathy 4 years after the completion of adjuvant therapy.[18]

1.5 Duration of adjuvant therapy

Trials in the 1990s demonstrated non-inferiority of 6 months of adjuvant therapy to 12 months of therapy. Chau and colleagues conducted a trial to further reduce the timeframe of adjuvant therapy, comparing 3 months of continuous infusion 5-FU to 6 months of monthly Mayo Clinic 5-FU and leucovorin.[20] The investigators reported that overall survival was not appreciably different between the two arms and the probability of 3 months of continuous infusion 5-FU being inferior was extremely low ($p < 0.005$). Given the neuropathy and other toxicities associated with FOLFOX therapy, a trial to test the ability to reduce the total number of cycles of adjuvant FOLFOX is highly relevant. However, in order to test non-inferiority with acceptable confidence bounds, a very large sample size is required. Two trials in Europe that include a hypothesis regarding duration of FOLFOX therapy are currently open and accruing patients. The TOSCA trial in Italy (PI: Alberto Sobrero) is randomizing 3450 high-risk stage II and stage III patients to 3 versus 6 months of FOLFOX with an option for additional randomization to bevacizumab in stage IIIC patients. The SCOT trial in the UK (PI: James Cassidy) is randomizing 9500 stage II and III colon and rectal cancer patients to 3 versus 6 months of FOLFOX/XELOX. In addition, the GERCOR and AIO trials each will be enrolling 2000-2500 patients with stage III colon cancer to a trial of 3 versus 6 months of FOLFOX. As a result, the IDEA (International Duration Evaluation of Adjuvant Chemotherapy) steering committee (PI: Dan Sargent and Axel Grothey) was formed to develop a prospective pooling of data from each of these and the current trial to test for non-inferiority of 6 treatments versus 12 treatments of adjuvant FOLFOX.

1.6 CYCLOOXYGENASE AND COLON CANCER

Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) have long been studied as agents that may influence cancer development and progression.[21] In particular, data from observational studies and intervention trials consistently demonstrate that usage of these agents reduces the risk of colorectal adenomas and/or cancer.[22-24] Hypotheses for the mechanism of action of these agents include inhibition of the cyclooxygenase (COX) family of enzymes (increasing arachidonic acid which stimulates the conversion of sphingomyelin to ceramide that mediates apoptosis as well as altering prostaglandin production that decreases angiogenic factors), inhibition of the activation of nuclear factor-κ-B, interference of the binding of peroxisome-proliferator–activated receptor (PPARδ) to DNA and other potential non-COX-mediated pathways.[25] Regardless of the precise mechanism, the data is so consistent that causality is generally accepted.

In the Nurses' Health Study, an over thirty-year ongoing prospective observational study of 121,000 women, a dose- and duration-dependent protective effect of aspirin on colorectal cancer incidence was demonstrated.[26] Among women who regularly used aspirin (at least 2 standard 325 mg tablets per week), the multivariate relative risk (RR) for colorectal cancer was 0.77 (95% confidence interval [CI], 0.67-0.88) compared to non-regular users. A statistically significant risk reduction required more than 10 years of use. The strongest benefit was seen in subjects using more than 14 aspirins per week (RR 0.68 [95% CI, 0.49-0.95]). In a randomized placebo-controlled study of aspirin in patients with prior colorectal adenomatous polyps, aspirin reduced the risk of advanced adenomas at 3 years by 30%.[21] Similarly, in CALGB 9270, a phase III trial of aspirin versus placebo in patients with prior history of colorectal cancer, treatment with aspirin decreased subsequent adenomatous polyp formation by 35% in the 3 year follow-up period.[27]

The discovery of the second isoform of cyclooxygenase, COX-2, resulted in extensive research on the different roles of COX-1 and COX-2 in normal and abnormal cell function.[28,29] Studies emerged that suggested that COX-2 was induced by inflammation and COX-1 was more constitutive, particularly in the gastrointestinal tract. The subsequent conclusion was that inhibitors specific to COX-2 could have more therapeutic specificity with less gastrointestinal toxicity (as well as minimizing inhibition of platelet aggregation by having a more modest effect on thromboxane A_2 synthase). Thus, there was great enthusiasm to develop COX-2-specific inhibitors. In less than 10 years since the initial discovery of COX-2, celecoxib and rofecoxib were approved by the Food and Drug Administration for arthritis.[30]

Given the consistent observational data and two randomized trials on aspirin and colorectal adenomas and/or colorectal cancer, three trials were initiated to test the role of COX-2 inhibitors in polyp prevention.[31-33] The designs of each of these trials were similar. Patients were eligible if they had a colonoscopy in which at least one adenomatous polyp was fully excised (in the celecoxib trials, the polyp had to be 5-6 millimeters in size or multiple). Patients were randomized to placebo or COX-2 inhibitor. Colonoscopies were performed at 1 and 3 years after randomization. Aspirin usage was allowed for cardioprotection, but limited to less than 100 milligrams daily. All the trials demonstrated statistically significant reductions in cumulative incidence of subsequent adenomas at three years, with relative risks ranging from 0.55 – 0.76 (all having upper limits of confidence intervals < 1.0).

Table 1. Polyp prevention trials with COX-2 inhibitors

	Total number of subjects	Treatment arms	Cumulative incidence of adenoma by year 3
Adenoma Prevention with Celecoxib (APC)[33]	2035	Placebo 200 mg celecoxib twice a day 400 mg celecoxib twice a day	61 % 43 % 38 %
Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP)[31]	1561	Placebo 400 mg celecoxib daily	49% 34 %
Adenomatous Polyp Prevention on Vioxx (APPROVe)[32]	2587	Placebo 25 mg rofecoxib daily	55 % 41 %

A randomized controlled trial, Vioxx in Colorectal Cancer Therapy: Definition of Optimal Regime (VICTOR), evaluating the role of rofecoxib for stage II and III colon cancer was initiated in the United Kingdom in April 2002.[35] The trial was designed to enroll 7,000 patients with stage II or III colorectal cancer who completed adjuvant therapy and were randomized to placebo (1/2 of the patients), 25 mg daily of rofecoxib for 2 years (1/4 of the patients) or 25 mg daily of rofecoxib for 5 years (1/4 of the patients). The trial was terminated in September 2004 due to cardiac concerns with rofecoxib which eventually led to withdrawal of the drug from the market. Prior to termination, 2,434 patients were enrolled with 1,217 patients randomized to rofecoxib and 1,217 patients randomized to placebo. Patients were only on study medication for a median of 7.4 months (95% CI 3.1-14). Fifteen patients in the rofecoxib group had a cardiothrombotic event during or within 14 days after the treatment period compared to 6 patients in the placebo arm. During the treatment period and within the 2 years after closure of the trial, 21 patients in the rofecoxib arm and 14 patients in the placebo arm had a cardiothrombotic event.[35] The median follow-up of patients was 36.5 months. The hazard ratio for DFS was 0.90 (95% CI, 0.77-1.06) favoring rofecoxib amongst stage II and III patients. Since patients were treated for considerably less time than anticipated (nearly 50% were on study for < 6 months), point estimates for DFS can be considered only hypothesis-generating, but at least support that the point estimate may be beneficial.

1.7 Aspirin and NSAIDs in early stage colon cancer

Fuchs and colleagues reported that consistent users of aspirin in CALGB 89803 have >50% improvement in disease-free survival compared to non-users. A similar impact was demonstrated with use of COX-2 inhibitors.[34] Chan et al. found a 35% reduction in colorectal cancer-specific mortality for women with colon cancer in the Nurses' Health Study who regularly used aspirin after diagnosis.[299] In a randomized study of 635 colorectal cancer survivors (CALGB 9270), Sandler and colleagues found that patients randomized to take a daily dose of 325 mg of aspirin experienced an adjusted reduction in risk of adenomatous polyps of 35%.[27]

1.8 Celecoxib and cardiovascular risks

Celecoxib is more potent than aspirin or other NSAIDs in experimental models of colon tumor formation.[36] Use of celecoxib would have the advantage of permitting patients who are taking aspirin for other indications to be eligible for enrollment in this trial. There have been associations between celecoxib and risk of cardiovascular disease. However, in the Prevention of Spontaneous Adenomatous Polyps (PreSAP) trial utilizing the proposed dose of celecoxib for this intervention (400 mg daily), the

risks of cardiovascular events were only modestly above placebo and not statistically significant (HR 1.5 [95% CI, 0.9-2.4] for any cardiovascular event, HR 1.3 [95% CI, 0.6-2.5] for composite endpoint of death from cardiovascular causes, nonfatal MI or stroke, hospitalization for heart failure and HR 0.7 [95% CI, 0.2-2.7] for death from cardiovascular causes). In recent analyses of primary data from 6 randomized trials of celecoxib, the daily dosing of celecoxib appears considerably safer than twice a day dosing. It should also be noted that these studies did not have strict cardiovascular exclusion criteria and thus one can assume that with the exclusion criteria in this trial, the rare but observed differences in cardiovascular events between the intervention and control arm will be attenuated.

Table 2. Event rates per 1000 patient-years and pooled hazard ratios with 95% confidence intervals for the principal composite endpoint of cardiovascular death, myocardial infarction, stroke, heart failure, or thromboembolism for each individual trial, for each dose regimen, and for all the trials combined, adjusted for baseline cardiovascular risk[37].

Study	Median follow-up time (months)	Events/ Participants		Event rate/ 1000 pt years		Hazard ratio	95% CI	Relative weight
		Placebo	Celecoxib	Placebo	Celecoxib			
400 mg qd								
PreSAP	36	12/628	23/933	7.2	9.4	1.3	(0.6, 2.5)	7.9
Selenium/ Celecoxib	21	8/410	7/414	11.8	10.3	0.9	(0.3, 2.4)	3.7
Pooled 400 mg qd	***35***	***20/1038***	***30/1347***	***8.6***	***9.6***	***1.1***	***(0.6, 2.0)***	
200 mg bid								
ADAPT	24	8/1083	18/726	8.6	12.8	1.5	(0.8, 2.9)	9.0
APC	37	8/679	20/685	3.9	9.7	2.5	(1.1, 5.7)	5.7
CDME	15	3/47	0/39	54.3	0.0			
Pooled 200 mg bid	***36***	***29/1809***	***38/1450***	***6.9***	***10.8***	***1.8***	***(1.1, 3.1)***	
400 mg bid								
APC	37	8/679	27/671	3.9	13.4	3.6	(1.6, 8.0)	6.2
MA27	5	3/817	6/818	8.7	17.2	1.8	(0.4, 7.3)	2.0
Pooled 400 mg bid	***11***	**11/1496**	**33/1489**	**4.6**	**13.9**	***3.1***	***(1.5, 6.1)***	
Pooled all doses	***31***	**52/3664**	**101/4286**	***7.5***	***11.2***	**1.6**	**(1.1, 2.3)**	

1.9 Rationale for the current trial

This randomized phase III trial will potentially reduce the total amount of cytotoxic chemotherapy and improve upon existing disease-free survival rates. Given the issues of neuropathy associated with oxaliplatin as well as the costs (human, economic and resources) of adjuvant therapy, potential reduction in number of required treatments without impacting on efficacy is a critical question. Furthermore, there are still an appreciable number of patients with stage III colon cancer recurring despite recommended therapy, and thus continued testing of an agent for superiority of efficacy is critical. Celecoxib will be initiated at the start of FOLFOX chemotherapy (i.e., concurrent administration) and continued for 3 years total.

1.10 Inclusion of women and minorities

This study will be available to all eligible patients, regardless of race, gender, or ethnic origin. There is no information currently available regarding differential effects of oxaliplatin, 5-fluorouracil or celecoxib-based treatment in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences exist. Therefore, although the planned analysis will look for differences in treatment effect based on racial and gender groupings, the sample size is not increased in order to

provide additional power for ethnic subset analyses. In CALGB 89803, 12% (148/1264) of patients were classified as minorities by race and 45% (562/1264) of patients were women. No race or gender differences were observed in CALGB 89803.

Accrual Targets						
Ethnic Category	**Sex/Gender**					
	Females		**Males**		**Total**	
Hispanic or Latino	66	+	112	=	178	
Not Hispanic or Latino	1034	+	1288	=	2322	
Ethnic Category: Total of all subjects	1100	+	1400 (B1)	=	2500	(C1)
Racial Category						
American Indian or Alaskan Native	16	+	21	=	37	
Asian	39	+	49	=	88	
Black or African American	143	+	140	=	283	
Native Hawaiian or other Pacific Islander	11	+	14	=	25	
White	891	+	1176	=	2067	
Racial Category: Total of all subjects	1100	+	1400 (B2)	=	2500	(C2)

2.0 OBJECTIVES

2.1 Primary objective

To compare disease-free survival of patients with stage III colon cancer randomized to standard chemotherapy only (FOLFOX) or standard chemotherapy (FOLFOX) with 3 years of celecoxib 400 mg daily.

2.2 Secondary objectives

2.2.1 To contribute to an international prospective pooled analysis that will compare disease-free survival of patients with stage III colon cancer randomized to 6 treatments of adjuvant FOLFOX chemotherapy or 12 treatments of adjuvant FOLFOX chemotherapy.

2.2.2 To compare overall survival of patients with stage III colon cancer randomized to standard chemotherapy only (FOLFOX) or standard chemotherapy (FOLFOX) with 3 years of celecoxib 400 mg daily.

2.2.3 To contribute to an international prospective pooled analysis that will compare overall survival of patients with stage III colon cancer randomized to 6 treatments of adjuvant FOLFOX chemotherapy or 12 treatments of adjuvant FOLFOX chemotherapy.

2.2.4 To assess toxicities of celecoxib as maintenance adjuvant therapy in patients with stage III colon cancer.

2.2.5 To assess differences in cardiovascular-specific events with celecoxib versus placebo in a population of stage III colon cancer survivors.

2.2.6 To evaluate differences in toxicities, particularly cumulative peripheral neuropathy, for patients treated with 6 treatments of FOLFOX compared to those treated with 12 treatments of FOLFOX.

2.2.7 See Appendices III-V for substudy objectives.

3.0 ON-STUDY GUIDELINES

This clinical trial can fulfill its objectives only if patients appropriate for this trial are enrolled. All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate. Although they will not be considered formal eligibility (exclusion) criteria, physicians should recognize that the following may seriously increase the risk to the patient entering this protocol:

- Psychiatric illness that would prevent the patient from giving informed consent.
- Patient is not deemed a candidate for FOLFOX or celecoxib based on overall condition and co-morbidities.
- A medical condition such as active/uncontrolled infection that would make this protocol unreasonably hazardous for the patient in the opinion of the treating physician.
- Inability to take oral medications.

4.0 ELIGIBILITY CRITERIA

All questions regarding eligibility criteria should be directed to the CALGB or SWOG Study Chair. Please note that the Study Chair cannot grant waivers to eligibility requirements.

4.1 Requirements for tumor parameters

4.1.1 Histologically documented adenocarcinoma of the colon. The gross inferior (caudad) margin of the primary tumor must be at least 12 centimeters from the anal verge (i.e., patients with rectal cancer are not eligible).

4.1.2 Tumors must have been completely resected. Surgeon confirmation that the entire tumor was above the peritoneal reflection is only required in cases where it is important to establish if the tumor is a rectal or colon primary. In patients with tumor adherent to adjacent structures, en bloc R_o resection must be documented in the operative report.

4.1.3 At least one pathologically confirmed positive lymph node (note: AJCC version 6 defines extramural tumor nodules of any size with smooth contours to be counted as replaced regional lymph nodes).

4.1.4 No evidence of residual involved lymph node disease or metastatic disease at the time of registration.

4.1.5 Patients with synchronous colon cancers are eligible and staging for stratification will be based on higher N stage of the more advanced primary tumor. However, patients with synchronous colon and rectal primary tumors are not eligible.

4.2 Patients are ineligible if they use NSAIDs at any dose or aspirin at more than 325 mg at least three times per week, on average. Low-dose aspirin not exceeding 100 mg/day is permitted.

4.3 No previous or concurrent malignancy, except treated basal cell or squamous cell cancer of skin, treated in situ cervical cancer, treated lobular or ductal carcinoma in situ in one breast, or any other cancer for which the patient has been disease-free for at least 5 years.

4.4 No neurosensory or neuromotor toxicity ≥ grade 2 at the time of registration.

4.5 No known allergy to platinum compounds.

4.6 No prior allergic reaction or hypersensitivity to sulfonamides, celecoxib or NSAIDs.

4.7 No history of upper gastrointestinal ulceration, bleeding, or perforation within the past 3 years.

4.8 No symptomatic pulmonary fibrosis or interstitial pneumonitis ≥ grade 2.

4.9 No cardiac risk factors including:

- Uncontrolled high blood pressure (systolic blood pressure > 150).
- Unstable angina.
- History of documented myocardial infarction or cerebrovascular accident.
- New York Heart Association class III or IV heart failure.

4.10 Non-pregnant and not nursing. Men and women of childbearing potential must agree to employ adequate contraception for the duration of chemotherapy and for as many as 8 weeks after the completion of chemotherapy due to the unknown teratogenic effects of FOLFOX on the developing fetus.

4.11 ECOG performance status 0, 1, or 2.

4.12 Age at least 18 years.

4.13 Required initial laboratory values

Granulocytes	≥ 1,500/μL
Platelet count	≥ 100,000/μL
Creatinine	≤ 1.5 x upper limit of normal
Bilirubin	≤ 1.5 x upper limit of normal

5.0 REGISTRATION/RANDOMIZATION, STRATIFICATION, AND DATA AND SAMPLE SUBMISSION

5.1 Registration requirements

- **Informed consent:** the patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedures to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Human protection committee approval of this protocol and a consent form are required.

5.2 Patient registration/randomization

This study uses the CALGB Web-based Patient Registration system. Randomization will be accepted only through CALGB Main Member Institutions, selected affiliate institutions and CCOPs using the Web-based Patient Registration system. Registration must occur prior to the initiation of therapy.

Confirm eligibility criteria (Section 4.0). Complete the Registration Worksheet. Access the Web-based Patient Registration system via the Patient Registration tab on the CALGB Member Website at www.calgb.org. If the study does not appear on the list of studies in the Patient Registration system, the registration must be performed by the CALGB Registrar via phone or fax. If the registering CRA requires assistance, he/she may consult the on-line help file at the bottom of the screen or call the IS Help Desk at 1-888-44CALGB. If further assistance is required, the registering CRA may call the CALGB Registrar (919)-668-9396, Monday-Friday, 9 AM – 5 PM, Eastern Time. Enter the following information:

CALGB patient ID #, if applicable
NCI investigator number
Study
Name of group (CALGB)
Name of institution where patient is being treated
Name of treating physician
Name of person in contact with the patient record (responsible contact)
Protocol IRB approval date
Date of signed consent
Treatment Start Date
Date [of] HIPAA authorization signed by the patient
Patient's initials (L, F, M)
Patient's Social Security #, date of birth, hospital ID #, and survival status
Patient's gender
Patient's race
Patient's ethnicity
ECOG performance status
Patient's height (cm) and weight (kg)
Type of insurance (Method of Payment)
Patient's postal code
Disease, type and stage, if applicable
Eligibility criteria met (no, yes)
Companion studies patient has consented

When the patient is registered, a CALGB patient identification number will be generated. Please write the number in your records. Registration to any mandatory or optional companion studies will be done at the same time as registration to the treatment study. Registration to both treatment and companion studies will not be completed if eligibility requirements are not met for all selected trials (treatment and companions).

After registration is complete the patient may be randomized. The patient is randomized according to the stratification factors indicated in Section 5.4 below, which must be entered to obtain a (blinded) treatment assignment. Treatment is to begin within 14 days of randomization.

No blinded starter supplies will be available for this study. Initial blinded, patient-specific clinical supplies of celecoxib/placebo will be shipped from the Pharmaceutical Management Branch (PMB) to the registering investigator at the time of patient randomization and should arrive within 7 to 10 days of randomization (see Section 9.7).

The Main Member Institution and registering institution will receive a Confirmation of Registration and a Confirmation of Randomization. Please check both confirmations for errors. Submit corrections in writing to the data coordinator at the CALGB Statistical Center, Data Operations, 2424 Erwin Rd, Ste 802 Hock Plaza, Durham, NC 27705, or fax to 919-668-9397.

5.3 Registration to companion studies

There are three substudies within CALGB/SWOG C80702. These correlative science studies **must be offered to all patients** enrolled on CALGB/SWOG C80702 (although patients may opt to not participate). These substudies do not require separate IRB approval. The substudies included within CALGB/SWOG C80702 are:

- CALGB 150911: Correlative science companion studies for CALGB/SWOG C80702 (Appendix III)
- CALGB 60905: Pharmacogenetic companion studies for CALGB/SWOG C80702 (Appendix IV)
- Diet and Lifestyle substudy (Appendix V)

If a patient answers "yes" to "I agree that my specimen may be used for the research studies described above", question #2 in the model consent, they have consented to participate in the studies described in Appendix III. The patient should be registered to CALGB 150911 at the same time they are registered to the treatment trial (CALGB/SWOG C80702). Samples should be submitted per Sections 5.6.1-5.6.4.

If a patient answers "yes" to "I agree that my blood may be used for the genetic research studies described above", question #3 in the model consent, they have consented to participate in the studies described in Appendix IV. The patient should be registered to CALGB 60905. Samples should be submitted per Section 5.6.5.

If a patient answers "yes" to "I choose to take part in the Diet and Lifestyle study and agree to complete the diet and lifestyle questionnaire", question #1 in the model consent, they have consented to participate in the Diet and Lifestyle study described in Appendix V. There is no separate registration for this substudy.

5.4 Stratification

5.4.1 Stratification for the duration of adjuvant chemotherapy randomization

- Number of positive lymph nodes (1-3 vs. 4 or more)

5.4.2 Stratification for the celecoxib randomization

- Number of positive lymph nodes (1-3 vs. 4 or more)
- Current regular low dose aspirin usage (Yes vs. No)

5.5 Data submission: Forms should be submitted to the CALGB Statistical Center, Data Operations in compliance with the Data Submission schedule below. There are three options for submitting forms that use the Teleform barcode and cornerstones:

- The preferred method is to submit the forms electronically using the "Submit to CALGB" button located at the bottom of the last page of each form. Forms submitted electronically should not be submitted by fax or mail.
- The forms may be faxed at 919-416-4990. Please note that the four cornerstones and the form id ("bitmap") must appear on the form. Copies must be 100% of the original form size.
- The forms may be mailed to the CALGB Statistical Center, Data Operations, Hock Plaza, 2424 Erwin Rd, Suite 802, Durham, NC 27705. Please note that the four cornerstones and the form id ("bitmap") must appear on the form. Copies must be 100% of the original form size.

For the most up-to-date data forms, please visit the CALGB website at www.calgb.org.

Form*		**Submission Schedule**
	Baseline	
C-1953 Report Report	80702 On-Study Operative and Pathology reports CT/X-ray reports	Within one month of registration
	Treatment	
C-1954 C-1955	80702 Treatment Form** 80702 Adverse Event Form***	Every 2 cycles (4 weeks) while on FOLFOX, then every 3 months while on celecoxib/placebo alone.
C-1956	80702 Follow-up Form	Every 3 months while on celecoxib/placebo alone
	Follow-up (after end of protocol treatment)	
C-1956	80702 Follow-up Form	Every 3 months for year 1, then every 6 months for years 2-3, then every year for years 4-6 for a total of 6 years of follow-up from the date of registration.#
Report	CT/X-ray reports	At time of progression

*Use CALGB Remarks Addenda (C-260) if additional comments are necessary or additional writing space is needed.

If patient never starts treatment, submit the baseline data, a C-1954 80702 Treatment Form to report the reason for ending treatment, and all follow-up data.

**S-067 80702 Medication Calendar is provided for patient and institutional use. This form does not need to be submitted to the Statistical Center.

***Submit AE form until all protocol treatment related events have resolved or until non-protocol treatment begins. If patient death is reported via AdEERS report Grade 5 event on AE form even if patient is off protocol treatment.

Submit at months 3, 6, 9, 12, 18, 24, 30, 36, 48, 60, 72. Follow-up form must be submitted prior to the first follow-up imaging to prevent patients from appearing on the delinquency list.

5.6 Specimen submission for correlative and pharmacogenomic substudies

All participating institutions must ask patients for their consent to participate in the components of the correlative **(CALGB 150911)** and pharmacogenomic **(CALGB 60905)** substudies planned for CALGB/SWOG C80702, although patient participation is optional. Rationale and methods for the scientific components of these studies are described in Appendices III and IV.

Type of specimen	Pre-treatment
Tumor*	1 paraffin block
EDTA Plasma (lavender top)*	1 x 10 mL
Serum (red top)*	1 x 10 mL
Whole (venous) Blood (EDTA/lavender top)**	1 x 10 mL
[Total blood volume]	[30 mL]

* For patients who answer "yes" to consent question #2 (CALGB 1509011).

** For patients who answer "yes" to consent question #3 (CALGB 60905).

Instructions for the collection and shipping of samples are included below. Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary.

Shipment on Monday through Friday by overnight service to assure receipt is encouraged. If shipping on Friday, FedEx or UPS must be used and the air bill must be marked "For Saturday delivery." Do not ship specimens on Saturdays.

All specimens should be sent to the following address:

CALGB Pathology Coordinating Office
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967

Copies of all forms and reports should also be sent to the CALGB Statistical Center, Data Operations.

5.6.1 Submission of paraffin blocks of archived colorectal tumors (CALGB 150911)

For patients who consent to question #2, tumor blocks will be used for the correlative studies described in Appendix III. Paraffin blocks of tissue obtained from archival colorectal tumor specimens from primary site should be sent to the CALGB Pathology Coordinating Office.

The CALGB has instituted special considerations for the small percentage of institutions whose policies prohibit release of any blocks. If, due to institutional policy, a block cannot be sent, please call 614-293-7073 to obtain a protocol for submission of representative tissue from your institution.

The goal of the PCO is to provide investigators with quality histology sections for their research while maintaining the integrity of the tissue. All paraffin blocks that are to be stored at the PCO will be vacuum packed to prevent oxidation and will be stored at 4ºC to minimize degradation of cellular antigens. For these reasons it is preferred that the PCO bank the block until the study investigator requests thin sections. Please contact the PCO if additional assurances with your hospital pathology department are required.

5.6.2 Plasma collection procedures (CALGB 150911)

For patients who consent to consent question #2, plasma samples will be used for the studies described in Appendix III.

1. Collect blood in 10 mL lavender top tube. After collection, gently mix the blood by inverting the tube 8 to 10 times. Store vacutainer tubes upright at 4°C until centrifugation. Blood samples should be centrifuged within four hours of blood collection.
2. Centrifuge blood samples in a horizontal rotor (swing-out head) for 10 to 15 minutes at 1100-1300 g at room temperature. Warning: Excessive centrifuge speed (over 2000 g) may cause tube breakage and exposure to blood and possible injury. If needed, relative centrifugal force (RCF) for a centrifuge can be calculated. For an on-line calculator tool, please refer to: http://www.changbioscience.com/cell/rcf.html.
3. After centrifugation, plasma layer will be at the top of the tube. Mononuclear cells and platelets will be in a whitish layer, called the "buffy coat", just under the plasma and above the red blood cells.
4. Carefully collect the plasma layer with an appropriate transfer pipette without disturbing the buffy coat layer. Pipette the plasma into the labeled cryovials (recommended cryovials are described in Section 5.6.4). Aliquot volume is to be 500 μL. Close the caps tightly and place on ice. This process should be completed within 1 hour of centrifugation.
5. Check that all aliquot vial caps are secure and that all vials are labeled.
6. Place all aliquots upright in a specimen box or rack in an -80°C or colder freezer. All specimens should remain at -80°C or colder prior to shipping. The samples should not be thawed prior to shipping. Plasma should be shipped on dry ice according to the shipping procedures in Section 5.6.4.

5.6.3 Serum collection procedures (CALGB 150911)

For patients who consent to consent question #2, serum samples will be used for the studies described in Appendix III.

1. Collect blood in 10 mL red top tube. After collection, tubes ("vacutainers") should sit upright after the blood is drawn at room temperature for a minimum of 30 to a maximum of 60 minutes to allow the clot to form. Note: Use red top (serum) tubes (silicon-coated)—no additives and not SST (serum separator tubes).
2. Centrifuge the blood sample at the end of the clotting time (30-60 minutes) in a horizontal rotor (swing-out head) for 10-15 minutes at 1100-1300 g at room temperature.
3. Use a pipette to transfer the serum (Recommendation: do not pour). Pipette serum into the labeled cryovials (recommended cryovials are described in Section 5.6.4). Aliquot volume is to be 500 μL. Close the cap on the vial tightly. This process should be completed within 1 hour of centrifugation. Note: Be very careful not to pick up red blood cells when aliquoting. This can be done by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube.
4. Check that all aliquot vial caps are secure and that all vials are labeled.
5. Place all aliquots upright in a specimen box or rack in an -80°C or colder freezer. All specimens should remain at -80°C or colder prior to shipping. The samples should not be thawed prior to shipping. Serum should be shipped on dry ice according to the shipping procedures in Section 5.6.4.

5.6.4 Plasma and Serum Processing Procedures

The CALGB strongly recommends the usage of 2 mL cryovials for storage of plasma and serum specimens. Acceptable cryovials include:

Company name	Catalog number
Nalgene	03-337-7Y (through Fisher) NNI No.: 5012-0020
Fisher brand	05-669-57 (through Fisher)
Corning	03-374-21 (through Fisher) CLS430659 (through Sigma) Corning: 430488
VWR	16001-102

All samples should be labeled with the patient's initials, CALGB treatment study number (CALGB/SWOG C80702), CALGB patient ID#, date and time of collection, and sample type. The sample should be shipped on dry ice to the CALGB PCO.

5.6.5 Whole blood submission for the pharmacogenomic substudy (CALGB 60905)

For patients who consent to question #3, whole blood will be used for the pharmacogenomic studies described in Appendix IV. This sample should be collected prior to the initiation of protocol treatment.

Draw 10 mL of venous blood in a lavender top (EDTA coagulant) tube and keep refrigerated until shipped overnight to the CALGB PCO. Label the tube with the patient's initials, CALGB/CTSU patient ID number, CALGB treatment number (CALGB/SWOG C80702) and date of collection. The sample should be shipped the same day on a cold pack by overnight mail to the CALGB PCO.

5.7 Data submission for Diet and Lifestyle Companion

At registration, patients may elect to enroll on the diet and lifestyle questionnaire companion study (Appendix V). Surveys will be sent by Devin Wigler (see below) to individual treatment centers within 2 weeks of registration. Patients should complete the first survey within 6 weeks of randomization. Patients should also complete questionnaires 16-18 months from randomization. It is recommended that the surveys be completed during a physician visit, likely during the infusion of chemotherapy. Questions regarding the survey should be directed to Devin Wigler, 617-632-3687. Completed surveys should be mailed by the institutional CRA to the following address:

Dr. Meyerhardt
c/o Devin Wigler
Diet and Lifestyle Survey Coordination Office
Dana-Farber Cancer Institute
44 Binney Street
Boston, MA 02115

If the patient refused participation in the diet and lifestyle study, the questionnaire should be returned to Dr. Meyerhardt with the notation that the "patient refused" participation.

6.0 REQUIRED DATA

Pre-Study Testing Intervals

To be completed within 16 DAYS before registration:

- All bloodwork, history and physical, and pregnancy test.

To be completed within 42 DAYS before registration:

- CT abdomen and pelvis or PET/CT scan without evidence of metastatic disease
- CT chest or chest X-ray without evidence of metastatic disease

	Prior to Registration*	Day 1 of each tx w/ FOLFOX*	During tx w/ Celecoxib/ Placebo only**	Post-tx Follow Up***
Tests & Observations				
History and Physical Examination	X	A	X	X
Pulse, Blood Pressure	X	A	X	X
Height	X			
Weight/Body Surface Area ¢	X	X	X	X
Performance Status	X	A	X	X
Drug Toxicity Assessment		X	X	X
Monthly Celecoxib/Placebo Count		A	B	
Laboratory Studies				
CBC, Differential, Platelets	X	X		
Serum Creatinine and BUN	X	C	X	
AST, Alk Phos., Bilirubin	X	C	X	
PT/INR	D	D	D	
Pregnancy Test (UCG) ǂ	X			
CEA	X		E	E
Staging				
Chest x-ray, PA & Lateral or Chest CT	X		E	E
Abdominal Imaging: U/S or CT or MRI	X		E	E
Companion Studies Ψ				
Diet & Lifestyle / Other Meds / Comorbidities Questionnaire	*To be completed w/in first 6 weeks of randomization and 16-18 months after randomization.*			
Tumor Block	*To be collected pre-treatment (see Section 5.6).*			
Whole Blood, Plasma, and Serum	*To be collected pre-treatment (see Section 5.6).*			

* Pre-registration labs may be used for Day 1 of Cycle 1 tests if obtained within 7 days prior to Day 1 of Cycle 1. For subsequent cycles, labs and history & physical may be obtained within 48 hours prior to day of treatment.

** Every 3 months until 3 years after randomization or until disease progression, whichever comes first.

*** Every 6 months until 6 years after randomization or until disease progression, whichever comes first.

A While on FOLFOX, patients should have a physical exam and report capsule counts of celecoxib/placebo every 4 weeks (i.e., prior to cycles 3, 5, 7, etc.)

B Once FOLFOX is completed, patients should keep monthly diaries and return them every 3 months at time of routine visits.

C Every 4 weeks while on FOLFOX.

D Only for those patients receiving coumadin or warfarin: PT/INR should be monitored weekly.

E For patients who were randomized to 6 treatments of FOLFOX, the first post-treatment CEA and imaging should occur within 4 months after completion of FOLFOX. For patients who were randomized to 12 treatments of FOLFOX, the first post-treatment CEA and imaging should occur within 6 weeks of FOLFOX (see Section 11.0). Then, for all patients, every 6 months until 3 years after randomization, and then yearly for 3 years or until disease progression.

¢ It is not necessary to change the doses of chemotherapy unless the calculated dose changes by ≥10%.

ǂ For women of child-bearing potential.

Ψ For those patients who consent to participate in the substudies.

7.0 TREATMENT PLAN

Treatment is to begin between 21 and 56 days after definitive surgical resection of primary tumor and within 14 days of randomization. Questions regarding treatment should be directed to the CALGB or SWOG Study Chair.

This is a randomized, double-blind trial. No blinded starter supplies will be available for this study. Initial blinded, patient-specific clinical supplies of celecoxib / placebo will be shipped from the Pharmaceutical Management Branch (PMB) to the registering investigator at the time of patient randomization and should arrive within 7 to 10 days of randomization (see Section 9.7).

One cycle will be defined as 14 days of treatment. Cytotoxic chemotherapy will consist of 6 or 12 cycles of FOLFOX and daily celecoxib/placebo. Celecoxib/placebo will start on Day 1 of the first cycle of FOLFOX.

7.1 FOLFOX, every 2 weeks.

- **Oxaliplatin** 85 mg/m^2 IV over two hours, **followed by**
- **Leucovorin** 400 mg/m^2 IV over two hours. Alternatively, leucovorin may be administered (via separate infusion lines) concurrently with oxaliplatin, **followed by**
- **5-FU** 400 mg/m^2 IV bolus, then 2400 mg/m^2 continuous IV infusion over 46-48 hours.

Patients receiving oxaliplatin on this study should be counseled to avoid cold drinks, chewing of ice chips, and exposure to cold water or air because the neurotoxicity often seen with oxaliplatin appears to be exacerbated by exposure to cold. The period of time during which the patient is at risk for these cold-induced sensory neuropathies is not well documented. Patients should exercise caution regarding cold exposure during the treatment period. Peripheral sensory neuropathies can occur at any time after receiving oxaliplatin therapy.

7.2 Celecoxib or Placebo

Patients will be randomized to celecoxib 400 mg or placebo administered by mouth, once daily. The first dose of celecoxib/placebo will be given on Day 1 of the first cycle of FOLFOX (in clinic). Patients will self-administer celecoxib/placebo at about the same time every day with food. Celecoxib/placebo will continue for 3 years or until progression of disease or unacceptable toxicity.

8.0 DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY

- If a dose reduction beyond Level -3 is required for oxaliplatin, oxaliplatin will be discontinued. Continue celecoxib/placebo and 5-FU/leucovorin.
- If a dose reduction beyond level -3 is required for 5-FU, discontinue FOLFOX. Continue celecoxib/placebo.
- If more than one dose reduction applies, use the most stringent (i.e., the greatest dose reduction.)
- If FOLFOX is delayed for >4 weeks for toxicity, discontinue FOLFOX. Continue celecoxib/placebo.

8.1 Dose Levels

Agent	Level 0	Level –1	Level –2	Level –3
Oxaliplatin	85 mg/m^2	65 mg/m^2	50 mg/m^2	40 mg/m^2
5-FU Bolus	400 mg/m^2	320 mg/m^2	270 mg/m^2	230 mg/m^2
5-FU Infusion	2400 mg/m^2 over 46-48 hrs	1920 mg/m^2 over 46-48 hrs	1600 mg/m^2 over 46-48 hrs	1360 mg/m^2 over 46-48 hrs

Leucovorin dose is always 400 mg/m^2. If 5-FU is skipped, leucovorin must also be skipped.

The dose of **celecoxib** is always 400 mg. Celecoxib may be interrupted or discontinued according to the dose modifications provided below, but the dose is not reduced.

8.2 Hematologic toxicities

Dose modifications for hematologic toxicities are based on CBC on Day 1 of each cycle of FOLFOX (or within prior 48 hours). If FOLFOX is delayed for neutropenia or thrombocytopenia, continue celecoxib/placebo. If FOLFOX is delayed for neutropenia for 2 consecutive cycles, administer G-CSF or GM-CSF or pegfilgrastim after all subsequent cycles.

8.2.1 For ANC 1000-1199: Delay FOLFOX until ANC ≥ 1200, then resume at the previous doses of oxaliplatin and 5-FU.

8.2.2 For ANC < 1000: Delay FOLFOX until ANC ≥ 1200, then resume with one dose level reduction of oxaliplatin and 5-FU for all subsequent cycles.

8.2.3 For febrile neutropenia (defined as ANC < 1000 and T ≥ 38.5°C): Delay FOLFOX until fever has resolved and ANC ≥ 1200, then resume FOLFOX with one dose level reduction of oxaliplatin and 5-FU for all subsequent cycles.

8.2.4 For platelets 50,000 – 74,999: Delay FOLFOX until platelets ≥ 75,000, then resume at the previous dose levels of oxaliplatin and 5-FU.

8.2.5 For platelets < 50,000: Delay FOLFOX until platelets ≥ 75,000, then resume with one dose level reduction of oxaliplatin and 5-FU for all subsequent cycles.

8.3 Gastrointestinal toxicities

8.3.1 For ≥ grade 2 diarrhea: Delay FOLFOX until diarrhea improves to < grade 2. Continue celecoxib/placebo.

- **Following grade 2 diarrhea at any time during a cycle:** Continue FOLFOX at the previous dose levels of oxaliplatin and 5-FU.

- **Following grade 3 diarrhea at any time during a cycle:** Continue FOLFOX with one dose level reduction of 5-FU for all subsequent cycles and the previous dose level of oxaliplatin.
- **Following grade 4 diarrhea at any time during a cycle:** Continue FOLFOX with one dose level reduction of oxaliplatin and 5-FU for all subsequent cycles.

8.3.2 For ≥ grade 2 oral mucositis present on Day 1 of a cycle: Delay FOLFOX until mucositis improves to < grade 2. Continue celecoxib/placebo.

- **Following grade 2 oral mucositis**: Resume FOLFOX at the previous dose levels.
- **Following grade 3 or 4 oral mucositis:** Resume FOLFOX with one dose level reduction of 5-FU and the previous dose level of oxaliplatin.

8.3.3 For ≥ grade 2 vomiting present on Day 1 of a cycle despite antiemetic therapy: Delay FOLFOX until vomiting impoves to < grade 2. Continue celecoxib/placebo.

- **Following grade 2 vomiting**: Resume FOLFOX at the previous dose levels.
- **Following grade 3 vomiting**: Resume FOLFOX with one dose level reduction of oxaliplatin and the previous dose level of 5-FU.
- **Following grade 4 vomiting:** Resume FOLFOX with one dose level reduction of oxaliplatin and 5-FU.

8.3.4 For GI bleeding not associated with thrombocytopenia (platelets < 75,000) or for GI ulceration, discontinue celecoxib/placebo. Continue FOLFOX.

8.4 Pulmonary toxicities

For ≥ grade 3 cough, dyspnea, hypoxia, pneumonitis, or pulmonary infiltrates, skip oxaliplatin until interstitial lung disease is ruled out. Continue 5-FU/leucovorin and celecoxib/placebo. Discontinue all protocol therapy if interstitial lung disease is confirmed.

8.5 Thrombotic microangiopathy

For ≥ grade 3 hemolytic uremic syndrome (HUS): Discontinue oxaliplatin. Continue 5-FU/leucovorin and celecoxib/placebo.

8.6 Neurotoxicity

Toxicity Scale for the Sensory Neuropathies Associated with Oxaliplatin

	Symptoms
Grade 1	Paresthesias/dysesthesias* of short duration that resolve and do not interfere with function.
Grade 2	Paresthesias/dysesthesias* interfering with function, but not with activities of daily living (ADL)
Grade 3	Paresthesias/dysesthesias* with pain or with functional impairment that also interfere with ADL.
Grade 4	Persistent paresthesias/dysesthesias* that are disabling or life threatening.
	* May be cold-induced

8.6.1 For grade 2 neurotoxicity persisting between treatments: Continue FOLFOX with one dose level reduction of oxaliplatin for all subsequent cycles and the previous dose level of 5-FU. Continue celecoxib/placebo.

8.6.2 For grade 3 neurotoxicity resolving to ≤ grade 2 between treatments: Continue FOLFOX with one dose level reduction of oxaliplatin for all subsequent cycles and the previous dose level of 5-FU. Continue celecoxib/placebo.

8.6.3 For grade 3 neurotoxicity persisting between treatments: Discontinue oxaliplatin. Continue 5-FU/leucovorin and celecoxib/placebo.

8.6.4 For grade 4 neurotoxicity: Discontinue oxaliplatin. Continue 5-FU/leucovorin and celecoxib/placebo.

8.6.5 For pharyngolaryngeal dysesthesia: Increase the duration of oxaliplatin infusion to 6 hours for all subsequent cycles. See also Section 8.8.1.

8.7 Extravasation

Extravasation of oxaliplatin has been associated with necrosis; if extravasation is suspected, the infusion should be stopped and the drug administered at another site. Extravasation should be treated according to institutional guidelines.

8.8 Allergic Reactions

- **For grade 1 allergic reactions**: Decrease the infusion rate by 50% until symptoms resolve, then resume at the initial planned rate.
- **For grade 2 allergic reactions**: Stop infusion. Administer H_1 and/or H_2 blockers, and/or steroids according to institutional policy. Restart the infusion when symptoms resolve and pretreat before all subsequent doses. Treat according to institutional policy.
- **For grade 3 or grade 4 allergic reactions or anaphylaxis:** Stop the infusion. Discontinue FOLFOX. Continue celecoxib/placebo.

8.8.1 Oxaliplatin-induced pharyngolaryngeal dysesthesia

Should a patient develop oxaliplatin-induced pharyngolaryngeal dysesthesia, her/his oxygen saturation should be evaluated via a pulse oximeter; if normal, an anxiolytic agent may be given and the patient observed in the clinic until the episode has resolved. Increase the duration of oxaliplatin to 6 hours for all subsequent treatments. Some overlap may exist between the manifestations of pharyngolaryngeal dysesthesia and hypersensitivity reactions. A table comparing the two is presented below.

Comparison of the Symptoms and Treatment of Pharyngo-Laryngodysesthesias and Platinum Hypersensitivity Reactions

Clinical Symptoms	Pharyngo-Laryngeal Dysesthesias	Platinum Hypersensitivity
Dyspnea	present	present
Bronchospasm	absent	present
Laryngospasm	absent	present
Anxiety	present	present
O_2 saturation	normal	decreased
Difficulty swallowing	present (loss of sensation)	absent
Pruritis	absent	present
Urticaria/rash	absent	present
cold-induced symptoms	yes	no
BP	normal or increased	normal or decreased
Treatment	anxiolytics, observation in a controlled clinical setting until symptoms abate or at the physician's discretion	oxygen, steroids, epinephrine, bronchodilators; fluids and vasopressors, if appropriate

8.9 Cardiovascular toxicities

8.9.1 For grade 3 or 4 cardiac ischemia/infarction: Discontinue all protocol therapy (FOLFOX and celecoxib/placebo).

8.9.2 For grade 3 or 4 cerebrovascular ischemia: Discontinue all protocol therapy (FOLFOX and celecoxib/placeo).

8.10 Other non-hematologic toxicities for FOLFOX

For other grade 3 or 4 non-hematologic toxicities considered related to FOLFOX, delay FOLFOX until toxicity resolves to ≤ grade 1, then resume FOLFOX at one dose level reduction of oxaliplatin and 5-FU. Continue celecoxib/placebo.

8.11 Other non-hematologic toxicities for celecoxib/placebo

For other grade 3 or 4 non-hematologic toxicities considered related to celecoxib/placebo, interrupt celecoxib/placebo for a maximum of 14 days until toxicity improves to ≤ grade 1, then resume celecoxib/placebo at the previous dose.

For recurrence of the same grade 3 or 4 non-hematologic toxicity considered related to celecoxib/placebo, discontinue celecoxib/placebo. Continue FOLFOX.

For persistent grade 2 non-hematologic toxicity considered related to celecoxib/placebo that the patient finds unacceptable, interrupt celecoxib/placebo for a maximum of 14 days until toxicity improves to ≤ grade 1, then resume celecoxib/placebo at the previous dose. Continue FOLFOX.

For recurrence of unacceptable grade 2 non-hematologic toxicity considered related to celecoxib/placebo, or if grade 2 toxicity does not improve after 2 weeks, discontinue celecoxib/placebo. Continue FOLFOX.

8.12 Dose modifications for obese patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, **all dosing is to be determined solely by actual weight without any modification** unless explicitly described in the protocol. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. **Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation.** Physicians who are uncomfortable with calculating doses based on actual body weight should recognize that doing otherwise would be a protocol violation.

The actual weight on the day of registration or the first day of treatment may be used for cycle 1 unless the change in the weight results in a change in calculated dose ≥10%, in which case the weight on the day of treatment should be used. Over the course of treatment it is not required to change the doses of 5-FU, leucovorin or oxaliplatin due to changes in weight unless the calculated dose changes by ≥10%.

9.0 DRUG FORMULATION, AVAILABILITY, AND PREPARATION

9.1 Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.

9.2 Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.

9.3 The total administered dose of cytotoxic chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose.

9.4 It is not necessary to change the doses of 5-FU, leucovorin or oxaliplatin due to changes in weight unless the calculated dose changes by ≥10%.

9.5 Oxaliplatin [Eloxatin] (NSC #266046)

Availability

Oxaliplatin is commercially available as an aqueous solution in vials containing 50 mg and 100 mg at a concentration of 5 mg/mL. The vials do not contain any preservative and they are intended for single use.

Storage and Stability

Intact vials should be stored at room temperature. Solutions diluted in D5W are stable for 6 hours at room temperature or 24 hours under refrigeration.

Preparation

The calculated dose of oxaliplatin should be diluted for infusion with 250 mL to 500 mL D5W. Oxaliplatin should not be diluted with a sodium chloride solution. Needles, syringes, catheters or IV administration sets containing aluminum should not be used with oxaliplatin. As with other platinum compounds, contact with aluminum may result in a black precipitate.

Administration

Oxaliplatin will be administered by intravenous infusion over 120 minutes prior to or concurrent with leucovorin. Infusion time may be prolonged (up to 6 hours) in patients experiencing pharyngolaryngeal dysesthesia.

Oxaliplatin is unstable in the presence of chloride or alkaline solutions. **Do NOT** mix or administer oxaliplatin with saline or other chloride-containing solutions. **Do NOT** administer other drugs or solutions in the same infusion line. Flush IV lines/catheters with Dextrose 5% in Water both before and after oxaliplatin administration.

Toxicity

The most commonly observed oxaliplatin toxicities include neurotoxicity, GI toxicity, and myelosuppression. Three neurotoxicity syndromes have been seen: acute sensory neuropathy develops within hours to 2 days after oxaliplatin administration. Symptoms include, paresthesias, dysesthesias, and hypothesia of the hands, feet and perioral region. Jaw spasm, abnormal tongue sensation, dysarthria, eye pain and a sensation of chest pressure have also been noted. Acute sensory neuropathy symptoms may be exacerbated by exposure to cold temperature or cold objects. Symptoms are reversible, usually resolving within 14 days and commonly recurring with further dosing. This syndrome has been observed in about 56% of patients receiving oxaliplatin with 5-FU and leucovorin.

Acute pharyngolaryngeal dysesthesia is reported to occur in 1-2% of patients. This syndrome is characterized by a subjective sensation of difficulty breathing or

swallowing without laryngospasm or bronchospasm or objective evidence of hypoxia. Avoidance of cold drinks, food and air is suggested in order to minimize pharyngolaryngeal dysesthesia. Antianxiety agents (e.g., lorazepam) may be used to treat pharyngolaryngeal dysesthesias once oxygen saturation has been documented to be normal.

Peripheral neuropathy persisting > 14 days is characterized by paresthesias, dysesthesias, and hypothesia. Abnormalities in proprioception may also be seen. Symptoms of persistent neuropathy may improve upon discontinuation of oxaliplatin.

Various agents have been used in an attempt to minimize neurotoxicity of oxaliplatin (e.g. carbamazepine, Mg+, Ca++). Calcium and magnesium infusions appear to be beneficial in preventing neurotoxicity. Contrary to preliminary findings described in 2007, calcium and magnesium do not appear to interfere with tumor response to FOLFOX. Calcium and magnesium infusions are generally given before and after oxaliplatin, and should not be prepared in the same infusion solution as FOLFOX components.

Gastrointestinal toxicities include nausea, vomiting (oxaliplatin is considered to be moderately emetogenic) and diarrhea.

Neutropenia is reported in 73% of patients receiving oxaliplatin with 5-FU and leucovorin (44% grade 3 or 4). Grade 3 or 4 thrombocytopenia is reported to occur in 4% of patients receiving the combination.

Allergic reactions, similar to those seen with other platinum compounds, have also been observed in patients treated with oxaliplatin. Reactions range from rash to anaphylaxis.

Rarely, oxaliplatin has been associated with pulmonary fibrosis, which may be fatal. Oxaliplatin should be discontinued in the presence of unexplained pulmonary symptoms (e.g. nonproductive cough, dysphagia) or pulmonary infiltrates until interstitial lung disease or pulmonary fibrosis have been ruled out.

Recent reports of oxaliplatin extravasation suggest that tissue necrosis may result and that oxaliplatin should be considered a vesicant. No standard treatment exists for oxaliplatin extravasation although heat and sodium thiosulfate have both been suggested.

Veno-occlusive disease (VOD) of the liver is a rare complication associated with oxaliplatin and 5-FU. Clinical manifestations of VOD include hepatomegaly, ascites, and jaundice. Histologically, VOD is characterized by diffuse damage in the centrilobular zone of the liver. Sequelae of VOD include hepatomegaly, splenomegaly, portal hypertension, and esophageal varices. A recent analysis of resected liver metastases in 153 patients indicated histological findings consistent with VOD in 6/27 patients who received 5-FU alone, 4/17 patients who received 5-FU and irinotecan, 20/27 patients who received 5-FU and oxaliplatin, and 14/16 who received 5-FU, oxaliplatin and irinotecan. The remaining 66 patients had not received chemotherapy prior to resection. There were no such findings in these patients.

For more information on toxicities associated with oxaliplatin, please see the package insert.

9.6 5-Fluorouracil (5-FU; fluorouracil)

Please refer to the package insert for complete product information.

Availability

5-FU is commercially available as a 50 mg/mL solution for injection in 10 mL, 20 mL, 50 mL and 100 mL vials.

Preparation

Inspect for precipitate; if found, agitate or gently heat in water bath. Bolus injections are prepared using undiluted drug.

46-48 hour infusion of 5-FU should be prepared for administration via ambulatory infusion pump according to the individual institution's standards. These solutions may be prepared in D5W or 0.9% NaCl. 5-FU should not be mixed in the same solution with most parenteral antiemetics.

Storage and Stability

Intact vials should be stored at room temperature and protected from light. Slight yellow discolor does not usually indicate decomposition. Stability in ambulatory pumps varies according to the pump, manufacturer of drug, concentration and diluent. Please refer to appropriate reference sources for additional information.

Administration

In this study, 5-FU is administered as a 400 mg/m^2 IV bolus followed by 2400 mg/m^2 by IV infusion over 46 to 48 hours. The bolus is administered after leucovorin, and the 46-48 hour infusion follows immediately after the bolus.

Toxicity

Nausea, diarrhea, vomiting (mild); stomatitis: 5-8 days after treatment initiation; myelosuppression: granulocytopenia (9-14 days); thrombocytopenia (7-14 days); Alopecia; loss of nails; hyperpigmentation; photosensitivity; maculopapular rash; palmar–plantar erythrodysethesias: (42-82% receiving continuous infusion); CNS effects: cerebral ataxia (rare); cardiotoxicity: MI, angina; asymptomatic S–T changes 68%; ocular effects: excessive lacrimation and less commonly, tear duct stenosis.

Drug Interactions

Leucovorin enhances the cytotoxicity of 5-FU by forming a more stable tertiary complex with thymidylate synthase. Concommitant adminstration of 5-FU with warfarin has been reported to result in increased INR/prolonged prothrombin time. Patients receiving both drugs should be followed with weekly INRs.

9.7 Leucovorin Calcium (Folinic Acid) (calcium folinate; citrovorum factor; N 5-formyltetrahydrofolate; 5-formyl-FH4; folinic acid)

Please refer to the package insert for complete product information.

Availability

Leucovorin calcium is commercially available in: 50 mg, 100 mg, 200 mg, 350 mg and 500 mg vials for reconstitution, and as a solution for injection in 50 mL vials at a concentration of 10 mg/mL.

Storage and Stability

Intact vials should be stored at room temperature and protected from light. Solutions reconstituted with BWI are stable for at least 7 days at room temperature. Solutions diluted for infusion are stable for 24 hours at room temperature and 4 days under refrigeration.

Preparation

Leucovorin may be reconstituted with Bacteriostatic Water for Injection (BWI), Sterile Water For Injection, or bacteriostatic NaCl or NaCl. Solutions should be further diluted in D5W, 0.9% NaCl or Ringers solution for infusion over two hours.

Administration

Leucovorin will be administered as a 400 mg/m^2 IV infusion over 2 hours after oxaliplatin administration and immediately before 5-FU. Leucovorin may also be administered concurrently with oxaliplatin as a separate IV infusion.

Toxicity

The only adverse reactions associated with leucovorin are allergic reactions. These are extremely uncommon.

9.8 Celecoxib (Celebrex) / Placebo (NSC #719627, CALGB IND #107051)

Availability

Celecoxib (NSC 719627) and matching Placebo will be provided free of charge by Pfizer and distributed by the Pharmaceutical Management Branch (PMB), Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI).

Celecoxib and matching Placebo will be supplied in bottles containing 100 – 400mg capsules (Celecoxib) or 100 – 0mg capsules (Placebo) with a child-resistant cap and a tamper-evident seal. Each blinded, patient-specific bottle will be labeled with:

- the protocol number (i.e., "CALGB-80702")
- the bottle number (i.e., "Bottle 1 of 2", "Bottle 2 of 2")
- the number of capsules (i.e., "100 capsules")
- the patient ID number (e.g., "999999", where "999999" represents a unique patient identifier assigned by CALGB at registration)
- the patient initials (i.e., last initial, first initial, middle initial [e.g., "L,FM"])
- the agent identification (i.e., "Celecoxib 400 mg or Placebo")
- a blank line for the pharmacist to enter the patient's name
- administration instructions (i.e., "Take one capsule once daily with food.")
- storage instructions (i.e., "Store at room temperature (15ºC to 25ºC, 59ºF to 77ºF).")
- emergency contact instructions
- a Julian date

The Julian date indicates the day the bottle was labeled and shipped and is composed of the last two digits of the calendar year (e.g., 2009 = 09, 2010 = 10) and a day count (e.g., January 1 = 001, December 31 = 365). For example, a bottle labeled and shipped on January 1, 2009 would have a Julian date of '09001' and a bottle labeled and shipped on December 31, 2010 would have a Julian date of '10365'. The Julian date will be used by PMB for recalls. When a lot expires, PMB will determine the last date the expired lot was shipped and will recall all bottles (i.e., both Celecoxib and Placebo) shipped on or before that date thus eliminating any chance of breaking the blind.

Questions about drug orders, transfers, returns, or accountability should be addressed to the PMB by calling 301-496-5725 Monday through Friday between 8:30AM and 4:30PM Eastern Time or by emailing <PMBAfterHours@mail.nih.gov> anytime.

Drug Ordering

No blinded starter supplies will be available for this study. Blinded, patient specific clinical supplies will be sent to the registering investigator at the time of randomization and should arrive within approximately 7 to 10 days. This randomization will be performed by the CALGB Statistical Center in Durham, NC. The assigned CALGB patient ID number must be recorded by the registering institution for proper bottle dispersion. Once a patient has been registered, the CALGB Statistical Center will electronically transmit a clinical drug request for that patient to the PMB. This request will be entered and transmitted by the CALGB Statistical Center the day the patient is registered and will be processed by the PMB the next business day and shipped the following business day. Shipments within the United States will be sent by US Priority Mail (generally two to three day delivery) and shipments to Canada will be sent by FedEx (generally one to two day delivery). Thus, if a patient is registered on Monday, CALGB would enter a clinical drug request for that patient on Monday and PMB would process that request on Tuesday and ship the drug on Wednesday. United States sites could expect to receive their order

approximately Friday or Monday and Canadian sites could expect to receive their order either Thursday or Friday. Shipments to United States sites can be expedited (i.e., receipt on Thursday in example above) by the provision of an express courier account name and number to the CALGB Statistical Center at the time the patient is randomized.

The initial request will be for **2 – 100 capsule bottles** (a 6 month supply at a dose of one capsule once daily) of Celecoxib or matching Placebo. After five months (one month before needed), sites may reorder an additional **2 – 100 capsule bottles** (a 6 month supply at a dose of one capsule once daily) by completing an NCI Clinical Drug Request form and faxing it to the PMB at 301-480-4612. The NCI Clinical Drug Request form is available on the CTEP home page (http://ctep.cancer.gov). The protocol number (i.e., CALGB-80702), the assigned patient ID number (e.g., "999999"), the patient initials (e.g., "L,FM"), and the number of bottles remaining from the previous shipment should be entered on each order. **Please note that a maximum of six shipments (12 bottles of 100 capsules), a sufficient quantity to complete three years of therapy, will be provided for each patient.**

All drug orders will be shipped directly to the registering physician at the shipping address listed on their most recent Supplemental Investigator Data Form (IDF) on file with CTEP. The registering investigator must maintain an active investigator status with CTEP, DCTD through the annual submission of an FDA Form 1572 (Statement of Investigator), a Curriculum Vitae, a Supplemental Investigator Data Form (IDF), and a Financial Disclosure Form (FDF) (http://ctep.cancer.gov/InvestigatorResources/investigator_registration.htm).

CALGB-80702 Shipment Schedule

Patient Randomized with CALGB	Initial e-Order Transmitted by CALGB	Initial e-Order Received and Approved by PMB	Initial Order Shipped By PMB	Initial Order Received at Site**
Monday	Monday	Tuesday	Wednesday	Monday
Tuesday	Tuesday	Wednesday	Thursday	Tuesday
Wednesday	Wednesday	Thursday	Friday	Wednesday
Thursday	Thursday	Friday	Monday	Thursday
Friday	Friday	Monday	Tuesday	Friday

**** arrival time approximate / shipments sent by US Priority Mail**

How Supplied

"Celecoxib" and matching "Placebo" are supplied as a size 0 white to off-white opaque hard gelatin capsule for oral administration. Each tamper-evident, child-resistant, 180ml, square, white, opaque, high-density polyethylene (HDPE) bottle contains 100 capsules. For “Celecoxib”, each capsule contains 400mg of celecoxib with croscarmellose sodium, edible inks, gelatin, lactose monohydrate, magnesium stearate, povidone, and sodium lauryl sulfate. For “Placebo”, each capsule contains croscarmellose sodium, edible inks, gelatin, lactose monohydrate, magnesium stearate, povidone, and sodium lauryl sulfate.

Storage and Stability

"Celecoxib" and matching "Placebo" are shipped at room temperature by US Priority Mail. The capsules should be stored at controlled room temperature (15ºC to 25ºC, 59ºF to 77ºF). The intact bottles of 100 capsules are stable for five years from date of manufacture when stored at controlled room temperature.

Route of Administration

Oral. Celecoxib/placebo at doses of 400mg orally once daily should be administered with food to improve absorption.

Drug Transfers

Bottles may NOT be transferred from one patient to another patient or from one protocol to another protocol. All other transfers (e.g., a patient moves from one participating clinical site to another participating clinical site, the registering investigator for a patient changes) must be approved in advance by the PMB. To obtain an approval for transfer, investigators should complete and submit to the PMB (fax number 301-402-0429) a Transfer Investigational Agent Form available on the CTEP home page (http://ctep.cancer.gov). The patient ID number (e.g., "999999"') and the patient initials (e.g., "L,FM") must be entered in the "Received on NCI Protocol No." and the "Transferred to NCI Protocol No." fields in addition to the protocol number (i.e., "CALGB-80702"). The julian date / order number (e.g., 10365-9999) should be entered in the "Lot Number" field.

Drug Returns

Only undispensed clinical supplies should be returned to the PMB. When it is necessary to return study drug (e.g., sealed bottles remaining when a patient permanently discontinues protocol treatment, expired bottles recalled by the PMB), investigators should return the study drug to the PMB using the NCI Return Drug List available on the CTEP home page (http://ctep.cancer.gov). The patient ID number (e.g., "999999"), the patient initials (e.g., "L,FM"), and the julian date / order number (e.g., 10365-9999) should be entered in the "Lot Number" field. A separate line item is required for each patient ID number (e.g., "999999") being returned. **Dispensed bottles with remaining tablets should be documented in the patient-specific NCI Investigational Agent Accountability Record (i.e., logged is as "returned by patient" and logged out as "destroyed on site") and destroyed on site in accordance with institutional policy.**

Drug Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return of all drugs received from the PMB using the NCI Investigational Agent Accountability Record available on the CTEP home page (http://ctep.cancer.gov). A separate NCI Investigational Agent Accountability Record must be maintained for each patient ID number (e.g., "999999") on this protocol. The combination julian date / order number in the upper right hand corner of the patient-specific bottle label (e.g., 10365-9999) should be recorded as the lot number.

Unblinding Procedures

Unblinding can be done only in the case of an emergency. Follow the directions below to unblind patient treatment. Please note that, if treatment is unblinded due to an emergency, the patient must permanently discontinue all protocol therapy.

Emergency Unblinding Procedures

Examples of emergencies include …

1) a life threatening unexpected adverse event that is at least possibly related to the investigational agent and for which unblinding would influence treatment decisions

OR

2) a medication error, such as an accidental overdose.

Expected adverse events are listed in the "Toxicities" section below.

Contact a CALGB Approving Physician (i.e., Executive Officer) by calling the pager number, 773-652-0098. If an Executive Officer cannot be reached, contact the CALGB Statistical Center at 1-877-442-2542 and the Statistical Center will contact an Approving Physician. Note: The Statistical Center cannot give permission for unblinding; only a CALGB Approving Physician can authorize emergency unblinding.

The following information will be required when contacting the CALGB Approving Physician:

- CALGB study number (i.e., "CALGB-80702")
- CALGB patient ID number (e.g., "999999")
- Patient initials (e.g., "L,FM")
- Institution name
- Name and telephone number of treating physician
- Name and telephone number of person requesting the unblinding procedure
- Name and telephone number of contact person to inform of treatment assignment
- Reason for emergency unblinding

After authorization by a designated CALGB Approving Physician, the treatment assignment will be provided to the contact person by the CALGB Statistical Center.

Toxicities

Celecoxib/placebo is generally well tolerated. The most common toxicity reported in arthritis trials is a headache. Other possible toxicities include peripheral edema, insomnia, dizziness, skin rash, dyspepsia, diarrhea, abdominal pain, nausea, flatulence, back pain, upper respiratory tract infection, sinusitis, pharyngitis, and rhinitis.

The incidence of GI ulcers documented by endoscopy in arthritis trials is 7%. GI bleeding is more likely to occur in patients with a history of peptic ulcer disease and/or GI bleeding. The risk of GI ulceration, bleeding or perforation with celecoxib is increased with concomitant use of aspirin. Chronic use of aspirin (>100 mg/day) or other NSAIDs is not allowed on this trial (see Section 4.2).

As is the case with non-selective NSAIDs, celecoxib may be associated with nephrotoxicity in patients in whom renal prostaglandins are important in maintenance of renal blood flow. Specifically, patients with pre-existing renal dysfunction, heart failure, liver dysfunction or dehyration, elderly patients, or patients taking diuretics or ACE inhibitors are at the greatest risk for significant inhibition of renal blood flow and nephrotoxicity. In addition, long-term use of NSAIDs has been associated with renal injury, including renal papillary necrosis. Unlike other non-selective NSAIDs, celecoxib does not appear to inhibit platelet aggregation.

A safety analysis performed in December, 2004, of several long-term celecoxib trials was conducted following the removal of rofecoxib from the market because of an increased risk of adverse cardiovascular events. The celecoxib analysis resulted in the suspension of drug use for patients enrolled in the Adenoma Prevention with Celecoxib (APC) trial. In this trial, the risk of cardiovascular death, myocardial infection or stroke in the celecoxib groups was 2-3 times higher than the risk in the placebo group. This increased hazard with celecoxib was not observed in another long-term trial.

Drug Interactions

Celecoxib is metabolized in the liver by cytochrome P450 2C9. Drugs that inhibit (e.g., fluconazole) or induce (e.g., rifampin) the 2C9 isoenzyme might be expected to result in increased toxicity or decreased effect of celecoxib, respectively. In addition, celecoxib is reported to inhibit the 2D6 isoenzyme, potentially enhancing the effects of drugs metabolized by this isoenzyme.

The following describes possible drug interactions:

ACE-inhibitors: NSAIDs may diminish the antihypertensive effect of ACE-inhibitors.

Coumadin: Celecoxib does not alter PT/INR; there have been reports of prolonged prothrombin time and bleeding in elderly patients taking both coumadin and celecoxib.

Fluconazole: Concomitant administration of celecoxib and fluconazole results in a two-fold increase in celecoxib levels.

Lithium: Concomitant administration of celecoxib and lithium results in an increase in steady-state lithium levels.

10.0 ANCILLARY THERAPY

10.1 Patients should receive *full supportive care*, including transfusions of blood and blood products, epoietins, antibiotics, antiemetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on Form C-1954.

10.2 Treatment with *hormones or other chemotherapeutic* agents may not be administered except for steroids given for adrenal failure or hypersensitivity reactions; hormones administered for non-disease-related conditions (e.g., insulin for diabetes); and intermittent use of dexamethasone as an antiemetic.

10.3 Loperamide: For symptoms of diarrhea and/or abdominal cramping that occur at any time during a treatment cycle, patients will be instructed to begin taking loperamide. Loperamide should be started at the earliest sign of (1) a poorly formed or loose stool or (2) the occurrence of 1 to 2 more bowel movements than usual in 1 day or (3) an increase in stool volume or liquidity. Loperamide should be taken in the following manner: 4 mg at the first onset of diarrhea, then 2 mg every 2 hours around the clock until diarrhea-free for at least 12 hours. Patients may take loperamide 4 mg every 4 hours during the night. The maximum daily dose of loperamide is 16 mg/day. Patients should be advised to obtain loperamide at the initial treatment visit so that they have sufficient supply on hand in case antidiarrheal support is required. Additional antidiarrheal measures may be used at the discretion of the treating physician. Patients should be instructed to increase fluid intake to help maintain fluid and electrolyte balance during episodes of diarrhea.

10.4 Anticoagulants: Patients who are taking warfarin or coumadin may participate in this study; however, the prothrombin time/INR should be monitored weekly. Subcutaneous or low molecular weight heparin is permitted.

11.0 CRITERIA FOR RESPONSE, PROGRESSION, AND RELAPSE

To minimize potential bias of differential post-treatment surveillance between the two arms, CEA testing and imaging requires standardization. For patients who have signs or symptoms in which imaging is clinically indicated, the decision of timing of radiographic studies remains at the discretion of the treating clinician. However, for asymptomatic surveillance after the completion of FOLFOX, the timing of CEA testing and radiograph imaging must be similar. As such, for patients randomized to 6 treatments of FOLFOX, the first post-treatment CEA and imaging should occur 4 months after completion of FOLFOX. For patients randomized to 12 treatments of FOLFOX, the first post-treatment CEA and imaging should occur within 6 weeks of completion of FOLFOX.

At the time of each evaluation, patients will be classified in the following manner:

11.1 No evidence of disease (NED)

11.2 Recurrence of disease (REC)

Recurrence must be confirmed by imaging and/or biopsy, with the colonoscopy and pathology reports submitted. Elevated CEA levels only or physical findings only will not be accepted as evidence of recurrence.

12.0 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

12.1 Duration of treatment

Patients with documented disease progression at any time during therapy will be removed from protocol treatment.

12.2 Extraordinary medical circumstances

If at any time, the constraints of this protocol are detrimental to the patient's health and/or patient no longer wishes to continue protocol therapy, protocol therapy should be discontinued and the study chair and CALGB should be notified. The reason for discontinuation needs to be documented and patient should be asked if he/she can still be followed for recurrence and survival.

13.0 STATISTICAL CONSIDERATIONS

13.1 Sample size and power estimates

The primary endpoint of this trial is DFS measured from study entry (time of randomization) until documented progression or death from any cause. The superiority hypothesis of celecoxib use will be tested in this patient population using the DFS endpoint. Non-exponential survival is expected. Based on the findings from NSABP C07 and the MOSAIC trials, the DFS distribution under the null hypothesis assumes probabilities of failure by year for years 1 through 6 to be 0.10, 0.12, 0.06, 0.05, 0.03 and 0.02, respectively. It is anticipated that 2,500 patients will be enrolled in 3.125 years (800 patients per year) with a follow-up period of 3 years.

The number of expected DFS events at the conclusion of the trial (n=775) is estimated using the method proposed by Schoenfeld. A hazard ratio of 0.79 in favor of celecoxib is assumed; this corresponds to an increase in the probability of being disease-free at 3 years from 0.72 to 0.77. With 775 events observed, this difference can be detected with power of approximately 0.91 (2-sided α=0.05).

Overall survival (OS) measured from study entry (time of randomization) until death from any cause will be studied as a secondary endpoint.

In addition, a prospective international effort is underway to pool patient level data from multiple trials that will test the duration assumption (the IDEA trial). Our goal is for the United States Intergroup to contribute 2500 patients with stage III colon cancer to this effort. The current statistical assumptions for the IDEA trial is to pool 10,500 patients with stage III colon cancer and declare non-inferiority if the 2-sided 95% confidence interval for the hazard ratio comparing 3 to 6 months of therapy lies entirely below 1.10. The disease-free survival endpoint in C80702 for the duration question is thus a secondary endpoint since 2500 patients will not adequately address non-inferiority (i.e., patients from this trial will contribute to the pooling but we do not plan to report the results for the duration question as a primary endpoint in C80702). Secondary endpoints of toxicity will also be reported for the 2500 patients in this trial. Data provided to the IDEA trial will be fully de-identified. The CALGB Statistical Center will maintain the data link between the coded patient numbers provided in the de-identified dataset and the CALGB patient identification numbers. Any interim transfer of data must be approved by the CALGB Data and Safety Monitoring Board.

13.2 Interim monitoring

Formal interim analyses of the primary endpoint, DFS, will begin when approximately 20% ($\geq$ 155/775) of the total expected failures has occurred. Subsequently, interim analyses will be conducted every 6 months to coincide with scheduled meetings of the CALGB DSMB. Three interim analyses are expected during the accrual period and five during the follow-up period. The 2-sided, α=0.05 Lan-DeMets analogue of the O'Brien-Fleming boundaries, will be used to test for efficacy

for the celecoxib hypothesis at each interim. Interim analyses are expected when approximately 20%, 29%, 40%, 52%, 64%, 74%, 83%, and 90% of data are available. A futility analysis will also be conducted for the celecoxib hypothesis at each interim analysis. If the adjusted lower confidence bound for the DFS hazard ratio at an interim analysis is greater than 0.79, consideration will be given to curtail further accrual or follow-up. The CALGB Statistical Center will submit quarterly reports to CTEP by electronic means using the Clinical Data Update System (CDUS).

14.0 ADVERSE EVENT REPORTING (AER)

Investigators are required by Federal Regulations to report serious adverse events as defined below (in both the table and text). CALGB investigators are required to notify the Investigational Drug Branch (IDB), the CALGB Central Office, the Study Chair, and their Institutional Review Board if a patient has a reportable serious adverse event. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

All reactions determined to be "reportable" in an expedited manner must be reported using the NCI Adverse Event Expedited Reporting System (AdEERS).

14.1 CALGB/SWOG C80702 Reporting Requirements for <u>all arms of the study</u>:

Phase 2 and 3 Trials: Adeers Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of Treatment Under a CTEP IND or CALGB IND:

	Grade 1	**Grade 2**	**Grade 2**	**Grade 3**		**Grade 3**		**Grades 4 & 5**	**Grades 4 & 5**
				Unexpected		**Expected**			
	Unexpected and Expected	**Unexpected**	**Expected**	**with Hospitali-zation**	**without Hospitali-zation**	**with Hospitali-zation**	**without Hospitali-zation**	**Unexpected**	**Expected**
Unrelated Unlikely	Not Required	Not Required	Not Required	Not Required	Not Required	Not Required	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	Not Required	Not Required	Not Required	Not Required	Not Required	Not Required	10 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment with an agent under a CTEP IND or CALGB IND require reporting as follows:**

AdEERS 10 calendar day report:

- Grade 4 unexpected events
- Grade 5 expected or unexpected events

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Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:
  - "10 calendar days" - A complete AdEERS report on the AE must be submitted within <u>10 calendar days</u> of the investigator learning of the event.
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND or CALGB IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

14.2 Additional Instructions or Exclusion to AdEERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent Under a CTEP IND or CALGB IND:

- CALGB/SWOG C80702 uses a drug under a CALGB IND. The reporting requirements in the table and text should be followed for both arms in this trial.
- Deaths occurring greater than 30 days after the last dose of treatment that are due to disease progression do not require AdEERS expedited reporting.
- All grade 4 events that are unexpected and that are at least possibly related to treatment must be reported via AdEERS within 10 calendar days.
- Grade 4 events that are expected with chemotherapy (FOLFOX) that occur during FOLFOX chemotherapy do not require AdEERS expedited reporting, even if they result in hospitalization.
- Grade 4 events that are expected with chemotherapy (FOLFOX) that OCCUR DURING CELECOXIB/PLACEBO MONOTHERAPY must be reported via AdEERS within 10 calendar days.
- GRADES 2-5 CARDIOVASCULAR EVENTS (CARDIAC ISCHEMIA, CEREBROVASCULAR ISCHEMIA, VENOUS THROMBOMBOLIC EVENTS, LEFT VENTRICULAR SYSTOLIC DYSFUNCTION, HEART FAILURE) THAT OCCUR WITHIN 30 DAYS OF THE LAST DOSE OF ANY/ALL PROTOCOL TREATMENT MUST BE REPORTED VIA ADEERS WITHIN 10 CALENDAR DAYS.
- Treatment expected adverse events include those listed in Section 9.0 and in the package insert or celecoxib Investigator's Brochure.
- AdEERS reports should be submitted electronically to the CALGB Central Office (calgb@uchicago.edu). Faxed copies of the AdEERS paper template, available at the AdEERS web page, will be accepted (312-345-0117), but electronic submission is preferred.
- All adverse events reported via AdEERS (i.e., serious adverse events) should also be forwarded to your local IRB.
- The reporting of adverse events described above is in addition to and does not supplant the reporting of adverse events as part of the report of the results of the clinical trial (e.g., study forms).
- New primary malignancies should be reported using study form C-1001. Secondary AML/MDS should be reported using the NCI/CTEP Secondary AML/MDS Report Form.

14.3 Comprehensive Adverse Events and Potential Risks list (CAEPR) for Oxaliplatin (NSC 266046)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' *http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_adeers* for further clarification. *Frequency is provided based on 1141 patients.* Below is the CAEPR for oxaliplatin.

Version 2.2, March 11, 2010[1]

Adverse Events with Possible Relationship to Oxaliplatin (CTCAE 4.0 Term) [n= 1141]			**EXPECTED AEs FOR ADEERS REPORTING Agent Specific Adverse Event List (ASAEL)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	***Expected***
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia***
	Disseminated intravascular coagulation		***Disseminated intravascular coagulation***
	Febrile neutropenia		***Febrile neutropenia***
	Hemolysis		***Hemolysis***
		Thrombotic thrombocytopenic purpura	
CARDIAC DISORDERS			
	Atrial fibrillation		***Atrial fibrillation***
	Atrial flutter		***Atrial flutter***
	Paroxysmal atrial tachycardia		***Paroxysmal atrial tachycardia***
	Sinus bradycardia		***Sinus bradycardia***
	Sinus tachycardia		***Sinus tachycardia***
	Supraventricular tachycardia		***Supraventricular tachycardia***
	Ventricular arrhythmia		***Ventricular arrhythmia***
	Ventricular fibrillation		***Ventricular fibrillation***
	Ventricular tachycardia		***Ventricular tachycardia***
EAR AND LABYRINTH DISORDERS			
	Hearing impaired		***Hearing impaired***
	Middle ear inflammation		***Middle ear inflammation***
EYE DISORDERS			
	Conjunctivitis		***Conjunctivitis***
	Dry eye		***Dry eye***
	Eye disorders - Other (amaurosis fugax)		***Eye disorders - Other (amaurosis fugax)***
	Eye disorders - Other (cold-induced transient visual abnormalities)		***Eye disorders - Other (cold-induced transient visual abnormalities)***
	Eyelid function disorder		***Eyelid function disorder***
	Papilledema		***Papilledema***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain***
	Ascites		***Ascites***
	Colitis		***Colitis***
	Constipation		***Constipation***
Diarrhea			***Diarrhea***
	Dry mouth		***Dry mouth***
	Dyspepsia		***Dyspepsia***
	Dysphagia		***Dysphagia***
	Enterocolitis		***Enterocolitis***
	Esophagitis		***Esophagitis***
	Flatulence		***Flatulence***
	Gastritis		***Gastritis***

		Gastrointestinal disorders – Other (pneumatosis intestinalis)	
	Gastrointestinal hemorrhage[2]		***Gastrointestinal hemorrhage[2]***
	Gastrointestinal necrosis[3]		***Gastrointestinal necrosis[3]***
	Gastrointestinal ulcer[4]		***Gastrointestinal ulcer[4]***
	Ileus		***Ileus***
	Mucositis oral		***Mucositis oral***
Nausea			***Nausea***
	Pancreatitis		***Pancreatitis***
	Small intestinal obstruction		***Small intestinal obstruction***
Vomiting			***Vomiting***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills***
	Edema face		***Edema face***
	Edema limbs		***Edema limbs***
Fatigue			***Fatigue***
	Fever		***Fever***
	Gait disturbance		***Gait disturbance***
	General disorders and administration site conditions - Other (Hepato-renal syndrome)		***General disorders and administration site conditions - Other (Hepato-renal syndrome)***
	Injection site reaction		***Injection site reaction***
	Non-cardiac chest pain		***Non-cardiac chest pain***
HEPATOBILIARY DISORDERS			
		Cholecystitis	
	Hepatic failure		***Hepatic failure***
	Hepatobiliary disorders - Other (hepatic enlargement)		***Hepatobiliary disorders - Other (hepatic enlargement)***
	Hepatobiliary disorders - Other (veno-occlusive liver disease)		***Hepatobiliary disorders - Other (veno-occlusive liver disease)***
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		***Allergic reaction***
INFECTIONS AND INFESTATIONS			
	Infection[5]		***Infection[5]***
INVESTIGATIONS			
	Activated partial thromboplastin time prolonged		***Activated partial thromboplastin time prolonged***
Alanine aminotransferase increased			***Alanine aminotransferase increased***
	Alkaline phosphatase increased		***Alkaline phosphatase increased***
Aspartate aminotransferase increased			***Aspartate aminotransferase increased***
	Blood bilirubin increased		***Blood bilirubin increased***
	Creatinine increased		***Creatinine increased***

	GGT increased		***GGT increased***
	INR increased		***INR increased***
	Lymphocyte count decreased		***Lymphocyte count decreased***
	Neutrophil count decreased		***Neutrophil count decreased***
Platelet count decreased			***Platelet count decreased***
	Weight gain		***Weight gain***
	Weight loss		***Weight loss***
	White blood cell decreased		***White blood cell decreased***
METABOLISM AND NUTRITION DISORDERS			
	Acidosis		***Acidosis***
	Anorexia		***Anorexia***
	Dehydration		***Dehydration***
	Hyperglycemia		***Hyperglycemia***
	Hyperuricemia		***Hyperuricemia***
	Hypoalbuminemia		***Hypoalbuminemia***
	Hypocalcemia		***Hypocalcemia***
	Hypoglycemia		***Hypoglycemia***
	Hypokalemia		***Hypokalemia***
	Hypomagnesemia		***Hypomagnesemia***
	Hyponatremia		***Hyponatremia***
	Hypophosphatemia		***Hypophosphatemia***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia***
	Back pain		***Back pain***
	Bone pain		***Bone pain***
	Myalgia		***Myalgia***
	Trismus		***Trismus***
NERVOUS SYSTEM DISORDERS			
	Ataxia		***Ataxia***
	Depressed level of consciousness		***Depressed level of consciousness***
	Dizziness		***Dizziness***
	Dysgeusia		***Dysgeusia***
	Dysphasia		***Dysphasia***
	Extrapyramidal disorder		***Extrapyramidal disorder***
	Headache		***Headache***
	Intracranial hemorrhage		***Intracranial hemorrhage***
	Ischemia cerebrovascular		***Ischemia cerebrovascular***
	Nerve disorder[6]		***Nerve disorder[6]***
	Nervous system disorders - Other (multiple cranial nerve palsies)		***Nervous system disorders - Other (multiple cranial nerve palsies)***
	Peripheral motor neuropathy		***Peripheral motor neuropathy***
Peripheral sensory neuropathy			***Peripheral sensory neuropathy***
	Seizure		***Seizure***
PSYCHIATRIC DISORDERS			
	Anxiety		***Anxiety***
	Confusion		***Confusion***
	Depression		***Depression***
	Insomnia		***Insomnia***

RENAL AND URINARY DISORDERS			
		Acute kidney injury	***Acute kidney injury***
	Hematuria		***Hematuria***
	Renal hemorrhage		***Renal hemorrhage***
	Urinary frequency		***Urinary frequency***
	Urinary retention		***Urinary retention***
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
	Hematosalpinx		***Hematosalpinx***
	Ovarian hemorrhage		***Ovarian hemorrhage***
	Prostatic hemorrhage		***Prostatic hemorrhage***
	Spermatic cord hemorrhage		***Spermatic cord hemorrhage***
	Testicular hemorrhage		***Testicular hemorrhage***
	Uterine hemorrhage		***Uterine hemorrhage***
	Vaginal hemorrhage		***Vaginal hemorrhage***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Adult respiratory distress syndrome	***Adult respiratory distress syndrome***
	Allergic rhinitis		***Allergic rhinitis***
	Bronchopulmonary hemorrhage		***Bronchopulmonary hemorrhage***
	Bronchospasm		***Bronchospasm***
	Cough		***Cough***
	Dyspnea		***Dyspnea***
	Hiccups		***Hiccups***
	Pneumonitis		***Pneumonitis***
	Pulmonary fibrosis		***Pulmonary fibrosis***
	Sinus disorder		***Sinus disorder***
	Voice alteration		***Voice alteration***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		***Alopecia***
	Dry skin		***Dry skin***
	Hyperhidrosis		***Hyperhidrosis***
		Palmar-plantar erythrodysesthesia syndrome	***Palmar-plantar erythrodysesthesia syndrome***
	Pruritus		***Pruritus***
	Rash maculo-papular		***Rash maculo-papular***
	Urticaria		***Urticaria***
VASCULAR DISORDERS			
	Flushing		***Flushing***
	Hot flashes		***Hot flashes***
	Hypertension		***Hypertension***
	Hypotension		***Hypotension***
	Phlebitis		***Phlebitis***
	Thromboembolic event		***Thromboembolic event***
	Vascular disorders - Other (hemorrhage with thrombocytopenia)		***Vascular disorders - Other (hemorrhage with thrombocytopenia)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Doudenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Gastrointestinal necrosis includes Anal necrosis, Esophageal necrosis, Gastric necrosis, Pancreatic necrosis, Peritoneal necrosis, and Rectal necrosis under the GASTROINTESTINAL DISORDERS SOC.

[4]Gastrointestinal ulcer includes Anal ulcer, Colonic ulcer, Duodenal ulcer, Esophageal ulcer, Gastric ulcer, Ileal ulcer, Jejunal ulcer, Rectal ulcer, and Small intestine ulcer under the GASTROINTESTINAL DISORDERS SOC.

[5]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[6]Nerve disorder includes Abducens nerve disorder, Accessory nerve disorder, Acoustic nerve disorder NOS, Facial nerve disorder, Glossopharyngeal nerve disorder, Hypoglossal nerve disorder, IVth nerve disorder, Oculomotor nerve disorder, Olfactory nerve disorder, Trigeminal nerve disorder, and Vagus nerve disorder under the NERVOUS SYSTEM DISORDERS SOC.

[7]Gastrointestinal perforation includes Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Ileal perforation, Jejunal perforation, Rectal perforation, and Small intestinal perforation under the GASTROINTESTINAL DISORDERS SOC.

Also reported on oxaliplatin trials but with the relationship to oxaliplatin still undetermined:

CARDIAC DISORDERS - Heart failure; Left ventricular systolic dysfunction; Myocardial infarction; Pericardial effusion
EYE DISORDERS - Eye pain
GASTROINTESTINAL DISORDERS – Gastrointestinal perforation[7]
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Injury to superior vena cava; Vascular access complication
INVESTIGATIONS - Cardiac troponin I increased; Lipase increased; Serum amylase increased
METABOLISM AND NUTRITION DISORDERS - Hypercalcemia; Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Generalized muscle weakness
NERVOUS SYSTEM DISORDERS - Syncope
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Hypoxia
VASCULAR DISORDERS - Visceral arterial ischemia

Note: Oxaliplatin in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

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16.0 MODEL CONSENT FORM

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

You are being asked to take part in this study because you have cancer of the colon, which has been surgically removed, but has spread to lymph nodes.

Why is this study being done?

This study is being done to evaluate the effects (good and bad) of different chemotherapy treatments. One of the common combinations of chemotherapy drugs used to treat your type of cancer includes 5-fluorouracil (also called 5-FU), leucovorin and oxaliplatin, and is also called "FOLFOX". At the present time, the Food and Drug Administration (FDA) has approved each of these drugs as treatment for colon cancer. FOLFOX is a standard treatment used to prevent colon cancer from coming back (recurrence).

In this study, we will evaluate the effects (good and bad) of an oral drug called celecoxib when given in combination with FOLFOX chemotherapy. Celecoxib is approved by the FDA to treat arthritis. It is also approved to help prevent colon polyps in families with a genetic risk for colon cancer. The addition of celecoxib to FOLFOX chemotherapy is considered investigational. One of the purposes of this study is to determine if giving patients celecoxib (by mouth) and chemotherapy decreases the risk of colon cancer recurrence.

This study will also look at whether receiving FOFLOX chemotherapy for 6 treatments (12 weeks) is as good as 12 treatments (24 weeks) in preventing recurrence of colon cancer. Currently, the standard of care for your stage of colon cancer is 12 treatments with FOLFOX. That was the number of treatments tested in previous research studies. However, it is not known if fewer treatments would be as helpful in preventing your cancer from coming back. In this trial, we will explore whether 6 treatments are as effective as 12 treatments and whether side effects can be reduced with fewer treatments. If you participate in this study, you may be assigned to the group receiving only 6 treatments, which would be fewer treatments than the standard of care (12 treatments).

How many people will take part in the study?

About 2,500 people will take part in this study.

What Will Happen if I Take Part in the Research Study?

Before you begin the study . . .

You will need to have the following exams, tests or procedures to find out if you can be in the study. These exams, tests or procedures are part of regular cancer care and may be done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. This will be up to your study doctor.

- Medical history and physical examination;
- Blood tests, a pregnancy test, liver function tests;
- CT, MRI scan, or ultrasound of the abdomen and chest CT or X-ray.

If the exams, tests and procedures show that you can be in the study and you choose to take part, then you will be "randomized" into one of the study groups described below. Randomization means that you are put into a group by chance. A computer program will place you in one of the study groups. Neither you nor your doctor can choose the group you will be in. You will have an equal chance of being placed in one of the four groups. The four treatment groups are:

Group 1 (also called "Arm A") FOLFOX for 12 treatments (24 weeks) plus placebo (also known as a “sugar capsule”) given by mouth every day for three years.

Group 2 (also called "Arm B") FOLFOX for 12 treatments (24 weeks) plus celecoxib given by mouth every day for three years.

Group 3 (also called "Arm C") FOLFOX for 6 treatments (12 weeks) plus placebo (also known as a “sugar capsule”) given by mouth every day for three years.

Group 4 (also called "Arm D") FOLFOX for 6 treatments (12 weeks) plus celecoxib given by mouth every day for three years.

You will be told if you are to get treatment with FOLFOX for 6 treatments (12 weeks) or 12 treatments (24 weeks). Neither you nor your physician will be told if you are to get celecoxib or a placebo (sugar capsule) that looks just like celecoxib. However, in case of an emergency, your doctor may be able to find out whether you are getting the celecoxib or the placebo. If this happens, you will be required to drop out of the study.

Even after you have completed study treatment, you and your doctor will not be told whether you received celecoxib or a placebo capsule.

During the study . . .

Each treatment group will receive intravenous treatment with FOLFOX every 2 weeks and treatment with celecoxib or placebo capsules every day. Each 2-week period is called "a cycle".

ARM A:

FOLFOX every 2 weeks **plus** **Placebo** every day for **12 treatments (24 weeks)**	**Then:**	**Placebo** alone every day for **3 years** total

ARM B:

FOLFOX every 2 weeks **plus** **Celecoxib** every day for **12 treatments (24 weeks)**	**Then:**	**Celecoxib** alone every day for **3 years** total

ARM C:

FOLFOX every 2 weeks **plus** **Placebo** every day for **6 treatments (12 weeks)**	**Then:**	**Placebo** alone every day for **3 years** total

ARM D:

FOLFOX every 2 weeks **plus** **Celecoxib** every day for **6 treatments (12 weeks)**	**Then:**	**Celecoxib** alone every day for **3 years** total

FOLFOX: For the FOLFOX combination, you will receive the following drugs: **Oxaliplatin** will be given by vein over a period of about 2 hours. Afterwards (or at the same time), **leucovorin** will be given by vein for 2 hours. **5-FU** will then be given as a shot through your vein, followed by an infusion which will take about 2 days. You can get the 2-day infusion as an outpatient.

Celecoxib or Placebo: Starting with the first day of treatment with FOLFOX you will start treatment with celecoxib or the placebo. You will take one capsule every day for three years. You will receive a new bottle of celecoxib or placebo capsules every 3 months. Try to take the capsule at about the same time every day with food. You will be asked to record the day, number

of capsules taken and time of each dose of celecoxib or placebo on a medication calendar. You will be asked to bring the calendar and any unused capsules with you each visit.

Tests and Procedures:

During the time that you are receiving the study treatment, you will need the following tests and procedures that are part of regular cancer care.

- Physical examinations (every 4 weeks during FOLFOX treatment and then every 3 months while taking celecoxib/placebo only),
- Blood tests (every 2 weeks during FOLFOX treatment and then every 3 months while taking celecoxib/placebo only), to look at the side effects of chemotherapy
- Blood tests for liver and kidney function (every 4 weeks during FOLFOX treatment and then every 3 months while taking celecoxib/placebo only),
- CT scans, MRI scans, ultrasound scans and chest x-rays to monitor your condition after the completion of the FOLFOX treatment (about every 6 months).

Other Medicines:

You should not take NSAIDs (e.g., ibuprofen or similar drugs) other than celecoxib/placebo study medicine. You should not take aspirin more than 3 times a week. If you take baby aspirin (100 mg or less), you should take no more than 1 per day. If you have any questions about these medicines, please ask your doctor.

How long will I be in the study?

You will be treated with the FOLFOX chemotherapy for up to 6 treatments (12 weeks) or 12 treatments (24 weeks) (depending on your treatment arm) and the celecoxib or placebo capsule for up to 3 years. Some or all of the treatments would be stopped if you become too sick to receive the therapy, if your doctor believes another treatment is appropriate, or if you no longer wish to continue with the study. Whether or not you remain on study treatment, the study doctor will continue to follow your progress at least every 6 months for up to 6 years after you started treatment.

Can I stop being in the study?

Yes. You can decide to stop at any time. Tell the study doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely.

It is important to tell the study doctor if you are thinking about stopping so any risks from the drugs can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest; if you do not follow the study rules; or if the study is stopped.

What side effects or risks can I expect from being in the study?

You may have side effects while on the study. Everyone taking part in the study will be watched carefully for any side effects. However, doctors don't know all the side effects that may happen. Side effects may be mild or very serious. Your health care team may give you medicines to help lessen side effects. Many side effects go away soon after you stop taking the drugs. In some cases, side effects can be serious, long lasting, or may never go away. There is also a risk of death.

You should talk to your study doctor about any side effects that you have while taking part in the study.

Risks and side effects related to the treatments being studied include:

FOLFOX plus Placebo (Arms A and C)

LIKELY:

- Lack of enough red blood cells (anemia which may make you short of breath, weak, fatigued, or tired)
- Diarrhea, which could lead to dehydration
- Nausea or the urge to vomit
- Vomiting
- Fatigue or tiredness
- Increased blood level of a liver enzyme (ALT/SGPT)
- Increased blood level of a liver enzyme (AST/SGOT)
- Decreased number of a type of blood cell (platelet) that helps to clot blood (may result in easy bruising or bleeding)
- Inflammation or damage of the peripheral nerves (those nerves outside of brain and spinal cord) causing numbness, tingling, burning
- Decrease in the total number of white blood cells (leukocytes); may make you more vulnerable to infection which could be serious or even life-threatening
- Decreased number of a type of white blood cell (lymphocyte)
- Decreased number of a type of white blood cell (neutrophil/granulocyte)
- Temporary hair loss
- Darkening of the skin. This happens most often in the palms of the hands or along the vein where 5-FU is given. This is not harmful, but it could be permanent.
- Soreness or painful ulcers in the mouth and/or throat (lasting a couple of days)
- Photosensitivity (exposure to sunlight can cause skin to be sensitive to sunburn). You should use a sunscreen.
- A sensation of pain or tingling in areas of the body that are exposed to cold air or cold liquid, such as your hands if placed in the refrigerator or your throat if exposed to a cold wind or drinking cold liquids (see the Additional Information section below).
- Dizziness (or sensation of lightheadedness, unsteadiness, giddiness, spinning or rocking)
- Changes in fingernails
- Loss of appetite

- Taste changes
- Headache (or head pain)

LESS LIKELY:
- Abnormal blood clotting and/or bleeding
- Fever associated with dangerously low levels of a type of white blood cell (neutrophils)
- Destruction of red blood cells
- Abnormally fast irregular heartbeat involving the upper chambers of the heart (atria)
- Abormally fast regular heartbeat involving the upper chambers of the heart (atria)
- Period of very rapid and regular heartbeats that begins and ends suddenly
- Slow heartbeat; regular rhythm
- Fast heartbeat; regular rhythm
- Fast heartbeat usually originating in an area located above the ventricles
- Irregular heartbeat resulting from an abnormality in one of the lower chambers of the heart (ventricle)
- Irregular heartbeat that involves the lower chambers of the heart (ventricles) that results in uncoordinated muscle movement of the ventricles making them tremble rather than contract properly; life-threatening, needs immediate attention
- Rapid heartbeat of one of the lower chambers (ventricle) of the heart; regular rhythm but potentially life-threatening, needs immediate attention
- Hearing loss
- Inflammation (swelling and redness) of the middle ear
- Inflammation (swelling and redness) of the conjunctiva (the outermost layer of the eye and the inner surface of the eyelids). Commonly called “pink eye”.
- Dry eye
- A situation in which one has temporary blindness of one eye, due to a blockage (or decreased blood flow) in the blood vessels leading to that eye
- Temporary vision problems caused by the cold
- Problem with eyelid
- Swelling around the nerve responsible for sight
- Belly pain
- Fluid collection in the abdomen
- Constipation
- Dry mouth
- Heartburn
- Difficulty swallowing
- Inflammation of the small and/or large bowel
- Inflammation of the esophagus (gullet or tube that goes from mouth to stomach through which food passes)
- Excess passing of gas
- Inflammation of the stomach lining
- Bleeding in some organ(s) of the digestive tract, for example, blood in your stool
- Death of tissue somewhere in the digestive tract
- Sore (ulcer) somewhere in the digestive tract
- Partial or complete blockage of the small and/or large bowel. With ileus, the bowel acts like it is blocked.

- Inflammation of the pancreas
- Blockage of the small bowel
- Chills
- Swelling of the face
- Swelling of the extremeties (arms and/or legs)
- Fever
- Limp or difficulty walking
- A condition in which both the liver and kidneys fail
- Inflammation (swelling and redness) or damage to the tissue surrounding where a drug was injected
- Chest pain not heart-related
- Liver failure
- Increase in size of the liver
- A condition in which there is blockage of the veins of the liver; leads to liver damage
- Allergic reaction: abnormal reaction of the body to substances, called allergens, that are contacted through the skin, inhaled in the lungs, swallowed, or injected
- Infection
- Test that shows a problem in blood clotting
- Increased blood level of a liver or bone enzyme (alkaline phosphatase)
- Increased blood level of a liver pigment (bilirubin) often a sign of liver problems
- Increased blood level of creatinine (a substance normally eliminated by the kidneys into the urine)
- Increased blood level of a liver enzyme (GGT)
- Increased INR (measure of the ability of the blood to clot properly) which increases the risk of bleeding
- Weight gain
- Weight loss
- More acid than normal in the blood
- Loss of appetite
- Dehydration (when your body does not have as much water and fluid as it should)
- Increased blood sugar level
- Increased blood level of uric acid, a waste material from food digestion
- Decreased levels of a blood protein called albumin
- Decreased blood level of calcium
- Decreased blood sugar level
- Decreased blood level of potassium
- Decreased blood level of magnesium
- Decreased blood level of sodium
- Decreased blood level of phosphate
- Joint pain
- Back pain
- Bone pain
- Muscle pain
- Difficulty or limitation in ability to open mouth
- Loss of muscle coordination; awkward, uncoordinated walking; unsteadiness when walking

- Sleepiness
- Speech problems
- Restless, repetitive, or involuntary movements and rapid speech
- Bleeding in the brain
- Decreased blood flow to the brain which may lead to stroke
- A malfunction of the nerves within the head and neck
- Paralysis of facial muscles due to problems with the nerves that supply them
- Weakness or paralysis (loss of muscle function) caused by damage to peripheral nerves (those nerves outside of brain and spinal cord)
- Convulsion or seizure
- Anxiety, feelings of dread or danger
- Confusion
- Feelings of sadness, worthlessness, thoughts of suicide or death (depression)
- Difficulty sleeping or falling asleep
- Blood in the urine
- Bleeding in the kidney
- Need to urinate often
- Difficulty emptying the bladder
- Presence of blood in the fallopian tube (tube between ovary to uterus [womb])
- Bleeding in the ovary
- Bleeding in the prostate
- Bleeding in the spermatic cord (a structure resembling a cord that suspends the testis within the scrotum and contains the vas deferens [the tube that carries sperm] and other vessels and nerves)
- Bleeding in the testis
- Bleeding in the uterus (womb)
- Bleeding in the vagina
- Stuffy or runny nose, sneezing
- Bleeding in the respiratory tract
- Throat tightness, shortness of breath, or a choking sensation (see the Additional Information section below)
- Cough
- Hiccups
- Inflammation of the lungs
- Scarring of the lungs that can cause shortness of breath and interfere with breathing
- Problem of the sinuses
- Voice change
- Dry skin
- Excess sweating
- Itching
- Skin rash with the presence of macules (flat discolored area) and papules (raised bump)
- Hives
- Sudden reddening of the face and/or neck
- Hot flashes
- High blood pressure (hypertension)
- Low blood pressure

- Inflammation of a vein; blood clot
- Formation of a blood clot that breaks loose and is carried by the blood stream to plug another blood vessel
- Swelling and redness of the skin on the palms of the hands and soles of the feet
- Kidney damage that could be severe (see the Additional Information section below)
- Watery eyes

RARE BUT SERIOUS:
- Formation of blood clots in small blood vessels around the body that leads to a low platelet (a type of blood cell that helps to clot blood) count
- Gas in the intestinal (bowel) wall
- Inflammation of the gallbladder possibly associated with gall stones
- Sudden or traumatic injury to the kidney
- Severe potentially life-threatening damage to the lungs which can lead to fluid in the lungs
- Clotted catheter or catheter infection
- Severe diarrhea that may be life threatening
- Heart problems (chest pain, heart attack)
- Accumulation of fluid around the heart

Celecoxib plus FOLFOX (Arms B and D)

LIKELY:
- Lack of enough red blood cells (anemia which may make you short of breath, weak, fatigued, or tired)
- Diarrhea, which could lead to dehydration
- Nausea or the urge to vomit
- Fatigue or tiredness
- Increased blood level of a liver enzyme (ALT/SGPT)
- Increased blood level of a liver enzyme (AST/SGOT)
- Decreased number of a type of blood cell (platelet) that helps to clot blood (may result in easy bruising or bleeding)
- Inflammation or damage of the peripheral nerves (those nerves outside of brain and spinal cord) causing numbness, tingling, burning
- Dizziness (or sensation of lightheadedness, unsteadiness, giddiness, spinning or rocking)
- Darkening of the skin. This happens most often in the palms of the hands or along the vein where 5-FU is given. This is not harmful, but it could be permanent.
- Temporary hair loss
- Decrease in the total number of white blood cells (leukocytes); may make you more vulnerable to infection which could be serious or even life-threatening
- Decreased number of a type of white blood cell (lymphocyte)
- Decreased number of a type of white blood cell (neutrophil/granulocyte)
- Soreness or painful ulcers in the mouth and/or throat (lasting a couple of days)
- Photosensitivity (exposure to sunlight can cause skin to be sensitive to sunburn). You should use a sunscreen.

- A sensation of pain or tingling in areas of the body that are exposed to cold air or cold liquid, such as your hands if placed in the refrigerator or your throat if exposed to a cold wind or drinking cold liquids (see the Additional Information section below).
- Changes in fingernails
- Vomiting
- Loss of appetite
- Taste changes
- Headache (or head pain)

LESS LIKELY:
- Abnormal blood clotting and/or bleeding
- Fever associated with dangerously low levels of a type of white blood cell (neutrophils)
- Destruction of red blood cells
- Abnormally fast irregular heartbeat involving the upper chambers of the heart (atria)
- Abormally fast regular heartbeat involving the upper chambers of the heart (atria)
- Period of very rapid and regular heartbeats that begins and ends suddenly
- Slow heartbeat; regular rhythm
- Fast heartbeat; regular rhythm
- Fast heartbeat usually originating in an area located above the ventricles
- Irregular heartbeat resulting from an abnormality in one of the lower chambers of the heart (ventricle)
- Irregular heartbeat that involves the lower chambers of the heart (ventricles) that results in uncoordinated muscle movement of the ventricles making them tremble rather than contract properly; life-threatening, needs immediate attention
- Rapid heartbeat of one of the lower chambers (ventricle) of the heart; regular rhythm but potentially life-threatening, needs immediate attention
- Hearing loss
- Inflammation (swelling and redness) of the middle ear
- Inflammation (swelling and redness) of the conjunctiva (the outermost layer of the eye and the inner surface of the eyelids). Commonly called “pink eye”.
- Dry eye
- A situation in which one has temporary blindness of one eye, due to a blockage (or decreased blood flow) in the blood vessels leading to that eye
- Temporary vision problems caused by the cold
- Problem with eyelid
- Swelling around the nerve responsible for sight
- Belly pain
- Fluid collection in the abdomen
- Constipation
- Dry mouth
- Difficulty swallowing
- Inflammation of the small and/or large bowel
- Inflammation of the esophagus (gullet or tube that goes from mouth to stomach through which food passes)
- Excess passing of gas
- Inflammation of the stomach lining

- Bleeding in some organ(s) of the digestive tract, for example, blood in your stool
- Death of tissue somewhere in the digestive tract
- Sore (ulcer) somewhere in the digestive tract
- Partial or complete blockage of the small and/or large bowel. With ileus, the bowel acts like it is blocked.
- Inflammation (swelling and redness) of the pancreas
- Blockage of the small bowel
- Chills
- Swelling of the face
- Swelling of the extremeties (arms and/or legs)
- Fever
- Limp or difficulty walking
- A condition in which both the liver and kidneys fail
- Inflammation (swelling and redness) or damage to the tissue surrounding where a drug was injected
- Chest pain not heart-related
- Liver failure
- Increase in size of the liver
- A condition in which there is blockage of the veins of the liver; leads to liver damage
- Allergic reaction: abnormal reaction of the body to substances, called allergens, that are contacted through the skin, inhaled in the lungs, swallowed, or injected
- Infection
- Test that shows a problem in blood clotting
- Increased blood level of a liver or bone enzyme (alkaline phosphatase)
- Increased blood level of a liver pigment (bilirubin) often a sign of liver problems
- Increased blood level of creatinine (a substance normally eliminated by the kidneys into the urine)
- Increased blood level of a liver enzyme (GGT)
- Increased INR (measure of the ability of the blood to clot properly) which increases the risk of bleeding
- Weight gain
- Weight loss
- More acid than normal in the blood
- Loss of appetite
- Dehydration (when your body does not have as much water and fluid as it should)
- Increased blood sugar level
- Increased blood level of uric acid, a waste material from food digestion
- Decreased levels of a blood protein called albumin
- Decreased blood level of calcium
- Decreased blood sugar level
- Decreased blood level of potassium
- Decreased blood level of magnesium
- Decreased blood level of sodium
- Decreased blood level of phosphate
- Joint pain
- Back pain

- Bone pain
- Muscle pain
- Difficulty or limitation in ability to open mouth
- Loss of muscle coordination; awkward, uncoordinated walking; unsteadiness when walking
- Sleepiness
- Speech problems
- Restless, repetitive, or involuntary movements and rapid speech
- Bleeding in the brain
- Decreased blood flow to the brain which may lead to stroke
- A malfunction of the nerves within the head and neck
- Paralysis of facial muscles due to problems with the nerves that supply them
- Weakness or paralysis (loss of muscle function) caused by damage to peripheral nerves (those nerves outside of brain and spinal cord)
- Convulsion or seizure
- Anxiety, feelings of dread or danger
- Confusion
- Feelings of sadness, worthlessness, thoughts of suicide or death (depression)
- Difficulty sleeping or falling asleep
- Blood in the urine
- Bleeding in the kidney
- Need to urinate often
- Difficulty emptying the bladder
- Presence of blood in the fallopian tube (tube between ovary to uterus [womb])
- Bleeding in the ovary
- Bleeding in the prostate
- Bleeding in the spermatic cord (a structure resembling a cord that suspends the testis within the scrotum and contains the vas deferens [the tube that carries sperm] and other vessels and nerves)
- Bleeding in the testis
- Bleeding in the uterus (womb)
- Bleeding in the vagina
- Stuffy or runny nose, sneezing
- Bleeding in the respiratory tract
- Throat tightness, shortness of breath, or a choking sensation (see the Additional Information section below)
- Cough
- Hiccups
- Inflammation of the lungs
- Scarring of the lungs that can cause shortness of breath and interfere with breathing
- Problem of the sinuses
- Voice change
- Dry skin
- Excess sweating
- Itching
- Skin rash with the presence of macules (flat discolored area) and papules (raised bump)

- Hives
- Sudden reddening of the face and/or neck
- Hot flashes
- High blood pressure (hypertension)
- Low blood pressure
- Inflammation of a vein; blood clot
- Formation of a blood clot that breaks loose and is carried by the blood stream to plug another blood vessel
- Watery eyes
- Swelling and redness of the skin on the palms of the hands and soles of the feet
- Kidney damage that could be severe (see the Additional Information section below)

RARE BUT SERIOUS:

- Formation of blood clots in small blood vessels around the body that leads to a low platelet (a type of blood cell that helps to clot blood) count
- Gas in the intestinal (bowel) wall
- Inflammation of the gallbladder possibly associated with gall stones
- Sudden or traumatic injury to the kidney
- Severe potentially life-threatening damage to the lungs which can lead to fluid in the lungs
- Clotted catheter or catheter infection
- Severe diarrhea that may be life threatening
- Accumulation of fluid around the heart
- Heart problems (chest pain, heart attack, heart failure)
- Blood clots such as in your legs and lungs
- Kidney failure
- Bone marrow failure
- Stroke or mini-stroke

Unanticipated side effects which have not been previously reported may occur with any of these drugs. If you have any unusual symptoms, report them immediately to your doctor.

Reproductive risks: You should not become pregnant or father a baby while on this study and for at least two months after you stop taking the FOLFOX and celecoxib because the drugs may affect an unborn baby. Women should not breastfeed a baby while on this study and for at least two months after you stop taking FOLFOX and celecoxib. It is important you understand that you need to use birth control while on this study. Check with your study doctor about what kind of birth control methods to use and how long to use them. Some methods might not be approved for use in this study.

Additional Information about Risks

Additional information about the risks of oxaliplatin:

Exposure to cold (oxaliplatin): When receiving oxaliplatin, the nerves that affect your throat may be affected and cause a strange sensation when swallowing cold liquids. You should avoid cold beverages while you are participating in this study. You may also notice a tingling and

numbness or pain in your hands and feet that worsen on exposure to cold. Extra layers of clothing (gloves, mittens and warm socks) may help these symptoms be less severe.

If you should develop **throat tightness, shortness of breath, or a choking sensation**, contact your doctor immediately. In the patients treated with this drug, there have been 11 patients (of more the 50,000 who have be treated with oxaliplatin) who have had lung problems such as cough, shortness of breath, or trouble breathing. This caused scar tissue in the lungs and these events can be life threatening.

Inflammation of the nerves can become worse during the time you are receiving treatment, and the risk of developing it increases with the amount of oxaliplatin you receive. This inflammation usually goes away.

In some cases, the combination of oxaliplatin and 5-FU can cause a severe infection often associated with **diarrhea**. This infection is serious and can be life threatening. Contact your physician immediately if you are experiencing severe diarrhea (more than 7 or 8 times per day), fever, as well as numbness or tingling in your hands, feet or throat, or weakness.

A few patients treated with oxaliplatin have developed kidney damage. The damage to the kidneys may lead to the need for kidney dialysis usually on a temporary basis. You may also develop a condition associated with the dysfunction of your kidneys called Hemolytic Uremic Syndrome. This syndrome can be serious and may lead to seizures, problems with the central nervous system, or coma.

Platinum drugs like oxaliplatin have been known to cause **leukemia** in a small number of patients. It is not known whether risk of future development of leukemia is a side effect of oxaliplatin. One case of leukemia and one case of myelodysplastic syndrome, a condition which could lead to leukemia, have been seen following oxaliplatin chemotherapy, although it is not certain that oxaliplatin caused these blood disorders.

For more information about risks and side effects, ask the study doctor or contact _____.

Are there benefits to taking part in the study?

Taking part in this study may or may not make your health better. While doctors hope that adding celecoxib to FOLFOX may decrease the risk of cancer recurrence compared to FOLFOX alone, there is no proof of this yet. Furthermore, while doctors hope that the shorter course of FOLFOX (6 treatments) will be as effective a longer course of FOLFOX (12 treatments), there is no proof of this yet.

We do know that the information from this study will help doctors learn more about these drugs as a treatment for cancer. This information could help future cancer patients.

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

Your other choices may include:

- Getting treatment or care for your cancer without being in a study, which may involve using combinations of the drugs used in this study or different drugs
- Taking part in another study
- Getting no treatment, but this is not generally recommended for this stage of colon cancer.

Talk to your doctor about your choices before you decide if you will take part in this study.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The Cancer and Leukemia Group B (CALGB)
- The Southwest Oncology Group (SWOG)
- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people
- Pfizer pharmaceutical company, the makers of celecoxib.

The Cancer Trials Support Unit (CTSU) may also view your records if you are participating in this trial through one of their institutions.

The Cancer and Leukemia Group B has received a Certificate of Confidentiality from the federal government, which will help us to protect your privacy. The Certificate protects against the involuntary release of information about you collected during the course of the study. The researchers involved in this project may not be forced to identify you in any legal proceedings (criminal, civil, administrative, or legislative) at the federal, state, or local level. However, some information may be required by the Federal Food, Drug, and Cosmetic Act, the U.S. Department of Health and Human Services or for purpose of program review or audit. Also, you may choose to voluntarily disclose the protected information under certain circumstances. For example, if you or your guardian requests the release of information about you in writing (through, for example, a written request to release medical records to an insurance company), the Certificate does not protect against that voluntary disclosure.

What are the costs of taking part in this study?

You and/or your health plan/insurance company will need to pay for some or all of the costs of treating your cancer in this study. Some health plans will not pay these costs for people taking

part in studies. Check with your health plan or insurance company to find out what they will pay for. Taking part in this study may or may not cost your insurance company more than the cost of getting regular cancer treatment.

The cost of 5-FU, oxaliplatin, and leucovorin will be charged to you/your insurance company.

The celecoxib/placebo will be provided at no charge while you take part in this study.

Even though it probably won't happen, it is possible that the manufacturer may not continue to provide the celecoxib/placebo for some reason. If this would occur, other possible options are:

- You might be able to get the celecoxib/placebo from the manufacturer or your pharmacy but you or your insurance company may have to pay for it.
- If there is no celecoxib/placebo available at all, no one will be able to get more and the study would close.

If a problem with getting celecoxib/placebo occurs, your study doctor will talk to you about these options.

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

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

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

It is important that you tell your study doctor, _____ [investigator's name(s)], if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ [telephone number].

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

A Data Safety and Monitoring Board, an independent group of experts, will be reviewing the data from this research throughout the study.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

It may be necessary to contact you at a future date regarding new information about the treatment you have received. For this reason, we ask that you notify the institution where you received treatment on this study of any changes in address. If you move, please provide your new address to the following person:
(name) _____ (title)_____
(address)_____ (phone number) _____.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the study?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ [name(s)] at _____ [telephone number].

For questions about your rights while taking part in this study, call the _____ [name of center] Institutional Review Board (a group of people who review the research to protect your rights) at _____ (telephone number).

[Note to Local Investigator: Contact information for patient representatives or other individuals in a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can be listed here.]

* You may also call the Operations Office of the NCI Central Institutional Review Board (CIRB) at 888-657-3711 (from the continental US only).

RELATED STUDIES

Please note: The following section of the informed consent form is about additional research studies that are being done with people who are taking part in the main study. You may take part in these additional studies if you want to. You can still be a part of the main study even if you say "no" to taking part in any of these additional studies.

The results of these research studies will not be provided to you or your doctor, nor will the results have any effect on your treatment. It is unlikely that what we learn from these studies will have a direct benefit to you. However, the information learned from these studies may benefit other patients in the future.

The results from these studies may be published, but individual patients will not be identified in these publications.

There will be no charge to you for participating in these research studies. Your sample and information will only be used for research and will not be sold. The research done with your sample may help to develop new products in the future.

The greatest risk to you is the release of information from your health records. We will do our best to make sure that your personal information will be kept private. The chance that this information will be given to someone inappropriate is very small.

In the future, people who do research may need to know more about your health. While the Cancer and Leukemia Group B may give them reports about your health, it will not give them your name, address, phone number, or any other information that will let the researchers know who you are.

If you decide now to participate and then change your mind at any time about participating in these studies for any reason, you should contact your institution and let them know that you do not want the researchers to use your sample. The sample will then no longer be used for research. It will either be destroyed or returned to your institution for storage. The sample will also be returned to your institution upon request if needed for any other medical or legal reasons.

You can say "yes" or "no" to each of the following studies. Please mark your choice for each study. No matter what you decide to do, it will not affect your care.

Diet and Lifestyle Study

The study investigators would like to ask you to fill out a questionnaire about your diet and daily activities within the first six weeks of treatment and between 16 and 18 months after the start of treatment. This questionnaire should take about 30 minutes to complete. When you are presented with the questionnaire, you may choose whether or not you would like to fill it out.

1) I choose to take part in the Diet and Lifestyle study and agree to complete the diet and lifestyle questionnaire:

_____ Yes _____ No Initials _____

Studies on tissue and blood:

As part of this research study, we would like to request your permission to study cells from your tumor. The tumor samples were previously obtained when your disease was first diagnosed or when you had surgery. No additional biopsy will be required. These tumor samples will be used in a laboratory to investigate colorectal cancer. This will include looking at the cells in your tumor tissue to detect changes in genetic material that may have occurred. These types of changes are not usually passed down from generation to generation and are not considered to be inherited.

The researchers would like to keep some of the tissue that was left over for future research. If you agree, this tissue will be kept and may be used in research to learn more about cancer and other diseases. Please read the information sheet called "How is Tissue Used for Research" to learn more about tissue research. [This NCI information sheet is available at http://www.cancerdiagnosis.nci.nih.gov/specimens/patient.pdf]

In addition, the researchers would also like to collect additional samples of your blood. The researchers would like to investigate whether substances in your blood (sometimes called tumor markers) are related to the way that your body responds or doesn't respond to the chemotherapy you receive in this trial. Approximately 4 teaspoons of additional blood would be collected at the beginning of the study.

2) I agree that my specimens may be used for the research described above.

_____ Yes _____ No Initials _____

Genetic studies on blood cells:

Researchers at special CALGB laboratories wish to determine whether there is a relationship between genes and response to treatment and treatment outcomes, and side effects. No research studies will be performed that can knowingly reveal genetic information that might be of risk to you or to your family.

In order to study genes, the DNA must be removed from your blood sample. DNA is the substance that makes up your genes. Genes are the units of inheritance that are passed down from generation to generation. They are responsible for eye color, hair color, blood type, and hundreds of other traits.

New scientific tools will now allow researchers to look at your whole DNA, not just one part or one gene. This kind of research can provide information to researchers about the development of cancer and response to treatment. It can also provide information about a variety of other conditions and diseases, including heart disease, diabetes and Alzheimer's disease.

Because the information gained in these genetic studies can be very useful to the research community, the National Institutes of Health (NIH) has requested that these data be placed in a central database housed at the NIH. The goal is to speed up the process for discovery of new treatments, prevention and diagnosis of disease. Researchers must get approval from the NIH before they can access the research results and health-related information from your specimen. All information will be coded with a unique number. Researchers will not have access to your identity; they will only see coded information.

Participation in this additional research study would require an additional sample of blood (about 2 teaspoons).

The greatest risk to you is the release of information from your health records. We will do our best to make sure that your personal information will be kept private. The chance that this information will be given to someone is very small. Below are some of the steps we have taken to protect your privacy and confidentiality:

- Blood samples will be stored at a CALGB laboratory. The CALGB Statistical Center will perform all analyses of data and store all study results. Your blood sample will not be stored with your name on it. Instead, it will be labeled with a special CALGB identification number. The only location where your name and special identification number will be stored together is at the CALGB Statistical Center. The greatest effort will be made to see that all personal information that can identify you is kept under conditions that protect your privacy.
- Information about your participation in this study and results of any tests performed on your sample will be kept only at the CALGB Statistical Center. This information will not be made available to your doctors or to individual researchers at CALGB. Test results from this study will not be put in your medical records. All study information, including

test results, is stored under conditions that limit access in order to protect the privacy of the people participating in the study.

- The Cancer and Leukemia Group B has received a Certificate of Confidentiality from the federal government, which will help us to protect your privacy. More information about the Certificate can be found in the paragraph "Will my medical information be kept private?"
- A federal law (Genetic Information Non-Discrimination Act, GINA) will help lower the risk from health insurance or employment discrimination on the basis of genetic information. The federal law does not include other types of misuse by life insurance, long-term care or disability insurance. If you want to learn more about the GINA Law, you can find information about it on the internet or ask the study staff. In addition to the federal law, some states have laws that also help protect against genetic discrimination.

While we believe that the risks to you and your family are very low, we cannot tell you exactly what all of the risks are from taking part in genetic research studies. Your privacy and confidentiality will be protected to the fullest extent possible.

You have the right to receive the planned therapy on this study without participating in the proposed research study on your blood sample. Please read the sentence below and think about your choice. After reading the sentence, please mark your choice, sign your name, and provide the current date. **No matter what you decide to do, it will not affect your care.**

3) I agree that my blood may be used for the genetic research studies described above.

_____ Yes _____ No Initials _____

Storage of your specimens:

The researchers would also like to store any portion of the tissue and blood that is not used up by the related studies described above. These samples may be stored indefinitely. You can still take part in the treatment study, and the research study described above without giving your consent for your samples to be stored.

It is not possible for you or the CALGB to know what studies of cancer may be appropriate in the future. We ask that you give permission in advance for other studies to be performed using the tissue and blood without being re-contacted to give permission for each test.

4) My specimens may be kept for future unknown use in research to learn about, prevent, treat, or cure cancer.

_____ Yes _____ No Participant _____ Date _____

5) My specimens may be kept for research about other health problems (for example: causes of diabetes, Alzheimer's disease and heart disease.

_____ Yes _____ No Participant _____ Date _____

6) My doctor or someone from CALGB may contact me in the future to ask me to take part in more research.

_____ Yes _____ No Participant _____ Date _____

Where can I get more information?

You may call the National Cancer Institute's Cancer Information Service at:

1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

You may also visit the NCI Web site at http://cancer.gov/

- For NCI's clinical trials information, go to: http://cancer.gov/clinicaltrials/
- For NCI's general information about cancer, go to http://cancer.gov/cancerinfo/

You will get a copy of this form. If you want more information about this study, ask your study doctor.

Signature

I have been given a copy of all _____ [insert total of number of pages] pages of this form. I have read it or it has been read to me. I understand the information and have had my questions answered. I agree to take part in this study.

Participant _____
Date _____

APPENDIX I

CANCER TRIALS SUPPORT UNIT (CTSU) PARTICIPATION PROCEDURES

CANCER TRIALS SUPPORT UNIT (CTSU) PARTICIPATION PROCEDURES

To submit site registration documents:

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103
Phone: 1-888-823-5923
Fax: 215-569-0206

For patient enrollments:

CTSU Patient Registration
Phone: 1-888-462-3009
Fax: 1-888-691-8039
Hours: 9:00 AM – 5:30 PM Eastern Time, Monday – Friday (excluding holidays)

(Registrations received after 5:00 PM ET will be handled the next business day. For CTSU patient enrollments that must be completed within approximately one hour, or other extenuating circumstances, call 301-704-2376 between 9:00 AM and 5:30 PM.)

Submit study data directly to the Lead Cooperative Group unless otherwise specified in the protocol:

CALGB Statistical Center
Hock Plaza
2424 Erwin Road, Suite 802
Durham, NC 27705
Tel: 919-668-9350
Data Operations Fax: 919-668-9348
Teleform Fax: 919-416-4990

Sites should submit Teleforms via Fax or Mail. See Section 6.0 Data Submission Section for details on forms submission.

Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.

For patient eligibility or treatment related questions: Contact the CALGB Study Chair.

For questions unrelated to patient eligibility, treatment, or data submission contact the CTSU Help Desk by phone or e-mail:

CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.

The CTSU Web site is located at https://www.ctsu.org

REGISTRATION/RANDOMIZATION

Prior to the recruitment of a patient for this study, investigators must be registered members of the CTSU. Each investigator must have an NCI investigator number and must maintain an "active" investigator registration status through the annual submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature, and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, NCI. These forms are available on the CTSU Web site (enter credentials at https://www.ctsu.org; then click on the Register tab) or by calling the PMB at 301-496-5725 Monday through Friday between 8:30 AM and 4:30 PM Eastern time.

Each CTSU investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU members' area at https://www.ctsu.org.

All forms and documents associated with this study can be downloaded from the CALGB/SWOG C80702 Web page on the CTSU members' area of the website

(https://www.ctsu.org). Patients can be registered only after pre-treatment evaluation is complete, all eligibility criteria have been met, and the study site is listed as "approved" in the CTSU RSS.

Requirements for CALGB/SWOG C80702 site registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet

Prestudy requirements for patient enrollment on CALGB/SWOG C80702:
- Patient must meet all inclusion criteria, and no exclusion criteria should apply
- Patient has signed and dated all applicable consents and authorization forms
- All baseline laboratory tests and prestudy evaluations performed within the time period specified in the protocol.

CTSU Procedures for Patient Enrollment

1. Contact the CTSU Patient Registration Office by calling 1-888-462-3009 between 9:00 AM and 5:30 PM Eastern Time. Leave a voicemail to alert the CTSU Patient Registrar that an enrollment is forthcoming. For immediate registration needs (e.g., within one hour), call the registrar cell phone at 1-301-704-2376.

2. Complete the following forms:

   - CTSU Patient Enrollment Transmittal Form
   - CALGB/SWOG C80702 Eligibility Checklist
   - CALGB/SWOG C80702 Registration Worksheet Note

3. Fax these forms to the CTSU Patient Registrar at 1-888-691-8039 between the hours of 9:00 AM and 5:30 PM, Mon-Fri, Eastern Time (excluding holidays); however, please be aware that registrations received after 5:00 PM will be processed the next day. Registration is limited to operating hours of the CALGB Registration office (9am-5pm ET). The CTSU registrar will check the investigator and site information to ensure that all regulatory requirements have been met. The registrar will also check that forms are complete and will follow-up with the site to resolve any discrepancies.

4. Once investigator eligibility is confirmed and enrollment documents are reviewed for compliance, the CTSU registrar will contact the CALGB, **within the confines of CALGB's registration hours**, to obtain assignment of a treatment arm and assignment of a unique patient ID (to be used on all future forms and correspondence). The CTSU registrar will confirm registration by fax.

   Protocol treatment should begin within 7 days of registration.

DATA SUBMISSION AND RECONCILIATION

1. All case report forms (CRFs) and transmittals associated with this study must be downloaded from the CALGB/SWOG C80702 Web page located on the CTSU members' area of the website (https://www.ctsu.org). Sites must use the current form versions and adhere to the instructions and submission schedule outlined in the protocol.

2. Submit all completed CRFs (with the exception of patient enrollment forms), clinical reports, and transmittals directly to the CALGB (see contact table or section 5.5) unless an alternate location is specified in the protocol. Do not send study data to the CTSU. A completed CTSU-CALGB coversheet should accompany all data submissions.

3. The CALGB **Statistical Center** will send (general via e-mail but may be sent via postal mail or fax) query notices and delinquency reports directly to the site for reconciliation. Please send query responses and delinquent data to the CALGB Statistical Center (via postal mail or fax) and do not copy the CTSU Data Operations. Each site should have a designated CTSU Administrator and Data Administrator and **must keep their CTEP IAM account contact information current.** This will ensure timely communication between the clinical site and the CALGB Statistical Center.

SPECIAL MATERIALS OR SUBSTUDIES

- CALGB 150911: Correlative science companion studies for CALGB/SWOG C80702
- CALGB 60905: Pharmacogenetic companion studies for CALGB/SWOG C80702
- Diet and Lifestyle substudy

These substudies must be offered to all patients enrolled on CALGB/SWOG C80702 (although patients may opt not to participate).

SERIOUS ADVERSE EVENT (AE) REPORTING (SECTION 14.0)

1. CTSU sites must comply with the expectations of their local Institutional Review Board (IRB) regarding documentation and submission of adverse events. Local IRBs must be informed of all reportable serious adverse reactions.
2. CTSU sites will assess and report adverse events according to the guidelines and timelines specified in the protocol. You may navigate to the CTEP Adverse Event Expedited Report System (AdEERS) from either the Adverse Events tab of the CTSU members' area of the website (https://www.ctsu.org) or by selecting Adverse Event Reporting Forms from the document center drop down list on the CALGB/SWOG C80702 Web page.
3. Do not send adverse event reports to the CTSU.
4. Secondary AML/MDS/ALL reporting: Report occurrence of secondary AML, MDS, or ALL via the NCI/CTEP AML-MDS Report Form in lieu of AdEERS. Submit the completed form and supporting documentation as outlined in the protocol.

DRUG PROCUREMENT (SECTION 9.0)

Investigational agents: Celcoxib/placebo (distributed by PMB)
Commercial agents: 5-FU, leucovorin, oxaliplatin

1. Information on drug formulation, procurement, storage and accountability, administration, and potential toxicities are outlined in Section 9.0 of the protocol.
2. You may navigate to the drug forms by selecting Pharmacy Forms from the document center drop down list on the CALGB/SWOG C80702 Web page.

REGULATORY AND MONITORING

Study Audit

To assure compliance with Federal regulatory requirements [CFR 21 parts 50, 54, 56, 312, 314 and HHS 45 CFR 46] and National Cancer Institute (NCI)/ Cancer Therapy Evaluation Program (CTEP) Clinical Trials Monitoring Branch (CTMB) guidelines for the conduct of clinical trials and study data validity, all protocols approved by NCI/CTEP that have patient enrollment through the CTSU are subject to audit.

Responsibility for assignment of the audit will be determined by the site's primary affiliation with a Cooperative Group or CTSU. For Group-aligned sites, the audit of a patient registered through CTSU will become the responsibility of the Group receiving credit for the enrollment. For CTSU Independent Clinical Research Sites (CICRS), the CTSU will coordinate the entire audit process.

For patients enrolled through the CTSU, you may request the accrual be credited to any Group for which you have an affiliation provided that Group has an active clinical trials program for the primary disease type being addressed by the protocol. Per capita reimbursement will be issued by the credited Group provided they have endorsed the trial, or by the CTSU if the Group has not endorsed the trial.

Details on audit evaluation components, site selection, patient case selection, materials to be reviewed, site preparation, on-site procedures for review and assessment, and results reporting and follow-up are available for download from the CTSU Operations Manual located on the CTSU Member Web site.

Health Insurance Portability and Accountability Act of 1996 (HIPAA)

The HIPAA Privacy Rule establishes the conditions under which protected health information may be used or disclosed by covered entities for research purposes. Research is defined in the Privacy Rule referenced in HHS 45 CFR 164.501. Templated language addressing NCI-U.S. HIPAA guidelines are provided in the HIPAA Authorization Form located on the CTSU Web site.

The HIPAA Privacy Rule does not affect participants from outside the United States. Authorization to release Protected Health Information is NOT required from patients enrolled in clinical trials at non-US sites.

Clinical Data System–Web (CDS-Web) Monitoring

This study will be monitored by the Clinical Data System (CDS-Web). The sponsoring Group fulfills this reporting obligation by transmitting the CDS data collected from the study-specific case report forms, via the Web to the NCI Center for Biometrics (NCICB). Cumulative CDS data are submitted quarterly.

APPENDIX II

Collaborative Agreement Language

The celecoxib/placebo used in this protocol is provided to CTEP, DCTD, NCI under a Collaborative Agreement (CSA) between the Pfizer, Inc. (hereinafter referred to as "Collaborator") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the Intellectual Property Option to Collaborator (http://ctep.cancer.gov/industry/ipo.html) contained within the terms of award, apply to the use of the celecoxib/placebo in this study:

1. Celecoxib/placebo may not be used for any purpose outside the scope of this protocol, nor can it be transferred or licensed to any party not participating in the clinical study. Collaborator data for celecoxib are confidential and proprietary to Collaborator and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator, the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order.- Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data and Safety Monitoring Board (DSMB).

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator for advisory review and comment prior to submission for publication. Collaborator will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator's intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Regulatory Affairs Branch, CTEP, DCTD, NCI
   Executive Plaza North, Suite 7111
   Bethesda, Maryland 20892
   FAX 301-402-1584
   Email: anshers@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator. No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

APPENDICES III, IV, V
COMPANION STUDIES TO CALGB/SWOG C80702

Correlative Science Investigators:

Charles Fuchs, MD, MPH	*Dana-Farber Cancer Institute* 44 Binney St Boston, MA 02115 Tel: 617-632-5840
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Monica Bertagnolli, MD	*Brigham and Women's Hospital* 75 Francis Street Boston, MA 02115 Tel: 617-732-8910
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Todd Golub, MD	*Dana-Farber Cancer Institute* 44 Binney Street Dana Building 640C Boston, MA 02115 Tel: 617-632-4903
Shuji Ogino, MD, PhD	*Dana-Farber Cancer Institute* 44 Binney St Boston, MA 02115 Tel: 617-632-3978
Bruce Hollis, PhD	*Medical University of South Carolina* 173 Ashley Ave Charleston, SC 29403 Tel: 843-792-6854
Nader Rifai, PhD	*Children's Hospital of Boston* 300 Longwood Avenue Boston, MA 02115 Tel: 617-355-7173
Michael Pollak, MD	*Jewish General Hospital* 3755 Côte Ste. Catherine Road Montreal, Quebec, H3T 1E2 Tel: 514-340-8222

Pharmacogenomic Investigators:

Howard McLeod, MD	*University of North Carolina, Chapel Hill* Campus Box 7361, Genetic Medicine Building, rm 1094 Chapel Hill, NC 27599-7360 Tel: 919-966-0512

Correlative science projects are planned using various biospecimens:

Appendix III correlative science studies:

Tumor tissue

COX-2 expression
p21 expression
Microsatellite instability
VEGF expression and microvessel density
Interleukin (IL-6) expression
β-catenin expression and localization
FOXP3, CD3, CD8, CD45RO
15-PGDH

Vitamin D receptor (VDR) expression
1-α-hydroxylase expression
KRAS mutation analysis

Genome-wide expression profiling/DASL
CpG island methylation/CIMP
BRAF mutational status
LINE-1 methylation

Blood

Markers of inflammation including CRP, IL-6, sTNFα-R2
Plasma levels of 25-hydroxyvitamin D_3

Appendix IV pharmacogenetic studies:

Blood

Genotyping of vitamin D pathway genes (VDR, VDBP, CYP27B1, RXR, CYP24A1)
UGT1A6, CYP2C9, COX-2 (PTSG-2), COX-1 (PTSG-2), PPAR-gamma, thromboxane synthase, NF-kappa B1, prostacyclin synthase, and 5-lipoxygenase genotyping
AGXT 154C>T polymorphisms

INTRODUCTION TO COMPANION STUDIES CALGB 150911 AND CALGB 60905

Current concepts and clinical practice regarding prognosis and therapy for patients with colon cancer rest on the gross clinical/pathological staging. Identification of molecular determinants of drug efficacy and toxicity to chemotherapies might become important in the design of individualized chemotherapy based on the individual's molecular tumor and genomic profiles. The goals of these correlative projects are to identify germline variations and gene expression levels and arrays associated with clinical toxicity and outcome in patients treated with FOLFOX therapy with or without celecoxib.

These appendices describe pathways as well as state-of-the-art technologies at the time of protocol activation. However the knowledge base will increase significantly during the course of this study along with development of improved and novel technologies. Analyses of the samples collected within this protocol will incorporate new discoveries and new technologies. Tumor and blood samples will be collected and stored at the CALGB Pathology Coordinating Office (PCO) under the direction of Dr. Scott Jewel. The priority of molecular analyses as well as the laboratories chosen for these analyses will be identified by chair (Dr. Fuchs) of correlative science of this protocol who will closely work with participating investigators to assure the highest quality of analyses.

The molecular data generated from the C80702 samples will be sent, in a computer readable format using secure means to the CALGB Statistical Center. This transmission is not limited to pre-processed data generated by the lab. The raw (e.g., *.CEL, *.idat or *.sproc) files will be submitted to the CALGB Statistical Center. Along with this transmission, the lab will provide a table which at the minimum will provide the following information for each sample received from the repository:

- the Lab ID number that the Statistical Center can use to decode against the registration database to get patient ID number;
- the date at which the specimen was received from the repository;
- the date at which the sample was processed.

Additionally, the lab will also provide the complete results from any quality control measures carried out if requested by the CALGB Statistical Center. The lab will report any QC issues encountered directly to the CALGB Statistical Center. If a sample had to be redone (e.g., defective or poor quality), the lab will provide the results from both replications and add an appropriate column to the table.

The lab will commit to handle any molecular data generated from CALGB samples in a safe, secure (e.g., HIPAA compliant) and organized fashion.

APPENDIX III

CORRELATIVE SCIENCE COMPANION STUDIES: CALGB 150911

1.0 OBJECTIVES

1.1 To assess molecular features within the tumor that influence the efficacy of celecoxib as adjuvant therapy for stage III colon cancer.

1.2 To assess whether markers of systemic inflammation in blood can predict the efficacy of celecoxib as adjuvant therapy for stage III colon cancer.

1.3 To assess the influence of baseline plasma 25(OH)-vitamin D level on disease-free and overall survival in patients with stage III colon cancer.

1.4 To assess whether tumoral expression of vitamin D receptor (VDR) and 1-α-hydroxylase and KRAS mutational status modifies the relation between baseline plasma 25(OH)-vitamin D level on patient outcome.

1.5 To determine an mRNA expression signature that is predictive of disease-free survival among patients with stage III colon cancer

1.6 To determine an mRNA expression signature that predicts efficacy of celecoxib as adjuvant therapy for patients with stage III colon cancer

1.7 To determine if CpG island methylator phenotype (CIMP) is an independent predictor of PFS in patients with stage III colon cancer

2.0 BACKGROUND

2.1 Tumoral molecular alterations associated with the efficacy of COX-2 inhibition

Celecoxib, at least in part, prevents colorectal neoplasia through inhibition of cyclooxygenase-2 (COX-2), the rate-limiting step for the conversion of arachidonic acid to prostaglandins and related eicosanoids.[44,45] COX-2 promotes inflammation and cell proliferation,[46] and is overexpressed in the majority of human colorectal cancers.[47,48] Overexpression of COX-2 in tumor tissue has been associated with a poorer prognosis among colorectal cancer patients in some,[48-51] but not all studies.[52,53] In addition, intratumoral expression of COX-2, has been independently associated with tumor differentiation,[52] angiogenesis,[54,55] recurrence,[56] and metastasis.[57] Moreover, COX-2 expression has been correlated with worsened patient survival in some, [48-51,54] but not all studies.[52,53,58,59] In a large prospective cohort study, regular aspirin and NSAID use was found to be associated with a greater reduction in the risk of COX-2 overexpressing colorectal cancer, whereas regular aspirin and NSAID use only modestly reduced the risk of COX-2 negative tumors. Therefore, in the current study of patients with stage III colon cancer, it is hypothesized that the benefit associated with adjuvant celecoxib use will be greater for patients with COX-2 overexpressing cancers.[44]

Interleukin-6 (IL-6) and its receptor are highly expressed in colorectal carcinoma and colorectal cancer cell lines, but not in normal colon tissue. IL-6 inhibits apoptosis through downstream activation of STAT-1.[60] Administration of NSAIDs block IL-6 mediated STAT-1 activation.[60] This relationship between IL-6 and COX inhibition suggests the importance of examining interactions between IL-6 levels, aspirin and NSAID use, and cancer outcomes. It is hypothesized that the effect of COX inhibition on patient outcome may vary by the level of expression of IL-6 in the tumor. Preclinical models suggest that aspirin and NSAIDs induce the expression of p21, thereby influencing colorectal cancer tumorigenesis.[61] In mice, inactivation of p21

increased tumor formation in a gene-dose-dependent manner.[62] Moreover, inactivation of p21 completely eliminated the ability of NSAIDs to inhibit tumor formation.[61] It is therefore hypothesized that the efficacy of a COX inhibitor on patient outcome may be modified by the level of tumoral p21 expression.

Alternative molecular markers in COX-2-related pathways may also be informative. For example, aspirin may function to disrupt COX-2 through modulation of expression and localization of β-catenin.[63,64] COX-2 has also been hypothesized to mediate its neoplastic influence through the promotion of angiogenesis, potentially through expression of vascular endothelial growth factor (VEGF),[65-67] and as measured by microvessel density.[68] Thus, the association between intratumoral markers such as β-catenin, VEGF, and microvessel density will be examined to determine the importance of these alternative COX-2-related pathways in predicting celecoxib efficacy.

Recently, 15-prostaglandin dehydrogenase (15-PGDH), a prostaglandin degrading enzyme, was demonstrated to function as an endogenous inhibitor of the colonic COX-2 pathway and as a tumor suppressor gene.[69,70] Gene knock-out of 15-PGDH conferred near complete resistance to celecoxib colon tumor prevention in mice.[71] 15-PGDH is highly expressed in normal colon mucosa, but expression is lost in human colorectal cancers.[69,72] Preliminary data suggest that normal tissue 15-PGDH expression is an accurate predictor of celecoxib anti-tumor response.[71] Specimens containing normal rectal mucosa from 16 patients were examined on the APC trial, a study that randomized patient at high risk for colon adenoma development to treatment with celecoxib or placebo. In a subset of these patients, 2 mm biopsies of normal and adenomatous rectal mucosa were obtained. Measurement of pre-treatment 15-PGDH transcript levels by real-time PCR showed a 5.4-fold variation from lowest to highest 15-PGDH mRNA expression across these 16 individuals (median: 3.4, mean: 4.4, range: 2.1-11.4). Post-treatment colonoscopy showed that four of these patients were resistant to celecoxib, as evidenced by development of new adenomas. All of the patients failing treatment had colonic 15-PGDH levels below the median of the cohort ($p=0.008$).[71] Virtually identical results were obtained when these tissues were examined using IHC or RT-PCR to detect 15-PGDH levels in FFPE tissue. In summary, it was found that 15-PGDH activity can determine sensitivity or resistance to the selective COX-2 inhibitor, celecoxib, and preliminary data from a human clinical trial indicate that low levels of 15-PGDH in disease target tissue predict failure of celecoxib anti-tumor efficacy. These observations imply that measurement of 15-PGDH may be clinically useful in selecting patients most likely to benefit from treatment with COX-2 inhibitors. It is predicted that patients treated with celecoxib in the adjuvant setting who have low levels of 15-PGDH in their normal intestinal tissue at baseline will have reduced DFS and OS compared to patients with high levels of 15-PGDH. In each patient, 15-PGDH levels will be examined by both IHC and gene expression, as described below.

It has been known for many years that lymphocytic infiltrate surrounding primary colorectal cancer is associated with improved prognosis.[73] Although the mechanism remains unclear, the adaptive immune system is thought to play an important role in suppressing the progression of this disease. A high density of $CD8^+$ T cells has been associated with the absence of tumor invasion, earlier stage, and improved patient survival.[74,75] Using CD3 as a universal marker of T cells, the ratio of T-cell density at the advancing tumor margin compared with the central core was recently proposed as having stronger prognostic significance than conventional TNM staging.[76] In addition, $CD45RO^+$ cells include both $CD4^+$ and $CD8^+$ lymphocytes that have been exposed to antigen. Pages et al.[74] subsequently demonstrated that a high density of $CD45RO^+$ cells within the tumor was associated with decreased invasiveness, lower stage, and improved survival. Finally, regulatory T cells (Tregs) suppress the activity of cytotoxic T cells; the most specific Treg cell marker identified to date is the nuclear transcription factor known as FOXP3.[77,78] A high density of tumor-infiltrating $FOXP3^+$ Tregs has been associated with outcome in patients with colorectal cancer.[73] The

interrelationship between COX inhibition, T cell infiltrate, and patient survival has not been comprehensively investigated. It is hypothesized that the influence of adjuvant celecoxib on patient outcome may be modified by the inflammatory infiltrative pattern (CD3, CD45RO, CD8 and FoxP3 staining).

Lastly, Ruschoff et al. observed, *in vitro*, a marked reduction in microsatellite instability (MSI) during exposure to aspirin or sulindac.[79,80] The effect was reversible, dose dependent, and independent of cyclooxygenase function. The mechanism appeared to be via a genetic selection that enhanced apoptosis in cells undergoing MSI. It is therefore hypothesized that the benefit associated with celecoxib would be greater in microsatellite unstable (MSI-high) tumors.

2.2 Plasma inflammatory factors and colon cancer

Chronic inflammation is characterized by abnormal production of cytokines and inflammatory factors which have been causally linked to obesity, diabetes, and cancer. In humans, cytokines such as C-reactive protein (CRP), interleukin-6 (IL-6), and soluble tumor necrosis factor-α -receptor 2 (sTNFα-R2) not only mediate the inflammatory response, but also serve as potential biomarkers of chronic inflammation and inflammation-related diseases. CRP levels correlate with the metabolic syndrome, triglyceride levels, obesity, fasting glucose, insulin sensitivity,[81] diabetes mellitus,[82] as well as major dietary patterns that have been associated with a higher risk of colorectal cancer.[83] Elevated baseline plasma CRP levels have been associated with an increased risk of colorectal neoplasia in some, though not all studies.[84-88] Two studies found that elevated prediagnostic CRP predicted a 2-fold increase in colorectal cancer,[85,87] while two studies found no association.[86,88] Interleukin-6 (IL-6) is a related inflammatory cytokine that may also be associated with colorectal cancer risk.[87] As described above, administration of NSAIDs block IL-6 mediated STAT-1 activation.[60] This relationship between IL-6 and COX inhibition suggests the importance of examining interactions between IL-6 levels, aspirin and NSAID use, and cancer outcomes. It is hypothesized that stage III colon cancer patients with elevated baseline circulating levels of C-reactive protein (CRP), interleukin-6 (IL-6), and soluble tumor necrosis factor-α-receptor 2 (sTNFα-R2) will experience a greater benefit with use of adjuvant celecoxib.

2.3 Influence of plasma 25(OH)D levels on colon cancer

The best indicator of vitamin D status is plasma 25(OH)D, since it reflects not only skin exposure to ultraviolet-B (UV-B) light and total vitamin D intake, but also cholecalciferol production in the skin and hydroxylation of all sources of cholecalciferol in the liver.[89] Prospective studies have shown that individuals with higher plasma levels of 25(OH)D experience a significant reduction in risk of colorectal cancer when compared to those with low plasma level.[90-95] A meta-analysis of five epidemiologic studies found a 51% decrease in the risk of colorectal cancer associated with plasma 25(OH)D levels in the highest quintile compared to those in the lowest quintile ($P<0.0001$).[96] Furthermore, a randomized placebo-controlled trial of vitamin D and calcium supplementation in postmenopausal women demonstrated a 60% decrease in all-cancer risk (including colorectal cancer) in the intervention arm ($P<0.03$).[97]

In contrast, the influence of vitamin D on survival of patients with established colorectal cancer remains uncertain. A large observational study in Norway found that people diagnosed with colorectal cancer in the summer and autumn, when 25(OH)D concentrations are highest, had a significantly better survival than those diagnosed in the winter.[98,99] The authors speculated that a high circulating 25(OH)D at the time of diagnosis, and possibly during initial treatment, may improve cancer prognosis. However, this study was limited by its use of season of diagnosis – an indirect indicator of vitamin D status – as the primary exposure.

In a recently completed prospective analysis of 304 colorectal cancer patients in the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS), Ng et

al. found that increasing circulating levels of 25(OH)D were associated with a significant reduction in overall mortality (P for trend = 0.02).[100] Compared with levels in the lowest quartile, patients with 25(OH)D levels in the highest quartile had an adjusted hazard ratio (HR) of 0.52 (95% confidence interval [CI], 0.29 to 0.94) for overall mortality. The results remained unchanged after excluding patients diagnosed within five years of blood collection (P for trend = 0.04); the adjusted HR for overall mortality comparing extreme quartiles was 0.45 (95% CI, 0.19 to 1.09). Moreover, the benefit associated with vitamin D appeared greater among patients with more advanced disease. Therefore, the influence of plasma levels of 25(OH)D on disease-free and overall survival will be examined in a large population of patients with stage III colon cancer who are enrolled in a clinical trial of adjuvant chemotherapy.

2.4 Tumoral vitamin D receptor expression and KRAS mutational status

The vitamin D receptor (VDR) and 1-α-hydroxylase (CYP27B1) are frequently expressed in colon cancer cells. Well-differentiated colon cancer cell lines have higher VDR expression,[101] and the antiproliferative effects of vitamin D may only occur in cell lines expressing high levels of VDR.[102] Expression of VDR and CYP27B1 increases in the early stages of colorectal tumorigenesis, but appears to decline in poorly-differentiated tumors and metastases.[103]

Point mutations in the KRAS oncogene occur in approximately 40% of colorectal cancers.[104-106] Recent data indicate that KRAS mutations may be associated with lack of response to epidermal growth factor receptor (EGFR)-targeting agents.[107-110] Interestingly, the vitamin D pathway may interact with KRAS signaling. In a RAS-transformed cell line of human keratinocytes, malignant cells were found to be resistant to the growth-inhibitory effects of $1,25(OH)_2D$.[111,112] Furthermore, VDR expression appears to be down-regulated in KRAS-mutated cell lines,[113] and vitamin D's ability to affect apoptosis may vary by KRAS status.[114]

These data suggest that the improved patient survival associated with higher circulating vitamin D may be greater in patients with tumoral VDR and CYP27B1 overexpression and diminished for those with KRAS-mutated tumors. It will be assessed whether the relationship between vitamin D and patient survival is modified by VDR and CYP27B1 expression and KRAS mutational status.

2.5 Gene expression profiling

A technical challenge facing a more thorough exploration of gene expression profiling approaches to colon cancer outcome prediction has been the lack of suitable patient material for such genomic analyses. Current genome-wide expression profiling methods require frozen tissue for analysis, whereas tissue banks accompanied by long-term clinical outcome are generally populated only by formalin-fixed specimens that are not amenable to microarray-based expression profiling. Even today, the vast majority of patient specimens are formalin-fixed; the collection of frozen tissues has yet to permeate routine clinical practice. Thus, a genomic profiling method suitable for fixed tissues would have the potential for significant translational and clinical impact. Whole genome mRNA expression profiles in colon tumor tissue will be interrogated to identify patterns of genes dysregulated in tumors. A novel, validated platform, the Illumina cDNA-mediated Annealing, Selection, Extension, and Ligation (DASL) system will be applied to interrogate expression of 24,000 genes using RNA extracted from tumor tissue.[115,116] The Illumina system uses the 96-well Sentrix Array Matrix and incorporates a DASL platform designed specifically for the expression profiling of formalin-fixed, paraffin-embedded (FFPE) tumor samples. The formalin fixation process can degrade mRNA into randomly fragmented pieces of 80-100 nucleotides. Because DASL uses random priming in the cDNA synthesis without depending on an intact poly-A tail for oligo-d(T) priming, and targets a relatively short sequence of about 50 nucleotides, it works well on severely degraded RNA in FFPE tissues.[116] In the protocol, RNA is converted to cDNA using random primers, and a pair of oligonucleotide probes, designed to interrogate each gene, are annealed. The

gap between probes is filled by extension and ligation to create a PCR template. The PCR products are hybridized to capture sequences on the microarray. Genes to be interrogated are defined by a pool of the oligonucleotide pool (DAP). High fidelity amplification of each locus is achieved because of the uniform length of the amplicons and the use of a single set of generic primers for the PCR reaction.[115,116]

Gene expression profiling has proven to hold great potential as part of a path toward personalized medicine. The DASL-based discovery method described here should be distinguished from RT-PCR-based candidate gene profiling methods, such as those used in the commercially available OncoTypeDx test for breast cancer prognosis. Whereas standard RT-PCR methods can measure a small number of transcripts in FFPE samples, unbiased, genome-wide discovery studies are not feasible using those methods.

DASL will be performed on tumor specimens in the laboratory of Dr. Todd Golub (Broad Institute, Cambridge, MA). Dr. John Quackenbush (Dana-Farber Cancer Institute), a recognized expert in computational biology with extensive experience in the analysis of gene expression data will assist with the analysis and interpretation of the DASL expression data.

The laboratory of Todd Golub (Broad Institute, Boston, MA) has tested and validated the DASL method on over 1,000 FFPE tissue blocks, and has found the assay to be highly reproducible (R^2 on average >0.96 in replicate experiments) with an overall success rate of approximately 90%, including the testing of blocks as old as 24 years of age. In a recent analysis of FFPE samples from 307 patients with hepatocellular cancer, Golub et al. identified a gene expression signature that reproducibly predicted patient survival.[117] To date, RNA for the DASL assay has been extracted from FFPE blocks of 348 colorectal cancer blocks. Among those, QC RT-PCR was performed on 65 cases, and 63 cases (97%) showed acceptable quality of RNA. For quality control, SYBR green (Applied Biosystems) quantitative polymerase chain reaction assay for a housekeeping gene (RPL13A), is used to estimate RNA quality. RNA with crossover threshold <30 cycles is considered good quality.

In separate analyses, Drs. Golub, Quackenbush and Illumina also tested both the quantity of RNA necessary and the overall reproducibility of the assay. Using multiple replicate assays with various quantities of starting material, they found excellent reproducibility showing correlation between hybridization of two independent 50 ng aliquots. Average linkage hierarchical clustering of individual samples demonstrated that technical variability in the assay is far smaller than inter-sample biological variability for large (200 ng), mixed (200 vs. 50 ng) and small (5 ng, 10 ng, 50 ng) quantities. Further analysis indicated that as little as 10 ng of initial material could yield useful results.

2.6 CpG island methylation

Aberrant DNA methylation of CpG islands has been widely observed in both benign and malignant human colorectal tumors and is associated with gene silencing when it occurs in promoter areas. A subset of colorectal tumors has been described to have an exceptionally high frequency of methylation of some CpG islands, leading to the definition of a distinct trait referred to as “CpG Island Methylator Phenotype”, or “CIMP”.[118] However, the lack of a consistent definition of CIMP has contributed to conflicting reports of its existence.[118-120] An improved panel of five markers to classify CIMP+ tumors has been identified and it was found that CIMP represents a distinct trait with a remarkably tight association with somatic mutation of the BRAF oncogene and that sporadic cases of mismatch repair deficiency occur almost exclusively as a consequence of CIMP-associated methylation of MLH1.

The CpG Island Methylator Phenotype was first proposed by Dr. Jean-Pierre Issa’s group in 1999 for a distinct subset of colorectal tumors with an exceptionally high frequency of methylation of “Type C” loci, which were defined as loci methylated in cancer, but not in normal tissues.[118] In essence, CIMP represents concordant methylation of a subset of CpG islands in a subset of tumors. In subsequent studies,

the CIMP trait was found to be associated with a variety of clinical, histopathological and epidemiological characteristics,[121] but the initially reported bimodal distribution of methylation frequency was often not observed. Furthermore, several carefully conducted studies concluded that cancer-specific DNA hypermethylation occurs across a continuous frequency spectrum and that the designation of a distinct CIMP subgrouping would be arbitrary.[118-120] These discrepant results stem largely from the use of varying sets of methylation markers used to screen the colorectal tumors.

The initial definition of CIMP was based on concordant methylation of Type C loci, and specifically excluded markers that showed evidence of age-associated methylation in normal tissues, referred to as "Type A" loci.[118] However, the distinction between Type C and Type A loci has not held up particularly well, with some Type A loci showing Type C methylation behavior in other tissues,[122-124] and many Type C loci showing detectable methylation in normal tissues.[122,125-128] More importantly, some authentic Type C loci do not show concordant methylation with classic CIMP markers,[129-131] suggesting that CIMP is not an indiscriminate increase in global CpG island hypermethylation, but may represent one or more distinct defects in epigenetic control, each affecting only a subset of CpG islands in a subset of tumors.[121,132] If this is indeed the case, then unsupervised two-dimensional clustering of large numbers of markers and tumor samples would reveal the existence of these distinct correlated subsets. An automated real-time PCR based MethyLight technology has been established to determine clustered DNA methylation behavior and a final panel of five markers has been determined for CIMP classification. MLH1 showed detectable methylation in normal mucosae using MethyLight. It is evident that the CIMP+ cases display an increased frequency and intensity of cancer-associated DNA methylation.

Since the assembly of our new five-marker panel contributed to the original CIMP classification, the assessment of its performance compared to our temporary standard could be biased. The new panel of five markers performs very well in all cross-panel comparisons. It outperforms the panel of classic CIMP loci in every comparison. It even gives lower misclassification error than the classic panel against a panel of 14 markers that includes the classic panel, but excludes the new panel.

An alternative strategy to evaluate the performance of panels is to compare their associations with characteristics of colorectal cancer that have previously been reported to be associated with CIMP+ status. It is assumed that if this association reflects an important underlying biological relationship, then a superior CIMP classification would result in a tighter association. A significant correlation between CIMP+ and location of the tumor, BRAF, KRAS and MSI status could be demonstrated. Of note, MSI has been associated with an improved survival in colorectal cancer whereas mutations in BRAF have been associated with an inferior survival.[133] Moreover, beyond methylation at CpG islands in promoter regions (CIMP), global DNA methylation, as measured by methylation in repetitive long interspersed nucleotide element-1 (LINE-1) elements, may have an independent effect on patient outcome. Using 643 colon cancers in two independent prospective cohorts, DNA methylation in repetitive long interspersed nucleotide element-1 (LINE-1) elements was quantified using Pyrosequencing, as an indicator of global DNA methylation level.[134] LINE-1 hypomethylation was associated with a significant increase in colon cancer–specific mortality ($P_{trend} < .001$) and overall mortality ($P_{trend} = .002$). The association was independent of MSI, CIMP and BRAF status.[134] As such, it will be interesting to dissect the separate clinical and etiological features associated with mismatch repair deficiency, CIMP, LINE-1 methylation, proximal tumor location, and BRAF mutation status.

A recent study demonstrated that CIMP+ was an independent predictive marker for survival benefit from 5-FU chemotherapy in colorectal cancer.[308] The folate status is critically important to the provision of methyl groups and may be relevant to the CpG-island methylator phenotype (CIMP).[309] Preliminary data suggest that the folate pool in colorectal cancers has been associated with promoter-specific DNA hypermethylation and polymorphisms within the MTHFR gene. Recent data

suggested that the haplotype with low enzyme activity of MTHFR is linked with promoter hypermethylations and modifies the risk of the CIMP+ proximal colon cancer development in the Japanese people.[310] Thus, an opportunity exists to perform comprehensive pharmacogenetic investigations and integrate the genetic folate status and DNA repair capacity in conjunction with MSI and CIMP status as predictors of tumor recurrence.

3.0 HYPOTHESES

3.1 The benefit associated with adjuvant celecoxib in patients with stage III colon cancer is significantly modified by tumoral COX-2, VEGF, p21, IL-6, beta-catenin, 15-PGDH, T cell overexpression, higher microvessel density, and microsatellite instability.

3.2 Higher baseline circulating levels of C-reactive protein (CRP), interleukin-6 (IL-6), and soluble tumor necrosis factor-α-receptor 2 (sTNFα-R2) is associated with a greater benefit with use of adjuvant celecoxib in stage III colon cancer.

3.3 Higher baseline levels of plasma 25-hydroxyvitamin D_3 [25(OH)D] are associated with improved disease-free and overall survival in patients with stage III colon cancer.

3.4 The improved patient survival associated with higher circulating 25(OH)D is greater in patients with tumoral VDR and CYP27B1 overexpression, and diminished for those with KRAS-mutated tumors.

3.5 Unique mRNA expression signatures are predictive of disease-free survival among patients receiving adjuvant chemotherapy for stage III colon cancer.

3.6 Unique mRNA expression signatures will predict stage III colon cancer patients most likely to benefit from celecoxib.

3.7 CpG island methylator phenotype (CIMP) is an independent predictor of tumor recurrence in patients with stage III colon cancer.

4.0 METHODS

4.1 Tumoral molecular alterations associated with the efficacy of COX-2 inhibition

Tissue blocks procurement: Formalin-fixed/paraffin-embedded tissue blocks and the corresponding pathology slides will be acquired from patients enrolled on C80702 who consent to CALGB 150911. The tissue blocks will be initially inspected by PCO pathologists to ensure that the pathology report and tissue blocks are adequately identified and logged into the CALGB database. After review of the pathology report, a set of blocks will be selected which provide appropriate specimens for a) preparation of routine H and E slides for microdissection and DNA extraction; b) preparation of routine H and E slides for microdissection and RNA extraction for DASL; c) construction of tissue microarrays and immunohistochemical evaluation; d) establishment of a bank of TMA blocks and slides for future molecular and cellular studies; and e) establishment of a DNA bank for future assays.

Tissue DNA and RNA extraction: In the laboratory of Dr. Shuji Ogino (Dana-Farber Cancer Institute, Boston, MA), H&E stained slides will be reviewed, areas comprising at least 70% neoplastic cellularity will be marked and scraped from 15μm unstained slides under direct visualization, and DNA and RNA will be extracted.

Determination of Microsatellite Instability (MSI): Previously published methods of DNA fragment analysis will be used to determine MSI (Bethesda 10 marker panel).[135] PCR and DNA fragment analysis for all of the markers except for D2S123, D5S346, and D17S250, will be performed in duplicate. "High degree of MSI" (MSI-H) will be defined as having instability in 30% or more of the markers. "MSI-low (MSI-L)" will be defined as having instability in less than 30% of the markers, and "microsatellite stability (MSS)" as having no unstable marker.

Construction of tissue microarrays (TMAs): The use of TMAs allows us to perform a high throughput screen of all available colon cancers in our study population. Construction will be conducted in the PCO. TMAs will be constructed using the Beecher Automated Arrayer. Two 0.6 mm tissue cores each from a tumor and normal mucosa is placed in each TMA block, and four duplicate blocks will also be constructed. Each TMA block will have a total of 400 cores (100 tumors).

Immunohistochemical staining and interpretation: Immunohistochemical analyses will be performed in the laboratory of Dr. Ogino. For p21, β-catenin, IL-6, and VEGF, monoclonal antibodies will be applied to the tumor sections: monoclonal anti-p21 (Pharmingen) dilution 1:50; β-catenin clone 14 (Transduction Laboratories) dilution 1:400; polyclonal antisera (R & D Systems, Abingdon, UK) directed against IL-6, or a polyclonal antisera directed against VEGF 165 (Santa Cruz Antibodies). Biotinylated rabbit anti-mouse antibody (DAKO; code E354) will be used as secondary antibody, and the immunoreaction will be visualized by avidin-biotin complex. Tumors with >10% nuclear expression of p21/WAF1/CIP1 will be considered positive. The κ coefficient between the two observers was 0.62 for p21 ($p<0.0001$; N=179), indicating substantial agreement.

For IL-6, the distribution will be scored according to the numbers of positive cells: none (not stained), 0; focal (<one-third of cells stained), 1; multifocal (<two-thirds of cells stained), 2; and diffuse (most cells stained), 3. The staining intensity will be scored as: none (not stained), 0; mild (between 0 and 2), 1; and strong, 2. The distribution and intensity scores are then added to produce the following grades for the staining: 0, negative; 1 and 2, intermediate; and 3, 4 and 5, positive.

The fraction of cells demonstrating cytoplasmic staining for VEGF will be measured and recorded. Tumors with >10% VEGF staining cells will be considered positive. Microvessel density will be determined in the leading edge of the tumor in an area of apparent highest vessel density following anti-CD31 staining.[136] For this analysis, microvessel density will be coded as a dichotomous variable. Tumors with greater than 28 vessels per 100X field will be considered high.

For β-catenin, normal colonic epithelial cells serve as an internal positive control with membrane staining. Cytoplasmic and nuclear expressions will be recorded separately as either no expression (0), weak expression (1+), or moderate/strong expression (2+). β-catenin activation score will be calculated as the sum of nuclear score (0-2), cytoplasmic score (0-2) and membrane score (0 if membrane staining was positive, +1 if membrane expression was lost), as originally described by Jass et al.[137]. Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically-stained slides were examined by one of the investigator unaware of other data. A random sample of 402 tumors was examined for β-catenin by a second observer unaware of other data, and the concordance between the two observers for β-catenin activation (inactive vs. active) was 0.83 ($\kappa=0.65$, $p<0.0001$).[138] In addition, it was observed that COX-2 overexpression correlated with cytoplasmic β-catenin expression but not nuclear β-catenin, supporting the role of cytoplasmic β-catenin in stabilizing COX-2 mRNA.[138]

For COX-2, monoclonal antibody will be applied to the tumor sections: COX-2 (Cayman), dilution 1:300. A positive and negative control (tumors with known expression status of each of the selected proteins) will be included in each staining batch. COX-2 expression will be scored for both the proportion of cells staining: none (not stained), 0; focal (<33%), 1; multifocal (33-67%), 2; and diffuse (≥67%), 3. The staining intensity will be scored as: none, 0; mild, 1; and strong, 2. The distribution and intensity scores are added: 0, negative; 1 and 2, intermediate; and 3, 4 and 5, positive. In our previous work, the κ coefficient between the two observers was 0.62 for COX-2 ($p<0.0001$; N=108), indicating substantial agreement.

Immunohistochemistry for FOXP3, CD3, CD8 and CD45RO will also be performed at the Dana-Farber Harvard Cancer Center Immunohistochemistry Core Facility. Antibodies and dilutions are as follows: FOXP3 (BioLegend, San Diego CA; clone

206D, 1:50), CD3 (DAKO, Carpinteria, CA; clone A0452, 1:250), CD8 (DAKO, clone C8/144B, 1:100) and CD45RO (DAKO, clone UCHL1, no dilution). Immunostained TMA (tissue microarray) slides will be scanned by the Ariol instrument SL-50 (Applied Imaging, Grand Rapids, MI), and immunostaining will be quantitatively recorded as both the number of nuclei with staining and the intensity of staining. Positive cells in tumor areas and stromal areas will be counted separately in a given TMA tissue core. Each tumor has been sampled at least twice in a TMA block. For each tumor, scores will be calcuted for each marker in tumor area, stromal area and whole tissue core area. Scores that will be calculated include average positive cell count per tissue core, positive cell density (i.e., positive cell count / surface area) and average intensity of staining.

Quality control for tumor block analyses: In all immunohistochemical analyses, appropriate positive and negative controls will be included in each run of immunohistochemical assay. In addition, a random sample of more than 200 cases will be re-examined by a second pathologist in Dr. Ogino's laboratory to assess inter-rater agreement. A kappa measure of agreement proposed by Kraemer will be used to estimate the agreement between raters for each marker. If agreement is unacceptable, further training and monitor agreement will be instituted in the next set of 200 samples.

Analysis of 15-PGDH: 15-PGDH will be assessed in the laboratory of Dr. Sanford Markowitz. Methods for measuring 15-PGDH in FFPE tissues include IHC using an anti-15-PGDH monoclonal antibody, raised against purified recombinant human 15-PGDH protein by the Markowitz laboratory. Stained slides will be reviewed independently by two GI pathologists, and staining graded semi-quantitatively (scale 0 to +3; 0 = non-reactive, 1+ = weak staining, 2+ = moderate staining, 3+ = strong staining). RNA will be extracted from slides of FFPE colonic tissue and converted to cDNA prior to real-time PCR measurement of 15-PGDH using the Applied Biosystems human 15-PGDH Taqman Probe/Primer kit Hs00168359_ml. Results will be in the form of numerical averages from three independent reverse transcription reactions. The utility of this assay for quantifying 15-PGDH expression levels in FFPE samples from mucosal biopsies as small as 2mm in diameter has been confirmed.[69]

4.2 Circulating inflammatory factors and patient outcome

Plasma will be collected in one 5 mL lavender top tube, centrifuged for 10-15 minutes at 1,300 g and aliquoted into 2 mL cryovials at 0.5 mL/vial. Samples will be frozen and shipped on ice or dry ice. Multiple masked quality control samples will be interspersed among the case samples, and all laboratory personnel will be blinded to patient outcomes.

Plasma levels of C-reactive protein, interleukin-6, and soluble tumor necrosis factor-α-receptor II (sTNF-α-R2) from blood samples obtained at study enrollment will be examined. These inflammatory factors will be measured in the laboratory of Dr. Nader Rifai (Clinical Chemistry Lab, Children's Hospital of Boston). Dr. Rifai is an expert in the measurement of these plasma analytes from large population studies and his laboratory provides high assay precision. Baseline plasma C-reactive protein levels will be measured via a high-sensitivity latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring). Plasma interleukin-6 will be measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay), and sTNF-α-R2 levels by an ELISA kit utilizing immobilized monoclonal antibody to human sTNF-α-R2 (Genzyme). The CVs are 3.8% for CRP, 5.9% for IL-6, and 6.2% for TNF-αR2. In addition, in a cohort of women, the ICC for samples drawn from the same subject one year apart was 0.95 for CRP and 0.66 for TNF-αR2. In a cohort of men, plasma CRP and IL-6 was also assessed 1-3 years apart in the same participants and yielded ICC's from 0.6-0.85, indicating that plasma measurements of these analytes are relatively stable over time.

The investigators will work closely with Dr. Rifai, who will help interpret the assays and update the team with recent developments. Precision will be monitored by

routinely adding approximately 5% of repeated quality control samples as blinded specimens. Should a decrease in precision be noted, the investigators will work with the laboratory to address the problem and suspend additional analysis until the problem is resolved.

4.3 Vitamin D measurement

Plasma will be collected in one 5 mL lavender top tube, centrifuged for 10-15 minutes at 1,300 g and aliquoted into 2 mL cryovials at 0.5 mL/vial. Samples will be frozen and shipped on ice or dry ice. Multiple masked quality control samples will be interspersed among the case samples, and all laboratory personnel will be blinded to patient outcomes. 25(OH)D concentrations will be measured by radioimmunoassay in the laboratory of Dr. Bruce Hollis (Medical University of South Carolina, Charleston, SC), as described previously.[139] In prior analyses, the mean coefficient of variation of the assay was ~10%.[93] For the current trial, plasma 25(OH)D levels will be assessed only at study baseline; nonetheless, our prior studies suggest that within each individual, plasma 25(OH)D remain relatively stable over time. To assess the intraperson stability of 25(OH)D over time, plasma levels of 25(OH)D were measured in 144 men who donated repeated blood specimens four years apart. The Pearson correlation coefficient was 0.70 for the two 25(OH)D measurements ($P < 0.0001$).[140]

4.4 Vitamin D related tumor assays

Vitamin D immunohistochemistry and KRAS mutational status will be performed in the laboratory of Dr. Shuji Ogino.

Tissue Block Procurement: Formalin-fixed/paraffin-embedded (FFPE) tissue blocks and slides will be examined. A set of blocks will be selected for preparation of routine hematoxylin and eosin (H&E) slides for microdissection and DNA extraction for analysis of KRAS mutations. CALGB pathologists will also construct tissue microarrays (TMAs) and Dr. Ogino will perform immunohistochemical (IHC) evaluation of VDR and 1-α-hydroxylase (CYP27B1).

Tissue DNA Extraction: H&E stained slides will be reviewed, areas comprising ≥70% neoplastic cellularity will be marked and scraped from 15μm unstained slides under direct visualization, and DNA will be extracted. DNA of sufficient quality for polymerase chain reaction (PCR) was obtained from >95% of colorectal cancers.

Sequencing of KRAS: Utilizing Pyrosequencing technology, KRAS mutations at codons 12 and 13 will be assessed.[141] Pyrosequencing is highly sensitive to detect a small amount of KRAS mutant alleles in paraffin-embedded tumor tissue.[141] Using this methodology, KRAS mutational status was previously assessed in 508 patients participating in an NCI-sponsored trial of adjuvant chemotherapy for stage III colon cancer (CALGB 89803). KRAS mutations were detected in 178 tumors (35%) by Pyrosequencing. When compared to patients with wild-type KRAS, those with a mutation in KRAS did not experience any difference in disease-free (DFS), recurrence-free (RFS), or overall survival (OS) (log-rank P>0.56 for DFS, RFS, and OS) (manuscript under review).

IHC Staining and Interpretation: IHC analyses will be performed in the laboratory of Dr. Ogino. The following antibodies will be applied: polyclonal rabbit anti-VDR (C-20; Santa Cruz, Cat# SC-1008; dilution 1:200) and monoclonal anti-CYP27B1 (1-α-hydroxylase) (clone H-90; The Binding Site # sc-67261; dilution 1:400). The intensity of staining (absent, weak, moderate/strong) will be recorded in each relevant cellular compartment (nucleus, cytoplasm, and/or membrane) as well as fraction of tumor cells with staining. For each antibody, published cell lines have been identified to serve as controls and cells have been embedded into paraffin blocks for incorporation into TMAs. For each antibody tested, positive controls have been successfully stained while noting loss in all negative controls. For each new TMA tested, controls will be included for quality control. In preliminary analysis, 619 colorectal tumors in our laboratory were evaluated for VDR expression, with 233 (38%) showing

overexpression. A similar proportion of patients demonstrate 1-α-hydroxylase overexpression.

Quality Control for Tumor Block Analyses: For analyses of KRAS, Pyrosequencing has been designed to confirm the presence of a mutation by artificial frameshifting in pyrograms (with extra fluorescence peaks), and/or by a second Pyrosequencing primer. Approximately 5% repeated QC samples will be added as blinded specimens; they will be randomly nested in the sample sets with coded IDs. In all IHC analyses, appropriate positive and negative controls will be included in each run of IHC assay. In addition, a random sample of >100 cases will be re-examined by a second pathologist in Dr. Ogino's laboratory to assess inter-rater agreement using a kappa measure of agreement (κ) proposed by Kraemer. If agreement is unacceptable, further training and monitor agreement will be instituted in the next set of 200 samples. Thus far, the concordance rate and κ coefficient between the two pathologists is 82% (κ=0.62; n=139) for VDR.

4.5 Whole genome expression

As described above, a novel, validated platform, the Illumina cDNA-mediated Annealing, Selection, Extension, and Ligation (DASL) system will be applied to interrogate expression of 24,000 genes using RNA extracted from colon cancer tissue.[115,116] The Illumina system uses the 96-well Sentrix Array Matrix and incorporates a DASL platform designed specifically for the expression profiling of formalin-fixed, paraffin-embedded (FFPE) tumor samples. The formalin fixation process can degrade mRNA into randomly fragmented pieces of 80-100 nucleotides. Because DASL uses random priming in the cDNA synthesis without depending on an intact poly-A tail for oligo-d(T) priming, and targets relatively short sequence of about 50 nucleotides, it works well on severely degraded RNA in FFPE tissues.[116] In the protocol, RNA is converted to cDNA using random primers, and a pair of oligonucleotide probes, designed to interrogate each gene, are annealed. The gap between probes is filled by extension and ligation to create a PCR template. The PCR products are hybridized to capture sequences on the microarray. Genes to be interrogated are defined by a pool of the oligonucleotide pool (DAP). High fidelity amplification of each locus is achieved because of the uniform length of the amplicons and the use of a single set of generic primers for the PCR reaction.[115,116]

Whereas standard RT-PCR methods can measure a small number of transcripts in FFPE samples, unbiased, genome-wide discovery studies are not feasible using those methods. The DASL approach has been extended to 24,000 genes. The ability to discover and implement gene expression signatures on FFPE colon cancer blocks will allow us a unique opportunity to identify genes involved in celecoxib-mediated inhibition of colorectal carcinogenesis. In the future, genes identified in these analyses will be functionally characterized.

4.6 CpG Island Methylator Phenotype (CIMP)

A subset of colorectal adenocarcinomas displays an unusually high frequency and concordance of CpG island hypermethylation. This subset was first described by Toyota et al. as CIMP (CpG Island Methylator Phenotype) colorectal adenocarcinomas.[139] The definition of CIMP was recently refined, and was shown to underly the majority of sporadic mismatch repair deficient colorectal adenocarcinomas and to be very tightly associated with BRAF mutation.[139] An improved panel of five markers (CACNA1G, IGF2, NEUROG1, RUNX3, and SOCS1) has been established to classify the CIMP phenotype, which outperforms other commonly used panels of CIMP in sensitivity and specificity.[142]

CpG Island Methylator Phenotype (CIMP) will be analyzed using the MethyLight procedure at the USC Epigenome Center under supervision by Dr. Peter W. Laird and Dr. Heinz Josef Lenz as described.[142-144] MethyLight technology utilizes real-time PCR analysis for bisulfite-based DNA methylation analysis. The USC Epigenome Center uses a non-methylation-dependent control reaction designed for a consensus Alu

repetitive element sequence.[141] This control reaction is a sensitive measure of very low DNA amounts, and is less prone to quantitation errors introduced by gene-copy anomalies, such as aneuploidy, gene amplification, deletions, etc., compared to a single-copy control reaction. MethyLight data are reported as a ratio between the value obtained for a methylation-specific reaction and that obtained for the methylation-independent Alu control reaction, normalized against similar measurements obtained for a fully methylated reference sample. The resulting output is expressed as "Percent of Methylated Reference (PMR)". The USC Epigenome Center has extensive experience with high-throughput DNA methylation analysis, and has established numerous quality control procedures. All reagents are prepared with dedicated or disposable vessels, solutions, and pipettes. Positive displacement pipettes or air-displacement pipettes with aerosol-resistant tips are used. Plate preparation is performed on a dedicated custom-built automated robotic platform based on the Qiagen BioRobot 3000. 10% of samples are repeated in duplicate randomly intermixed with the other samples as an additional quality control. Each bisulfite-converted DNA sample is evaluated prior to MethyLight analysis using the Alu control reaction to ensure that sufficient bisulfite-DNA amounts are utilized in each CIMP-specific MethyLight assay.

Sequencing of BRAF: Utilizing Pyrosequencing technology, BRAF mutations at codon 600 will be assessed in Dr. Ogino's laboratory.[141,113] Pyrosequencing is highly sensitive to detect a small amount of BRAF mutant alleles in paraffin-embedded tumor tissue.[141,133] Using this methodology, BRAF mutational status was previously assessed in 640 colon cancer patients.[133] BRAF mutations were detected in 105 tumors (17%) by Pyrosequencing.

LINE-1 methylation: To accurately quantify global DNA methylation, Pyrosequencing technology will be utilized in Dr. Ogino's laboratory.[134] PCR and subsequent Pyrosequencing for LINE-1 will be performed using the PyroMark kit (Biotage, Uppsala, Sweden). The PCR condition will be 45 cycles of 95C for 20 sec, 50C for 20 sec and 72C for 20 sec, followed by 72C for 5 min. The biotinylated PCR product will be purified and made single-stranded to act as a template in a pyrosequencing reaction, using the Pyrosequencing Vacuum Prep Tool (Biotage). Pyrosequencing reactions will be performed in the PSQ HS 96 System (Biotage). The nucleotide dispensation order will be: ACT CAG TGT GTC AGT CAG TTA GTC TG. Complete conversion of cytosine at a non-CpG site ensures successful bisulfite conversion. The amount of C relative to the sum of the amounts of C and T at each CpG site will be calculated as percentage. The average of the relative amounts of C in the 4 CpG sites will be used as overall LINE-1 methylation level in a given sample. Pyrosequencing to measure LINE-1 methylation has been previously validated.[134]

5.0 DATA ANALYSES

The primary efficacy variable for analyses will be disease-free survival (DFS). Secondary efficacy endpoints include recurrence-free survival (RFS) and overall survival.

It is hypothesized that specific tumor alterations will modify the effect of adjuvant celecoxib on patient survival. For the analysis of tumoral alterations (**Objective 1.1**), all patients who had a tumor block available will be included in the analysis. Within each of binary category of COX-2, VEGF, p21, beta-catenin, 15-PGDH, T cell overexpression, higher microvessel density, and microsatellite instability, the effect of celecoxib vs. placebo on DFS and OS will be examined. Cox proportional hazards models will be used to calculate HRs and 95% CIs for DFS and OS, adjusted for other prognostic factors. Tests for statistical interaction will be performed by entering into the model the cross-product term of the relevant biomarker with treatment assignment (celecoxib vs. placebo). Moreover, in Cox models, the main independent effect of each biomarker on DFS and OS will be assessed.

For plasma analytes (**Objective 1.2**: CRP, IL-6, and sTNF-α-R2), whether the effect of celecoxib vs. placebo on DFS differs according to baseline levels of each plasma

inflammatory factor will be assessed. For primary analyses of survival, patients who died within three months of plasma collection will be excluded to minimize any bias due to occult cancer recurrence or preclinical illness. For these stratified analyses, each plasma analyte will be categorized into tertiles to maximize statistical power. Within each tertile of the specific plasma inflammatory factor, the effect of celecoxib vs. placebo on DFS and OS will be examined. Cox proportional hazards models will be used to calculate HRs and 95% CIs for DFS and OS, adjusted for other prognostic factors. Tests for statistical interaction will be performed by entering into the model the cross-product term of the plasma level as a continuous variable with treatment assignment (celecoxib vs. placebo).

In addition, the main effect of each plasma inflammatory factor on DFS and OS will be examined. For the main effect, plasma levels will be divided into quintiles for the analysis. Baseline characteristics of patients will be compared according to quintiles of the biomarker using Wilcoxon signed rank tests for continuous variables and chi-squared tests for categorical variables. For primary analyses of survival, patients who recurred or died within three months of plasma collection will be excluded to minimize any bias due to occult cancer recurrence or preclinical illness. In sensitivity analyses, the possibility of reverse causation will be assessed by allowing different lag times between plasma assessment and cancer recurrence or death. The log-rank test and Kaplan-Meier curves will be used to compare DFS OS by quintile of plasma level. Cox proportional hazards models will be used to control for multiple confounders. The two-tailed *P* value for the linear trend test across categories will be calculated using the plasma level as a continuous variable, consistent with prior studies.

The primary efficacy variable for analyses will be disease-free survival (DFS). Secondary efficacy endpoint will be overall survival.

For the main effect of plasma 25(OH)D (**Objective 1.3**), plasma 25(OH)D levels will be divided into quintiles for the analysis. Baseline characteristics of patients will be compared according to quintiles of the biomarker using Wilcoxon signed rank tests for continuous variables and chi-squared tests for categorical variables. For primary analyses of survival, patients who recurred or died within three months of plasma collection will be excluded to minimize any bias in the 25(OH)D level due to occult cancer recurrence or preclinical illness. In sensitivity analyses, the possibility of reverse causation will be assessed by allowing different lag times between 25(OH)D assessment and cancer recurrence or death. The log-rank test and Kaplan-Meier curves will be used to compare DFS OS by quintile of 25(OH)D level. Cox proportional hazards models will be used to control for multiple confounders. The two-tailed *P* value for the linear trend test across categories will be calculated using the 25(OH)D level as a continuous variable, consistent with prior studies. In secondary analyses, we will examine how the relationship between 25(OH)D level and patient outcome is modified by relevant covariates such as ECOG performance status, treatment assignment, physical activity and body mass index, among others. Tests for statistical interaction will be performed by entering into the model the cross-product term of the plasma 25(OH)D as a continuous variable with the covariate as a continuous or binary variable.

For the analysis of tumoral alterations in vitamin D and related pathways (**Objective 1.4**), all patients who had a tumor block available for analysis of VDR expression, 1-α-hydroxylase, and KRAS mutation and data on circulating 25(OH)D concentration will be included. Tumoral protein overexpression will be defined as a) moderate/strong staining in any fraction of cells, or b) ≥50% of tumor cells with weak staining. All tumors will be categorized as having VDR and 1-α-hydroxylase overexpression versus no overexpression, and KRAS mutated versus wild type. Within each of category of VDR expression, 1-α-hydroxylase expression, and KRAS mutational status, we will examine the influence of plasma levels of 25(OH)D divided into tertiles on DFS and OS. Tertiles will be utilized instead of quintiles of plasma 25(OH)D to maximize the ability to detect an association within each subgroup. Cox proportional hazards models will be used to calculate HRs and 95% CIs for DFS and

OS, adjusted for other prognostic factors. Tests for statistical interaction will be performed by entering into the model the cross-product term of plasma 25(OH)D as a continuous variable with the molecular alteration as a binary variable (overexpressed versus not; mutated versus wild type).

In describing analyses related to DASL, the genes/transcripts on the DASL chip will be referred to as features. The terms feature, feature expression and expression will also be used interchangeably. The maximum number of features on the DASL chip is 24,000. In these discussions, K will denote the number features (among the 24,000) which pass the non-phenotypic filters. The expressions, which are summary measures, are obtained following pre-processing. The background correction algorithm implemented by the BeadStudio software will be employed and quantile normalization will be applied to the background adjusted intensities.

Objective 1.5 is to identify the features on the DASL chip which are associated with DFS. As outliers in both the time-to-event observations and gene expressions may be encountered, the length of follow-up varies among patients, and multiple markers are considered, methods which are robust, are able to incorporate the censoring mechanism, and allow for incorporation of multiplicity adjustments will be employed. The association between the time-to-event endpoint and each of the features will be quantified using the non-parametric rank-covariance measure.[145] This method is a non-parametric counterpart to Cox regression where the time-to-event variable is regressed on the ranks of the expression. The family-wise error rate (FWER) adjusted exact permutation P-values will be calculated using B=10,000 permutation replicates. Features will be considered significant at the 0.05 FWER adjusted level.

We are interested in the association between each feature and DFS. Kendall's tau will be used to quantify these pairwise associations. This quantity is uniquely determined by the copula generating the joint (bivariate) distribution between each feature and DFS and as such is invariant to marginal distributions. It will be assumed that the marginal effects are continuous so as to ensure uniqueness of the generating copula.

An additional objective of DASL (**Objective 1.6**) is to identify those features for which there is expression by celecoxib interactions with respect to DFS. These analyses will be carried within the framework of two-way multiplicative Cox models.

The analyses outlined for the primary endpoint, will be carried out for OS and AE as well. For the adverse event endpoints, the two-sample Wilcoxon test will be employed to investigate the association between each feature and outcome.[146] For this endpoint, conditional inference tree and random forests methods for binary outcomes will be used.

The classification models for DFS, OS and adverse events in the previous models were based on molecular data. As additional exploratory analyses, the inclusion of clinical or demographic co-variables will be considered.

Functional annotation of discovered signature will be performed by Gene Set Enrichment Analysis (GSEA)16 using the Molecular Signature Database (MSigDB, http://www.broad.mit.edu/gsea/msigdb/). Survival data analyses will be performed using the log-rank test and multivariate Cox regression. All analyses were performed using GenePattern18 (http://www.broad.mit.edu/cancer/software/genepattern/) or the R statistical package (http://www.r-project.org).

For the analysis of CIMP (**Objective 1.7**), the primary efficacy variable for analyses will be disease-free survival (DFS). Secondary efficacy endpoints include recurrence-free survival (RFS) and overall survival (OS).

Baseline characteristics of patients will be compared according to CIMP-high vs. CIMP-low/0 using Wilcoxon signed rank tests for continuous variables and chi-squared tests for categorical variables. The log-rank test and Kaplan-Meier curves will be used to compare DFS and OS by CIMP-high vs. CIMP-low/0. Cox proportional hazards models will be used to control for multiple confounders including MSI, BRAF, and LINE-1 status. In secondary analyses, we will examine how the

relationship between CIMP status and patient outcome is modified by relevant covariates such as age, ECOG performance status and treatment assignment, MSI, BRAF, and LINE-1 status among others. Tests for statistical interaction will be performed by entering into the model the cross-product term of the CIMP status (binary variable) with the covariate as a continuous or binary variable. The main independent effect of BRAF and LINE-1 status on DFS will also be assessed using the aforementioned COX models.

6.0 STATISTICAL CONSIDERATIONS

The three clinical endpoints are adverse events (e.g., neuropathy), disease-free survival (DFS) and survival (OS). The exact definitions for these outcomes will be equivalent to those specified in the treatment protocol (CALGB/SWOG C80702). The treatment by biomarker (or genotype) interactions will be tested for the celecoxib hypothesis. If an interaction is present, relevant hypotheses will also be tested in the treatment groups of patients receiving and not receiving celecoxib. If no interaction is present, the treatment groups will be combined (celecoxib + no celecoxib) for analysis. Power estimates for specified differences in interaction are given in Table 3. Table 4 contains power estimates for DFS comparisons of biomarker and genotype within treatment group (celecoxib; no celecoxib) by marker prevalence, detectable hazard ratio and the percent of samples available. Table 5 provides power estimates for testing the prognostic value of a marker when no treatment by marker interaction is present. Table 6 illustrates detectable differences in DFS for biomarkers quantified in quartiles. Table 7 illustrates the difference detectable with the specified power for a biomarker quantified in quartiles versus a dichotomous marker; for example, Vitamin D levels measured by plasma 25(OH)D versus K-ras mutational status. Tables 8 and 9 describe the power achieved to detect the illustrated difference in Grade 3+ toxicity rates versus a biomarker quantified in quartiles. Examples are Grade 3+ neutropenia and diarrhea versus body mass index.

Table 3. Approximate power to detect the specified hazard ratio for treatment (no celecoxib; celecoxib) by biomarker interactions with 60% (n=1,500) and 80% (n=2,000) of samples obtained (2-sided α=0.1) assuming exponential survival, a dichotomous biomarker, equal sample sizes per group, and an overall event rate of 0.31 (775/2500).

n (events)	Hazard Ratio-Interaction	Power
	1.5	
1500 (465)		0.34,0.58,0.71
2000 (620)		0.47,0.71,0.81
	1.6	
1500 (465)		0.48,0.71,0.81
2000 (620)		0.63,0.83,0.90
	1.7	
1500 (465)		0.60,0.81,0.88
2000 (620)		0.76,0.91,0.95

Moderate to large interaction hazard ratios are detectable with adequate power.

Table 4. Power estimates for DFS comparisons of a dichotomous biomarker or genotype within treatment groups (e.g., patients receiving celecoxib) by marker prevalence, detectable hazard ratio and the percent of samples available (log rank test, 2-sided α=0.01, 0.05, 0.10). Exponential survival, equal numbers of samples per treatment arm, and an overall event rate of 0.31 (775/2500) are assumed.

	Detectable Hazard Ratio	1.4	1.5	1.6
Marker/ Genotype Prevalence	**n (number of events)**			
0.5				
	750 (233)	0.49,0.72,0.82	0.69,0.87,0.92	0.84,0.94,0.97
	1000 (310)	0.65,0.84,0.90	0.83,0.94,0.97	0.94,0.98,0.99
0.2				
	750 (233)	0.30,0.53,0.65	0.46,0.69,0.79	0.61,0.81,0.88
	1000 (310)	0.41,0.65,0.76	0.61,0.81,0.88	0.76,0.91,0.95
0.1				
	750 (233)	0.15,0.33,0.45	0.23,0.45,0.58	0.33,0.57,0.69
	1000 (310)	0.21,0.42,0.55	0.33,0.57,0.69	0.46,0.69,0.79

Adequate power is achieved to detect moderate to large hazard ratios for dichotomous biomarkers or genotypes with prevalence close to 50%. The power estimates at 50% prevalence are approximately correct for biomarkers or genotypes with prevalence ≥ 30% and ≤ 70%. For biomarkers or genotypes with prevalence near 20% and, in particular, 10%, larger differences are detectable with adequate power.

Table 5. Power estimates for DFS comparisons of a dichotomous biomarker or genotype by marker prevalence for all patients, detectable hazard ratio, and the percent of samples available (log rank test, 2-sided α=0.01, 0.05, 0.10). Exponential survival and an overall event rate of 0.31 (775/2500) are assumed.

	Detectable Hazard Ratio	1.2	1.3	1.4	1.5
Marker/ Genotype Prevalence	**n (number of events)**				
0.5					
	1500 (465)	0.27,0.50,0.62	0.59,0.80,0.88	0.85,0.95,0.97	0.96,0.99,0.99
	2000 (620)	0.37,0.62,0.73	0.75,0.90,0.94	0.94,0.98,0.99	0.99,0.99,0.99
0.2					
	1500 (465)	0.15,0.34,0.47	0.37,0.61,0.73	0.62,0.82,0.89	0.82,0.93,0.96
	2000 (620)	0.22,0.44,0.56	0.51,0.74,0.83	0.78,0.91,0.95	0.92,0.98,0.99
0.1					
	1500 (465)	0.08,0.21,0.32	0.18,0.39,0.52	0.034,0.58,0.70	0.51,0.74,0.83
	2000 (620)	0.11,0.27,0.38	0.26,0.49,0.62	0.47,0.71,0.80	0.67,0.85,0.91

Adequate power is achieved to detect moderate to large hazard ratios for dichotomous biomarkers or genotypes with prevalence close to 50%. The power estimates at 50% prevalence are approximately correct for biomarkers or genotypes with prevalence ≥ 30% and ≤ 70%. For biomarkers or genotypes with prevalence near 20% and, in particular, 10%, larger differences are detectable with adequate power.

Table 6. Approximate DFS hazard ratios (maximum versus minimum hazard under "least favorable configuration") detectable with 70%, 80%, and 90% power for comparisons of a continuous biomarker categorized by quartiles for all patients and the percent of samples available (2-sided α=0.01, 0.05, 0.10). The chi-square non-centrality parameters (ncp) are 20.66, 16.74, 14.16, respectively, 70%, 80% and 90% power, with α=0.01; 16.21, 12.73, 10.46, respectively, for α=0.05; and 8.67, 10.77, and 14.02, respectively, for α=0.1. Exponential survival and an overall event rate of 0.31 (775/2500) are assumed.

	Detectable Hazard Ratio (DFS)		
Power	0.7	0.8	0.9
n (number of events)			
1500 (465)	1.64,1.53,1.47	1.72,1.60,1.54	1.82,1.70,1.64
2000 (620)	1.54,1.45,1.40	1.60,1.50,1.45	1.68,1.58,1.53

With 60 and 80% of samples studied, adequate power is achieved to detect moderate to large hazard ratios for biomarkers categorized by quartiles.

Table 7. Difference in median DFS detectable for a continuous marker categorized by quartiles (Biomarker 1) versus a second dichotomous biomarker (Biomarker 2) with approximate powers of 0.44, 0.68, 0.78, respectively, for 2-sided α=0.01, 0.05, 0.10, with 60% of patients submitting samples and approximate powers of 0.61, 0.81, 0.88, respectively, for α=0.01, 0.05, 0.10, with 80% of patients submitting samples (logrank test). Exponential survival, equal sample sizes per group, and an overall event rate of 0.31 (775/2500) are assumed.

	Median DFS (years)			
Biomarker 1	Quartile(1)	Quartile(2)	Quartile(3)	Quartile(4)
Biomarker 2				
Absent	8.0	9.0	10.0	11.0
Present	9.0	8.0	7.0	6.0

Moderate to large interaction hazard ratios are detectable with adequate power.

Table 8. Power estimates to detect the difference illustrated in Table 9 for a continuous marker categorized by quartiles versus toxicity (< Grade 3+; ≥ Grade 3+) for 60% (ncp=9.2) and 80% (ncp=12.3) of patients submitting samples (chi-square, 2-sided α=0.01, 0.05).

	Significance Level	
n	α=0.01	α=0.05
1500 (60% of patients)	0.67	0.85
2000 (80% of patients)	0.82	0.93

Table 9. Difference in proportions of patients by a continuous marker categorized in quartiles versus toxicity (< Grade 3+; ≥ Grade 3+) that is detectable with power given in Table 8. A Grade 3+ toxicity prevalence of 0.3 is assumed.

Biomarker	Quartile(1)	Quartile(2)	Quartile(3)	Quartile(4)	**Total**
Adverse Event					
< Grade 3+	0.1875	0.18	0.175	0.1625	0.7
≥ Grade 3+	0.0625	0.075	0.075	0.0875	0.3
Total	0.25	0.25	0.25	0.25	1.0

For DASL analyses, we will assume that 775 events are expected in the clinical study. This corresponds to an event rate of 0.31. We will assume that 70% will provide consent and usable samples for these analyses. The corresponding sample size and number of events to be used for the power calculations are 1750 and 542 respectively. For these simulations, we will assume that the DFS distribution is mixture of exponentials, with rates -log(0.72)/3 and -log(0.77)/3 respectively, and that the censoring distribution is uniform. The parameters on the latter are chosen so as to set the expected event rate is 0.31. The power, at the two-sided 0.01 FWER Bonferroni adjusted level, is illustrated in Table 10 based on a Kendall tau coefficient of 0.11, 0.12 and 0.13 under the Gaussian, Frank and Gumbel copulas. Each illustration is based on 10,000 Monte Carlo simulations. As we have pointed out, we will sharpen these FWER bounds through permutation resampling for the analyses.

Table 10. Power illustration for the DASL study at the two-sided 0.05 FWER level

tau	**Copula**	**Power**
0.11	Normal	0.79
0.12		0.91
0.13		0.97
0.11	Frank	0.82
0.12		0.92
0.13		0.98
0.11	Gumbel	0.54
0.12		0.71
0.13		0.84

A molecular classification model will be constructed using conditional inference trees with binary splits.[38] These trees will allow for direct incorporation of the censoring mechanism, which is important given that the length of follow-up varies among patients, as well as adjustment for multiplicity. The overall error rate will be adjusted at the 0.2 level using permutation resampling. The terminal nodes of these trees will be considered "risk" groups (classified by the trees). Kaplan-Meier estimates of the hazard profiles for these risk groups will be produced and presented graphically. We will also consider the random forests for survival data.[39-41] One of the key advantages of the two methods is that they implicitly incorporate interactions among the variables.

7.0 STATISTICAL SOFTWARE AND COMPUTING HARDWARE

The statistical environment R along with extension packages from the Bioconductor project will be used for carrying out the statistical analyses.[42,43] When appropriate, bindings with other languages such as Python and C++ will be used. The CALGB Bioinformatics Unit maintains a dedicated 4-way quad-core (16 cores) AMD Opteron 8384 processors with 64GB of RAM, 3TB of usable hard-drive space with RAID 10 redundancy running the Debian GNU/Linux stable AMD64 operating system. The machine can be connected to the CALGB SAN through an iSCSI interface for additional storage. Drs. Niedzwiecki and Owzar also have access to an 8-way Socket F AMD Opteron server with dual core 3.0 Ghz 2222SE processors (total of 16 cores) with 64GB of RAM running Debian GNU/Linux stable AMD64 administered by the Duke Comprehensive Cancer

Center Information Science (CCIS). By virtue of their academic affiliations with Department of Biostatistics and Bioinformatics, at the Duke University Medical Center (for whom Dr. Owzar serves as the chair of the computing committee), will have access to departmental computing resources as well. These include two 8-way Socket F AMD Opteron servers each with dual core 3.0 Ghz 2222SE processors (total of 16 cores) with 64GB of RAM and one 4-way Socket F AMD Opteron server each with dual core 3.0 Ghz 2222SE processors (total of 8 cores) with 32GB of RAM. Additionally, through a collaborative agreement with the Duke HPC group, Drs. Niedzwiecki and Owzar also have access to GPU (graphics processor unit) computing facilities.

APPENDIX IV

PHARMACOGENETIC COMPANION STUDIES: CALGB 60905

1.0 OBJECTIVES

1.1 To assess the influence of genetic variations in the cyclooxygenase and related pathways on the efficacy of celecoxib as adjuvant therapy for stage III colon cancer

1.2 To assess the influence of germline variation in vitamin D pathway genes on disease-free and overall survival in patients with stage III colon cancer.

1.3 To investigate the potential association between the AGXT 154C>T polymorphisms and neuropathy in the Caucasian population.

1.4 To identify specific SNPs and/or copy number variations that are associated with the prevalence of oxaliplatin-related peripheral neuropathy.

1.5 To identify specific SNPs and/or copy number variations that are associated with DFS and OS.

2.0 BACKGROUND

Candidate gene, pathway analyses and whole genome scans are common approaches for the identification of germline polymorphisms that contribute to a given phenotype. A candidate gene approach focusing on drug metabolizing enzymes and drug targets is proposed. In addition, a genome wide single nucleotide polymorphism (SNP) and copy number variant (CNV) scan will be performed to provide more definitive assessment of the genomic contribution to variation in drug effect (see below).

2.1 Genetic variation in COX-2 and related pathways

It is hypothesized that germline polymorphisms in COX related pathway will influence the effect of celecoxib on patient survival. Aspirin and NSAIDs may influence colorectal carcinogenesis, in part, through the inhibition of cyclooxygenase-2 (COX-2; PTSG-2) and COX1 (PTSG-1).[147,148] COX is the rate-limiting enzyme for the metabolic conversion of arachidonic acid to prostaglandins and related eicosanoids. COX-2 promotes inflammation and cell proliferation and is overexpressed in human colorectal cancer. Polymorphisms in COX-2 (PTGS2) have been associated with the risk of colorectal cancer.[149,150] In a randomized trial of adjuvant rofecoxib in colorectal cancer patients that was terminated early, 3 COX-2 SNPs (rs10911907, rs11583191, and rs2179555) which lie 5' to the COX2 gene significantly influenced the effect of rofecoxib.[151] The effect of rofecoxib was significantly greater in patients with at least one variant allele at each of the three SNP sites (p, interaction = 0.04). Similarly, COX-1 polymorphisms have been linked to the colorectal adenoma risk and appear to modify the influence of aspirin/NSAID use on adenoma risk.[152] 5-lipoxygenase (5-LOX) represents an alternative pathway to COX in arachidonate metabolism. Polymorphism in the promoter region of 5-LOX (ALOX5) has been associated with increased inflammation,[153,154] levels of C-reactive protein,[155] aspirin-intolerant asthma,[153] and colorectal cancer.[156] Prostacyclin synthase is a downstream enzyme from COX. A variable number tandem repeat polymorphism in the prostacylin synthase gene has been associated with altered transcriptional activity and increased risk of hypertension and stroke.[157-159] Thromboxane synthase is an alternative pathway that is downstream from COX. The T(-386)G polymorphism has been associated with an increased risk of myocardial infarction.[160] PPARγ, a member of the steroid receptor/transcription factor family, is a critical regulator of adipogenesis and a target of the adenomatous polyposis coli (APC) gene.[161-163] PPARγ may also be a potential target of aspirin and NSAIDs.[164,165] Animal and clinical studies demonstrate

that PPARγ also has a role in insulin signaling, insulin resistance, development of type 2 diabetes and may function as a tumor suppressor gene.[163] A common variant in the PPARγ gene (Pro12Ala) reduces the promoter affinity by approximately 50% and is associated with a reduced risk of type 2 diabetes.[166] Carriers of the PPARγ 12Ala variant allele were at reduced risk of colorectal neoplasia.[167,168] Nuclear factor-κB (NF-κB) is a ubiquitous transcription factor involved in the regulation of inflammation, apoptosis, and carcinogenesis.[169,170] Various proinflammatory cytokines (IL-1-beta, IL-8, TNF-alpha) activate NF-κB, and, in turn, activated NF-κB promotes colorectal cell proliferation and other inflammatory genes including COX-2.[171-175] NF-κB is constitutively activated in human colorectal cancer, and aspirin-induced suppression of NF-κB impairs colorectal tumorigenesis. Polymorphisms in NF-κB have been associated with the increased risk of inflammatory disorders,[176-178] diabetes mellitus,[179,180] and myeloma.[181]

The genes involved in celecoxib metabolism will also be examined. CYP2C9 metabolizes both celecoxib and NSAIDs, and variant CYP2C9 genotypes modified the effect of aspirin on adenoma risk in one study.[182] Thus, the efficacy of celecoxib on colon cancer recurrence may be influenced by the metabolism of the agent.

2.2 Germline variations in the vitamin D pathway

The pathway through which vitamin D exerts transcriptional effects is complex. An editorial in the Journal of the National Cancer Institute recommended that future investigations consider the interrelationships of vitamin D and associated genetic polymorphisms within the vitamin D pathway.[183] In CALGB/SWOG C80702, we will examine the joint effect of vitamin D with polymorphisms in five critical genes in the vitamin D pathway.

The cellular effects of 1-25-dihyroxycholecalciferol [1,25$(OH)_2$D] – the active metabolite of vitamin D – are principally mediated through the vitamin D receptor (VDR), which regulates the transcription of genes involved in cellular differentiation and inhibition of proliferation.[184,185] Well-differentiated human colon cancer cell lines have higher VDR expression[101] and the antiproliferative effects of vitamin D only occur in cell lines expressing high levels of VDR.[102] Several common polymorphisms (FokI, ApaI, TaqI and BsmI) have been identified in the VDR gene, although functional studies of these polymorphisms have shown contradictory results.[186-194] Moreover, associations between these polymorphisms and the risk of colorectal neoplasia remain inconsistent.[195-202] To better define genetic variation in the VDR gene, Nejentsev developed high-resolution SNP, haplotype and linkage disequilibrium (LD) maps of VDR in a multiethnic cohort.[203] A total of 24-26 TagSNPs were required to tag the three haplotype blocks ($r^2 \geq 0.8$). The authors observed that FokI was not in LD with any other common SNP in their study. In addition, ApaI, TaqI and BsmI missed a large fraction of common variation. Therefore, a comprehensive examination of VDR, plasma 25(OH)D, and patient outcome will be performed using these 26 TagSNPs.

Vitamin D binding protein (VDBP, GC) transports vitamin D and its plasma metabolites to target tissues and may also be involved in intracellular metabolism of vitamin D.[204,205] Two common VDBP polymorphisms (Glu432Asp and Thr436Lys) have been associated with osteoporosis,[203] COPD,[204] chronic mucus hypersecretion,[208] diabetes,[204,205] and Grave's disease.[209] In contrast, genotyping in 24 unrelated Caucasian individuals of 33 other SNPs with a minor allele frequency >5% revealed little LD across VDBP. Consequently, the investigators will focus on the Glu432Asp and Thr436Lys VDBP polymorphisms.

1-α-hydroxylase (CYP27B1) catalyzes the 1α-hydroxylation of 25$(OH)_2$D to 1,25$(OH)_2$D.[210] Beyond the proximal renal tubules, the enzyme is present in both normal human colonic mucosa and colorectal adenocarcinomas.[210-214] 1-α-hydroxylase polymorphisms have been associated with the risk of Addison's disease, Hashimoto's thyroiditis, Graves' disease, and type 1 diabetes mellitius.[215,216] We will

examine five SNPs with a minor allele frequency >5% (all noncoding) identified in a multiethnic cohort.[217]

Retinoid X receptor (RXR) functions as a heterodimer with the VDR, forming a VDR/RXR complex that regulates transcription of several target genes.[211,218] Seven SNPs with a minor allele frequency >5% (6 noncoding and one coding, Ser327Ile) will be examined in a multiethnic cohort.[219]

25-hydroxyvitamin D-24-hydroxylase (CYP24A1) initiates degradation of $1,25(OH)_2D$ and 25(OH)D. CYP24A1 mRNA and protein levels are significantly upregulated in cancers relative to normal tissues, suggesting CYP24A1 may be an oncogene.[220] Polymorphisms in the promoter region of CYP24A1 enhance both basal and vitamin D_3-stimulated promoter activity.[221] Six SNPs in the CYP24A1 promoter will be examined.[222]

2.3 AGXT 154C>T polymorphisms and neuropathy

There is increasing evidence which suggests that germline polymorphisms related to anticancer therapeutics metabolism, transport, and resistance correlate with drug response; furthermore, germline polymorphisms related to therapeutic targets and/or therapeutic pathways might also help predict therapeutic outcomes.[223,224]

This study offers an excellent opportunity to evaluate the role of genetic variants in relevant genes influencing the pharmacology of 5-fluorouracil, oxaliplatin, and celecoxib, which might influence the eventual drug response and/or toxicity of these agents.

The introduction of oxaliplatin to 5-fluorouracil has had a major impact on the treatment of colorectal cancer. However, the efficacy of oxaliplatin-based therapy is often compromised because of the substantial risk for severe toxicities, including neurotoxicity.[225] Oxaliplatin has a direct "pharmacologic" effect on the excitability of sensory neurons and muscle cells that has not previously been described with other platinum agents. Neurotoxicity can result in both acute and chronic debilitation. Moreover, colorectal cancer patients treated with oxaliplatin more often discontinue therapy due to peripheral neuropathy than for tumor progression, potentially compromising patient benefit. Numerous methods to prevent neurotoxicity have so far proven unsuccessful.[225] In order to circumvent this treatment-altering side effect, while taking advantage of the antitumor activities of oxaliplatin, efforts to identify mechanism-based biomarkers are underway.

The neurotoxic effects of oxaliplatin are not seen with exposure to the cytotoxic metabolite, DACH platinum, but rather with the oxalate metabolite. A promising biomarker for risk of oxaliplatin-associated peripheral neuropathy is AGXT, which encodes alanine-glyoxylate aminotransferase. Gamelin et al. proposed that key components of the oxalate synthesis pathway could be associated with platinum-drug neurotoxicity. In a study of 145 patients treated with oxaliplatin, a C to T change in AGXT nucleotide 154 was associated with risk of chronic neurotoxicity.[226] Patients with a C/C genotype (~70% of Caucasians) had a significantly lower incidence of grade 2+ neurotoxicity (~5%) compared to the C/T and T/T patients (~30% of patients, expected incidence of grade 2+ neurotoxicity would be 30%). AGXT is a major enzyme of glyoxylate and oxalate metabolism and 154C>T has a partially reduced activity. These patients seem unable to cope with a brutal inflow of oxalate after oxaliplatin infusion, whose elimination becomes predominantly urinary. Its intracellular concentration increases and it interferes with sodium channels and generates acute neurotoxicity. Our primary hypothesis is that patients with AGXT 154 C/T or T/T will have a significantly greater incidence of grade 2+ peripheral neuropathy, compared with 154 C/C patients.

Finally, in addition to specific hypothesis testing for the above candidate genes, this study will also provide the framework for hypothesis generation investigations of genotype and/or haplotype in additional candidate genes of putative importance to drug response of the agents being evaluated in this study.

2.4 Genome-wide association studies

Most pharmacogenetic analyses have taken a candidate gene approach that utilizes biological data to guide the selection of drug response genes in a pathway. This approach is limited by our knowledge of the mechanisms underlying the phenotypes. In the case of drug response phenotypes, most candidate gene studies have focused on drug metabolizing enzymes and transporters, thus limiting the chance of discovering causal SNPs not involved in mediating drug levels.[227,228] In contrast, a genome-wide approach collects SNP data across the entire human genome and has significant power to detect common variants that confer a modest risk for a complex phenotype.[229] Genome-wide studies capitalize on the large number of SNPs (more than 10 million available in dbSNP) that have been localized and validated across the genome, a majority of which have resulted from the HapMap project.[230] This valuable collection of publicly available, validated SNPs has provided the framework for performing genome-wide association studies. Recent technological advancements in genotyping platforms have also enabled the development of genome-wide associations. Searching the whole genome in an association study requires genotyping of anywhere between 10^5 to 10^6 markers across the genome.[231-234] Until recently, this approach was fiscally prohibitive and impractical. However, new gene chip platforms from Affymetrix and Illumina have made large-scale genotyping feasible and cost effective. The Illumina HumanHap550 chip that will be used in this study has the capacity to genotype over 555,000 SNPs simultaneously. In addition, there are 4,300 SNPs in regions of copy number variations (CNVs), thus allowing for the detection of CNVs as well. This new capability represents a paradigm shift in the number of genotypes that can be evaluated in any given individual with one genotyping assay and provides a platform for the identification of novel genes involved in the response to and toxicity associated with 5-fluorouracil, oxaliplatin, and celecoxib.

An increasing number of reports of significant findings from genome-wide association studies in cancer are being published. To date, these have all focused on SNPs associated with risk of developing cancer, and include studies in prostate,[235-239] colorectal,[240-242] lung,[243] and breast cancer.[244-246] The success of these studies illustrates the power and validity of this approach for identifying genetic causes of disease. To date, there are no published reports of genome-wide association analyses in cancer pharmacogenetics. The relatively large size of CALGB/SWOG C80702 and robust response and toxicity phenotype data make it an ideal sample set for whole genome analysis. The identification of SNPs that contribute to response and toxicity of the three widely used drugs studied in CALGB/SWOG C80702 will lead to additional studies to understand the mechanism for these associations and to investigate the application of genetic information for the optimization of cancer therapy. The whole genome SNP data will also be useful for biological associations, including those detailed in the correlative science portions of this protocol.

3.0 HYPOTHESES

3.1 Genetic variation in COX-2 and related pathway genes will influence the efficacy of adjuvant celecoxib on cancer recurrence.

3.2 Germline variation in vitamin D pathway genes (VDR, VDBP, CYP27B1, RXR, CYP24A1) will impact disease-free and overall survival in stage III colon cancer patients. Moreover, polymorphisms in these genes modify the effect of plasma 25(OH)D on patient outcome.

3.3 Patients with AGXT 154 C/T or T/T will have a significantly greater incidence of grade 2+ peripheral neuropathy, compared with 154 C/C patients.

3.4 Novel SNPs and/or copy number variations will be identified that are associated with the prevalence of oxaliplatin-related peripheral neuropathy as well as DFS and OS.

4.0 METHODS

DNA will be available from patients who consented to the pharmacogenomic companion study (CALGB 60905) and provided blood samples (10 mL in EDTA tube). Samples will be banked by the PCO. DNA will be isolated and DNA quality will be assessed by UV spectrophotometry and by agarose gel electrophoresis. Phenotypic data will be extracted from research databases by the CALGB statistical group.

4.1 Genetic analysis of candidate genes

For genetic analyses of the candidate genes such as COX-2, vitamin D (VDR, VDBP, CYP27B1, RXR, CYP24A1), and AGXT pathway genes, assays will be performed using previously published methods such as Taqman allelic discrimination or pyrosequencing assays. If more efficient alternative genotyping methods become available in the future, the PET committee will change the genotype approach to optimize resources. Output from the genotyping platforms is entered in a database and forwarded to the CALGB Statistical Center where it will be correlated with clinical outcomes.

4.2 Whole-genome genotyping

Illumina's HumanHap550 Genotyping BeadChip enables whole-genome genotyping of over 555,000 single nucleotide polymorphisms (SNPs) loci efficiently and accurately on a single BeadChip. The HumanHap550 BeadChip is powered by the Infinium™ II assay, which uses a single-tube, whole-genome amplification method that does not require PCR and enables intelligent SNP selection using tagSNPs. TagSNPs are loci that can serve as proxies for many other SNPs. The information and power from a larger number of SNPs can be gathered by genotyping only a subset of loci. TagSNPs on the HumanHap550 BeadChip were selected from the recently completed International HapMap Projected. The Illumina's HumanHap550 Genotyping BeadChip is one of the platforms that might be used for this study. However, additional platforms, including high-throughput resequencing, might also be used to interrogate the germline genomic variation of patients.

5.0 DATA ANALYSES

5.1 Genetic variation of COX and related pathways

For analyses of germline variation in COX-2 and related pathway genes (objective 1.1), there are several strategies for choosing variants to genotype in specific candidate genes, ranging from selection of a single SNP with proven or potential functional significance, to haplotype- or linkage-disequilibrium tagging approaches. We have chosen the strategy for each gene that we feel can be best justified at this time, taking note of the fact that this is a fast-moving research area. Thus, for most candidate genes, the SNP(s) of proven function, or SNPs with probable function that have been previously associated with the disease, have been chosen. For the most part, the choices are conservative on a gene-by-gene basis, and are the variants that are most likely to interact with the environmental factors proposed, given current knowledge. Although some of the proposed SNPs have been investigated in functional studies, the functional roles of many other SNPs, especially those in the noncoding regions, have been minimally studied.

Candidate SNPs as Effect Modifiers of Celecoxib Efficacy: All polymorphisms will be individually evaluated and categorized as variant or wild type. Baseline characteristics of patients will be compared according to genotype. We will also investigate whether COX-2 and related pathway genotypes modify the relationship between celecoxib use (vs. placebo) and patient outcome. Cox proportional hazards models will be used to calculate multivariable-adjusted HRs and 95% CIs for DFS and OS by celecoxib vs. placebo for each SNP: those with the variant genotype and those with wild type. Tests for statistical interaction will be performed by entering into the model the cross-product term of celecoxib vs. placebo with the genotype as a

binary variable. The main effect of each genotype on DFS and OS will also be examined. The main effect analyses will also utilize Cox proportional hazards models to calculate multivariable-adjusted HRs and 95% CIs for DFS and OS by genotype. Multivariate models will be adjusted for confounding variables.

5.2 Germline variations in the vitamin D pathway

For analyses of germline variation in vitamin D pathway genes (objective 1.2), there are several strategies for choosing variants to genotype in specific candidate genes, ranging from selection of a single SNP with proven or potential functional significance, to haplotype- or linkage-disequilibrium tagging approaches. We have chosen the strategy for each gene that we feel can be best justified at this time, taking note of the fact that this is a fast-moving research area. Thus, for most candidate genes, the SNP(s) of proven function, or SNPs with probable function that have been previously associated with the disease have been chosen. For the most part, our choices are conservative on a gene-by-gene basis, and are the variants that are most likely to interact with the environmental factors proposed, given current knowledge. Although some of the proposed SNPs have been investigated in functional studies, the functional roles of many other SNPs, especially those in the noncoding regions, have been minimally studied. In addition, in future analyses, an agnostic approach will be employed and genome-wide association studies will be conducted to identify SNPs that may be associated with plasma 25(OH)D concentrations and patient survival.

Candidate SNPs as Main Effects: All polymorphisms will be individually evaluated and categorized as variant or wild type. Baseline characteristics of patients will be compared according to genotype. The main analyses will utilize Cox proportional hazards models to calculate multivariable-adjusted HRs and 95% CIs for DFS and OS by genotype. Multivariate models will be adjusted for confounding variables. It will be examined whether genetic variation in the vitamin D pathways is associated with vitamin D levels using linear regression. All patients who have available plasma 25(OH)D and genotype data will be included; analyses will be age- and season of blood draw-adjusted. We will also investigate whether vitamin D pathway genotypes modify the relationship between vitamin D levels and patient outcome. Cox proportional hazards models will be used to calculate multivariable-adjusted HRs and 95% CIs for DFS and OS by tertile of 25(OH)D for each SNP: those with the variant genotype and those with wild type. Tertiles will be used instead of quintiles for these analyses in order to maximize power. Tests for statistical interaction will be performed by entering into the model the cross-product term of 25(OH)D as a continuous variable with the genotype as a binary variable.

5.3 AGXT 154C>T polymorphism and neuropathy

The primary statistical objective for the pharmacogenetic companion of this study is to investigate the potential association between the AGXT 154C>T polymorphism and neuropathy in the Caucasian population.[226] Specifically, it is hypothesized that the presence of the T allele is associated with higher incidence oxaliplatin-related peripheral neuropathy.

The primary statistical endpoint will be the realization of a grade 2 or higher neuropathy event. Let D denote the corresponding event and pi=P(D=1) denote the relative prevalence of the event in the population. The study will be powered assuming that the model is non-decreasing with respect to the T allele. In other words, it is assumed that pi0<=pi1<=pi2, where pi0=P(D=1|CC), pi1=P(D=1|CT) and pi2=P(D=1|TT). Assuming Hardy-Weinberg, the event probability is expressible as the mixture pi=(1-q)^2* pi0 + 2*q(1-q)*pi1+q^2*pi2.

The clinical study plans to contribute 2,500 patients to this intergroup study. It is assumed that 85% of these patients will provide consent and usable samples for this companion study. The analysis population will consist of those self-reported as Caucasian on the CRF form. The expected proportion for this population is at least

0.85. The companion study will be powered based on a sample size of 1806 (=2500*0.85*0.85).

The putative allelic relative frequency for the risk T allele is q=0.3. The power of the Cochran-Armitage test, at the two-sided 0.05 level, assuming a dominant model (DOM: pi1=pi2=pi0*GRR) and an additive model (ADD: pi1=pi0*GRR, pi2=pi0*GRR*GRR) is illustrated in Table 1 for GRR>1. Here GRR denotes the Genotype Relative Risk.

pi	GRR	Power	
		DOM	ADD
0.30	1.15	0.42	0.73
	1.20	0.62	0.92
	1.25	0.80	0.98
0.33	1.15	0.46	0.78
	1.20	0.69	0.95
	1.25	0.85	0.99

Table 1: Power illustration for the genotype test, at the two-sided 0.05 level, assuming a dominant or additive model.

It is noted that this is a hypothesis of association (gene by outcome) regardless of the number of cycles of oxaliplatin received. Any potential effect due to amount of therapy received will be examined using regression techniques.

Secondary SNPs of interest include potential predictive markers of outcome (OS or DFS) with respect to celecoxib (e.g., COX-2 and pathways) and germline variation in the vitamin D pathway.

The addition of other important clinical and demographic co-variables will be considered. Multivariable models, with molecular, clinical and demographic variables, will be constructed using conditional inference trees and random forests. All secondary and exploratory objectives will be tested at an unadjusted two-sided level of 0.05.

5.4 Genome-wide association studies

5.4.1 Pre-processing

For pre-processing (QC and genotype calls) the Illumina chips, we will use the commercial program Bead Studio developed by Illumina. Although, Illumina does not provide a Linux port of Bead Studio, one can run the software on VMWARE running on a Linux host. A two CPU dual core (four cores) AMD Opteron Socket F workstation with 16GB of RAM will be available for this purpose (the statistical analyses will be carried out on a Linux server with four quad-core Opteron CPUs (16 cores) with 64GB of RAM [expandable to 128GB if needed]).

5.4.2 Analyses to assess genotyping quality and population stratification

Initial quality studies will be conducted to identify SNPs that have generated sufficiently poor quality genotype data and should be removed from analyses. Call rate, patterns of missing data, and departures from Hardy-Weinberg equilibrium (HWE) assessed using an exact test will all be scrutinized to identify markers that will not be used in analysis. In general, SNPs with call rates <95% and those with highly significant departures from HWE ($p<10^{-7}$) will not be included in analyses. Non-random patterns of missing data are sometimes encountered in data generated on high-throughput genotyping platforms; the most common non-random missing data problem is that heterozygous genotypes are more likely to be assigned as missing than either homozygous genotype. We will perform analyses using blind duplicates as well as analyses assessing the relationship between heterozygous call rates and missing data to identify any SNPs in which data are clearly not missing at random. Depending on the number and degree of

difficulty observed, we will either remove problematic SNPs from analysis, or assign quality scores to reflect the extent of the non-random missing data.

Additional preliminary quality control analyses will be conducted to ensure that the sample does not include duplicated samples or closely related individuals. These analyses can be rapidly conducted using PLINK.[300] Duplicated samples (or unrecognized identical twins) will be reduced to a single sample for further analyses. Although we do not expect to have closely related individuals included in this sample, only one member of any set of first-degree relatives will be included in subsequent analysis.

Population structure that is not appropriately recognized and accommodated can lead to both false positive and false negative results in association studies. We will conduct studies using structure[301] to estimate ancestry proportions using 10,000 SNPs chosen for having no pairwise LD with unrelated individuals from the HapMap CEU, YRI and CHB+JPT samples used to model the ancestral populations. Substantial previous research has shown this to be a rapid and effective approach to defining historical geographic ancestry. Although self-identified race/ethnicity is usually highly correlated with estimated historical geographic ancestry, there are often a few individuals who appear to be misclassified with self-defined labels, and it is the genetically defined ancestry that is critical to correctly accommodate to ensure robust results from association studies. Each individual will then have estimates of European, African and Asian ancestry. For individuals with high ancestry proportion for a single group (>98%), we will conduct further analyses with eigenstrat[302] using all SNPs to determine whether there are additional important sources of variation among individuals leading to detectable stratification by allele frequencies (reflecting, for example, differences in ethnic make-up within individuals of European descent from different U.S. cities from which subjects for the trial were obtained). Primary analyses, described below, will be conducted within groups defined by historical geographic ancestry. Secondary analyses will be conducted using logistic regression with ancestry proportions (and any additional stratification identified using eigenstrat) as covariates.

5.4.3 Feature discovery

The association between the genotype call (say AA, AB or BB) for each autosomal SNP and the clinical outcome [for example, adverse event (AE) or no AE] will be investigated within the framework of 2 by 3 contingency table stratified by ancestry. Fisher's exact test (i.e., randomized conditional counterpart to Fisher's test for 2 x 3 tables)[303] will be used for carrying out inference on these tables. A feature (SNP) will be considered significant if the corresponding nominal unadjusted two-sided P-value is less than 0.05/K, where K is number of features which pass the pre-processing step. Needless to say, this approach may be conservative. It does however guarantee strict type I error control.

For the sake of discussion, let B denote the risk allele with an assumed relative allelic frequency of q. Under the Hardy-Weinberg equilibrium assumption, the genotypes AA, AB or BB will have relative genotypic frequencies of $(1-q)^2$, $2q(1-q)$ and q^2, respectively. Let D denote the binary clinical outcome (D=1 if the AE event occurs or =0 otherwise) and define the probability of an AE occurrence given the copies of the risk allele on the genotype, to be denoted by G, as $p_g=P[D=1|G=g]$, for g=0,1 or 2. The relationship between the event probability p=P[D=1] in the general population is then expressible as the following mixture $p=(1-q)^2p_0+2*q*(1-q)p_1+q^2p_2$. The effect size in the context of genome-wide association studies is typically quantified using the genotype relative risk (GRR) whose definition depends on the disease model. Under the recessive disease model, $p_0=p_1$ and $p_2=GRRp_0$ while under the dominant disease model $p_1=p_2=GRRp_0$. Finally, under the multiplicative (log-additive model), $GRR=p_1/p_0=p_2/p_1$. Under these disease model, the event probability in the population, p, can then be reformulated as the

mixture $p=(1-q)^2p_0w_0+2*q*(1-q)p_0w_1+q^2p_0w_2$, where $w=(w_0,w_1,w_2)=(1,1,GRR)$, for the recessive model, $=(1,GRR,GRR)$ for the dominant model and $=(1,GRR,GRR^2)$ for the multiplicative model.

The clinical study plans to contribute 2,500 patients to this intergroup study. It is assumed that 85% of these patients will provide consent and usable samples for this companion study. The analysis population will consist of those self-reported as Caucasian on the CRF form. The expected proportion for this population is at least 0.85. The companion study will be powered based on a sample size of 1806 (=2500*0.85*0.85). The power, at the two-sided 0.05/600000 level (i.e., assume K=600,000 autosomal SNP markers pass through the pre-processing step) is 0.9, for a range of relative allele frequencies (q) assuming the event probability is P[D=1]=0.3 under recessive, dominant and multiplicative models assuming HWE.

5.4.4 Submission of molecular data

The laboratory of Dr. Yusuke Nakamura will submit the Illumina *.idat image files using secure means to the CALGB Statistical Center. The lab will also submit a table along with this transmission, which at the minimum will provide the following information for each sample received from the repository.

The lab ID number provided by the repository.

The experimental ID, a concatenation of the plate, well and replicate information, generated by the lab.

The idat file names (the file string name will contain the Lab ID).

The md5sum signature of the idat files to ensure data integrity.

The date the specimen was received from the repository.

The date the sample was analyzed by the RIKEN laboratory.

Additionally, the lab will also provide the complete results from any quality control measures carried out. If a sample had to be redone (e.g., defective or poor quality array), the lab will provide all replicate idat files and add an appropriate column to the supplementary table. The molecular data generated for this aim may not be shared with other investigators or used for any analysis not specified in the protocol until a formal approval from the CALGB Statistical Center is obtained.

5.4.5 Secondary objectives

Logistic regression models and conditional inference trees (or more generally conditional random forests) will be used to construct multi-variable models based on the SNPs identified as interesting. These models also allow for inclusion of other potentially relevant clinical and demographic variables.

The Illumina Human610 Quad chip contains 184.064 SNPs in regions with common copy number variants (CNVs). Given the complex structure of CNVs, it is not always clear how to define the genotype of a CNV. Instead of categorizing copy numbers into genotypes, we will estimate relative genomic abundance probe intensities. This approach allows for the consideration of other CNVs beyond deletions, including duplications and combinations of both. For notational brevity, we shall refer to these as CNV markers.

For each objective, the association between each CNV marker and the clinical AE endpoint, will be assessed using the Wilcoxon two-sample test. The family-wise error rate will be controlled at the 0.05 level using permutation resampling (based on B=100,000 replicates).

Regression methods, as in the case of the SNP markers, will be employed to construct multivariable models based on the CNV markers.

Secondary relevant clinical endpoints include other adverse events (e.g., proteinuria and hypertension), progression-free and overall survival. For censored time-to-event outcomes, the stratified log-rank test will be primarily used for assessment of significance.

A risk analysis will be carried out by comparing the genotypic distributions of the SNPs from the CALGB/SWOG C80702 data to those from controls (thought to not to have cancer). The SNP data from the controls will be obtained from public databases.

In addition to conducting analyses on all features directly assessed on the high-throughput platform used in these studies, we will also interrogate all additional HapMap SNPs that are not in strong pairwise LD with any genotyped SNP but for which there is sufficient multi-locus LD to SNPs on the high-throughput platform. TUNA (Testing UNtyped Alleles) is a robust approach for conducting such analyses that provides inexpensive in silico follow up to the initial analysis and allows us to more efficiently design any follow up genotyping studies.[304,305] For example, use of Illumina HumanHap300 enables direct testing of 270K-450K SNPs, and indirect testing of 750K-1.5M additional SNPs (i.e., these SNPs are so highly correlated with SNPs that are directly tested for association that testing them would provide little additional information). The ranges given above bracket the expectations for different human populations, with European populations at the high end of the range, and populations of recent African descent at the lower end. Use of TUNA enables interrogation of an additional 100K-250K SNPs that are neither on the platform nor highly correlated with any individual SNP on the platform. Note that use of TUNA will facilitate comparisons to genome-wide association studies on potentially related phenotypes (e.g. clinical trials of the same or related drugs) conducted using other high-throughput platforms or candidate gene studies utilizing SNPs not directly genotyped on the high-throughput platform chosen for our studies.

Finally, we note that the methodology field for the analysis of genome-wide SNP data is in its infancy. We will consider the employment of "newer" methods if they are deemed to be statistically sound and enable us to better interrogate, and more importantly, understand the data.

5.4.6 Statistical software

The R statistical environment[306] and Bioconductor[307] packages will be used for all of the primary statistical analyses relating features to phenotypes. Specialized statistical genetics software, including PLINK,[300] structure,[301] eigenstrat,[302] and TUNA[304,305] will be used for some of the quality or secondary analyses, and R will be used for logistic regression analyses allowing for ancestry covariates.

APPENDIX V

DIET AND LIFESTYLE COMPANION

The influence of diet and other exogenous factors on disease-free survival, overall survival and treatment-related toxicity among patients with stage III colon cancer will be assessed. Patients enrolled on CALGB/SWOG C80702 will be asked to complete a food-frequency questionnaire within the first 6 weeks of start of randomization and 16-18 months after randomization. The questionnaire has been extensively validated among large populations and provides comprehensive data on 131 food items and over 100 micronutrients. The instrument will also ascertain leisure-time physical activity, cigarette smoking, height and weight, aspirin and non-steroidal anti-inflammatory drug use, vitamin/supplement use, and alternative medicine use. It is suggested that patients are given the questionnaire in clinic, completed in clinic/infusion and then mailed back by the research assistant/nurse. Patients who recur are not required to complete the questionnaire.

1.0 OBJECTIVES

1.1 Assess the influence of diet, body mass index, physical activity and other lifestyle habits on disease-free and overall survival among patients with stage III colon cancer.

1.2 Assess the influence of baseline plasma C-peptide, insulin-like growth factor binding protein-1 (IGFBP-1), insulin-like growth factor-1 (IGF-1), IGFBP-3, and adiponectin on disease-free survival in patients with stage III colon cancer.

1.3 Assess whether tumoral expression of phospo-Akt (pAkt) and fatty acid synthase (FASN) and KRAS and PI3K mutational status modifies the relation between measures of energy balance (e.g., dietary insulin index, obesity, physical activity, plasma C-peptide) and patient survival.

1.4 In exploratory analyses, assess the influence of diet, obesity, physical activity, and other lifestyle habits on the risk of toxicity associated with chemotherapy.

2.0 BACKGROUND

Epidemiologic and scientific research indicates that diet and other lifestyle factors have a significant influence on the risk of developing colon cancer. Consumption of red meat [247,248], alcohol [249,250], calcium [251,252], fiber [253], aspirin [254,255], and folic acid [250,253,256], obesity [257-264], physical activity [261,262,264], and cigarette smoking [259,265-267] are among factors that have been suggested to influence the risk of developing colorectal cancer.

Not until recently have there been data accessing the influence of these factors on patients with established cancer. In CALGB 89803 (trial of adjuvant therapy for stage III colon cancer comparing 5-FU/LV to irinotecan/5-FU/LV), a self-completed questionnaire was utilized to access diet, physical activity, smoking, medication use and family history. Multiple important findings have stemmed from this trial. However, these data require confirmation. Furthermore, the trial utilized an adjuvant therapy regimen that is now not standard of care and thus the influence of these factors on patients receiving FOLFOX would be very important.

3.0 PROPOSED HYPOTHESES

3.1 Lower dietary insulin index and regular physical activity are associated with an improved disease-free survival, and the benefit of lower dietary insulin index and regular physical activity are greater among tumors with wild-type KRAS, wildtype PI3K, and reduced expression of pAkt and FASN.

In prospective and retrospective studies, obesity is associated with an increased risk of colon cancer, whereas regular physical activity confers a reduced risk. Recently, several studies among patients with stage II and III colon cancer suggest that obesity has been associated with a reduced disease-free and overall survival, whereas physical activity was associated with an improved patient survival, including a recent study conducted within an adjuvant chemotherapy trial in stage III colon cancer patients (CALGB 89803). Recent hypotheses have linked physical activity, obesity, and adipose distribution to circulating insulin and free insulin-like growth factor 1 (IGF-1),[268,269] which is determined by the integrated actions of circulating IGF-1 and IGF binding proteins (BPs). Indeed, colon cancer risk is elevated in individuals with higher circulating levels of insulin or C-peptide (a marker of insulin secretion) and IGF-1 or IGF-1/IGFBP-3 ratio. Pre-clinically, insulin stimulates pathways that increase levels of free IGF-1, and both insulin and IGF-1 promote cell proliferation and inhibit apoptosis in colon cancer cells.

The relation between hyperinsulinemia and colon cancer suggests that a diet inducing an elevated insulin response may contribute to tumor growth. A Western pattern diet has been associated with hyperinsulinemia and hypertriglyceridemia. Among stage III colon cancer patients participating in CALGB 89803, Meyerhardt et al. found that increasing consumption of a Western pattern diet was associated with a significant increase in cancer recurrence and mortality. Brand-Miller et al. have therefore developed a novel insulin index for foods, which represents the incremental area under the insulin curve after feeding 1000 kjoules of a test food, divided by the insulin response to 1000 kjoules of white bread. A database for the insulin index of foods with the Willet dietary questionnaire that will allow the calculation of a dietary insulin index for each patient has been developed and validated. The insulin index will be used to assess the relation with patient outcome in the clinical trial.

Several studies suggest that insulin and IGF-1 act synergistically with activation of the K-ras/MAP-kinase pathway.[270-272] Inactivation of ras blocks insulin- and IGF-1-induced cell proliferation, and K-ras cannot transform mouse fibroblasts that are devoid of the IGF-1 receptor. It is hypothesized that the relation between dietary insulin index, physical activity and patient survival may be stronger among patients with K-ras-wildtype tumors. Similarly, the growth-promoting and anti-apoptotic effect of sedentary lifestyle, and insulin appears to be mediated principally through activation of phosphatidylinositol 3-kinase (PI 3-kinase), which in turn activates Akt/protein kinase B (PKB) via phosphorylation. Phosphorylation of Akt (phospho-Akt) results in cell proliferation and escape from apoptosis. Approximately 20% of patients have mutations in PI3K, and it was recently found that such mutations confer a reduced survival in a large population of stage I-III colon cancer patients.[273] Moreover, 45% of colorectal cancers overexpress phospho-Akt. Finally, fatty acid synthase (FASN) is the major enzyme required for the anabolic conversion of dietary carbohydrate to fatty acids. FASN is overexpressed in ~25% of colon cancers and was associated with a significantly improved survival among colon cancer patients. Moreover, the effect of FASN was significantly modified by obesity.[274] In light of these data, we will assess whether expression of phospho-Akt and FASN or mutations in PI3k and KRAS modify the influence of dietary insulin index and exercise on colon cancer recurrence and mortality.

3.2 Elevated baseline plasma C-peptide, IGF-1 and leptin and reduced plasma IGFBP-3 and adiponectin are associated with reduced disease-free survival among patients with stage III colon cancer.

Among stage I-III colon cancer patients, Wolpin et al. recently found that higher prediagnosis plasma C-peptide (a long term measure of insulin secretion) and reduced plasma IGFBP-1 (inversely associated with insulin secretion) conferred a significant increase in cancer-specific and all-cause mortality, independent of plasma levels of IGF-1 and IGFBP-3 (an antagonist to IGF-1).[275] Ongoing trials are assessing antibodies to the IGF-1 receptor (IGF1R) in patients with metastatic colorectal cancer. Further, plasma adiponectin has been associated with the risk of developing colorectal cancer and is a marker of insulin sensitivity.[276] Therefore, the influence of plasma levels of these factors and cancer recurrence and mortality among patients with stage III colon cancer will be examined. In exploratory analyses, it will also be assessed whether the effect of the plasma factors are modified by tumoral mutations in KRAS, PI3K, and expression of pAkt and FASN.

3.3 Higher intake of a Western dietary pattern, as manifested by higher red meat and total fat intake and lower n-3 polyunsaturated fatty acids, fruit and vegetable intake, is associated with increased cancer recurrence and mortality.

Western-style diets have been hypothesized as contributing to the development of colon cancer.[277] A factor analysis was conducted and two major dietary patterns: "prudent" and "Western" were identified. The prudent pattern was characterized by higher intakes of fruits, vegetables, legumes, fish, poultry, and whole grains, while the Western pattern, by higher intakes of red and processed meats, sweets and desserts, french fries, and refined grains. Increasing consumption of a Western diet was associated with an increased risk of colon cancer whereas increasing consumption of a prudent diet was associated with a reduced risk.[83,278] In mouse models, a Western-style diet accelerates colon cancer progression and mortality.[279-281] Dietary factors have been associated with the risk of developing colon cancer but the influence of diet on patients with established disease is unknown. The association of dietary patterns with cancer recurrences and mortality was examined in 1009 patients with stage III colon cancer who were enrolled in a randomized adjuvant chemotherapy trial (CALGB 89803). A higher intake of a Western dietary pattern after cancer diagnosis was associated with a significantly worse disease-free survival (colon cancer recurrences or death). Compared with patients in the lowest quintile of Western dietary pattern, those in the highest quintile experienced an adjusted hazard ratio (AHR) for disease-free survival of 3.25 (95% confidence interval [CI], 2.04-5.19; P for trend <.001). The Western dietary pattern was associated with a similar detriment in recurrence-free survival (AHR, 2.85; 95% CI, 1.75-4.63) and overall survival (AHR, 2.32; 95% CI, 1.36-3.96]), comparing highest to lowest quintiles (both with P for trend <.001). The reduction in disease-free survival with a Western dietary pattern was not significantly modified by sex, age, nodal stage, body mass index, physical activity level, baseline performance status, or treatment group.[282]

The influence of a Western pattern diet on the outcome of stage III colon cancer patients will be examined in the clinical trial. Moreover, the influence of pre- and post-diagnosis intakes of red meat, n-3 polyunsaturated fatty acids, and fruits and vegetables on survival will be examined.

3.4 In exploratory analyses, the effect of diet on chemotherapy-induced toxicity will be examined.

The influence of Western and prudent pattern diets, physical activity, and dietary insulin index on chemotherapy-based toxicity will be assessed and, in additional secondary analyses, other dietary determinants for toxicity will be explored.

4.0 METHODS

4.1 Assessment of diet and lifestyle factors

In this companion study, patients participating in the treatment trial will be asked to complete a 131-item validated, food-frequency questionnaire within first 6 weeks of randomization and 16-18 months after randomization. The questionnaire proposed, designed by Dr. Walter Willett and colleagues for the Nurses' Health Study, has been extensively validated among both health professional and lay populations, and provides comprehensive data on over 100 micro-nutrients, with and without supplement use. This questionnaire can be self-administered. Within the questionnaire, a series of questions about leisure-time physical activity, smoking habits, alcohol intake, and other habits that have also been validated in large populations will be included. Height and weight will also be obtained as part of the adjuvant therapy. A similar study was initiated in the preceding CALGB adjuvant therapy trial (CALGB 89803) and more than 90% of eligible patients completed the questionnaire.

Validation of the Semi-quantitative Food Frequency Questionnaire: The current version of the questionnaire consists of 131 food items plus vitamin and mineral supplement use that collectively account for over 90% of the intake of the nutrients assessed.[283-287] For each food, a commonly used unit or portion size (e.g., one egg or slice of bread) is specified, and participants are asked how often, on average over the past year, they consumed that amount of each food. There are nine possible responses, which range from never to six or more times per day. The nutrient intakes will be computed by multiplying the frequency of consumption of each food by the nutrient content of the specified portions, using composition values from Department of Agriculture sources supplemented with other data, including the components of specific vitamins and breakfast cereals. All nutrients will be adjusted for total energy intake by the residuals method.[288]

In 1980, the food frequency questionnaire was administered twice to 173 individuals at an interval of approximately one year, and four one-week diet records for each subject were collected during that period. Diet records probably are the best measures of current, short-term food intake. Since the seven-day record provides information for a relatively short period of time, four one-week diet records in different seasons were collected. The mean calorie adjusted intakes from the four one-week diet records and those from the questionnaire were well-correlated.[285-287] In the 1986 diet validation study, the correlation between folate calculated from the semi-quantitative food frequency questionnaire (SFFQ) and red cell folate level was 0.55.[250] Nutrients calculated from the expanded SFFQ were correlated with other corresponding biochemical indicators: plasma beta-carotene (r = 0.30-0.42),[289,290] plasma vitamin E (r = 0.41-0.53),[289,290] adipose linoleic acid (r = 0.35-0.37),[291,292] adipose trans fatty acid (r = 0.51),[291,292] and adipose N-3 fatty acids (r = 0.48-0.49).[291,292] To evaluate further the capability of the revised 131-item questionnaire to discriminate among subjects, Willett and colleagues asked 127 individuals to complete two weeks of diet records and the semi-quantitative food frequency questionnaire in 1986. The mean calorie adjusted intakes from the diet records and those from the questionnaire were well-correlated.[285]

The validity of this 131-item SFFQ will be separately assessed in 200 patients with colorectal, breast, or neuroendocrine cancer undergoing treatment with cytotoxic chemotherapy.[293] The Pearson correlation coefficients for various carotenoids as measured by the questionnaire, with the corresponding measurements in plasma specimens, ranged from 0.33 to 0.44 (all P < .001), adjusted for total energy intake, body mass index, age, sex, smoking status, and total plasma cholesterol. Similarly, the adjusted correlation between self-reported total vitamin E intake and plasma alpha-tocopherol was 0.34 (P < .001). Correlations between questionnaire and plasma measurements of trans-fat, eicosapentaenoic acid, and docosahexaenoic acid were 0.55, 0.29, and 0.42 (all P < .001), respectively. These levels of correlation were

consistent with those reported in similar studies of self-reported diet in otherwise healthy populations. Thus, among patients with cancer receiving cytotoxic chemotherapy, questionnaire-based measurements of various micronutrients and dietary factors appeared to predict meaningful differences in the corresponding measurements in plasma specimens.

These data indicate that the proposed self-administered dietary questionnaires provides highly informative and biologically relevant measurement of a wide variety of nutrients, thus allowing one to address the dietary hypotheses outlined in the specific aims.

In terms of other measures from the survey, Wolf et al. reported on a detailed validation study of the physical activity questionnaire among a sample of 325 participants in the parallel Nurses' Health Study II (NHS II) (241 random cohort sample and 84 random sample of African American participants).[294] Participants completed four 1-week activity recalls and four 7-day activity diaries over one year and then repeated the NHS II activity questionnaire. For the total activity score, the correlations of the last activity questionnaire with the diaries was 0.64 for the total cohort sample and 0.59 for the African American sample. Within the Health Professionals Follow-up Study, a parallel study of men, validity of the physical activity questionnaire was assessed among 238 randomly selected participants by comparisons with four 1-week activity diaries, four 1-week activity recalls, and resting and post exercise pulse rates.[295] Correlations with the activity diaries were 0.41 for inactivity (sitting) and 0.58 for vigorous physical activity. Vigorous activity assessed by the questionnaire was correlated with resting pulse (r = -0.45) and post-exercise pulse (r = -0.41).

4.2 Analysis of the Growth Factor Blood Markers

Serum will be collected in a red top tube prior to start of any chemotherapy. The blood is allowed to clot for 30 minutes at room temperature. The clotted blood is centrifuged for 10-15 minutes at 1,300 g and serum is aliquoted into two 2 mL cryovials, frozen and stored. Assays for C-peptide, IGFBP-1, IGF-1, and IGFBP-3 will be performed in the laboratory of Dr. Michael N. Pollak (Lady Davis Research Institute of the Jewish General Hospital, McGill University). Plasma levels of C-peptide were assayed by RIA (Linco Research), an assay with little or no cross-reactivity with proinsulin. IGFBP-1, IGF-1, and IGFBP-3 ELISAs will be assayed using reagents from Diagnostic Systems Laboratory (Webster, TX). The mean intra-assay CVs for C-peptide, IGFBP-1, IGF-1, and IGFBP-3 were each <10%.[275] Plasma adiponectin will be measured in the laboratory of Dr. Nader Rifai (Children's Hospital of Boston) by competitive RIA using a commercial reagent set (Linco Research), utilizing a highly purified antibody (intra-assay CVs 2-6%). All assays will be carried out by laboratory personnel blinded to patient outcome. In addition, masked quality control duplicate samples will be interspersed among the case samples.

4.3 Tumor based analyses

pAkt and FASN immunohistochemistry and KRAS and PI3K mutational status analyses will be performed in the laboratory of Dr. Shuji Ogino.

Tissue Block Procurement: Formalin-fixed/paraffin-embedded (FFPE) tissue blocks and slides will be examined. A set of blocks will be selected for preparation of routine hematoxylin and eosin (H&E) slides for microdissection and DNA extraction for analysis of KRAS and PI3K mutations. CALGB pathologists will also construct tissue microarrays (TMAs) for immunohistochemical (IHC) evaluation of pAkt and FASN.

Tissue DNA Extraction: H&E stained slides will be reviewed, areas comprising ≥70% neoplastic cellularity will be marked and scraped from 15μm unstained slides under direct visualization, and DNA will be extracted. DNA of sufficient quality for polymerase chain reaction (PCR) will be obtained from >95% of colorectal cancers.

Sequencing to detect KRAS and PIK3CA mutations: Utilizing pyrosequencing technology, KRAS mutations at codons 12 and 13,[141] and PIK3CA mutations in exons 9 and 20 will be assessed as previously described.[296] Pyrosequencing is highly sensitive to detect a small amount of KRAS mutant alleles in paraffin-embedded tumor tissue.[141]

Using this methodology, KRAS mutational status was previously assessed in 508 patients participating in an NCI-sponsored trial of adjuvant chemotherapy for stage III colon cancer (CALGB 89803). KRAS mutations were detected in 178 tumors (35%) by pyrosequencing. When compared to patients with wild-type KRAS, those with a mutation in KRAS did not experience any difference in disease-free (DFS), recurrence-free (RFS), or overall survival (OS) (log-rank P>0.56 for DFS, RFS, and OS) (manuscript under review).

In addition, among 450 resectable colon cancers (stage I to III) in two independent prospective cohorts, the PIK3CA mutation was detected in 82 tumors (18%) by pyrosequencing. Compared with patients with PIK3CA wild-type tumors, those with PIK3CA-mutated tumors experienced an increase in colon cancer-specific mortality according to univariate analysis (HR = 1.64; 95% CI, 0.95 to 2.86), which persisted after adjusting for other known or potential risk factors for cancer recurrence (including MSI; multivariate HR = 2.23; 95% CI, 1.21 to 4.11). The effect of PIK3CA mutation on cancer survival seemed to differ according to KRAS mutational status. Among patients with KRAS wild-type tumors, the presence of PIK3CA mutation was associated with a significant increase in colon cancer-specific mortality (HR = 3.80; 95% CI, 1.56 to 9.27). In contrast, PIK3CA mutation conferred no significant effect on mortality among patients with KRAS-mutated tumors (HR = 1.25; 95% CI, 0.52 to 2.96).[270]

IHC Staining and Interpretation: IHC analyses will be performed in the laboratory of Dr. Ogino. For FASN immunohistochemistry, primary antibody against FASN (BD Biosciences, Mississauga, ON, Canada) (dilution 1:100) will be applied for 60 min at room temperature. Then, Multilink secondary antibody (BioGenex) (20 min) and then streptavidin horseradish peroxidase (BioGenex) will be applied (20 min). Sections were visualized by diaminobenzidine (DAB) (5 min) and methyl-green counterstain. FASN expression will be interpreted as negative, weak (1+), positive (2+), and strongly positive (3+), using normal colonic epithelial cells and adipose tissue as reference. Appropriate positive and negative controls are included in each run of immunohistochemistry. All immunohistochemically stained slides will be interpreted by a pathologist blinded to other data. Random samples of 246 colorectal cancer have previously been examined for FASN by a second observer unaware of other data, and the concordance between the two observers was 0.93 for FASN (= 0.57, P < .0001). Using a database of 647 patients with colon cancer, FASN overexpression was detected in 84 tumors (13%) by immunohistochemistry.[274] FASN overexpression was associated with a significant reduction in colon cancer-specific mortality (adjusted HR, 0.41; 95% CI, 0.19 to 0.89). Notably, the effect of FASN expression on mortality differed according to body mass index (BMI; P(interaction) = .019); the adjusted HR of overall mortality for FASN overexpression was 0.63 (95% CI, 0.39 to 1.02) among patients with BMI less than 27.5 kg/m^2 and 2.91 (95% CI, 1.19 to 7.12) among those with BMI ≥27.5 kg/m^2. Moreover, the adverse effect of moderate overweight/obesity on overall survival was limited to FASN-positive tumors (adjusted HR, 4.10; 95% CI, 1.14 to 14.8; BMI ≥27.5 kg/m^2 v < 27.5 kg/m^2).

For phospho-AKT, monoclonal antibody will be applied to the tumor sections: phospho-Akt (Ser473: Cell Signaling Technology) dilution 1:50. A positive and negative control (tumors with known expression status of each of the selected proteins) will be included in each staining batch. Using the method of Itoh et al., phospho-Akt will be recorded as: 0, nearly no positive cells; 1+, 5-25% of tumor cells showing reactivity; 2+, 25-50% of cells showing reactivity; or 3+, >50% showing reactivity.[297] Tumors scoring 0-1 are considered as normal whereas those with 2-3

are considered as over-expression. In a prior analysis, 46% of colorectal cancers showed a high level (2+ or 3+) of phospho-Akt.[297,298]

Quality Control for Tumor Block Analyses: For analyses of KRAS and PIK3CA, pyrosequencing has been designed to confirm the presence of a mutation by artificial frameshifting in pyrograms (with extra fluorescence peaks), and/or by a second pyrosequencing primer. Approximately 5% repeated QC samples will be added as blinded specimens; they will be randomly nested in the sample sets with coded IDs. In all IHC analyses, appropriate positive and negative controls will be included in each run of IHC assay. In addition, a random sample of >100 cases will be re-examined by a second pathologist in Dr. Ogino's laboratory to assess inter-rater agreement using a kappa measure of agreement (κ) proposed by Kraemer. If agreement is unacceptable, further training and monitor agreement will be instated in the next set of 200 samples.

4.4 Data Analyses

The primary efficacy variable for analyses will be disease-free survival (DFS). Secondary efficacy endpoints include recurrence-free survival (RFS) and overall survival.

Exposure definitions: For all dietary exposures (**Objective 1.1**) including dietary insulin index, intakes will be categorized into energy-adjusted quintiles, consistent with our previous studies. In addition, physical activity will be categorized into categories of MET-hours as previously defined in prior work. Body mass index (kg/m^2; Aim 2) will be divided into World Health Organization categories of underweight, normal weight, overweight and obesity.

For the main effect of plasma analytes (**Objective 1.2**; C-peptide, IGFBP-1, IGF-1, IGFBP-3, and adiponectin), plasma levels will be divided into quintiles for the analysis. Baseline characteristics of patients will be compared according to quintiles of the biomarker using Wilcoxon signed rank tests for continuous variables and chi-squared tests for categorical variables. For primary analyses of survival, patients who recurred or died within three months of plasma collection will be excluded to minimize any bias in the due to occult cancer recurrence or preclinical illness. In sensitivity analyses, the possibility of reverse causation will also be assessed by allowing different lag times between plasma assessment and cancer recurrence or death. The log-rank test and Kaplan-Meier curves will be used to compare DFS and OS by quintile of plasma level. Cox proportional hazards models will be used to control for multiple confounders. The two-tailed P value for the linear trend test across categories will be calculated using the plasma level as a continuous variable, consistent with prior studies. In secondary analyses, we will examine how the relationship between a specific plasma analyte level and patient outcome is modified by relevant covariates such as ECOG performance status, treatment assignment, physical activity and body mass index, among others. Tests for statistical interaction will be performed by entering into the model the cross-product term of the plasma level as a continuous variable with the covariate as a continuous or binary variable.

It is hypothesized that specific tumoral alterations modify the effect of measures of energy balance (e.g., dietary insulin index, obesity, physical activity, plasma C-peptide) on patient survival. For the analysis of tumoral alterations (**Objective 1.3**), all patients who had a tumor block available for analysis and data on the relevant exposure or plasma analyte will be included. All tumors will be categorized as having FASN and pAkt overexpression versus no overexpression, and KRAS and PIK3CA mutated versus wild type. Within each of binary category of FASN expression and pAkt expression, and KRAS and PIK3CA mutational status, we will examine the influence of an exposure or plasma analyte divided into tertiles on DFS and OS. Tertiles will be utilized instead of quintiles of to maximize the ability to detect an association within each subgroup. Cox proportional hazards models will be used to calculate HRs and 95% CIs for DFS and OS, adjusted for other prognostic factors. Tests for statistical interaction will be performed by entering into the model the cross-

product term of the relevant exposure or plasma analyte as a continuous variable with the molecular alteration as a binary variable (overexpressed versus not; mutated versus wild type).

In exploratory analyses (**Objective 1.4**), the influence of diet and other factors on toxicities associated with adjuvant therapy will be assessed. Data from the questionnaire obtained during adjuvant therapy will be used for these analyses. Using logistic regression models, odds ratios and 95% CIs for the specific toxicity will be the measure of association with an exposure, adjusted for multiple potential confounders simultaneously.

Update #01
11/1/10

CANCER AND LEUKEMIA GROUP B

PROTOCOL UPDATE TO CALGB/SWOG C80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (CALGB IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

X Revision	**X Amendment**	**Status Change**
X Change of participants		Activation
X Editorial, administrative changes		Closure
X Scientific changes **(IRB approval)**		Suspension
X Therapy changes **(IRB approval)**		Reactivation
X Eligibility changes **(IRB approval)**		
X Informed Consent changes **(IRB approval)**		
Other:		

This update must be approved by your Institutional Review Board within 90 days.
Full board review of this update is recommended. Please follow your local IRB guidelines.

AMENDMENTS/REVISIONS:

Cover page (pp. 1-3)
- Contact information for the protocol coordinator has been changed.
- The contact information for the Protocol Coordinator, Central Office, CALGB Pathology Coordinating Office, Statistical Center, etc. and the CTSU registration instructions have been separated on pages 2 and 3.
- Contact information for the endorsing groups (SWOG, NCIC CTG, and NCCTG) is now listed on page 3.
- Endorsement Plus procedures have been provided on the bottom of page 3.

Schema (p. 4)
Under patient eligibility, "or NIC disease as defined in AJCC version 7", has been added to "At least pathologically confirmed positive lymph node".

Table of Contents (p. 5)
- The Table of Contents has been updated due to repagination.
- Appendix V has been modified to "Cancer Prevention (Diet and Lifestyle) Studies".
- A sixth appendix has been added as "Cancer Prevention (Colonoscopy) Studies".

Section 4.0 *Eligibility Criteria* (p. 12)
The following statement has been added to eligibility criterion 4.1.2: "Treatment must begin no less than 21 days and no more than 56 days after definitive surgical resection of the primary tumor." Eligibility criterion 4.1.3 has been updated with AJCC version 7.0. The parenthetical statement now reads: "or the AJCC version 7 designation of N1C defined as tumor deposit(s) in the subserosa, mesentery, or nonperitonealized pericolic or perirectal tissues without regional lymph node metastases."

Section 5.3 *Registration to companion studies* (p. 15)
A third bullet point has been added after the first paragraph which states, "CALGB 71002: Cancer prevention companion studies for CALGB/SWOG C80702 (Appendices V and VI)". The fourth paragraph has been modified to read: "If a patient answers 'yes' to 'I choose to take part in the cancer prevention studies and agree to complete the diet and lifestyle questionnaire', question #1 in the model consent, they have consented to participate in the cancer prevention studies described in Appendices V and VI. Questionnaires and reports should be submitted per Section 5.7."

Section 5.5 *Data submission* (p. 16)
The Data Submission Schedule has been changed to include additional rows titled "Other": "Colonoscopy reports" and "Pathology reports." The submission schedule for these reports states: "For patients enrolled to CALGB 71002. See Section 5.7."

Section 5.6 *Specimen submission for correlative and pharmacogenomic substudies* (p. 17)
Specimen tracking instructions have been added to this section.

Section 5.7 *Data submission for cancer prevention companion studies (CALGB 71002)* (p. 20)
- The section title has been updated.
- The first paragraph has been modified to include: "…cancer prevention studies (Appendices V and VI) by consenting to question #1. These studies will require patients to complete a Diet and Lifestyle survey and agree to the submission of their colonoscopy and pathology reports as described below. Diet and Lifestyle Survey:…"
- A third and fourth paragraph entitled "Colonoscopy and pathology reports" has been added.

Section 6.0 *Required Data* (p. 21)
- Under Pre-study testing intervals, the scans that require completion 42 DAYS before registration has been updated to: "CT or MRI abdomen/pelvis or PET-CT scan without evidence of metastatic disease."
- The second row under "Staging" in the table has been updated to read: "Abdominal/pelvic Imaging: (CT, MRI, or PET-CT scan)."
- The table has been modified to include the submission of colonoscopy and pathology reports under "Companion Studies."

Section 7.0 *Treatment Plan* (p. 22)
The last sentence of the third paragraph now reads: "Celecoxib/placebo will start by Day 15 of the first cycle of FOLFOX."

Section 7.1 *FOLFOX, every 2 weeks* (p. 22)
The parenthetical statement in the instructions for leucovorin has been changed to "via separate infusion containers."

Section 7.2 *Celecoxib or Placebo* (p. 22)
The second sentence now reads: "The first dose of celecoxib/placebo will be given by Day 15…" A new fourth, fifth, and sixth sentences have been added, which read: "Doses should only be made up if missed within 12 hours of the regularly scheduled dose. Vomited doses should only be made up if the entire capsule can be seen in vomit. Missed doses should be taken with food."

Section 15.0 *References* (pp. 60-61)
New references have been added as 311 – 322.

Section 16.0 *Model Consent Form, During the Study…* (pp. 64-65)
The Celecoxib/Placebo section has been modified to include new fifth, sixth, and seventh sentences which read: "Doses should only be made up if missed within 12 hours of the regularly scheduled dose. Vomited doses should only be made up if the entire capsule can be seen in your vomit. Missed doses should be taken with food."

Section 16.0 *Model Consent Form, What side effects or risks can I expect from being in the study?* (pp. 66-69)
The FOLFOX and celecoxib risks have been consolidated and are now listed separately:

FOLFOX, Likely:
- The risks "Nausea or the urge to vomit" and "Vomiting" have been combined in the risk "Nausea or vomiting".
- The risks "Increased blood level of a liver enzyme (ALT/SGPT)" and "Increased blood level of a liver enzyme (AST/SGOT)" have been replaced with "Abnormal liver function as seen on a blood test: ALT, AST".
- The words "Inflammation or damage of the peripheral" and "(those nerves outside of brain and spinal cord)" have been removed from the phrase which now reads: "Damage to nerves causing numbness, tingling, burning".
- The risk "Decrease in the total number of white blood cells (leukocytes); may make you more vulnerable which could be serious or even life threatening" has been removed.
- The phrase "that can lead to infection" has been added to the risks "Decreased number of a type of white blood cell (lymphocyte)" and "Decreased number of another type of white blood cell (neutrophil/granulocyte)."
- The risk "Soreness or painful ulcers in the mouth and/or throat (lasting a couple of days)" now reads: "Sores in the mouth and/or throat".
- The phrase "or sensation of lightheadedness, unsteadiness, giddiness, spinning or rocking" has been removed from "Dizziness".
- The phrase "or head pain" has been removed from "Headache".

FOLFOX, Less Likely
- The following risks have been removed: "Abnormally fast irregular heartbeat involving the upper chambers of the heart (atria)", "Abnormally fast regular heartbeat involving the upper chambers of the heart (atria)", "Period of very rapid and regular heartbeats that begins and ends suddenly", "Slow heartbeat; regular rhythm", "Fast heartbeat; regular rhythm", "Fast heartbeat usually originating in an area located above the ventricles", "Irregular heartbeat resulting from an abnormality in one of the lower chambers of the heart (ventricle), "Irregular heartbeat that involves the lower chambers of the heart (ventricles) that results in uncoordinated muscle movement of the ventricles making them tremble rather than contract properly; life-threatening, needs immediate attention", "Rapid heartbeat of one of the lower chambers (ventricle) of the heart; regular rhythm but potentially life-threatening, needs immediate attention". These risks have been replaced with the risk "Abnormal heart rhythm".
- The words "swelling and redness" and "middle" have been removed from the phrase "Inflammation in the ear".

- The risk “Inflammation (swelling and redness) of the conjunctiva (the outermost layer of the eye and the inner surface of the eyelids). Commonly called ‘pink eye’” has been changed to “Swelling and redness of the eye and eyelids.”
- The following risks have been removed: “A situation in which one has temporary blindness of one eye, due to a blockage (or decreased blood flow) in the blood vessels leading to that eye”, “Inflammation of the small and/or large bowel”, and “Blockage of the small bowel”.
- The risk “Inflammation of the esophagus (gullet or tube that goes from the mouth to the stomach through which food passes” has been replaced with “Sores in the GI tract, including esophagus or intestines”.
- The risk “Inflammation of the stomach lining” has been changed to “Irritation of the stomach”.
- The risk “Partial or complete blockage of the small and/or large intestine. With ileus, the bowel acts like it is blocked” has been changed to “Blockage of the intestines with severe constipation.”
- The phrase “that can cause belly pain and may be serious” has been added to the risk “Inflammation of the pancreas.”
- The risks “Swelling of the face” and “Swelling of the extremeties (arms and/or legs)” has been combined to read: “Swelling of the face, arms, or legs.”
- The word “limp or” has been removed from the risk “Difficulty walking”.
- The risk “Inflammation (swelling and redness) or damage to the tissue surrounding where a drug was injected” has been changed to “Irritation at the site of the IV”.
- The risk “A condition in which there is blockage…” has been replaced with “Blockage of the veins in the liver leading to liver damage”.
- The phrase “abnormal reaction of the body to substances, called allergens, that are contacted through the skin, inhaled in the lungs, swallowed, or injected” has been removed from the risk “allergic reaction”.
- The following risks have been removed: “Test that shows a problem in blood clotting”, “Increased blood level of a liver or bone enzyme (alkaline phosphatase)”, “Increased blood level of a liver pigment (bilirubin) often a sign of liver problems”, “Increased blood level of creatinine (a substance normally eliminated by the kidneys into the urine)”, “Increased blood level of a liver enzyme (GGT)”, and “Increased INR (measure of the ability of the blood to clot properly) which increases the risk of bleeding”. These risks have been replaced with the following: “Slow blood clotting as seen on a blood test: PTT, INR”, “Abnormal kidney function as seen on a blood test: creatinine”, “Abnormal liver function as seen on a blood test: alkaline phosphatase, bilirubin, GGT”, and “Abnormal test of bone health: alkaline phosphatase”.
- The risk “More acid than normal in the blood” has been removed.
- The phrase “when your body does not have as much water and fluid as it should” has been removed from “Dehydration”.
- The word “decreased” has been added to “Increased or decreased blood sugar level”. The risk “Decreased blood sugar level” has been removed.
- The word “blood” has been removed from “decreased levels of a protein called albumin”.
- The following risks have been removed: “Increased blood level of uric acid, a waste material from food digestion”, “Decreased blood level of calcium”, “ “Decreased blood level of potassium”, “Decreased blood level of magnesium”, “Decreased blood level of sodium”, and “Decreased blood level of phosphate”. These risks have been replaced with “Abnormal blood chemistries that could lead to abnormal heart, kidney, or nerve function: blood acid, uric acid, calcium, potassium, magnesium, sodium, phosphate”.
- The risks “Joint pain”, “Back pain”, “Bone pain”, and “Muscle pain” have been combined in the risk “Pain including joint, back, bone, and muscle”.
- The risk “Loss of muscle coordination; awkward, uncoordinated walking; unsteadiness when walking” has been removed.
- The risk “Restless, repetitive, or involuntary movements and rapid speech” has been changed to “Abnormal or involuntary movements.”
- The risk “Decreased blood flow to the brain which may lead to stroke” has been replaced with “Stroke or mini-stroke (TIA)”.
- The phrase “due to problems with the nerves that supply them” has been removed from the risk “Paralysis of facial muscles”.

- The risk "Weakness or paralysis (loss of muscle function)…" has been changed to "Weakness or paralysis caused by damage to nerves."
- The words "feelings of dread or danger" have been removed from the risk "Anxiety".
- The phrase "feelings of sadness, worthlessness, thoughts of suicide or death (depression)" has been replaced with "depression".
- The following risks have been removed: "Presence of blood in the fallopian tube (tube between ovary to uterus [womb]", "Bleeding in the ovary", "Bleeding in the prostate", "Bleeding in the spermatic cord (a structure resembling a cord that suspends the testis within the scrotum and contains the vas deferens [the tube that carries sperm] and other vessels and nerves", "Bleeding in the testis", "Bleeding in the uterus (womb)", and "Bleeding in the vagina". These risks have been replaced with "Bleeding in male or female organs".
- The word "wheezing" has been added to the risk "Cough".
- The phrase "with the presence of macules (flat discolored area) and papules (raised bump)" has been removed from the risk "Skin rash".
- The phrase "hypertension" has been removed from the risk "High blood pressure".
- The phrase "blood clot" has been removed from the risk "Inflammation of a vein".
- The risk "Formation of a blood clot" has been changed to "Formation of a blood clot that could break loose and be carried by the blood stream to block another blood vessel."
- The phrase "that can be serious" has been added to the risk "Swelling and redness of the skin on the palms of the hands and soles of the feet".

FOLFOX, Rare but Serious
The words "fluid in the lungs" have been replaced with "difficulty breathing" in the risk "Severe potentially life-threatening damage to the lungs which can lead to…"

Risks for celecoxib have been listed separately. The risks in the "**Less Likely**" category include "Headache", "Heartburn", "Diarrhea", "Belly Pain", and "Bleeding in some organ(s) of the digestive tract, for example, blood in your stool." The risks in the "**Rare but Serious**" category include "Sudden or traumatic injury to the kidney", "Heart attack", and "Stroke". There are no "**Likely**" risks associated with celecoxib.

Section 16.0 *Model Consent Form, Cancer Prevention and Diet and Lifestyle Study* (75)
This section, including consent question #1, has been updated with information regarding the new cancer prevention companion study.

Appendix I *Cancer Trials Support Unit (CTSU) Participation Procedures, CTSU Procedures for Patient Enrollment (p. 82)*
The sentence after item number 4 has been modified to state: "Protocol treatment should begin within 14 days of registration".

Appendix V *Cancer Prevention Companion Studies (Diet and Lifestyle): CALGB 71002 (p. 118-124)*
The appendix has been renamed.

Appendix VI *Cancer Prevention Companion Studies (Colonoscopy): CALGB 71002 (p. 125-128)*
New cancer prevention companion studies have been added as Appendix VI.

Due to extensive repagination, a replacement protocol has been issued.

ATTACH TO THE FRONT OF EVERY COPY OF THIS PROTOCOL

Update #02
07/15/11

CANCER AND LEUKEMIA GROUP B

PROTOCOL UPDATE TO CALGB/SWOG C80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (CALGB IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

X Revision	**X Amendment**	**Status Change**
X Change of participants		Activation
X Editorial, administrative changes		Closure
X Scientific changes **(IRB approval)**		Suspension
X Therapy changes **(IRB approval)**		Reactivation
X Eligibility changes **(IRB approval)**		
X Informed Consent changes **(IRB approval)**		
Other:		

This update must be approved by your Institutional Review Board within 90 days.
Full board review of this update is recommended. Please follow your local IRB guidelines.

AMENDMENTS/REVISIONS:

Cover page (pp. 1, 3)
- Xing (Cynthia) Ye hasreplaced Donna Hollis as the staff statistician for this study.
- NSABP has been added as an Endorsing Plus Group.

Schema (p. 4)
- The following changes have been made to the patient eligibility criteria on this page:
  - The third entry has been revised to, "At least one pathologically confirmed positive lymph node or N1c disease as defined in AJCC version 7 (see §4.1.3)".
  - The sixth entry has been revised to, "Patients are ineligible if they use NSAIDS at any dose more than 2 times a week on average or aspirin at more than 325 mg at least three times per week on average. Low-dose aspirin not exceeding 100 mg/day is permitted (see §4.2)".
  - The 11TH entry has been revised. It now reads, "No history of upper gastrointestinal ulceration, upper gastrointestinal bleeding, or upper gastrointestinal perforation within the past 3 years (see §4.7)".
- The diagram (schema) has been updated. The "Arms" now read "Arm A", "Arm B", "Arm C" and "Arm D" from left to right and top to bottom; instead of, "Arm A", "Arm B", "Arm A" and "Arm B" from left to right and top to bottom.

Table of Contents (p. 5)
- The Table of Contents has been updated due to repagination.

Section 4.1 *Requirements for tumor parameters* (p. 12)
- Section 4.1.1 has been revised. It now reads, "Histologically documented adenocarcinoma of the colon. The gross inferior (caudad) margin of the primary tumor must lie above the peritoneal reflection (i.e., patients with rectal cancer are not eligible). Surgeon confirmation that the entire tumor was above the peritoneal reflection is only required in cases where it is important to establish if the tumor is a rectal or colon primary."
- Section 4.1.2 has been updated to now read, "Tumors must have been completely resected. In patients with tumor adherent to adjacent structures, en bloc R_o resection must be documented in the operative report. Near or positive radial margin are not exclusions as long as en bloc resection was performed. Positive proximal margin or distal margin is an exclusion."
- Section 4.1.3 has been revised to, "Node positive disease (N1 or N2) as designated in AJCC version 7. Either at least one pathologically confirmed positive lymph node or N1C (defined as tumor deposit(s) in the subserosa, mesentery, or nonperitonealized pericolic or perirectal tissues without regional lymph node metastases)."

Section 4.2 (p. 13)
- This section has been revised to, "Patients are ineligible if they plan on regular use of NSAIDS at any dose more than 2 times per week (on average) or aspirin at more than 325 mg at least three times per week, on average. Low-dose aspirin not exceeding 100 mg/day is permitted. Patients who agree to stop regular NSAIDs or higher dose aspirin are eligble and no wash out period is required."

Section 4.7 (p. 13)
- This section has been revised to, "No history of upper gastrointestinal ulceration, upper gastrointestinal bleeding, or upper gastrointestinal perforation within the past 3 years. Patients with ulceration, bleeding or perforation in the lower bowel are not excluded."

Section 5.2 *Patient registration/randomization* (p. 14)
- The IS Help Desk phone number in the second paragraph has been updated.

Section 5.4.2 *Stratification for the celecoxib randomization* (p. 15)
- A new sentence has been added at the end of this section. It reads, "Patients with N1C only disease (i.e., no positive nodes but N1C disease by AJCC 7 should be stratified to 1-3 nodes)."

Section 5.6 *Specimen submission for correlative and pharmacogenomic substudies* (p. 17)
- The table in this section has been modified. Under "Type of Specimen" & "Pre-treatment", the first row has been revised. It now reads "Tissue" under "Type of Specimen" and "2 paraffin blocks (1 tumor / 1 normal)" under "Pre-treatment".
- The first sentence in the fourth paragraph under the table has been slightly revised. This sentence now reads, "All submitted specimens must be labeled with the protocol number (CALGB/SWOG C80702), CALGB patient ID, patient's initials and date and type of specimen collected (e.g., FFPE tumor block, FFPE normal block, EDTA plasma, serum, whose (venous) blood)."

Section 5.6.1 *Submission of paraffin blocks of archived colorectal tissue (CALGB 150911)* (p. 18)
- The first paragraph has been revised and now reads, "For patients who consent to question #2, tissue blocks will be used for the correlative studies described in Appendix III. Paraffin blocks of tissue obtained from archival colorectal specimens from primary site should be sent to the CALGB Pathology Coordinating Office. Submit 1 block of tumor tissue and 1 block of normal tissue."

Section 5.7 *Data submission for cancer prevention companion studies (CALGB 71002)* (p. 20)

- The third sentence in the first paragraph below "Diet and Lifestyle Survey:" has been revised. It now reads, "Patients should also complete questionnaires 14 – 16 months from randomization."
- The fifth sentence in the first paragraph below "Diet and Lifestyle Survey:" has been revised. It now reads, "Questions regarding the survey should be directed to Devin Wigler, 617-632-3687 or 617-632-6855."
- The address below the "Diet and Lifestyle Survey:" has been updated.

Section 6.0 *Required Data* (p. 21)

- Below the "Pre-study Testing Intervals", the second bullet after "To be completed within 42 DAYS before registration:" has been revised. It now reads, "CT chest or chest X-ray (PET-CT including chest, is acceptable) without evidence of metastatic disease."
- The "Diet & Lifestyle/Other Meds/Comorbidities Questionnaire" row under the Companion studies column has been revised to: " To be completed w/in first 6 weeks of randomization and 14 – 16 months after randomization. See section 5.7."
- Footnote "D" under the table has been revised to: "Only for those patients receiving Coumadin or warfarin: PT/INR should be monitored weekly during FOLFOX. During celecoxib monotherapy, weekly PT/INR is required only for the first month, then as clinically indicated."

Section 7.0 *Treatment Plan* (p. 22)

- A new paragraph has been added to the end of this section. This paragraph now reads, "In the event of a leucovorin shortage, refer to the CALGB 80702 study page, which can be found on the CALGB and CTSU websites."

Section 7.1 *FOLFOX, every 2 weeks* (p. 22)

- The last sentence in third paragraph is this section has been revised to: "Celecoxib/placebo will start by Day 1 of cycle 2 of FOLFOX."

Section 7.2 *Celecoxib or Placebo* (p. 22)

- The second sentence in first paragraph is this section has been revised to: "The first dose of celecoxib/placebo will be given by Day 1 of the second cycle of FOLFOX (in clinic)."

Section 8.0 *Dose Modifications and Management of Toxicity* (p. 23)

- A new last bullet has been added to this section. It states, "If celecoxib/placebo held due to toxicity not deemed related to FOLFOX, continue FOLFOX therapy as scheduled."

Section 8.2 *Hematologic toxicities* (p. 23)

- The last sentence in the first paragraph has been revised to: "Growth factor support may be considered if neutropenia results in a delay of more than one week, at the discretion of the treating physician, prior to a delay of 2 consecutive cycles."

Section 8.3.2 *Oral Mucositis* (p. 24)

- This section has been renamed and modified for clarity.

Section 8.3.3 *Nausea or Vomitting* (p. 24)

- This section has been renamed and modified for clarity.

Section 8.6 *Neurotoxicity* (p. 25)

- The title of the table in this section has been revised to, "Toxicity Scale for the Sensory Neuropathies Associated with Oxaliplatin (using the Oxaliplatin Specific Neurotoxicity Scale)".

Section 8.8 *Allergic Reactions* (p. 25)

- The third bullet in this section has been modified in its entirety.

Section 9.7 *Leucovrin Calcium (Folinic Acid)* ... Availability (p. 30)

- A new last sentence has been added to this section. It now reads, "In the event of a leucovorin shortage, refer to the CALGB 80702 study page, which can be found on the CALGB and CTSU websites."

Section 13.1 *Sample size and power estimates* (p. 36)

- A new last sentence has been added to the second paragraph.

Section 14.2 *Additional Instructions or Exclusion to AdEERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent Under a CTEP IND or CALGB IND:* (p. 38)

- The last bullet in this section has been revised. It now reads, "New primary malignancies should be reported using study form C-1001. New malignancies, including secondary AML/MDS, should also be reported."

Section 16.0 Informed Consent, *During the Study... Celecoxib or Placebo* (p. 64)

- The first sentence of this section has been revised. It now reads, "Within the first 15 days of study treatment with FOLFOX you will start study treatment with celecoxib or the placebo."

Section 16.0 Informed Consent, *What side effects or risks can I expect from being in the study...FOLFOX...Less Likely* (pp. 67-68)

- "Sores in the GI tract, including esophagus or intestines" has been deleted.
- The following risk has been modified to now read, "Sore (ulcer) in the digestive tract, including esophagus or intestines."
- "Lose of appetite" has been removed from this section.
- "Paralysis of facial muscles" has been removed from this section.

Section 16.0 Informed Consent, *What side effects or risks can I expect from being in the study...Celecoxib...Less Likely* (p. 69)

- "Nasuea and vomiting", "hypertension", and "swelling in the arms and legs" have been added to this section.

Section 16.0 Informed Consent, *What side effects or risks can I expect from being in the study...Celecoxib...Rare But Serious* (p. 69)

- "Chest pain (angina)" and "blood clots" have been added to this section.

Section 16.0 Informed Consent, *What side effects or risks can I expect from being in the study...Celecoxib* (p.69)

- A sentence has been added at the end of the celecoxib section. This sentence reads, "Patients who continue to take the celecoxib/placebo after 1 year may be more likely to have a heart attack, stroke, or other cardiovascular problems."

Replacement Pages include: cover page (p. 1), 3-5, 12-18, 20-26, 30, 36-38, 64 and 67-69

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Update #03
03/15/12

CANCER AND LEUKEMIA GROUP B

PROTOCOL UPDATE TO CALGB/SWOG C80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (CALGB IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

X Revision	**Amendment**	**Status Change**
Change of participants		Activation
Editorial, administrative changes		Closure
Scientific changes **(IRB approval)**		Suspension
Therapy changes **(IRB approval)**		Reactivation
Eligibility changes **(IRB approval)**		
Informed Consent changes **(IRB approval)**		
X Other: Oxaliplatin CAEPR update		

IRB review and approval of this update is required within 90 days. Expedited IRB review and approval of this update is allowed. Please follow your local Institutional Review Board guidelines.

Section 14.2 Additional Instructions or Exclusion to AdEERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent Under a CTEP IND or CALGB IND, (p 38)
A sentence has been added to the 7th bullet in this section. The sentence reads: "NOTE: The ASAEL column of the oxaliplatin CAEPR has been replaced with the specific protocol exceptions to expedited reporting (SPEER) list. This list now includes 'expected' severity grades in addition to event terms."

Section 14.3 Comprehensive Adverse Events and Potential Risks List [CAEPR] for Oxaliplatin (NSC 266046), (pp 38-43):
An updated CAEPR of Oxaliplatin (Version 2.3, November 1, 2011) replaced the previous version.

Replacement Pages include: cover page (p. 1) and 38-43

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Update #04
06/15/12

CANCER AND LEUKEMIA GROUP B

PROTOCOL UPDATE TO CALGB/SWOG C80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (CALGB IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

X **Revision**	**_____ Amendment**	**_____ Status Change**
_____ Change of participants		_____ Activation
X Editorial, administrative changes		_____ Closure
_____ Scientific changes **(IRB approval)**		_____ Suspension
_____ Therapy changes **(IRB approval)**		_____ Reactivation
_____ Eligibility changes **(IRB approval)**		
_____ Informed Consent changes **(IRB approval)**		
X Other: Oxaliplatin CAEPR update		

IRB review and approval of this update is required within 90 days. Expedited IRB review and approval of this update is allowed. Please follow your local Institutional Review Board guidelines.

Cover Page (p. 1)
The Study Chair's address has been updated.

Section 4.1.2 (p. 12)
The second paragraph under this section has been removed.

Section 4.1.3 (p. 12)
A new sentence has been added to the end of this section. The sentence reads, "Patients with resected stage IV disease are not eligible."

Section 5.2, Patient registration/randomization (p. 15)
The last sentence of the second-to-last paragraph of this section has been revised. This sentence now reads, "Initial blinded, patient-specific clinical supplies of celecoxib/placebo will be shipped from the Pharmaceutical Management Branch (PMB) to the registering investigator at the time of patient randomization and should arrive within 7 to 10 days of randomization (see Section 9.8)."

Section 5.5, Data Submission (p. 16)

- In the submission table, the submission schedule for form C-1956 during Follow-up has been revised to read:

  "Every 3 months during year 1, then every 6 months during years 2-3, then every year during years 4-6 for a maximum of 6 years of follow-up from the date of registration.#"

- A sentence has been added (after the footnotes) at the bottom of the data submission section. It now reads, "This study will utilize the NCI Common Terminology Criteria for Adverse Events version 3.0 for routine reporting on study forms."

Section 6.0, Required Data (p. 21)

- At the top of the page, under Pre-Study Testing Intervals, the "To be completed…" section referring to CT scans has been changed to:

  "To be completed within 60 DAYS before registration:
  - CT or MRI abdomen/pelvis or PET-CT scan without evidence of metastatic disease
  - CT chest or chest X-ray (PET-CT including chest, is acceptable) without evidence of metastatic disease"

  from

  "To be completed within 42 DAYS before registration:
  - CT or MRI abdomen/pelvis or PET-CT scan without evidence of metastatic disease
  - CT chest or chest X-ray (PET-CT including chest, is acceptable) without evidence of metastatic disease".

- In the table, under **Tests & Observations**, "monthly" has been removed from "Celecoxib/Placebo Count".
- In the table, under **Tests & Observations**, a new footnote reference of "#" has been added next to "Celecoxib/Placebo Count#".
- Under the table, the second sentence of footnote "*" has been revised next to: "For subsequent cycles, labs and history & physical may be obtained within 72 hours prior to day of treatment."
- Under the table, the last sentence of footnote "E" has been revised next to: "Then, for all patients, every 6 months from last scan until at least 3 years after randomization, and then yearly for 3 years or until disease progression.
- Under the table, a new footnote "#" has been added. It reads, "Pill counts should include collection of monthly diaries as outlined above. Study staff should count remaining pills every 2 cycles during FOLFOX, then every 3 months while on celecoxib/placebo."

Section 7.0, Treatment Plan (p. 22)

The first paragraph of this section has been revised in its entirety.

Section 7.2, Celecoxib or Placebo (p. 22)

The follow new paragraph has been added to the end of this section:

"While celecoxib/placebo does not need to be held around surgical procedures (ie. port a cath removal or reversal of temporary colostomy), if celecoxib/placebo is held or delayed, treatment should not be held for greater than 28 days. Longer delays require notification of the CALGB or SWOG Study Chair. Repeated delays of > 21 days within 1 year also require notification of CALGB or SWOG Study Chair."

Section 8.0, Dose Modifications and Management of Toxicity (p. 23)

A new bullet has been added to the end of this section. It reads, "Missed doses of celecoxib/placebo (for any reason) are not made up."

Section 8.2, Hematologic Toxicities (p. 23)

The first paragraph of this section has been slightly modified. It now reads, "Dose modifications for hematologic toxicities are based on CBC on Day 1 of each cycle of FOLFOX (or within prior 72 hours). If FOLFOX is delayed for neutropenia or thrombocytopenia, continue celecoxib/placebo. If FOLFOX is delayed for neutropenia for 2 consecutive cycles, it is

recommended that G-CSF or GM-CSF or pegfilgrastim be administered after all subsequent cycles. Growth factor support may be considered if neutropenia results in a delay of more than one week, at the discretion of the treating physician, prior to a delay of 2 consecutive cycles."

Section 8.10, Other non-hematologic toxicities for FOLFOX (p. 26)
This section has been slightly modified to read, "For other grade 3 or 4 non-hematologic toxicities considered related to FOLFOX, delay FOLFOX until toxicity resolves to ≤ grade 1, then resume FOLFOX at one dose level reduction of oxaliplatin and 5-FU (if toxicity is hand-foot syndrome, dose reduction of only 5-FU bolus and continuous infusion of 5-FU is permitted). Continue celecoxib/placebo.

Section 8.11, Other non-hematologic toxicities for celecoxib/placebo (p. 26)
A few changes have been made to this entire section. Below are the four paragraphs of this section and the changes are underlined.

"For other grade 3 or 4 non-hematologic toxicities considered related to celecoxib/placebo, interrupt celecoxib/placebo for a maximum of 28 days until toxicity improves to ≤ grade 1, then resume celecoxib/placebo at the previous dose.

For recurrence of the same grade 3 or 4 non-hematologic toxicity considered related to celecoxib/placebo, or if toxicity does not improve after 28 days, discontinue celecoxib/placebo. Continue FOLFOX.

For persistent grade 2 non-hematologic toxicity considered related to celecoxib/placebo that the patient finds unacceptable, interrupt celecoxib/placebo for a maximum of 28 days until toxicity improves to ≤ grade 1, then resume celecoxib/placebo at the previous dose. Continue FOLFOX.

For recurrence of unacceptable grade 2 non-hematologic toxicity considered related to celecoxib/placebo, or if grade 2 toxicity does not improve after 28 days, discontinue celecoxib/placebo. Continue FOLFOX."

Section 10.4, Anticoagulants (p. 34)
This section has been revised to be consistent with the follow-up described in the Required Data Section (Section 6.0). It now reads, "Patients who are taking warfarin or coumadin may participate in this study. For patients receiving coumadin or warfarin, PT/INR should be monitored weekly during FOLFOX. During celecoxib monotherapy, weekly PT/INR is required only for the first month, then as clinically indicated. Subcutaneous or low molecular weight heparin is permitted."

Section 10.5, Non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin (p. 34-35)
This is a new section that has been added.

Section 16.0, Model Consent...Why is this study being done? (p. 62)
The second paragraph has been revised in its entirety.

Section 16.0, Model Consent...Tests and Procedures (p. 65)
The fourth bullet under this section has been slightly revised. It reads, "CT scans, MRI scans, ultrasound scans or chest x-rays to monitor your condition after the completion of the FOLFOX treatment (about every 6 months)."

Section 16.0, Model Consent...FOLFOX *Likely* **(p. 66)**
The following risk of, "Temporary hair thinning or loss" has been modified to be consistent with the language that was in the oxaliplatin CAEPR.

Section 16.0, Model Consent...FOLFOX *Less Likely* **(p. 67-68)**
- The risks,"Dry eye" and "Watery eye" have been combined to "Dry or watery eyes".
- "Problem with the eyelid" has been replaced by "Drooping eyelid". This is not a new risk. The language was modified to be consistent with the terms in the CAEPR.

- "Weight loss" and "Weight gain" have been combined. The risk now reads, "Weight gain or loss".
- "Skin rash" and "hives" have been combined. The risk now reads, "Skin rash or hives".
- "High blood pressure" and "low blood pressure" have been combined. The risk now reads, "High or low blood pressure".

Section 16.0, Model Consent...Celecoxib (p. 69)
The sentence, "Patients who continue to take the celecoxib/placebo after 1 year may be more likely to have a heart attack, stroke, or other cardiovascular problems", after the *Rare But Serious* risks has been removed.

Due to extensive repagination, a new protocol has been issued.

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Update #05
04/15/13

CANCER AND LEUKEMIA GROUP B

PROTOCOL UPDATE TO CALGB 80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational Agent: Sorafenib (IND# 69896, NSC# 724772) will be supplied by CTEP DCTD.

[X] **Update:**	[ ] **Status Change:**
[ ] Eligibility changes	[ ] Activation
[ ] Therapy/Dose Modifications/Study Calendar changes	[ ] Closure
[ ] Informed Consent changes	[ ] Suspension / temporary closure
[ ] Scientific / Statistical Considerations changes	[ ] Reactivation
[X] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other :	

IRB review and approval of this update is required within 90 days.
Expedited review is allowed. Please follow your local IRB guidelines.

Cover Page
Vance Erese has replaced Shivani Shah as the protocol coordinator for this study.

Table of Contents
The table of contents has been updated and reformatted to comply with the new CTEP PIO requirements for protocol submissions.

Section 4.0 Eligibility Criteria
The section headers of 4.1 through 4.6 have been revised with main and sub-headers to comply with the new CTEP PIO requirements for Table of Contents formatting.

Section 5.2 Patient registration/randomization
In the second to last paragraph in the section, the reference in the last line has been changed from "Section 9.8" to "Section 9.4." This was changed due to the reformatting of the protocol document.

Section 5.5 Data submission
- The second sentence in the opening paragraph has been deleted, as e-submission of all data forms is the mandatory for all sites. Supporting documentation and amended forms may be faxed or mailed. The three bullet points have been modified and combined into two bullet points to reflect these changes.
- The schedule for the submission of data forms during protocol treament section has been modified. Form C-1956 will be submitted on the same schedule as the form (C-1954), 80702 Treatment Form, and form C-1955, 80702 Adverse Event Form. The medication calendar (S-067) has also been added to this section, with a footnote (**) clarifying that it is for patient and institutional use only and does not need to be submitted to the Statistical Center.
- The submission schedule of C-1956, 80702 Follow-up Form, will now be submitted after the end of protocol treatment every 6 months for a maximum of 6 years from the date of registration. Footnote # has been removed to reflect these clarifications.

Sections 9.0 Drug Formulation, Availability, and Preparation
The headers for the previous sections 9.1, 9.2, and 9.3 have been removed. The information in these sections remains included at the beginning of Section 9.0.

Section 9.4 Celecoxib/placebo: Unblinding procedures
The emergency unblinding procedures have been updated to include the new pager number to be used to contact the Alliance Executive Officer.

Section 16.0 Model Informed Consent
- This section has been removed from the protocol document and is now a stand-alone document to comply with the new CTEP PIO requirements for submission of protocols.

UPDATES TO THE MODEL CONSENT:

No changes have been made.

A replacement protocol and model consent document have been issued.

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CANCER AND LEUKEMIA GROUP B

PROTOCOL UPDATE TO CALGB 80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (CALGB IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

[X] **Update:**	[ ] **Status Change:**
[ ] Eligibility changes	[ ] Activation
[ ] Therapy/Dose Modifications/Study Calendar changes	[ ] Closure
[ ] Informed Consent changes	[ ] Suspension / temporary closure
[ ] Scientific / Statistical Considerations changes	[ ] Reactivation
[X] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other :	

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Sections 9.0 Drug Formulation, Availability, and Preparation
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Section 9.4 Celecoxib/placebo: Unblinding procedures
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Section 16.0 Model Informed Consent
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UPDATES TO THE MODEL CONSENT:

No changes have been made.

A replacement protocol and model consent document have been issued.

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Update #06
02/15/14

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB 80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (Alliance IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

[X] **Update:**	[ ] **Status Change:**
[X] Eligibility changes	[ ] Activation
[X] Therapy/Dose Modifications/Study Calendar changes	[ ] Closure
[X] Informed Consent changes	[ ] Suspension / temporary closure
[ ] Scientific / Statistical Considerations changes	[ ] Reactivation
[X] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other :	

IRB review of this update is required within 90 days.
Expedited review is allowed. Please follow your local IRB guidelines.

UPDATES TO THE PROTOCOL:

- In keeping with new CTEP PIO requirements, the name of the lead group on the title page of the protocol has been updated from "Cancer and Leukemia Group B" to "Alliance for Clinical Trials in Oncology." All references to "CALGB" as an organization has been updated throughout the protocol document to "Alliance."
- The "CALGB Pathology Coordinating Office (PCO)" has undergone a name change to the "Alliance Biorepository at Ohio State University (OSU)." This has been revised throughout the protocol document.
- The "CALGB Statistical Center" is now titled "The Alliance Statistics and Data Center at Duke University." This change has been reflected throughout the protocol.

Cover Page

- Howard Hochster, M.D. has replaced Charles Blanke, MD as the SWOG GI Committee Chair. Contact information has been updated.
- The titles of "Faculty Statistician" and "Staff Statistician" have been updated to "Primary Statistician" and "Secondary Statistician," respectively.
- Katherine A. Guthrie, PhD has replaced Jacqueline Benedetti, PhD as the SWOG Primary Statistician. Contact information has been updated.
- On the second page, "CALGB Central Office" has been updated to "Alliance Protocol Operations Program Office." A web address to the Alliance website has also been added.
- "CALGB Patient Registrations" has been modified to "Alliance Patient Registration" with updated contact information.
- On the third page, the information regarding the NCI Cancer Trails Support Unit above the endorsing cooperative group section has been replaced with updated information regarding the CTSU.
- NCCTG has been removed from the table of endorsing cooperative groups, as NCCTG sites are now part of the Alliance.
- Also in the endorsing group table, "NSABP" has been updated to "NRG Oncology."
- Contact information for the SWOG Coordinating Office has been updated in the table of endorsing groups.
- Below the endorsing group table, the language regarding Endorsement Plus Groups has been deleted as this policy is no longer in affect.

Schema

- In the Required Initial Laboratory section, "Bilirubin" has been modified to "Total Bilirubin."
- Also, an asterisk has been placed next to "Total Bilirubin" with a footnote below that states "* In the absence of Gilbert's syndrome. If patient has Gilbert's syndrome: Direct Bilirubin ≤ 1.5 x ULN."
- The schema diagram has been modified for clarification purposes.
- Below the schema diagram, the sentence has been modified for clarification purposes to now read, "Celecoxib/placebo will be continued for a total of 3 years"

Section 1.9 Rationale for the current trial

In the last sentence of this section, the phrase "within two weeks of" have been inserted between the words "initiated" and "the start." It now reads, "Celecoxib will be initiated within two weeks of the start of FOLFOX chemotherapy (i.e., concurrent administration) and continued for 3 years total."

Section 2.2 Secondary objectives

References to Appendix VI have been added to the end of Objectives 2.2.1 and 2.2.3.

Section 4.1 Requirements for tumor parameters

- In criterion 4.1.2, the phrase "or otherwise confirmed by the surgeon" has been inserted at the end of the second sentence. It now reads, "In patients with tumor adherent to adjacent structures, en bloc Ro resection must be documented in the operative report or otherwise confirmed by the surgeon."
- Additionally in criterion 4.1.2, the word "not" in the third sentence has been capitalized for emphasis purposes.

Section 4.3 Patient history

In section 4.3.5, the word "not" in the second sentence has been capitalized for emphasis purposes.

Section 4.6 Required initial laboratory values
"Bilirubin" has been clarified to now state "Total Bilirubin." In addition, an asterisk has been placed next to bilirubin, with a footnote that states "*In the absence of Gilbert's disease. For patients with Gilbert's Syndrome: Direct Bilirubin ≤ 1.5 x upper limit of normal."

Section 5.2 Patient Registration/Randomization
All registrations and randomizations are completed via OPEN. Therefore, this section has been updated with new language describing OPEN registration and randomization procedures.

Section 5.5 Data submission
- The last paragraph in the section above the table now reads, "For the most up-to-date forms, please visit the CALGB website at www.calgb.org or the CTSU CALGB 80702 study page."
- Below the table, the following sentence has been added to the end of the last paragraph in this section, "However, adverse events reported via AdEERS must use CTCAE version 4.0 (See Section 14.0.)"

Section 5.6 Specimen submission for correlative and pharmacogenomics substudies
This section has been updated with information regarding the Alliance Biospecimen Management System (BioMS) including access location, logging and submission instructions and shipment procedures.

Section 5.7 Data submission for cancer prevention companion studies (CALGB 71002)
- In the "Diet and Lifestyle survey" section, Nathalie Fadel has replaced Devin Wigler as the contact person for this portion of the companion study.
- In the address found in the same section, the spelling of "Aven" has been corrected to "Avenue."
- In the first paragraph under the "Colonoscopy and pathology reports" section, the parenthentical portion of the second sentence has been modified to now read, "i.e., if the patient's initial colonoscopy at diagnosis was not a complete exam or the patient did not have a colonoscopy at diagnosis, guidelines suggest the first post-treatment colonoscopy be performed ~~within~~ sooner than 12 months after surgery – typically after completion of adjuvant FOLFOX).

Section 6.0 Required Data
- Above the table under the "Pre-Study Testing Intervals" section, the second interval guideline has been modified to now read, "To be completed within 80 DAYS before registration (preoperative scans are acceptable)."
- Under "Tests & Observations," the row header "Celecoxib/Placebo Count#" has been modified to now read, "Celecoxib/Placebo Adherence#."
- In the table under "Day 1 of each tx w/FOLFOX" and across from the now "Celecoxib/Placebo Adherence," the reference to footnote A has been changed to B.
- Under "Laboratory Studies," the third row has been modified from "AST, Alk Phos, Bilirubin" to "AST, Alk Phos, Total Bilirubin."
- In the table under "During tx w/Celecoxib/Placebo only**" and across from the rows"Serum Creatinine and BUN" and "AST, Alk Phos., Total Bilirubin," the X has been replaced with a new footnote, F. This new footnote states, "May be obtained 28 days in advance of follow-up appointment."
- In addition to labs, pre-registration history and physical, vital signs and clinical assessments may be used for day 1 of Cycle 1. Furthermore, H&P and labs during treatment may be obtained within 72 hours prior to day of treatment. Therefore, footnote "*" has been modified to now read, "Pre-registration H&P, vital signs, labs and clinical assessments may be used for Day 1 of Cycle 1. For subsequent cycles, H&P and labs may be obtained within 72 hours prior to day of treatment (96 hours if due to holidays)."

- For clarification purposes, footnote A has been modified to now read, "While on FOLFOX, patients should have a physical exam and report capsule counts of celecoxib/placebo prior to cycles 3 and 5 for study arms C and D and prior to cycles 3, 5, 7, 9 and 11 for study arms A and B. If treatment is held due to toxicity, physical exam and capsule count does not need to be repeated on the day that therapy is resumed."
- Patients should document celecoxib/placebo adherence throughout protocol treatment. Therefore, footnote B has been revised to now read, "Patients will record daily dosing of study medication on diary starting when the patients begins taking celecoxib/placebo (no later than cycle 2 day 1 of FOLFOX)."
- Also for clarification purposes, footnote C has been changed from "Every 4 weeks while on FOLFOX" to "Prior to cycles 3 and 5 for study arms C and D and prior to cycles 3, 5, 7, 9 and 11 for study arms A and B."
- In footnote D, the second sentence has been modified to now read, "During celecoxib/placebo monotherapy, PT/INR should be monitored at least every other week for the first month, then as clinically indicated."
- At the end of footnote E, the following sentence has been added: "CEA and scans may be performed +/- 1 months of next testing due date.
- Footnote "#" has been modified to now read, "Pill counts should include collection and review of monthly diaries as outlined in Section 7.3." This new section contain specific information previously stated in the footnote.

Section 7.1 FOLFOX, every 2 weeks
At the end of this section, above the statement regarding events of leucovorin shortage, the following sentence has been added: "Continuous infusion 5-FU should be administered as specified, though early pump shut-offs do not need to be reported on forms unless >10% of dose was not administered."

Section 7.3 Adherence
This section has been added to provide sites with specific instructions regarding the use and collection of pill diaries by patients. In addition, specifics on pill counts by the institution and dispensing of drug are outlined.

Section 8.0 Dose Modification and Management of Toxicity
The fourth bullet point has been modified for clarification purposes. It now reads, "If FOLFOX is delayed due to toxicity for ≥ 4 weeks, counting from the originally scheduled day of treatment that was held, discontinue FOLFOX. Continue celecoxib/placebo.

Section 8.2 Hematologic toxicities
- The first sentence in this section, "Dose modifications for hematologic toxicities are based on CBC on Day 1 of each cycle of FOLFOX (or within prior 72 hours)," has been split into two setences in order to provide sites clarifications on the time window for CBC collection. It now reads, "Dose modifications for hematologic toxicities are based on CBC on Day 1 of each cycle of FOLFOX. CBC may be collected within 72 hours of treatment (or 96 hours if due to holidays)."
- In the same paragraph, the last two sentences regarding use of growth factors have been deleted. This information can now be found in Section 10.6, "Use of Growth Factors."

Section 8.3 Gastrointestinal toxicities
Dose modification 8.3.4 has been updated to specify upper GI bleeding. It now reads, "For upper GI bleeding not associated with thrombocytopenia (platelets < 75,000) or for upper GI ulceration, discontinue celecoxib/placebo. Continue FOLFOX."

Section 8.10 Other non-hematologic toxicities for FOLFOX
In the parenthetical portion of the first sentence, the words "or stomatitis" have been inserted after "hand foot syndrome," and "dose reduction." It now reads, "For other grade 3 or 4 non-hematologic toxicities considered related to FOLFOX, delay FOLFOX until toxicity resolves to ≤ grade 1, then resume FOLFOX at one dose level reduction of oxaliplatin and 5-FU (if toxicity is hand-foot syndrome or stomatitis, dose reduction of only 5-FU bolus and continuous infusion of 5-FU is permitted)."

Section 9.4 Celecoxib (Celebrex) / Placebo (NSC #719627, Alliance IND #107051)

- The section header has been modified from "CALGB IND #107051" to "Alliance IND #107051."
- In the fourth paragraph under "Availability" that begins with "Questions about drug orders, transfers…," the phone number to the PMB has been changed from "301-496-5725" to "240-276-6575."
- Under the "Drug Ordering" section, all references to the "CALGB Statistical Center" have been updated to the "Alliance Statistics and Data Center."
- In the second paragraph under "Drug Ordering," the fax number to the PMB has been updated from "301-480-4612" to "240-276-7893."
- Under the "Drug Transfers" section in the third sentence, the fax number of the PMB has been updated from "301-402-0429" to "240-276-7893."

Section 10.2 Hormonal/other chemotherapeutic agents
This section has been modified to provide further details regarding exceptions. It now reads, "Treatment with hormones or other chemotherapeutic agents for cancer treatment may not be administered. Exceptions allowing usage of hormones and other chemotherapeutic agents include, but not limited to, steroid given for adrenal failure, hypersensitivity reactions or other non-cancer related conditions; hormones administered for non-disease-related conditions (e.g., but not limited to, insulin for diabetes); intermittent use of dexamethasone as an antiemetic; or methotrexate or other DMARDs (disease-modifying anti-rheumatic drugs) used for rheumatological conditions."

Section 10.4 Anticoagulants
The second sentence of this section has been modified to now read, "During celecoxib/placebo monotherapy, PT/INR should be monitored at least every other week for the first month, then as clinically indicated."

Section 10.6 Use of Growth Factors
This new section has been added to the protocol to provide institutions instructions regarding the use of growth factors during study treatment.

Section 11.0 Criteria for Response, Progression and Relapse

- In the fourth sentence of the first paragraph of this section, the word "approximately" has been inserted between the words "occur" and "4 months." It now reads, "As such, for patients randomized to 6 treatments of FOLFOX, the first post-treatment CEA and imaging should occur approximately 4 months after completion of FOLFOX."
- The following sentence has been added to the end of the first paragraph in this section: "CEA and scan may be performed +/- 1 month of next testing due date."

Section 13.1 Sample size and power estimates

- At the end of the first sentence of the fourth paragraph of this section, a reference to the new Appendix VI regarding the IDEA trial has been added. It now reads, "In addition, a prospective international effort is underway to pool patient level data from multiple trials that will test the duration assumption (the IDEA trial; see Appendix VI)."

- In the same paragraph, the third, fifth and sixth sentences have been deleted, as specific information regarding the IDEA trial may now be found in Appendix VI. These sentences read, "The current statistical assumptions for the IDEA trial is to pool 10,500 patients with stage III colon cancer and declare non-inferiority if the 2-sided 95% confidence interval for the hazard ratio comparing 3 to 6 months of therapy lies entirely below 1.10. The disease-free survival endpoint in C80702 for the duration question is thus a secondary endpoint since 2500 patients will not adequately address non-inferiority (i.e., patients from this trial will contribute to the pooling but we do not plan to report the results for the duration question as a primary endpoint in C80702). Secondary endpoints of toxicity will also be reported for the 2500 patients in this trial. Data provided to the IDEA trial will be fully de-identified."

Section 14.0 Adverse Event Reporting (AER)
The following paragraph has been inserted at the end of this section: "Please note: Adverse event reporting on the CALGB 80702 study forms uses CTCAE version 3.0. However, adverse events that require reporting via AdEERS utilize CTCAE version 4.0."

Section 14.2 Additional Instructions or Exclusion to AdEERs Expedited Reporting Requirements…
The eight bullet point, "AdEERS reports should be submitted electronically to the CALGB Central Office (calgb@uchicago.edu). Faxed copies of the AdEERS paper template, available at the AdEERS web page, will be accepted (312-345-0117), but electronic submission is preferred" has been deleted as this is no longer necessary.

Appendix I Cancer Trials Support Unit (CTUS) Participation Procedures
Because all enrollments to the study will now be conducted through OPEN and all other pertinent information in this appendix can now be found throughout the protocol, this appendix has been removed from the study. The subsequent appendices and their references in the protocol have been appropriately renumbered and updated.

Appendix I (Formerly Appendix II) Collaborative Agreement Language
In the sixth guideline of the Collaborative Agreement, the address and contact information to the Regulatory Affairs Branch has been updated.

Appendix IV (Formerly Appendix V) Cancer Prevention Companion Studies (Diet and Lifestyle): CALGB 71002

- In this now numbed IV appendix, in the second sentence of the first paragraph, "16-18" has been corrected to "14-16" months. This sentence now reads, "Patients enrolled on CALGB/SWOG C80702 will be asked to complete a food-frequency questionnaire within the first 6 weeks of start of randomization and 14-16 months after randomization."
- In the first sentence of the first paragraph of section 4.1 Assessment of diet and lifestyle factors, references to "16-18 months after randomization" have been corrected to "14-16 months of randomization."

Appendix V (Formerly Appendix VI) Cancer Prevention Companion Studies (Colonoscopy): CALGB 71002
In Section 3.3 Exposures, the reference to "16-18 months after randomization" in the second sentence has been corrected to "14-16 months after randomization." It now reads, "The questionnaire will be administered at 2 time points – within the first 6 weeks after randomization and within 14-16 months after randomization (~ 1.5 years after surgery)."

Appendix VI Idea Prospective Pooled Analysis
This new appendix has been added to provide further information regarding the data from CALGB 80702 being contributed to IDEA, an international prospective pooled analysis of stage III colon cancer patients on clinical trials.

UPDATES TO THE MODEL CONSENT:

- In keeping with new CTEP PIO requirements, the name of the lead group has been updated from "Cancer and Leukemia Group B" to "Alliance for Clinical Trials in Oncology." All references to "CALGB" as an organization has been updated throughout the model consent document to "Alliance."
- The "CALGB Statistical Center" is now titled "The Alliance Statistics and Data Center at Duke University." This change has been reflected throughout the model consent form.

Will My Medical Information Be Kept Private?

- In the second paragraph, the first bullet point has been updated from "The Cancer and Leukemia Group B (CALGB)" to "The Alliance for Clinical Trials in Oncology."
- In the first sentence of the fourth paragraph, "Cancer and Leukemia Group B" has been updated to "Alliance for Clinical Trials in Oncology."

Cancer Prevention and Diet and Lifestyle Study
In Update #02 of the protocol, the second sentence of the first paragraph of this section was updated to state that the questionnaire would be completed between 14 and 16 months after the start of treatment. This modification was inadvertently left off the cover change memo for that update. Please ensure this change has been made to your model consent.

Where can I get more information?
The first five lines of this section have been replaced with two paragraphs of updated information from the NCI informed consent template.

A replacement protocol and model consent document have been issued.

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Update #07
02/15/15

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB/SWOG 80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (Alliance IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

[X] **Update:**	[ ] **Status Change:**
[ ] Eligibility changes	[ ] Activation
[ ] Therapy/Dose Modifications/Study Calendar changes	[ ] Closure
[X] Informed Consent changes	[ ] Suspension / temporary closure
[ ] Scientific / Statistical Considerations changes	[ ] Reactivation
[ ] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[X] Other : CTEP-AERs	

IRB review of this update is required within 90 days. Expedited review is allowed.
Please follow your local IRB guidelines.

UPDATES TO THE PROTOCOL:

- References to the "Adverse Event Expedited Reporting System (AdEERS)" have been changed to "CTEP Adverse Event Reporting System (CTEP-AERS)" throughout the protocol.
- References to "CALGB patient number." "CALGB patient ID#," "CALGB/CTSU patient ID number" and "CALGB patient ID number" have been updated to "patient study ID number" throughout the protocol.

Cover Page

- The ClinicalTrials.gov Identifier has been added below the study title.
- A listing of the participating organizations have been added to the bottom of the page.

- Kylie J. Osterhus has replaced Kathe Douglas as the Data Coordinator. Contact information has been updated.
- The names of the NCIC-CTG and NRG Oncology Co-Chairs have been moved from the CTSU contact page to the front cover page.
- The web address to the CALGB web site has been removed on the second page. Also, the web address to the Alliance for Clinical Trials in Oncology website has been updated.
- The web address underneath "Adverse Event Reporting" has been updated.

Cancer Trials Support Unit (CTSU) Address and Contact Information
The formal endorsement system formerly used in the cooperative group system no longer exists in the new NCI National Clinical Trials Network. Therefore, underneath the CTSU contact information table, the following text has been deleted: "The following cooperative groups have formally endorsed this trial. Institutions from these groups must enroll patients and submit data via the CTSU." Additionally, the contact information for the SWOG, NCIC CTG and NRG Oncology coordinating offices have been deleted. The contact information for the co-chairs have been moved to the front cover page.

Schema
Beneath the schematic of the trial design, the line below the diagram has been revised as follows for clarification purposes: "Celecoxib/placebo will be continued for a total of 3 years from the day that study drug was initiated."

Section 1.9 Rationale for the current trial
In the last sentence of this section, the phrase "from the date when celecoxib (or placebo) was started" has been added. It now reads, "Celecoxib will be initiated within two weeks of the start of FOLFOX chemotherapy (i.e., concurrent administration) and continued for 3 years total from the date when celecoxib (or placebo) was started."

Section 5.0 Registration/Randomization, Stratification, and Data and Sample Submission
Former Sections 5.1 Registration Requirements and 5.2 Patient Registration/Randomization have been replaced with the following new sections:

- Section 5.1 CTEP Investigator Registration Procedures
- Section 5.2 CTEP Associate Registration Procedures / CTEP-IAM Account
- Section 5.3 CTSU Registration Procedures
- Section 5.4 Registration Requirements
- Section 5.5 Patient Registration/Randomization Procedures

These new sections provide new information regarding new NCTN procedures and use of the OPEN system for patient registration. Subsequent sections and their references have been renumbered and updated accordingly.

Section 5.8 (Formerly Section 5.5) Data Submission
In the paragraph above the form and submission schedule table, references to the CALGB web site have been updated to the Alliance web site. It now reads, "For the most up-to-date data forms, please visit the Alliance website at www.allianceforclinicaltrialsinoncology.org or the CTSU CALGB 80702 study page."

Section 5.9 (Formerly Section 5.6) Specimen submission for correlative and pharmacogenomics…
Below the table of specimen submission, the following has been added footnote "**": "Note: Specimen is requested to be drawn pre-treatment but can be drawn at any time while the patient is on study."

Section 5.9.5 (Formerly Section 5.6.5) Whole blood submission for the pharmacogenomics…
The following sentence has been added to the end of the first paragraph within this section: "Note: Specimen is requested to be drawn pre-treatment but can be drawn at any time while the patient is on study."

Section 5.10 Data submission for cancer prevention companion studies (CALGB 71002)
Under the "Diet and Lifestyle survey" header, the following sentence has been added as the second sentence in the first paragraph: "Versions of the surveys are available in Spanish and French – please email jmeyerhardt@parters.org requesting these versions if required for the patient."

Section 6.0 Required Data

- Below the table, footnote "**" has been modified as followed for clarification purposes: "Every 3 months until 3 years after initiation of celecoxib/placebo or until disease progression, whichever comes first.
- For clarification purposes, the word "randomization" has been updated to "initiation of celecoxib/placebo" in the third sentence of footnote "E." It now reads, "Then, for all patients, every 6 months from last scan until at least 3 years after initiation of celecoxib/placebo and then yearly for 3 years, or until disease progression. CEA and scans may be performed +/- 1 month of next testing due date."

Section 7.1 FOLFOX, every 2 weeks
In the final paragraph, reference to the CALGB web site has been updated to the Alliance web site. It now reads, "In the event of a leucovorin shortage, refer to the CALGB 80702 study page, which can be found on the Alliance and CTSU websites."

Section 7.2 Celecoxib or Placebo
The last sentence of the first paragraph has been modified as follows: "Celecoxib/placebo will continue for 3 years from the date of initiation of study drug (i.e. day 1 of celecoxib/placebo) or until progression of disease or unacceptable toxicity."

Section 8.10 Venous thrombosis events
This section has been added to provide dose modification insturctions for patients who develop vnous thrombosis while on study. It reads, "For patients who develop venous thrombosis (including deep venous thrombosis or port a cath clots) either during FOLFOX therapy or during celecoxib/placebo only treatment, therapy with either FOLFOX or celecoxib/placebo does not require treatment hold or dose reductions." Subsequent sections have been renumbered accordingly.

Section 9.0 Drug Formulation, Availability, and Preparation
The third paragraph under the section title, which previously read, "The total administered dose of cytotoxic chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose," has been deleted. It has been replaced with the following paragraphs: "For US sites, the total administered dose of cytotoxic chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose. For Canadian sites, the total administered dose of cytotoxic chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose for oxaliplatin and leucovorin, and 7% for 5FU."

Section 9.3 Leucovorin Calcium (Folinic Acid)…
In the second paragraph underneath the "Availability" section, reference to the CALGB website has been updated to the Alliance website. It now reads, "In the event of a leucovorin shortage, refer to the CALGB 80702 study page, which can be found on the Alliance and CTSU websites."

Section 9.4 Celecoxib (Celebrex) / Placebo (NSC #719627, Alliance IND #107051)

- In the fourth bullet underneath the second paragraph of the "Availability" section, the words "by CALGB" have been deleted. It now reads, "the patient ID number (e.g., "999999", where "999999" represents a unique patient identifier assigned at registration)."
- The following paragraph has been inserted as the second to last paragraph underneath the "Availability" header within this section: "The Alliance for Clinical Trials in Oncology holds the IND (#107051) for celecoxib/placebo for this trial. As such, the Alliance follows NCI CTMB policies and guidelines regarding drug distribution, repackaging and shipment of the drug."
- Under the "Drug Ordering" header, updated information has replaced the prior text. Updated ordering instrctions of Celecoxib/Placebo using the PMB OAOP application system, as well as a revised shipment schedule, has been provided.
- Under the "Drug Accountability" header, the entire paragraph has been replaced with new information to provide sites updated instructions on the required use of the Oral DARF for drug accountability purposes.

Section 14.2 Additional Instructions or Exclusion to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent Under a CTEP IND or Alliance IND

- The following text has been added as the fifth bullet point in this section: "Treatment expected adverse events include those listed in Section 9.0; in the package inserts for: 5-FU, leucovorin, oxaliplatin; and the Investigator's Brochure for celecoxib/placebo. Please see below for examples of expected events during FOLFOX chemotherapy: Nausea or vomiting, Diarrhea, Mucositis, Hand foot syndrome, Neuropathy, Acute pharyngolaryngeal dysesthesia, Allergic reaction, Dehydration, Fatigue, Alopecia, Hematosuppression (leukopenia, neutropenia, lymphopenia, anemia, and thrombocytopenia)."
- In the now sixth bullet point, the following underlined text has been added: "Grade 4 events that are expected with chemotherapy (FOLFOX) but unexpected with celecoxib/placebo and that OCCUR DURING CELECOXIB/PLACEBO MONOTHERAPY must be reported via CTEP-AERS within 10 calendar days."
- The previous seventh bullet point has been deleted, as the CAEPR for Oxaliplatin has been removed from the protocol. This bullet point read, "Treatment expected adverse events include those listed in Section 9.0; in the package inserts for: 5-FU, leucovorin, in the CAEPR for oxaliplatin; and the Investigator's Brochure for celecoxib/placebo. NOTE: The ASAEL column of the oxaliplatin CAEPR has been replaced with the specific protocol exceptions to expedited reporting (SPEER) list. This list now includes 'expected' severity grades in addition to event terms."

Section 14.3 Comprehensive Adverse Events and Potential Risks list (CAEPR) for Oxaliplatin (NSC 266046)

CTEP no longer sponsors an oxaliplatin IND and does not maintain a current CAEPR for this agent. Therefore, the CAEPR for Oxaliplatin has been removed from the protocol. Additionally, all references to this CAEPR throughout the protocol document have been removed.

UPDATES TO THE MODEL CONSENT:

Studies on tissue and blood

In the second paragraph, the following two sentences have been removed, as the hyperlink reference to the information sheet no longer valid: "Please read the information sheet called "How is Tissue Used for Research" to learn more about tissue research. [This NCI information sheet is available at http://www.cancerdiagnosis.nci.nih.gov/specimens/patient.pdf]."

Genetic studies on blood cells

- In the first bullet point underneath the sixth paragraph, the phrase "CALGB identification number" in the third sentence has been updated to read "identification number." It now reads, "Instead, it will be labeled with a special identification number."
- In the same section, references to the "Alliance Statistical Center" and "Alliance Data and Statistics Center" in the first and second bullet points have been updated to "Alliance Statistics and Data Center" for consistency and clarification purposes.

A replacement protocol and model consent document have been issued.

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Update #08
12/15/15

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB/SWOG 80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (Alliance IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

[X] **Update:**	[ ] **Status Change:**
[ ] Eligibility changes	[ ] Activation
[ ] Therapy/Dose Modifications/Study Calendar changes	[ ] Closure
[ ] Informed Consent changes	[ ] Suspension / temporary closure
[ ] Scientific / Statistical Considerations changes	[ ] Reactivation
[X] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other	

IRB approval (or disapproval) is required within 90 days. Expedited review is allowed. Please follow your local IRB guidelines.

UPDATES TO THE PROTOCOL:

Cover Page (Page 1)
- The activation date and update version number has been removed from the top left hand corner, as this information now be found in the document history table that has been added on the following page.
- Ryan Kuisle has replaced Kylie J. Osterhus as the data coordinator. Contact information has been updated. Additionally, "Data Coordinator" has been updated to "Data Manager."

Cover Page (Page 2)
- The address to the Alliance Protocol Operations Program Office has been updated.
- Data management services for this study has transferred from Duke University to Mayo Clinic. Therefore, the contact information for the Alliance Statistics and Data Center has been updated.

- A table that provides guidance on the appropriate contact for certain types of questions has been added.
- Additionally, a document history table has been added.

Cancer Trials Support Unit (CTSU) Address and Contact Information

The contact information table has been updated to provide the latest information regarding the CTSU, contacts for specific questions, and data submission.

Section 5.0 Registration/Randomization, Stratification, and Data and Sample Submission

The following sections have been updated with the latest procedures provided by CTSU:

- Section 5.1 CTEP Investigator Registration Procedures
- Section 5.2 CTEP Associate Registration Procedures / CTEP-IAM Account
- Section 5.3 CTSU Registration Procedures
- Section 5.4 Registration Requirements
- Section 5.5 Patient Registration/Randomization Procedures

Section 5.8 Data Submission

- All data should now be submitted to the Alliance Statistics and Data Center at Mayo Clinic. Therefore, the language at the beginning of this section above the forms table has been updated with modified instructions on how and where to submit case report forms for this study.
- Form C-113, "CALGB: Notification of Death Form," has been added to the table of required forms under the "Other" section.
- Additionally, a footnote reference, "#"has been added with Form C-113. This new footnote below the table reads: "Form C-113 cannot be submitted electronically and needs to be mailed to the Alliance Data Center (See above for mailing address)."

Section 5.9 Specimen submission for correlative and pharmacogenomic substudies

The Alliance Biorepository at Ohio State University does not accept Friday or Saturday shipments of specimens. As a result, the second to last paragraph of this section has been updated to reflect this.

Section 5.10 Data submission for cancer prevention companion studies (CALGB 71002)

- Alexandra Sorrentino has replaced Nathalie Fadel as the contact for the Diet and Lifestyle survey component of CALGB 71002. This section has been modified to reflect this change.
- As data management services has transferred from Duke University to Mayo Clinic, all colonoscopy and pathology reports should now be sent there. Therefore, the last paragraph of this section has been modified with updated instructions.

Section 6.0 Required Data

The following sentence has been added to the end of footnote "***" below the table for clarification purposes: "Once patient has progressive disease, survival follow-up is only required per the Follow-up (Post treatment) forms schedule in Section 5.8."

UPDATES TO THE MODEL CONSENT:

No changes have been made to the model consent document.

A replacement protocol and model consent document have been issued.

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Update #09
09/15/2016

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB/SWOG 80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (Alliance IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

[X] **Update:**	[ ] **Status Change:**
[ ] Eligibility changes	[ ] Activation
[ ] Therapy/Dose Modifications/Study Calendar changes	[ ] Closure
[ ] Informed Consent changes	[ ] Suspension / temporary closure
[X] Scientific / Statistical Considerations changes	[ ] Reactivation
[ ] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[X] Other: CTEP-AERS reporting requirements	

Expedited review is allowed. IRB approval (or disapproval) is required within 90 days. Please follow your IRB of record guidelines.

UPDATES TO THE PROTOCOL:

Cover Page

- The Protocol Coordinator contact information has been updated from Vance Erese to Alexandra LeVasseur.

Section 14.2

- Under "Additional Instructions or Exclusion to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent Under a CTEP IND or Alliance IND:," the second bullet has been removed that excluded deaths due to progressive disease from CTEP-AERS expedited reporting requirements. This is not correct, all deaths, even those due to progressive disease are required to be submitted as an adverse event via CTEP-AERS.

APPENDIX I

- On page 4 of Appendix I, under "Correlative science projects…", under the "Tissue" heading, the following changes have been made:
    - In the first list, "Next-generation sequencing (NGS)…" has been added as a correlative science study to be performed on collected tissue samples
    - In the second list, "KRAS mutation analysis," has been removed as a correlative science study to be performed on collected tissue samples.
    - In the third list, "DASL," has been removed as a correlative science study to be performed on collected tissue samples.

APPENDIX II

The following changes have been made:

- In Section 2.1:
    - A new second paragraph has been added that begins, "In light of the interaction…," to provide justification for the changes that have been made to the planned tissue correlative studies.
    - A new second-to-last paragraph has been added that begins, "At the same time, emerging…," to explain the importance of *Fusobacterium nucleatum* in colorectal cancer.
- In Section 2.5:
    - Part of paragraph 1 and all of paragraphs 2-4, starting with, "A novel, validated platform…," have been removed to reflect the changes in correlative studies that are to be performed.
    - Seven sentences have been added to the end of paragraph 1, starting with, "Various technologies have been…," to provide justification for the changes that have been made to the planned tissue correlative studies.
- In Section 4.1:
    - Paragraph 1, part b), the acronym "DASL" has been replaced with the phrase "transcriptomic analyses" to reflect the changes in correlative studies that are to be performed.
    - New third and fourth paragraphs have been added to explain the methods for the additional correlative studies that are to be performed.
- In Section 4.4:
    - The fourth paragraph has been removed that begins, "Sequencing of KRAS…," to reflect the changes in correlative studies that are to be performed.
    - In the last paragraph, "Quality Control for Tumor Block Analyses", the first sentence has been removed, "For analyses of KRAS...," to reflect the changes in correlative studies that are to be performed.
- In Section 4.5:
    - The first paragraph has been removed that begins, "As described above, a novel…," to reflect the changes in correlative studies that are to be performed.
    - A new first paragraph, "As described above, whole genome mRNA…" has been added to provide justification for the changes that have been made to the planned tissue correlative studies.
    - In the second paragraph, the first two sentences have been removed starting with, "Whereas standard RT PCR…," to reflect the changes in correlative studies that are to be performed.
- In Section 4.6:
    - The third paragraph beginning with, "Sequencing of BRAF…," has been removed to reflect the changes in correlative studies that are to be performed.

- In Section 5.0:
    - In the first sentence of the eighth paragraph, the acronym, "DASL," has been replaced with the phrase, "gene expression," to reflect the changes in correlative studies that are to be performed.
    - In the first sentence of the eighth paragraph, the phrase, "on the DASL chip," has been replaced with the phrase, "from RNA-seq," to reflect the changes in correlative studies that are to be performed.
    - In the third sentence of the eighth paragraph, the phrase, "on the DASL chip" has been removed to reflect the changes in correlative studies that are to be performed.
    - In the third sentence of the eighth paragraph, the word, "approximately," has been added before the number, "24,000", to more accurately describe the population size of genes/transcripts.
    - In the first sentence of the ninth paragraph, the phrase, "on the DASL chip" has been replaced with the phrase, "from whole transcriptome analysis", to reflect the changes in correlative studies that are to be performed.
    - In the first sentence of the eleventh paragraph, the acronym, "DASL", has been replaced with the phrase, "gene expression assessment", to reflect the changes in correlative studies that are to be performed.
- In Section 6.0:
    - In the text underneath Table 9, the acronym, "DASL", has been replaced with the phrase, "gene expression", to reflect the changes in correlative studies that are to be performed.
    - In the description for Table 10, the acronym, "DASL", has been replaced with the phrase, "gene expression", to reflect the changes in correlative studies that are to be performed.

APPENDIX IV

- In Section 4.3:
    - In the first sentence of the first paragraph, the text, "performed in the laboratory of Dr. Shuji Ogino", has been replaced with the text, "obtained from New Generation Sequencing (NGS) as described above", to reflect the changes in correlative studies that are to be performed.
    - Paragraphs 4 and 5, "Sequencing to detect KRAS…," have been removed to reflect the changes in correlative studies that are to be performed.
    - In the last paragraph, the first sentence has been removed, "For analyses of KRAS…," to reflect the changes in correlative studies that are to be performed.

UPDATES TO THE MODEL CONSENT:

No changes have been made to the model consent document.

A replacement protocol and model consent document have been issued.
This study remains closed to new patient accrual.

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Update #10
06/15/2017

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB/SWOG 80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

Investigational agent: Celecoxib/placebo, NSC #719627 (Alliance IND #107051), will be supplied by Pfizer, Inc., and distributed by CTEP, DCTD, NCI
Participation limited to U.S. and Canadian sites.

[X] **Update:**	[ ] **Status Change:**
[ ] Eligibility changes	[ ] Activation
[X] Therapy/Dose Modifications/Study Calendar changes	[ ] Closure
[ ] Informed Consent changes	[ ] Suspension / temporary closure
[X] Scientific / Statistical Considerations changes	[ ] Reactivation
[ ] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other:	

Expedited review is allowed. IRB approval (or disapproval) is required within 90 days. Please follow your IRB of record guidelines.

UPDATES TO THE PROTOCOL:

Cover Page

- The title for Dr. Jeffrey Meyerhardt has been updated from "Alliance Study Chair" to "Alliance Study Chair, Alliance GI Committee Co-Chair."
- Dr. Eileen O'Reilly has replaced Dr. Alan Venook as the Alliance GI Committee Chair, and the title for this position has been updated to now read "Alliance GI Committee Co-Chair."
- The institution and contact information for Dr. Charles Fuchs, the Alliance Correlative Science Co-Chair, has been updated.
- The title for Dr. Howard McLeod has been updated to "PPP Co-Chair" (previously, "PET Co-Chair"), and his institution and contact information have been updated as well.
- The email address for Dr. Philip Kuebler, the NRG Oncology Co-Chair, has been updated.

Protocol Contacts (Page 2)

- Contact information for the Pharmaceutical Management Branch has been added in the left column under the title "**Drug Distribution Contact**."
- The telephone number for the Regulatory Affairs Manager has been removed as all inquiries should be submitted via email.
- In the second table entitled "**Protocol-related questions may be directed as follows**," a new second to last row has been added for "Questions related to drug supply."

CANCER TRIALS SUPPORT UNTIL (CTSU) ADDRESS AND CONTACT INFORMATION

All text within the table has been updated with current CTSU boilerplate language.

Section 5.3 (CTSU Registration Procedures)

All text in this section has been updated with current CTSU boilerplate language.

Section 5.3.1 (Downloading Site Registration Documents)

All text in this section has been updated with current CTSU boilerplate language.

Section 5.3.2 (Requirements for CALGB 80702 Site Registration)

All text in this section has been updated with current CTSU boilerplate language.

Section 5.3.3 (Checking Your Site's Registration Status)

All text in this section has been updated with current CTSU boilerplate language.

Section 5.3.4 (Submitting Regulatory Documents)

All text in this section has been updated with current CTSU boilerplate language.

Section 6.0 (Required Data)

In the *** footnote, the second sentence has been replaced with new text to create second and third sentences for increased clarity that read: "Once a patient has progressive disease, submission of follow-up forms is still required for survival data. See forms schedule in Section 5.8" (previously, "Once patient has progressive disease, survival follow-up is only required per the Follow-up (Post treatment) forms schedule in Section 5.8").

Appendix II (Correlative Science Companion Studies: CALGB 150911)

- In Section 2.1, the last two sentences of the second paragraph have been removed (previously, "Current panels typically…" and "DNA is isolated from…") and replaced with six new sentences to describe the current technology available for these analyses. The new text begins with "Whole exome sequencing…."
- In the third paragraph in Section 4.1, the following changes have been made to describe the current technology available for the correlative science analyses:
    - In the first sentence, the phrase "have utilized" has been replaced with "will utilize."
    - The second, third, and fourth sentences (all remaining text in the paragraph) have been removed and replaced with five new sentences. The new text begins with "Extracted DNA is…."

UPDATES TO THE MODEL CONSENT:

No changes have been made to the informed consent document.

A replacement protocol and model consent document have been issued.
This study remains closed to new patient accrual.

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Protocol Update #11
12/01/2017

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB/SWOG 80702

A PHASE III TRIAL OF 6 VERSUS 12 TREATMENTS OF ADJUVANT FOLFOX PLUS CELECOXIB OR PLACEBO FOR PATIENTS WITH RESECTED STAGE III COLON CANCER

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[X] Informed Consent changes	[ ] Suspension / Temporary closure
[ ] Scientific / Statistical Considerations changes	[ ] Reactivation
[X] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other:	

Expedited review is allowed. IRB approval (or disapproval) is required within 90 days. Please follow your IRB of record guidelines.

UPDATES TO THE PROTOCOL:

Cover Page

- Dr. Qian Shi has replaced Dr. Donna Niedzwiecki as the Alliance Primary Statistician, and all contact information has been updated.
- The position of "Alliance Primary Statistician (PET)" has been removed, along with all contact information for Dr. Kouros Owzar, as the statistical responsibilities previously associated with this position will now be handled by the Alliance Primary Statistician.
- Tyler Zemla has replaced Xing (Cynthia) Ye as the Secondary Statistician, and all contact information has been updated.
- Christopher Bryhn has replaced Ryan Kuisle as the Data Manager, and all contact information has been updated.

Cover Page (pg. 2)

- A title has been added above the 1st table for clarity that reads: "**Study Resources**."
- Data submission for this study will now be performed using Medidata Rave®. Therefore, under "**Study Resources**," the website link for Medidata Rave® has been added in the right column.
- The fax number for Dr. Barbara Todaro has been removed as it had been included in error.

Cancer Trials Support Unit (CTSU) Address and Contact Information

All text in the 3rd column under the "**For study data submission**" heading has been replaced with updated information as data submission for this study will now be performed using Medidata Rave®.

Section 5.0 (Registration/Randomization, Stratification, and Data and Sample Submission)

- Section 5.1 (CTEP Investigator Registration Procedures) and Section 5.2 (CTEP Associate Registration Procedures / CTEP-IAM Account) have been removed and replaced with a new Section 5.1 entitled "**CTEP Registration Procedures**" which includes updated CTSU boilerplate language. Subsequent sections have been renumbered accordingly.
- All text in Section 5.2 (CTSU Registration Procedures) (formerly Section 5.3) has been completely revised to include updated CTSU boilerplate language.
- All text in Section 5.4 (Patient Registration/Randomization Procedures) (formerly Section 5.5) has been completely revised to include updated CTSU boilerplate language.

Section 5.7 (Data Submission)

- Data submission for this study will now be performed using Medidata Rave®, therefore the 1st paragraph and the subsequent bullet points have been removed and replaced with four new paragraphs that outline the new data submission procedures.
- In the "C-113" row of the table, the "#" symbol has been removed as well as the corresponding footnote below the table as this Form can now be submitted electronically via Medidata Rave®.
- In footnote **, the phrase "Alliance Statistics and Data Center" has replaced the phrase "Statistical Center" for consistency.

Section 5.9 (Data Submission for Cancer Prevention Companion Studies [CALGB 71002])

- Under the "Diet and Lifestyle Survey" heading, "Elizabeth Brighton" has replaced "Alexandra Sorrentino" as the research coordinator who will send and receive the surveys.
- In the 2nd paragraph below the "Colonoscopy and Pathology Reports" heading, the phrase "submitted via Medidata Rave®" has replaced the phrase "sent to the Alliance Statistics and Data Center at Mayo Clinic, Data Operations" in the 1st and 2nd sentences to reflect the new data submission process.

Section 10.1 (Supportive Care)

In the 3rd sentence of the 1st paragraph, the phrase "via Medidata Rave®" has been added to reflect the new data submission process.

Section 14.2 (Additional Instructions or Exclusion to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent Under a CTEP IND or Alliance IND)

In the 1st sentence of the 9th bullet, the phrase "via Medidata Rave®" has been added to reflect the new data submission process.

UPDATES TO THE MODEL CONSENT:

What side effects or risks can I expect from being in the study?
In the "LESS LIKELY" section under the "**Celecoxib**" heading, a new 5th bullet has been added for "Blockage of the bowels" to reflect current adverse event data for celecoxib.

A replacement protocol and model consent document have been issued.
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ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB/SWOG 80702

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[X] Scientific / Statistical Considerations changes	[ ] Reactivation
[ ] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other:	

If your site utilizes the CIRB as your IRB of record:
No recommended IRB level of review is provided by the Alliance since the CIRB is the IRB of record for this trial.
The site has 30 days after the posting of this amendment to implement it at their site. Please refer to the amendment application and CIRB guidelines for further instructions.

If your site utilizes a local IRB as your IRB of record:
Expedited IRB approval is allowed. The proposed changes in this amendment are minor and do not affect the overall risk/benefit ratio. IRB approval (or disapproval) is required within 90 days. Please follow your local IRB guidelines.

UPDATES TO THE PROTOCOL:

Cover Page (page 1)
-Dr. Philip Philip has replaced Dr. Howard Hochster as the SWOG GI Committee Chair. All contact information has been updated.

-Jessica Krier has replaced Christopher Bryhn as the Data Manager. All contact information has been updated.
-Katherine Guthrie's email address domain has been updated.
-The title "NCIC CTG Co-Chair" has been renamed "CCTG Champion" to reflect the Group's name change and in accordance with NCI policy.
-The title "NRG Oncology Co-Chair" has been renamed "NRG Oncology Champion" in accordance with NCI policy.
--In the **Participating Organizations** paragraph, the phrase "**NCIC CTG** / NCIC Clinical Trials Research Group" has been renamed "**CCTG** / Canadian Cancer Trials Group" to reflect the Group's name change.

Study Resources (page 2)
-The contact for questions regarding CTEP-AERS reporting has been updated to the Alliance Pharmacovigilance Inbox (previously, Regulatory Affairs Manager).
-The study document history table has been removed. This table now appears as a separate document on the protocol landing page, on the members' side of the Alliance website.

CTSU Contact Information (page 3)
The CTSU Address and Contact Information table has been updated in its entirety to align with the current CTSU boilerplate language.

Section 5.0 (Registration/Randomization, Stratification, and Data and Sample Submission)
-All text in Section 5.1, Section 5.2, Section 5.4 and Section 5.7 has been updated in its entirety to align with the current CTSU boilerplate language.
-Additionally in Section 5.7, the CTCAE version in the second sentence of the bolded paragraph below the table has been changed to 5.0 for expedited CTEP-AERS reporting (previously 4.0).

Section 13.1 (Sample Size and Power Estimates)
New fifth and sixth paragraphs have been added due to a slower than anticipated event rate. The power estimates and DFS rate have been revised to reflect the actual event rate. Two new in-text citations have been added as a result, and thus the References list in Section 15.0 has been updated accordingly.

Section 14.0 (Adverse Event Reporting [AER])
In the first paragraph, the phrase "until March 31, 2018" has been added to the end of the third sentence, and a new fourth sentence has been added to indicate that CTCAE version 5.0 is now required for expedited adverse event reporting via CTEP-AERS. The fifth and sixth sentences of the first paragraph, and the second sentence of the third paragraph have also been revised to reflect this version change.

Section 14.2 (Additional Instructions or Exclusions…):
-New tenth through fifteenth bullets have been added with expedited reporting instructions for secondary and second malignancies, death, pregnancy loss, and neonatal death.

UPDATES TO THE MODEL CONSENT:

No updates have been made to the model consent form.

A replacement protocol and model consent document have been issued.
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