THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

Molecular targeting of 15-LOX-1 for apoptosis induction in human colorectal tumors
DM02-592

Core Protocol Information

Short Title	Molecular targeting of 15-LOX-1 for apoptosis induction in human colorectal cancers
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Study Co-Chair(s):	Robert S. Bresalier Patrick Lynch
Study Manager:	Jennifer L. Guerra
Full Title:	Molecular targeting of 15-LOX-1 for apoptosis induction in human colorectal tumors
Public Description:	This study test the mechanisms by which celecoxib could prevent colon cancer
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Which Committee will review this protocol?

◉ The Clinical Research Committee - (CRC)

1.0 Objectives

To determine whether celecoxib downregulates GATA-6 expression to upregulate (15-lipoxygenase-1) 15-lox-1 expression, downregulate PPAR-delta (PPAR-d), and induce apoptosis in human rectal cancers. The current clinical study will measure GATA-6, PPAR-d, 15-LOX-1 expression, 13-S-hydroxyoctadecadienoic (13-S-HODE) levels, and apoptosis rates in paired normal and colorectal polyp epithelial tissues before and after 6 months of celecoxib treatment of patients with familial adenomatous polyposis (FAP).

2.0 Background

2.1.0 Colorectal Cancer as a Chemoprevention Target

Colorectal cancer is the second leading cause of cancer deaths in the United States (1). New strategies to prevent and treat colorectal cancers are therefore needed. Chemoprevention is one promising approach because it focuses on treating cancers at earlier stages to prevent them from becoming incurable. Colorectal cancer is a primary candidate as a target for developing chemopreventive agents because this disease is common and develops through a protracted, multistep process that allows targeting earlier stages for successful preventive interventions. Indeed, preclinical and early clinical studies suggest that this approach is quite feasible (2). NSAIDs are a very promising class of chemopreventive agents in colorectal cancers, as shown in epidemiologic, preclinical, and clinical studies (3). Nevertheless, the current estimate of the possible efficacy rate of NSAIDs as chemopreventive agents in the general population is 50% or less (2,3), so further improvement is needed. In addition, the molecular mechanisms through which NSAIDs exert their chemopreventive effects have yet to be fully defined (3). Identification of crucial molecular mechanisms for colonic tumorigenesis is important not only to better understand the mechanisms that underlie the chemopreventive activity of currently known agents, but more so to develop better molecularly targeted interventions.

2.2.0 Colorectal Chemoprevention by NSAIDs

NSAIDs act as chemopreventive agents against colorectal cancers in various experimental models (3). Furthermore, in clinical trials, sulindac and, more recently, celecoxib have suppressed the formation of adenomas in patients with familial Adenomatous polyposis syndrome (4,5). NSAIDs initially were considered to act by inhibiting prostaglandin synthesis; however, the role of prostaglandin synthesis inhibition as a basic mechanism for the chemopreventive effects of NSAIDs has been questioned (3). Apoptosis is diminished in human cancers; and reestablishment of apoptosis is being targeted as a mechanism for treating tumorigenesis (6). NSAIDs induce apoptosis in colorectal cancer cells independently of COX inhibition (7,8). Thus, COX-independent mechanisms for the chemopreventive effects of NSAIDs are being explored. One of these possible mechanisms is the NSAIDs' ability to modulate lipoxygenases (LOXs), a class of enzymes involved in polyunsaturated fatty acid metabolism. Like COXs, LOXs metabolize polyunsaturated fatty acids such as arachidonic acid to various biologically active metabolites (e.g., hydroxyeicosatetraenoic acids: 5-, 8-, 12-, and 15-*S*-HETE) (9). LOX metabolism and products are predominant in colonic epithelia compared with products of COX metabolism (10). This further suggests the importance of LOXs in colonic tumorigenesis. Indeed, several LOX metabolites of arachidonic acid, such as 12-HETE and LTB4, promote tumorigenesis (11,12). Like arachidonic acid, linoleic acid is another n-6 polyunsaturated fatty acid that forms biologically active products through oxidative metabolism. Furthermore, in human diets, the main form of n-6 polyunsaturated fatty acid intake is linoleic acid (13). Linoleic acid metabolism in relation to colonic carcinogenesis and chemoprevention has not received attention until recently (14).

2.3.0 Linoleic Acid, 13-*S*-HODE, and Colonic Carcinogenesis

Whereas arachidonic acid has multiple oxidative metabolic pathways in humans, linoleic acid is mainly limited to the 15-LOX-1 pathway, which produces 13-*S*-HODE (15,16). Several lines of evidence indicate that 13-*S*-HODE suppresses cellular proliferation and induces apoptosis (14). (a) We have found that 13-*S*-HODE levels and 15-LOX-1 expression were reduced in human colorectal cancers, and 13-*S*-HODE induced apoptosis and cell cycle arrest in transformed colonic cells (17). (b) Similarly, 15-LOX-1 is downregulated in human esophageal cancers (18). (c) Human osteosarcoma cells transfected with human 15-LOX-1 grow more than 50% slower with enzymatically active expression of 15-LOX-1 (19). Cell growth rates approach that observed in nonexpressing clones when 15-LOX-1 expression is lost. (d) The immediate and transient precursor of 13-*S*-HODE, 13-hydroxyperoxyoctadecadienoic acid, induces apoptosis in human T cells (20). (e) Induction of differentiation in transformed Caco-2 colonic cells and human tracheobronchial epithelial cells causes the expression of 15-LOX-1 in these cells, which is associated with the conversion of linoleic acid to 13-*S*-HODE (21,22). (f) Various histone deacetylase inhibitors, a class of putative antitumorigenic agents that is currently under extensive investigation (23), including trichostatin A and HC toxin, induce 15-LOX-1 expression in human colon cancer cell lines (24). (g) More recently, studies of skin tumorigenesis in a transgenic mouse model of epidermis-type 12-*S*-LOX indicated that 13-*S*-HODE production is associated with antitumorigenic effects (25). Thus, 13-*S*-HODE has antitumorigenic effects, and linoleic acid might promote colonic carcinogenesis through its conversion to arachidonic acid to from protumorigenic products (14). This concept is further supported by the finding that linoleic acid inhibits rather than promotes carcinogenesis in a mouse-skin tumorigenesis model (26) wherein linoleic acid is not converted into arachidonic acid but is converted into 13-*S*-HODE (27).

2.4.0. NSAID, upregulation of 15-LOX-1, and apoptosis

NSAIDs' ability to induce apoptosis in colorectal cancer cells plays an essential role in NSAID chemoprotective effects (7-8). Based on our earlier findings that 15-LOX-1 downregulation in colorectal cancer cells is linked to apoptosis loss in cancer cells, we evaluated whether NSAIDs restore 15-LOX-1 expression in colorectal cancer cell to induce apoptosis. We have found that in colorectal cancer cells, NSAIDs upregulated 15-LOX-1 expression and increased 13-*S*-HODE production during apoptosis induction and that these events were critical to the NSAIDs' ability to induce apoptosis (18,28,29). These findings were also confirmed *in vivo* study using xenograft models (Preliminary Data, Fig. 2 Appendix K). NSAIDs' induction of apoptosis through restoring 15-LOX-1 expression is not limited to colorectal carcinomas. Similar to what we had observed in colorectal cancers, NSAIDs restore 15-LOX-1 expression to induce apoptosis in human esophageal carcinomas (18). Next, we found that NSAIDs restored 15-LOX-1 expression and induced apoptosis independently of COX-2 inhibition (29). We therefore examined whether NSAIDs upregulate 15-LOX-1 expression by directly modulating 15-LOX-1 expression regulation rather than

through a substrate shift by inhibiting COX-2. Indeed, we found that NSAIDs upregulate 15-LOX-1 by modulating the transcriptional regulation of this enzyme (30). We next searched for candidate transcriptional factors that are involved in transcriptional regulation of 15-LOX-1.

2.5.0 GATA-6 and Regulation of 15-LOX-1 Expression in Colorectal Cancers

The GATAs are a family of six transcriptional regulation proteins. GATA-4, -5, and -6 are expressed in overlapping patterns during the development of endoderm-derived organs such as the intestine (31). In the intestinal crypt, GATA-6 expression is higher in the proliferating region than in the tips of the intestinal villi, where cells undergo differentiation and apoptosis (32). GATA-6 is also highly expressed in gastric, colonic, pancreatic, and prostatic cancer cell lines (33). Furthermore, induced terminal differentiation downregulates GATA-6 expression (32,34). We examined the effects of NSAIDs on the transcriptional regulation by GATA-6 of 15-LOX-1 expression after we had found that NSAIDs alter 15-LOX-1 expression at the transcriptional level (30). NSAIDs downregulated GATA-6 in a time-dependent manner in colorectal cancer cells, which preceded the upregulation of 15-LOX-1 that occurred at 24 hours (30). Ectopic GATA-6 overexpression blocked both 15-LOX-1 upregulation and apoptosis induction by NSAIDs (30), findings that indicate that GATA-6 plays an important role in regulating apoptosis through suppression of 15-LOX-1 transcription. Furthermore, we have recently found that GATA-6 is overexpressed in colorectal cancer epithelial tissues compared to paired normal epithelial tissues (preliminary data, Appendix K, Fig 3). All this evidence indicates that in colorectal cancer cells, GATA-6 has negative regulatory effects on differentiation and apoptosis by suppressing 15-LOX-1 expression and that NSAID downregulation of GATA-6 allows for 15-LOX-1 expression and restoration of apoptosis. The contribution of these events (i.e., NSAID downregulation of GATA-6 and subsequent upregulation of 15-LOX-1 expression) to the NSAIDs' induction of apoptosis in patients with colorectal cancers remains to be defined. Thus, our current proposed protocol aims to answer these questions by testing the following hypothesis in a clinical study: *NSAIDs downregulate GATA-6 expression to restore 15-LOX-1 expression, which increases 13*-S-*HODE production, thereby inducing apoptosis in human colorectal tumors.*

2.6.0 PPAR-, Colorectal Tumorigenesis, and NSAIDs' Chemopreventive Effects

PPARs act as nuclear receptors for polyunsaturated fatty acids (arachidonic and linoleic acids) and their metabolites (*1*). PPAR- appears to contribute to colonic tumorigenesis and the chemopreventive response to NSAIDs, although the role of PPAR-d is currently considered controversial, on the basis of preclinical data. PPAR-d is overexpressed in human colorectal cancers (*2*), PPAR-d knock-out profoundly suppresses tumorigenesis in xenografts of HCT-116 colon cancer cells in vivo (*3*), NSAIDs suppress PPAR-d activity in colorectal cancer cells to induce apoptosis in vitro (*4*), and PPAR- knock-out in mice attenuates sulindac's chemopreventive effects (*5*). The role of PPAR-d in colon tumorigenesis was questioned when PPAR-d knock- out in the Min mouse failed to show effects on intestinal tumorigenesis (*6*) and when high concentrations of sulindac sulfide (80 M) inhibited growth of PPAR-–null HCT-116 cells in vitro (*3*). We examined this relationship between NSAIDs' chemopreventive effects and PPAR-d further after finding that 13-*S*-HODE's binding to PPAR-d suppresses PPAR-d activity and expression (*7*). NSAIDs' induced expression of 15-LOX-1 downregulated PPAR-d in vitro and in vivo, and apoptosis induction and growth inhibition by celecoxib were significantly decreased in PPAR-null cells, thus indicating that PPAR- is an important signaling receptor involved with NSAID-induced apoptosis (*7*). A more recent study demonstrated that a PPAR- agonist promoted intestinal tumorigenesis in Min mice (*8*), whereas another study in those mice showed that PPAR- knock-out promoted polyp formation (*9*). Thus, the preclinical data are conflicting in terms of the role played by PPAR-, especially in the setting of the adenomatous polyposis coli (APC) gene mutation. Previous data have shown that PPAR- is upregulated in human colorectal cancers, but the role of PPAR- in human colorectal tumorigenesis remains unknown, especially in FAP patients with the APC mutation, which is simulated by the Min mouse model. Because animal models have limitations in simulating human tumorigenesis (*10*), clinical studies are needed to define the role of PPAR- in human colorectal tumorigenesis. We therefore propose, in this application, to examine whether the expression of PPAR- differs between polyp and normal tissues in patients with FAP and whether celecoxib modulates PPAR- expression in humans.

2.7.0 Rationale of the experimental design

The current proposal will explore the mechanistic role of GATA-6 and 15-LOX-1 in the apoptotic response to NSAIDs in the clinic. NSAIDs are a very promising class of chemopreventive agents, but the mechanisms of their chemopreventive effects remain unclearly defined. Defining these chemopreventive mechanisms is important not only to understand how NSAID works, but more so to identify molecular targets that can be used to develop better antitumorigenic agents against colorectal cancer. Loss of apoptosis is an important mechanistic event for human tumorigenesis development, and NSAIDs, like other antitumorigenic agents, exert their antitumorigenic effect through reestablishment of apoptosis. Our preclinical data have indicated that NSAIDs induce apoptosis through downregulation of GATA-6 to transcriptionally upregulate 15-LOX-1 expression. Validation of 15-LOX-1 as a molecular target for therapeutic induction of apoptosis in clinical studies is needed because of the differences between preclinical and clinical models and to establish its clinical importance as a molecular target for further drug development. We have selected celecoxib for testing in the current clinical study because celecoxib is a promising chemopreventive, as shown in preclinical models and in a clinical study of patients with FAP (5) and because we found in preliminary laboratory studies that celecoxib restored 15-LOX-1 expression to induce apoptosis at a markedly lower concentration than did other NSAIDs and that in a xenograft model, celecoxib's growth-inhibitory effects were dependent on 15-LOX-1 expression (preliminary data, appendix K, Figs. 1 and 2). The dosage selected for this study has been shown in a prior clinical study to have chemopreventive effects (5). Duration of the treatment was selected based on prior clinical study of FAP patients (5). The study will be limited to patients with FAP. FAP patients were selected because: a) Celecoxib in the dose selected for the study has been previously shown in a randomized trial to reduce polyp formation in FAP patients; and b) FAP patients have large numbers of polyps that require repeated surveillance colonoscopies during which biopsies can be obtained.

15-LOX-1 and GATA-6 will be measured using both Western blotting and immunohistochemical methods. Immunohistochemistry will help localize the expression to the epithelial tissue levels. The study will also provide data on the role played by PPAR-d in colonic tumorigenesis. This role has been difficult to understand because of conflicting preclinical data (section 2.6.0). 13-*S*-HODE is the primary product of 15-LOX-1, and the measurement of 13-*S*-HODE intracellular levels assesses 15-LOX-1 enzymatic activity. In contrast to the semiquantitative measurements of 15-LOX-1, PPAR-d and GATA-6 expression (Western blotting and immunohistochemistry), 13-*S*-HODE intracellular levels can be measured in human colonic epithelial tissues using a quantitative and reliable assay (17). We have therefore selected 13-*S*-HODE as the primary outcome variable. GATA-6 and 15-LOX-1 expression levels and apoptosis rates (TUNEL and activated caspase-3 assays) will be the co-primary outcome measures. COX-2 expression (western blotting assay) and PGE2 levels

[enzyme immunoassay (EIA) method] will be the secondary outcome variables to further examine relationship to 15-LOX-1 signaling pathway in-vivo.

3.0 SUMMARY OF STUDY PLAN

Design: Phase II study of 15-lox-1 molecular targeting by celecoxib in patients who have FAP.
Study Cohort: Patients with FAP that can be accessed endoscopically for tissue biopsies.
Study Drug: Celecoxib 400 mg po bid X 6 months.
Primary endpoints: 13-S-HODE, 15-LOX-1, GATA-6, PPAR-d, and apoptosis levels
Secondary endpoints: PGE2 and COX-2 expression levels
Duration of treatment: 6 months
Follow-up: Patients will be contacted by phone 72 hours after the first dose and every 2 weeks (+/- three days) for 6 months thereafter for toxicity assessment.
Sample size: 40 patients
Duration of the study: It is expected that accrual will be completed within 40 months from the date of the protocol modification and samples analyses will be finished by 48 months from that date.

4.0 STUDY SCHEMA

Pretreatment history and physical

Registration: Subjects will complete medication (Appendix G), demographic (Appendix E), and dietary food questionnaire (Appendix II) forms.

Colonoscopy - A total of 23 specimens will be obtained during each endoscopic procedure. One or two forceps biopsy specimens will be obtained from 10–20 separate polyps (the total number of colonic polyps sampled will be 20). The polyps with be sampled in a fashion that is representative of the size of the polyp population. Three additional specimens will be obtained from normal-appearing colonic mucosa. Small area with high density of colon or rectal polyps will be marked with India-ink tattoo. Colonoscopies will be videotaped.

Celecoxib 400 mg po bid x 6 months

History and physical for toxicity assessment

Repeat colonscopy and biopsies (20 biopsies from polyps, 3 from normal mucosa)

Videotape colonscopy with picture of previously tattooed area to assess the response to treatment.

Celecoxib serum levels before celecoxib treatment begins and again at the end of months 2, 4, and 6 (+/- seven days) of celecoxib treatment

5.0 ELIGIBILITY AND EXCLUSION CRITERIA

5.1.0 Eligibility

5.1.1 Diagnosis of familial adenomatous polyposis (patients should have colorectal remnant that can be biopsied. Patients who had total colorectal surgical resection are not eligible).

5.1.2 Adequate bone marrow function (ANC $\geq$ 1500 ml, platelet count $\geq$ 100,000/ml). Serum creatinine, total bilirubin, and ALT $\leq$ 1.5 upper limit normal.

5.1.3 Age of 16 or older.

5.1.4 Patient is able to give an informed consent.

5.1.5 Women of childbearing potential (women are considered to be of childbearing potential unless they are at 2 or more years post-menopausal/or surgically sterile), must:

a. Not be pregnant or lactating

b. Use adequate contraceptive measures (e.g. abstinence, IUD, birth control pills, Depo-Provera or diaphragm or condom with spermicidal gel) starting with last menses and throughout the study duration.

c. Have a negative serum pregnancy test within 14 days of starting celecoxib.

5.2.0 Exclusion criteria

5.2.1 Inflammatory bowel disease.

5.2.2 Intake of anti-inflammatory medications (e.g. non-steroidal, aspirin, and sulfasalazine) that can not be discontinued starting 3 days prior to the enrollment.

5.2.3 Chemotherapy or radiation therapy in less than three month from the time of the enrollment.

5.2.4 Individual who are taking Coumadin that can not be discontinued starting 7 days prior to the enrollment.

5.2.5 Individuals who have received an investigational chemopreventive agent during the month prior to the biopsies.

5.2.6 History of bleeding diathesis

5.2.7 History of sulfonamides (sulfa) allergies

5.2.8 History of cardiovascular diseases that might include the following: myocardial infraction, angina, coronary angioplasty, congestive heart failure, stroke, or coronary bypass surgery.

5.2.9 Uncontrolled hypertension (>135/>85 mm Hg) on three repeated measurements during the 6 weeks prior to enrollment on the study

5.2.10 Diagnosis of Diabetes

5.2.11 Cigarette smoking within the six months prior to the date of enrollment.

5.2.12 Uncontrolled hypercholesteremia (low-density lipoprotein cholesterol (LDL-C) > 130). Hypercholesteremia needs to be controlled following the updated National Cholesterol Education Program Adult Treatment Panel III Guidelines (appendix OO) for at least 3 months prior to enrollment on the study. Hypercholesteremia treatment needs to be continued during the enrollment on the protocol

5.2.13 Family history of premature coronary disease (i.e. onset <55 years of age)

5.2.14 Metabolic syndrome diagnosis in patients who are 30 years or older. (The diagnosis of metabolic syndrome is made when three or more of these risk factors are present):

a. Waist circumference: Men >102 cm (>40 in); Women >88 cm (>35 in).
b. Triglycerides =150 mg/dl (=1.69 mmol/L)
c. High-density lipoprotein cholesterol (HDL-C): Men <40 mg/dl (<1.03 mmol/L), Women <50 mg/dl (<1.29 mmol/L)
d. Blood pressure =130/=85 mm Hg
e. Fasting glucose =110 mg/dl (=6.1 mmol/L)

5.2.15 History of deep venous thrombosis, pulmonary embolism, systemic lupus erythematous, family history of protein S or C deficiencies or prior heparin-induced thrombocytopenia

6.0 DRUG INFORMATION

6.1.0 Name and dose of study drug: celecoxib 400 mg po bid X 6 months.

6.2.0 Formulation: Celecoxib is a diarly substituted pyrazole and chemically designated as 4-[5-(4-methylphenyl)-3(trifluoromethyl)-1H-pyraxol-1yl] benzene sulfonamide. Celecoxib molecular Formula is C17H14F3N3O2S. Celecoxib is supplied in hard, opaque, gelatin capsule(s) in either 200mg or 400 mg dose.

6.3.0 Administration: the drug will be taken orally at a dose of 400 mg (either two 200mg or one 400 mg capsule) with meals twice a day (morning and evening).

6.4.0 Source of the drug: Celecoxib is available by prescription from the Investigational Pharmacy at MDACC.

6.5.0 Packaging: The drug will be provided by prescription usually with one month supply with 6 refills.

6.6.0 Storage: The study drug will be stored at room temperature in a locked cabinet with restricted access. The medication supply will be protected from environmental extreme exposure.

6.7.0 Adherence monitoring: Adherence will be assessed by pill diary (Appendix L) and celecoxib serum level (measured before the treatment and at the end of months 2, 4, and 6 (+/- seven days) of celecoxib treatment). Celecoxib serum level will be measured as a send-out test through MD Anderson laboratory to Quest Diagnostics.

7.0 CLINICAL STUDY PLAN

7.1.0 Subject Recruitment

The study subjects will be selected from patients with a confirmed diagnosis of FAP who are will be undergoing celecoxib treatment and followed at MD Anderson Cancer Center.

7.2. 0 Pretreatment Evaluation

7.2.1 Pretreatment history (including medication history especially NSAID use) and physical examination will be performed. Physical examination should include heart, lung, and extremity examination. Study subjects are specifically asked regarding cardiovascular events (myocardial infarction, angina, stroke, arrhythmia or congestive heart failure) and cardiac symptoms (chest pain, palpitation, dyspnea, or edema).

7.2.2 Laboratory evaluation: fasting blood glucose and lipid profile (cholesterol, LDL, HDL and triglyceride). CBC, creatinine, bilirubin, and ALT.

7.2.3 Serum pregnancy test for women of childbearing age.

7.2.4 Pretreatment history, physical, and laboratory data are acceptable within 35 days prior to start of protocol therapy (celecoxib).

7.3.0 Enrollment on the study

The informed consent will be reviewed and discussed with subjects who are approached for enrollment on the study. A potential study candidate who is interested in enrollment can be provided with a copy of the informed consent to review at home. If after returning home, a potential study candidate expresses interest in enrolling in the study but is unable to be physically present at MD Anderson at the time of signing the informed consent due to physical distance, he or she may fax the signed consent form to a clinical study investigator and mail the original at the same time. The subject will be enrolled on the study once a clinical study investigator has signed the informed consent. In adddition, before the patient starts his or her colonic procedure the patient will then be re-consented by the clinical study investigator. After signing an informed consent, each subject will complete a demographic data forms including the following information: Age, Sex, diagnosis, registration number, telephone number and address. Subjects will also complete questionnaires regarding medications, vitamins, dietary food questionnaire, and nutritional supplements (Appendix H). The original copy of the consent form will be filed in the patient's medical records. A copy of the consent will be kept in the patient's study file, and a second copy will be given to the patient. In the event that the patient has an opportunity to qualify for other research studies, the patient may sign up two different consents. Once the patient is determined non-evaluable during the initial colonic procedure (having less than 20 polyps), the patient will be enrolled on an alternative research study that require the patient to have less than 20 polyps on their initial colonoscopy.

7.4.0 Celecoxib administration

Following the initial colonoscopy procedure, subject will start celecoxib at a dose of 400 mg po bid with meals. Celecoxib treatment will be continued for 6 months.

7.5.0 Sampling procedure during colonoscopies

A study investigator with clinical privileges to perform gastrointestinal endoscopic procedures will obtain a total of 23 specimens during each endoscopic procedure before celecoxib therapy starts and after 6 months of treatment. One or two forceps biopsy specimens will be obtained from 10–20 separate polyps (the total number of colonic polyps sampled will be 20). The polyps with be sampled in a fashion that is representative of the size of the polyp population. Three additional specimens will be obtained from normal-appearing colonic mucosa. If colorectal mucosa is carpeted with polyps, indigo carmine will be used to distinguish normal from adenomatous mucosa.

7.6.0 Sample handling, size of biopsy samples, assay needs, allocation and prioritization

Each specimen obtained via the biopsy forceps tissue collection procedure will weigh approximately 5 mg and contain at least 400 µg of protein. When the tissue is homogenized, a single biopsy specimen has been sufficient for performing three immunoassays in triplicate or for three or more Western blot analyses. We will use Tissue-Tek® OTC compound (Sakura Finetek, Torrance, CA) for embedding tissue samples for freezing. In our prior studies, we successfully used such frozen colonic tissues for various assays, including 13-*S*-HODE immunoassays and 15-LOX-1 Western blotting (*11*). Polyp tissue biopsy samples will be handled in the following order: 10 specimens for each assay will be frozen in OTC and processed for immunohistochemistry and 13-*S*-HODE enzyme immunoassay (EIA); 10 specimens for each assay will be frozen in liquid nitrogen and processed for 15-LOX-1, GATA-6, and PPAR- Western blotting. For normal tissues, one specimen for each assay will be frozen in OTC and processed for immunohistochemistry and 13-*S*-HODE EIA, and one specimen for each assay will be frozen in liquid nitrogen and processed for 15-LOX-1, GATA-6, and PPAR- Western blotting. Biopsy samples from the normal and polyp sites will placed in RNAlater (Ambion, Austin, TX) and then frozen for 15-LOX-1, PPAR-d, GATA-6, and nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1) mRNA expression studies by quantitative real time PCR analyses. Tissues to be frozen will be placed immediately (within 15 seconds) in OTC or liquid nitrogen and stored at –80C until use, when they will be transferred on dry ice to the Clinical Cancer Prevention laboratories.

7.7.0. Sample Tracking, Storage, and Analyses:

All tissue samples will be logged into a computer tracking system as described in the Data Management section. Samples will be transferred to the Clinical Cancer Prevention laboratories in the Naomi building (NA01.041) where they will be stored at -80C until the time of analyses. Dr Shureiqi and his research assistance will analyze the samples according to the methods that are detailed in the attached laboratory method section (appendix I). The principle investigator will enter sample analysis results into the study database.

7.8.0 Follow-up procedure

Patients will be contacted by phone 72 hours after the first celecoxib doses and every 2 weeks (+/- three days) thereafter for toxicity assessment for the entire period of the study (6 months). Subjects will be asked to maintain a treatment diary (Appendix L) of celecoxib intake. Study subjects are specifically asked regarding cardiovascular events (myocardial infarction, angina, stroke, arrhythmia or congestive heart failure) and cardiac symptoms (chest pain, palpitation, dyspnea, or edema). Serum celecoxib levels will be done at the end of months 2, 4, and 6 (+/- seven days) of celecoxib treatment.

7.9.0 Evaluation at the end of celecoxib treatment (6 month)

1. Focused history to evaluate for adverse events and medication use.
2. Focused physical exam. Physical examination includes heart, lung, and extremity examination. Study subjects are specifically asked regarding cardiovascular events (myocardial infarction, angina, stroke, arrhythmia or congestive heart failure) and cardiac symptoms (chest pain, palpitation, dyspnea, or edema).
3. Repeated colorectal biopsies (20 from each of polyps and 3 from normal mucosa). Colonscopies will be videotaped to assess response to celecoxib.
4. Adherence evaluation (treatment diary) and blood drawing for celecoxib serum level.

7.10.0 Blood Collection and sample processing for celecoxib serum level

A blood specimen (5 ml each sample) for celecoxib serum level will be collected in heparinized tubes (red top tubes) on the day before celecoxib treatment begins and at the end of months 2, 4, and 6 (+/- seven days) of celecoxib treatment. Blood sample specimen will be sent to the chemistry laboratory at MD Anderson for processing and shipment to Quest diagnostic for analyses.

7.11.0 Definition of an evaluable course

Completion of 6 months of celecoxib treatment (90% of celecoxib doses) and colorectal biopsies pre-celecoxib and after 6 months of celecoxib therapy as specified by the protocol.

7.12.0 Drop-out

New subjects will replace subjects who do not complete the study.

7.13.0 Financial Assistance for Travel Expenses for Financially Disadvantaged Patients:

Patients that might have financial difficulties with covering travel expenses to MD Anderson Cancer Center for the initial and follow up visit, a special fund has established by the Division of Cancer Prevention to assist these patients by covering travel expenses. Expenses covered are: air or ground transportation, lodging and meals. The subjects will be provided with the information regarding the program through the collaborating social worker (Camille Blundell, MSW). Information to contact Camille Blundell, MSW is included in the informed consent. The eligibility for the assistance will be determined by the social worker based on the following criteria:

Housing	Transportation
• Age 16-25 (and/or accompanied by parents) • Student • Unemployed • Income below DHHS poverty guidelines • Lack of health insurance • College students • Living outside Houston (100 mi) # of rooms/family members •	• Age 16-25 (and/or accompanied by parents) • Student • Unemployed • Income below DHHS poverty guidelines • Lack of health insurance • Lack of transportation

2006 HHS Poverty Guidelines			
Persons in family unit	Poverty Guideline for 48 States and DC	Poverty Guideline for Alaska	Poverty Guideline for Hawaii
1	$9,800	$12,250	$11,270
2	$13,200	$16,500	$15,180
3	$16,600	$20,750	$19,090
4	$20,000	$25,000	$23,000
5	$23,400	$29,250	$26,910
6	$26,800	$33,500	$30,820
7	$30,200	$37,750	$34,730
8	$33,600	$42,000	$38,640

For each additional person, add $3,400 (48 states and DC), $4,250 (Alaska), $3,910 (Hawaii)
From Federal Register, Vol. 71, No. 15, January 24, 2006, pp. 3848-3849

Expenses covered:
1. Transportation: Under 300 miles, subjects will be provided with bus tickets or paid 49 cents/mile if they drive to MD Anderson Cancer Center
Over 300 miles, subjects will be provided with airfare and shuttle expenses between MD Anderson and a Houston airport
2. Housing: Hotel room at the Rotary House for 1-2 nights

8.0 STUDY CALENDAR

Month	PreRx	**1**	**2**	**3**	**4**	**5**	**6**
MD Evaluation[a]	**X**						**X**
Laboratory Evaluation[e]	**X**						
Toxicity Evaluation[b]		**X**	**X**	**X**	**X**	**X**	**X**
Pregnancy test[c]	**X**						
colonoscopy and colorectal biopsies	**X**						**X**
Celecoxib Administration		**X**	**X**	**X**	**X**	**X**	**X**
Plasma for drug assay[d]	**X**		**X**		**X**		**X**

[a]MD will perform history and physical examination. Study subjects are specifically asked regarding CV events.
[b]Toxicity evaluation will be in person at the end of the study and by telephone 72 hours after initial intake then every 2 weeks for 6 months.
[c]Serum pregnancy test is performed in women who have childbearing potential.
[D]Plasma assay will be performed as in section 7.10.0.
[e]Laboratory evaluation are : fasting blood glucose, lipid profile, CBC, creatinine, bilirubin, and ALT

9.0 MANAGEMENT OF ADVERSE REACTIONS (ADRS)

9.1.0. Definition of Adverse Event

An adverse event is any condition that appears, worsens, or increases in frequency after starting the study drug. Investigators will attempt to define drug related versus non-drug related adverse events; however, all adverse events, whether thought to be drug related or not, will be noted in the records and reported to the IRB according to the guidelines for filing reports of adverse experiences at M. D. Anderson (Appendix B). Severe adverse events will be reported using the MDACC severe adverse event reporting form (Appendix J).

9.2.0 Adverse Event Severity grading

Adverse event toxicity will be graded according to the defined NCI Toxicity scales (Appendix C) and appropriate action taken. Adverse events not included in the defined NCI toxicity criteria will be graded according to their effects on the daily activity of the study subjects:
Grade 1: No limitation of daily activity
Grade 2: Some limitation of daily activity
Grade 3: Sever limitation of daily activity

9.4.0 Potential Expected Adverse events

The biopsies required for the study are not expect to appreciably increase the small risk of serious adverse events that might otherwise happen during or after rectal endoscopic procedures. These potential risks can include bleeding and bowel perforation. The risk of perforation is expected to be lower in the rectal region than to what expected with higher colonic biopsies.

Possible side-effects with celecoxib can include: diarrhea, indigestion, headache; nasal congestion, sore throat, rhinorrhea and/or upper respiratory tract infection; abdominal pain, stomach pain, peptic ulcer disease, gastrointestinal bleeding, gas, nausea, back pain; extremity edema, dizziness, insomnia, tinnitus, liver function test abnormalities, hepatitis, jaundice, and/or rash. Celecoxib at a dose of 800 mg daily was found to increase risk of major fatal and non-fatal cardiovascular events by 3.4-fold compared to subjects who were taking placebo in the Adenoma Prevention with Celecoxib (APC) trial (http://www.fda.gov/cder/drug/infopage/celebrex/celebrex-hcp.htm). One incident of rhambdomyolosis has been reported in a patient taking celecoxib. The possibility of celecoxib being the cause of this incident cannot be excluded.

9.5.0 Measures to monitor side effects and manage adverse events

Subjects will be observed for at least 60 minutes after biopsy procedures. Contact telephone numbers will be provided to ensure that medical staff may be easily reached. Subjects are asked to call in case of excessive bleeding or abdominal pain after of the procedure. If excessive bleeding is evident after the procedure, the lesions will be cauterized. Surgical backup is available at all times.

Study subjects will be contacted by phone 72 hr after the first celecoxib doses and every 2 weeks thereafter for the rest of the celecoxib treatment duration for toxicity assessment. In addition, subjects will undergo a history and physical to assess for toxicity during a clinical visit at the end of the treatment. All adverse events will be followed according to good medical practices. "Unacceptable toxicity for a chemoprevention agent is defined as any Grade 3 or higher toxicity (NIC Common Toxicity Scale) for any of the listed categories or Grade 2 or higher allergic reaction or cardiac toxicity." If a study investigator determines that an unacceptable toxicity [Grade 3 or higher toxicity for any of the NIC Common Toxicity Scale categories or Grade 2 or higher allergic reaction or cardiac toxicity) is possibly related to celecoxib, then celecoxib will be stopped and the study Chair be informed. The PI will meet with Drs.Lynch (study co-investigators) and Morris (study biostatistician) to perform a cumulative review of all adverse events and premature terminations every 6 months after study initiation or after completion of 15% of subject enrollment (enrollment of 6 patients). If any cardiovascular adverse event is identified for a follow-up period, Dr. Durand, the study collaborating cardiologist, will participate in the review of the cumulative toxicity. The patient will be followed up according to good medical practices. As a part of the follow-up in case of unacceptable toxicity development, a telephone contact will be made at 30 $\pm$ 7 days after celecoxib discontinuation to assess the resolution of the adverse event(s).

9.5.1 Clinical Research Compliance's Monitoring of the Conduct of the Study and the Safety of Participants

This monitoring schedule will occur once every 6 months or for every 15% of newly registered patients, whichever occurs first. The monitoring team will consist of one or more staff members from the Clinical Research Compliance, depending on the rate of accrual of patients. In addition, two or three faculty members with clinical research experience and expertise in the disease site of the protocol serve as the medical reviewers on the monitoring team. These medical reviewers, like the other members of the team, are independent and objective. They cannot be collaborators on the study or members of the same academic department as the PI, and they cannot have any other involvement with the study. (The members of the Clinical Research Compliance have no such conflicts because they are independent of all academic departments in the institution.)

The monitoring teams review the following elements for each study they monitor: informed consent forms, eligibility, pretherapy requirements, treatment administration, evaluations during the study and at follow-up, all toxic effects, response to therapy, and general data quality. In addition, the monitoring teams use the PDMS to generate a report so they can review all adverse events—serious, not serious, expected, and unexpected—reported by each study participant, not just those reported in the charts of participants selected for in-depth review. The information in this PDMS-generated report includes the date of onset of the event, its severity grade, its suspected causal relationship with the treatment, the date the event resolved, and whether the event required treatment.

A pre-activation meetings will be held with the PIs, their research teams, and representatives of the Clinical Research Compliance to discuss the data and safety monitoring plan for the trials. The monitoring plan is discussed, and templates for data collection are distributed. These templates are prepared by the Clinical Research Compliance case by case after their review of the study protocol. After the templates are completed by the research team, they become a permanent part of the study participants' medical records.

A report is prepared by the monitoring team after each monitoring visit. The completed report is given to the research team, which has 1 week to review the report and provide any data identified as missing by the monitoring team. A final monitoring report is then prepared. If the report does not indicate the occurrence of unexpected adverse events or issues of noncompliance with Current Good Clinical Practice, federal regulations, or institutional policies, it is reviewed by the Clinical Research Compliance Oversight Committee. If a monitoring report indicate such occurrence or issues, it is forwarded to the IRB for review and action.

9.6.0. Serious Adverse Events

Serious Adverse Experience -Any adverse drug experience that results in any of the following outcomes:
· Death

· A life-threatening adverse drug experience - any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.

· Inpatient hospitalization or prolongation of existing hospitalization

· A persistent or significant disability/incapacity - a substantial disruption of a person's ability to conduct normal life functions.

· A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

9.7.0 Reporting Serious Adverse Events

All serious adverse events, whether they appear to be related to the study medication or not, will be reported to the study chair and the MD Anderson IRB (via OPR) within 24 hours of knowledge of the event.

9.7.1 Reporting procedure for cardiovascular toxicity

If an adverse cardiovascular event is identified, the research nurse will notify the PI within 24 hours. The PI will report all grade 2 or more adverse cardiovascular events related to the study to the IRB within 1 working day of his notification about the event. All serious cardiac adverse events will be reported to the FDA through the FDA Medical products Reporting Program (Med Watch) using the on line reporting mechanism within 3 working days.

9.8.0 Early stopping role for cardiovascular toxicity

The proportion of subjects who developed serious cardiovascular adverse events while taking placebo was reported as 1.8 % in a large international study for the prevention of sporadic polyps, the Prevention of Spontaneous Adenomatous Polyps Trial. Based on this information, we have statistically estimated the numbers of cardiovascular events that will predict that celecoxib will have excessive cardiovascular toxicity (10.3.7). According to these calculations, we will suspend the study for excessive cardiovascular toxicity if 2 or more of the first 10 patients enrolled on the trial experience a grade 2 or greater cardiovascular toxicity, or if 3 or more of the first 31 patients experience a grade 2 or greater cardiovascular toxicity, or if at any time during the study a 4th patient experiences a grade 2 or greater cardiovascular toxicity. The study will be suspended by stopping enrollment of new subjects and celecoxib treatment for all enrolled patients. If the study is suspended, the IRB and NCI will be notified within 3 working days. The following study clinical investigators will review safety data: Drs. Shureiqi and Lynch in consultation with Dr. Morris, study biostatistician and Dr. Durand, collaborating cardiologist. A report of this safety review will be sent to MD Anderson IRB and the NCI. The study will be permanently stopped unless the MD Anderson IRB, NCI and study investigators all agree that the study can be safely resumed.

10.0 DATA ANALYSES

10.1.0. Data base management

10.1.1 Master Data Base

All clinical data for the protocol will be entered into The University of Texas MD Anderson Cancer Center Protocol Data Management System (PDMS). All laboratory data will be organized into a secure master database in Microsoft access. This data set will reside on the Intel machine in the department of Clinical Cancer Prevention.

Some of the data sets will collected at the subject level and some at the specimen level, so the appropriate index keys are used in each data set comprising the base to allow for efficient merging of the data. Following quality review, data will be merged into the master data set as it is entered (as described below).

10.1.2 Derived Data Sets

For each analysis, a data set will be built by merging appropriate sets from the master base (more than one analysis will use a given set). This data set will have records at the appropriate level of observation (e.g., subject), and will contain all of the transformations of original variables as specified above and as may be required. Provisions will be made to allow the production of derived data sets for graphics and reports that can be passed to a variety of programs on any of the major end-user platforms (DOS/Windows, Macintosh or UNIX).

10.1.3 Revisions and Corrections

All corrections to primary study documents will be initialed and dated. If computer-readable data is corrected by replacement of a data set, the replaced version of the data set will be retained in an archive. Corrections to electronic data will be stored in a tabular form; that is, for each data set within the master database, an auxiliary data set is retained that records each addition, deletion or correction to records in that data set. The collection of these auxiliary data sets represents an audit trail of corrections to the database. A single program uses the audit records to update the database as required.

10.1.4 Archives

In addition, to the standard system backup for PDMS for the clinical data of the protocol, the master database itself will be archived weekly to zip discs, which will be kept in a secure place. In addition, paper reports will be produced of all changes made to the data set. A paper listing of the data set will be produced periodically and kept off-site.

10.1.5 Security

Prior to registration, a registration checklist on-study form must be completed. It is added, along with the signed consent, to the individual subject's protocol research file. Each subject is assigned a unique study identification number, which will be used on all study forms and specimens. The study subjects' protocol research files and a coded list of subjects on the study will be kept in a key-locked filing cabinet in the department of Clinical Cancer Prevention. Confidentiality of the patients will be maintained and information kept on each patient will be made available only to identified investigators. Fields which could identify subjects, such as name, address, or social security number will not be stored electronically and will be stored as paper reports under lock and key at all times. All computer systems will be password-protected. Non-commercial software will not be

loaded onto the Intel-based computer dedicated to the data management. The redundant backups described above allow for quick restoration of data in the unlikely event that a security breach (or the more likely event of hardware failure) occurs.

10.2.0 Data Entry

10.2.1 Clinical Data Entry

Data will be entered into the PDMS database in case report forms after responses to all queries have been received. In case of missing data, a brief explanation is to be recorded as a comment.

10.2.2 Laboratory Data Entry

Laboratory data are recorded on forms and later entered into the Microsoft -access electronic database. The sample identification number should allow only individuals who have access to the clinical data to trace the sample to a specific patient. The sample identification number is however coordinated to ensure that sample identifiers internal to the laboratory can be unambiguously traced to a specific patient by the principle investigator. All questions of sample identity will be resolved before laboratory data are merged into the master database.

10.3.0 Data analyses

10.3.1 Hypotheses to be tested:

NSAIDs downregulate GATA-6 expression to restore 15-lox-1 expression that increases 13-S-HODE production, reduces PPAR-d expression, and thereby induce apoptosis in human colorectal tumors.

10.3.2 Predictor variables

a. Tissue disease status defined into one of two categories: normal-appearing mucosa, or polyp (tumor).
b. Treatment status: pre- versus post- celecoxib treatment.

10.3.3 Outcome variables

A. Primary outcome variable: 13-S-HODE, 15-LOX-1 primary product, levels (measured by ELISA assay).
B. Co-primary outcome variables: 15-LOX-1, PPAR-d, and GATA-6 levels (measured by western blotting and immunohistochemistry) and apoptosis (measured by TUNEL and casapse-3 immunohistochemistry).
C. Secondary endpoint: PGE2 levels (measured by EIA) and COX-2 expression by immunohistochemistry and western blotting

10.3.4 Confounding variables

Information regarding age, calcium, and folate supplement intake will be collected at the subject level and the effect of these variables will be assessed as potential confounders.

10.3.5 Statistical analysis:

(a) Primary end point analyses: The patients in the study will be dichotomized into responders and non-responders. Responders will be defined as patients with at least a 25% reduction in their number of colorectal polyps. The primary analysis will be to compare the mean change in 13 S HODE values from baseline to post-treatment between the responders and responders. To account for the correlation of multiple tumors within a single patient, mixed models (PROC MIXED in SAS) will be used to perform this test.

(b) Analysis for primary and secondary endpoint: 13 S HODE levels will be correlated with the ratio of normal to cancer tissue levels of 15 LOX 1, GATA 6, and TUNEL and caspase 3 using Pearson and Spearman Correlation, as well as mixed models. Additionally, multivariate analyses will be applied to investigate the relationship between these measurements and confounding variables.

10.3.6 Sample size justification

In this study, we will measure 13-*S*-HODE levels in triplicate in 10 polyps taken at baseline and in 10 polyps taken after 6 months of treatment with celecoxib from each of 40 patients. As previously mentioned, these polyps will be selected independent of size so that there is no systematic bias induced by selecting larger polyps, for example. This sample size will allow us to have at least 80% power to detect a 25% increase in mean 13-*S*-HODE levels from baseline to 6 months (from 0.63 to 0.79 ng/g protein, for a mean difference of = 0.16) using a paired *t* test with a two-sided significance level of 0.05, assuming that the standard deviation for the paired differences is = 0.355. These estimates are based on preliminary data from our laboratory on the 13-*S*-HODE levels of polyps, and the details of how we arrived at these values are given below. These calculations make the conservative assumption that there will be no correlation between the baseline and 6-month values. See the table below for details on the power calculations based on different values for the effect size, , and correlation between baseline and 6-month means from the same patient, . If the correlation is = 0.40, our power to detect a 25% increase in 13-*S*-HODE level from baseline to 6 months is 95%. With 40 patients and 10 polyps per patient, we have > 99.9% power to detect a change in 13-*S*-HODE level of at least 50% (from 0.63 to 0.94 ng/g protein, irrespective of the value of .

Table 1: Power for paired *t* test with two-sided significance level of 0.05 to detect significant change in mean 13-*S*-HODE level from baseline to 6 months as a function of effect size, , and standard deviation, , which depends on the correlation between baseline and 6-month measurements, .

Mean change in 13-*S*-HODE	Mean change from baseline to 6 mo ()		0	0.10	0.20	0.30	0.40	0.50

			0.355	0.337	0.318	0.297	0.275	0.251
25%	0.160	0.80	0.83	0.87	0.91	0.95	0.98	
50%	0.310	> 0.999	> 0.999	> 0.999	> 0.999	> 0.999	> 0.999	

Here we describe in detail the assumptions we made to arrive at our choice of effect size and standard deviation for the power calculations. First, we assume that a subset of patients with FAP will respond to celecoxib and that others will not respond. We define patients as responders if they experience a 25% reduction in polyp count from baseline to 6 months. Second, we assume that nonresponders will experience no change in their mean 13-*S*-HODE levels from baseline to 6 months. Our preliminary data showed that the mean 13-*S*-HODE level in patients' polyps before celecoxib treatment is 0.63 ng/g, so we assume that this will be the mean value at both baseline and 6 months for the nonresponders. Third, we compute the power under two assumptions for the responders: that they will experience a 50% increase or a 100% increase (i.e., a doubling) of 13-*S*-HODE level from baseline to 6 months. Assuming a baseline mean 13-*S*-HODE value of 0.63 ng/g means that the mean 6-month value for responders would be 0.94 or 1.26 ng/g, respectively. In our preliminary data, the mean 13-*S*-HODE level for normal tissue was 3.35 ng/g, and the mean ratio of 13-*S*-HODE between normal tissue and polyps was 5.27. Fourth, we assume that roughly half of the patients will be responders. In the study by Steinbach et al (*12*), 53% of the patients had a > 25% reduction in polyp counts and would have been considered responders by our definition. In Specific Aim 2, we compare the 13-*S*-HODE levels in responders and nonresponders, but in this Specific Aim, our analysis focuses on testing whether the mean 13-*S*-HODE level in polyps changes from baseline to 6 months for all patients, irrespective of response to treatment. Thus, we compute the power for an overall 25% or 50% increase in 13-*S*-HODE (from 0.63 ng/g to 0.79 or 0.94 ng/g), which corresponds to no change in the half of patients that are nonresponders and a 50% or 100% increase, respectively, in the half that are responders. Fifth, we assume that the variance in the mean 13-*S*-HODE level for a given patient (averaging values for 10 polyps, with 13-*S*-HODE measured in triplicate) at both baseline and 6 months is *s2* = 0.063, which is estimated using a variance component analysis on the preliminary data. In this variance component analysis, we estimated the patient-to-patient variation to be *s21* = 0.0563, the polyp-to-polyp variation to be *s22* = 0.0520 and the replicate-to-replicate variation to be *s23* = 0.0629. Routine statistical calculations show that the variance of the mean 13-*S*-HODE level for a patient (averaging values for 10 polyps, with triplicate measurements per polyp) is *s21 + s22/10 + s23/30* = 0.063. Sixth, we assume that the mean baseline and 6-month 13-*S*-HODE levels for a given patient will likely be correlated with each other. Routine statistical calculations, letting represent this correlation, show that the standard deviation of the difference between the mean 13-*S*-HODE levels at baseline and 6 months is given by $\sigma = \sqrt{2s^2(1-\rho)}$. Since we do not have pretreatment and posttreatment preliminary data, we do not know what should be, so we compute the power for values of from 0 to 0.50 in increments of 0.10. Our best guess is that should be close to the intraclass correlation between polyps within the same patient, which is given by *s21/(s21 + s22 + s23/3)* = 0.43. A value of = 0 is very conservative because we expect that there should be some positive correlation between the baseline and 6-month values

10.3.7 Estimation of celecoxib excessive cardiovascular toxicity

We will use the design of Thall and Simon (1994) to continuously monitor grade 2 cardiovascular toxicities, with the trial suspended if the rate is deemed too high, the data reviewed, and only opened again after review by the NCI and IRB. Let P represent the proportion of patients in the trial who experience at least one cardiovascular toxicity of grade 2 or higher during the 6 months on study drug. We will continuously monitor this quantity in the trial using the method of Thall and Simon (1994). We assume a diffuse prior distribution on P, specifically a Beta (0.02, 0.98), which has mean 0.02 and 95% credible interval (0,0.28). We will suspend accrual in the trial if, at any time during the trial, Pr (P>0.018)>0.90. That is, we will stop the trial early if, based on the data in the trial, there is strong evidence (probability > 0.90) that the grade 2 cardiovascular toxicity rate is greater than 0.018. Based on this criterion, the trial will be terminated early for excessive toxicity if 2 or more of the first 10 patients enrolled on the trial experience a grade 2 or greater cardiovascular toxicity, or if 3 or more of the first 31 patients experience a grade 2 or greater cardiovascular toxicity, or if at any time during the study a 4th patient experiences a grade 2 or greater cardiovascular toxicity. With these stopping rules, the study will suspend accrual of new patients and celecoxib treatment of enrolled patient with probability 0.85 if, in fact, P=0.10, and with probability 0.46 if, in fact, P=0.05. If the true proportion is P=0.01, then the trial will stop with probability 0.04.(see reference 35).

11.0 ETHICAL & REGULATORY CONSIDERATIONS

11.1 Institutional Review

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56). The protocol and informed consent form for this study must be approved in writing by the appropriate Institutional Review Board (IRB). The IRB must be from an institution which has a valid Multiple Project Assurance, Single Project Assurance or Cooperative Oncology Group Assurance on file with the Office for Protection from Research Risks, National Institutes of Health. The institution must be in compliance with regulations of the Food and Drug Administration and the Department of Health and Human Services.

Significant changes to the protocol, as well as a change of principal investigator, must also be approved by the Board and documentation of this approval provided to the study monitor. Records of the Institutional Review Board review and approval of all documents pertaining to this study must be kept on file by the investigator and are subject to FDA inspection at any time during the study. Periodic status reports must be submitted to the Institutional Review Board at least yearly, as well as notification of completion of the study and a final report within 3 months of study completion or termination. The investigator must maintain an accurate and complete record of all submissions made to the Institutional Review Board, including a list of all reports and documents submitted.

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