COVER PAGE

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Clinical study of the effect of combined treatment of aspirin and zileuton on biomarkers of tobacco-related carcinogenesis in current smokers

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SCHEMA

Clinical study of the effect of combined treatment of aspirin and zileuton on biomarkers of tobacco-related carcinogenesis in current smokers

Male and female current smokers with ≥ 20 pack years self-reported smoking history and an average use of ≥ 10 cigarettes/day

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Visit 1: Screening

Informed consent, clinical evaluation (medical history, vital signs, physical exam), clinical labs, conmeds and supplement use, Karnofsky performance status, tobacco use history.

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Washout

Minimum four-week washout of ASA, NSAIDs and leukotriene antagonist, if taken within the preceding 2 weeks

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Visit 2: Baseline Specimen Collection

Collection of nasal brushing (for gene expression analysis), urine (for PGE-M, LTE(4), and cotinine levels), blood (for plasma salicylate, ASA, zileuton, AA oxylipins), and buccal cells (as a reserved specimen for gene expression analysis and karyometric analysis).

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Randomization

1:1 to ASA 81 mg QD + zileuton (ZyfloCR) 2x 600 mg BID or placebos

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Intervention

ASA + Zileuton/placebos for 12 weeks.

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Visit 3: Wk 4 Interim Study Visit

Hepatic panel, adherence and AE evaluation, current tobacco use assessment.

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Visit 4: Wk 8 Interim Study Visit

Hepatic panel, adherence and AE evaluation, current tobacco use assessment.

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Visit 5: End-of-Intervention (week 12)

Clinical labs, adherence and AE evaluation, current tobacco use assessment, and collection of nasal brushing (for gene expression), urine (for PGE-M, LTE(4), cotinine), blood (for plasma salicylate, ASA, zileuton, AA oxylipins), and buccal cells (for banking and karyometric analysis).

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Visit 6: Follow up (week 14)

Hepatic panel, AE evaluation, and collection of nasal brushing (for gene expression), urine (for PGE-M, LTE(4)), blood (for plasma AA oxylipins), and buccal cells (for banking and karyometric analysis).

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Endpoints

Nasal epithelium gene expression
Urinary PGE-M and LTE(4)
Plasma AA oxylipins

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1. OBJECTIVES

1.1 Primary Objective

The primary objective of this study is to analyze the impact of combined treatment of ASA and zileuton on smoking-related gene expression signature in the nasal epithelium in current smokers and to analyze any difference between the ASA and zileuton intervention and placebo control.

1.2 Secondary Objectives

- To assess the impact of ASA and zileuton on three lung cancer gene signatures (an 80-gene bronchial signature, a PI3K pathway gene signature and a nasal diagnostic gene signature) and to compare this to placebo control.
- To determine whether the change in the smoking-related gene expression signature and the three lung cancer gene signatures of nasal epithelium persists 10-14 days off agent intervention.
- To measure urinary prostaglandin E metabolite (PGE-M) and leukotriene E(4) (LTE(4)) levels in current smokers after ASA and zileuton.
- To assess the safety in current smokers of 12 week exposure to ASA and zileuton.
- To evaluate a gender effect in the modulatory effects of ASA and zileuton on smoking-related-gene expression signature.
- To explore the effect of ASA and zileuton on the metabolomics profile of the arachidonic acid pathway.
- To explore, in a discovery-driven fashion, the effect of ASA and zileuton on whole-genome gene expression.
- To analyze the impact of ASA and Zileuton on karyometric analysis of buccal cells.

2. BACKGROUND

2.1 Lung Cancer

Lung cancer is the deadliest form of cancer in both men and women, accounting for 26% and 30% of all cancer –related deaths in U.S. women and men, respectively. Globally, lung cancer causes more than one million cancer deaths each year and is the leading cause of cancer-related mortality worldwide.[1] While prevention of tobacco use and tobacco cessation efforts remain the optimal means to prevent lung cancer, effective and well tolerated chemoprevention strategies for smokers and formers smokers who are at increased risk of developing lung cancer are urgently needed.

2.2 Study Agents

2.2.1 Aspirin as a Chemopreventive Agent

Aspirin (ASA) use has been associated with a reduced risk of a number of precancerous conditions and cancers; a meta-analysis of observational studies through 2011 reported the strongest favorable effect on risk of colorectal cancer (RR of 0.73) and other GI cancers (RR of 0.61), with greater support for risk reduction reported in case-control than in cohort studies. More modest risk reductions were reported for breast cancer (RR of 0.90) and prostate cancer (RR 0.90), while there was significant reduction in risk of lung cancer noted in case-control studies (RR 0.73) but not in cohort studies (RR 0.98).[2-4] An analysis of patient data from eight randomized trials of daily ASA versus no aspirin with a mean duration of ASA treatment of 4 years or longer reported a significant decrease in lung adenocarcinoma mortality associated with ASA use HR=0.68, 95% CI 0.50-0.92); no significant effect on mortality was noted for small cell and squamous cell lung carcinomas.[5] In

an analysis of a large cohort study (VITamin And Lifestyle study), total NSAID use (greater than 10 years) was associated with a borderline significant reduction in risk of lung cancer (HR 0.82; 95% C.I. 0.64-1.04), with the strongest association noted for adenocarcinoma (HR 0.59); this trend was limited to men (HR 0.66) and to long-term (≥ 10 years) former smokers (HR0.65). These trends did not differ for ASA (excluding low-dose ASA) versus total NSAID use.[6] This analysis, as well as an analysis of the Iowa Women's Health Study cohort which reported no significant trend for reduced lung cancer risk with ASA or non-ASA NSAID use (P_{trend} = 0.53, with no difference when analyzed by histologic subtype and smoking status), suggest a possible gender effect in the chemoprotective effect of ASA for lung cancer.[7] Proposed cellular/molecular mechanisms of action for the chemopreventive activity of ASA, which is an irreversible, non-selective COX-1 and -2 inhibitor, include inhibition of COX-2 and possibly COX-1, induction of tumor suppressor NAG-1, and other non-COX related induction of apoptosis and anti-angiogenic actions.[8-10]

Interest in ASA for lung cancer prevention also derives from the study of ASA in preclinical models of tobacco-and chemical-induced lung carcinogenesis. In a 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone (NNK) induced murine model of lung tumorigenesis, ASA inhibited tumorigenesis by 60%, and lowered PGE2 levels to basal levels.[11] In a 9,10-dimethylbenz(a)anthracene (DMBA) exposed murine model of lung carcinogenesis, ASA intervention decreased the incidence of lung tumors.[12] In contrast, ASA did not reduce incidence or multiplicity of lung tumors in tobacco smoke exposed A/J mice.[13]

2.2.2 Zileuton as a Chemopreventive Agent

Zileuton is a potent inhibitor of 5-lipoxygenase (5-LOX).[14, 15] It is FDA approved for the treatment of asthma based on the downstream suppression of leukotrienes (LTs) including LTB(4), LTC(4),LTD(4) and LTE(4), which are inflammatory mediators of asthma. 5-LOX and LTs appear to be relevant therapeutic targets for lung chemoprevention based on findings of higher levels of 5-LOX metabolites in a number of human solid tumors including lung cancer and leukemias, compared to normal tissues.[16] mRNA for 5-LOX and FLAP, which is required for activation of 5-LOX, is expressed in lung cancer cell lines and mRNA for 5-LOX is expressed in lung cancer tissues.[17] In preclinical studies, 5-LOX inhibitors including zileuton have inhibitory activity in a number of lung cancer models.[18, 18, 19] In an A/J mouse lung chemoprevention model using vinyl carbamate induction, the administration of zilueton 1200 mg/kg-diet (equivalent to a clinically relevant human dose) starting 2 weeks after carcinogen exposure caused a significant reduction in tumor multiplicity (24 % at 13 weeks and 28% at 43 weeks) and reduced the size of lung tumors. [20]

There are to date no epidemiologic studies showing that in asthmatics, who are at increased risk of lung cancer, long-term use of 5-LOX inhibitors have chemopreventive activity. Nonetheless, preclinical data cited above support the investigation of zileuton in the chemoprevention of lung carcinogenesis in high-risk individuals.

2.3 Rationale

The combination of ASA and zileuton may be additive or synergistic in inhibiting the arachidonic acid (AA) pathway of inflammatory mediators related to lung carcinogenesis. In an NNK-induced model of lung carcinogenesis, the combination of ASA with the 5-LOX inhibitor A-79175 was more effective than either drug alone, suggesting that concurrent inhibition of both 5-LOX and COX is more effective than inhibition of either pathway alone.[21] A study of the combination of zileuton and the selective COX-2 inhibitor celecoxib in smokers reported a significant reduction of the levels of PGE-M and LTE(4), suggesting that the combination resulted in inhibition of both LOX and COX pro-inflammatory pathways of AA metabolism. [22] The combination of celecoxib to zileuton led to a 62% reduction in PGE-M levels compared to an 18% reduction in PGE-M with zileuton alone; furthermore, the addition of celecoxib to zileuton did not affect the inhibition of LTE(4) by zileuton alone.[22]

We propose to evaluate the impact of combined treatment of ASA and zileuton on smoking-related gene-expression signature in nasal epithelium. Additionally, we intend to analyze the persistence of gene expression modulation by ASA in the continuous and intermittent ASA dosing arms by comparing the smoking-related gene expression signature sampled immediately after last dose ASA with that sampled after 10-14 days off ASA and zileuton or double placebo. Dr. Spira's group has identified a robust smoking-related signature in the bronchial epithelium of smokers in which genes involved in the regulation of oxidant stress, xenobiotic metabolism and oncogenesis are induced and genes involved in regulation of inflammation and tumor suppression are suppressed; this signature has been identified in the nasal epithelium, which appears to be a valid surrogate tissue for bronchial epithelium.[23, 24] The signature is composed of 119 genes whose expression is altered in both the bronchial and nasal epithelium of current versus never smokers. The first principal component from a principal component analysis across the 119 genes and nasal data from Zhang et al. [25] was used to compute a smoking signature score for each of the samples in Zhang et al. and in an independent cohort of 130 nasal samples from current and former smokers. The nasal smoking signature was able to significantly separate never or former smokers from current smokers ($p<0.01$, see Figure 1).

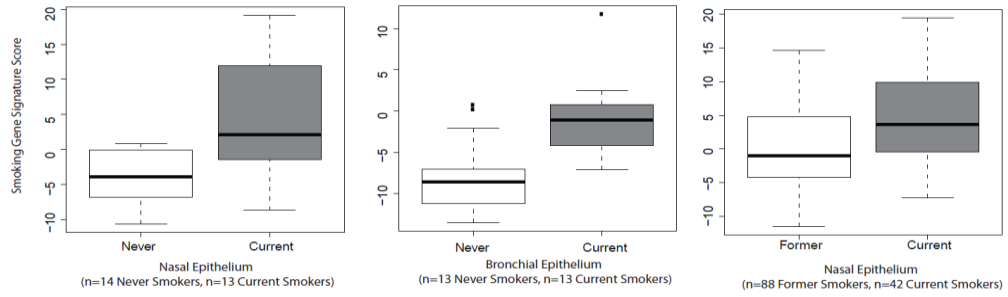

Figure 1. The smoking gene signature score separates never or former smokers from current smokers. The score is significantly different between the two groups in all three analyses by the Wilcoxon rank sum test ($p<0.01$).

Also of high interest is the effect of ASA and zileuton on three lung cancer gene signatures including an 80-gene signature from bronchial epithelium that distinguishes smokers with and without lung cancer[26], an early lung cancer signature derived from bronchial epithelium involving activation of the phosphatidylinositol 3-kinase (PI3K) pathway[27]; and a nasal gene signature derived from nasal epithelial brushing collected from smokers undergoing bronchoscopy for suspect lung cancer (personal communication). This latter gene signature can distinguish smokers diagnosed with lung cancer vs. those with alternate benign disease of the chest. The signature is enriched among genes that change with lung cancer in the bronchial airway from these same subjects. Dr. Spira's group is in the process of validating this nasal diagnostic gene expression signature in an independent test set.

Of additional interest is the impact of ASA and zileuton on whole-genome gene expression in nasal epithelium, which would involve a discovery driven approach looking at all genes on the array to discover those impacted by ASA and zileuton intervention.

We also plan to explore the effect of ASA and zileuton on inflammatory mediators derived from multiple AA metabolism pathways. It is recognized that there is complex crosstalk between the three branches of AA metabolism (COX, LOX, and cytochrome P450 (CYP)).[28] The inhibition of single or multiple branches may result in the shunting of AA metabolism through other branches. In an LPS-challenged murine model of inflammation, the selective inhibition of the COX pathway using ASA significantly reduced the production of PGE(2) and TXB(2) but also inhibited the CYP and LOX pathways as evidenced by decreased levels of DHETs (CYP pathway metabolites) and 5-HETE and 15-HETE (LOX pathway metabolites). In this model, the selective inhibition of the LOX pathway using the FLAP inhibitor MK 866 reduced the production of 5-HETE and 15-HETE but also reduced PGE(2) and TXB(2); notably, there was a dramatic increase in DHETs, indicating shunting of AA metabolism through the CYP pathway in the face of LOX inhibition.[28] In

a human study the administration of a COX-2 inhibitor caused shunting of AA metabolism into the pro-inflammatory LOX pathway in smokers with high baseline levels of urinary PGE-M.[29]

It is therefore of high interest to analyze the effect of ASA and zileuton intervention broadly on the AA oxylipidome, given the potential for shunting of AA metabolism. This is of particular interest given that metabolites of AA, individually and in concert, have distinct anti-and pro-inflammatory effects and importantly, different effects (adverse or favorable) on organ systems including the cardiovascular system.[30] The importance of comprehensive profiling of AA metabolism in evaluating effects of AA inhibitors on organ systems was shown by a study of oxylipin profiling in a murine model administered rofecoxib, a selective COX-2 inhibitor that has been associated with adverse cardiovascular events and stroke in humans.[31] Treated mice had dramatically decreased bleeding time, reflecting increased platelet aggregation; plasma metabolomic profiling of 27 oxylipins showed a >120-fold increase in 20-HETE in treated mice. Direct infusion of 20-HETE resulted in decreased BT in mice, implicating 20-HETE as a possible mediator of increased platelet aggregation by rofecoxib in this model and suggests its role as a biomarker of risk for CV events with COX-2 inhibition.[32] A comprehensive analysis of AA metabolism can be done by measuring the effect of an intervention on the oxylipin metabolome as measured by ultra- high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS).[33] An analysis of oxylipin profiling in bronchioalveolar lavage fluid of asthmatics and healthy normals exposed to inhaled particulates showed differences between the groups at baseline in 9 oxylipins, and delineated a differential response to the exposure.[34] A study of metabolomic profiling using LC-MS/MS analysis to assess the effect of a single dose of ASA on eiconasoids in 6 healthy volunteers showed a significant decrease in plasma concentration of TXB(2).[35] We propose to measure the effect of ASA and zileuton on targeted urinary AA metabolites, PGE-M and LTE(4), biomarkers of the COX and proinflammatory 5-LO pathway[36], respectively. Both PGE-M and LTE(4) are at higher basal levels in current smokers compared to never smokers.[29] Additionally, we propose to analyze the effect of ASA and zileuton on a comprehensive panel of AA metabolites emanating from COX, LOX and CYP-mediated metabolism. [33]

We will assess the safety profile of ASA given in combination with zileuton. We will collect and store buccal cells pre- and post- study intervention (end of intervention and 10-14 days post intervention) for biobanking and for karyometric analysis of nuclear chromatin in order to quantitatively measure changes in nuclear abnormality with study intervention. We will compare any differences in reduction of nuclear abnormalities between study intervention arms. We will randomize an equal number of men and women to each study arm in order to assess in a preliminary fashion a possible gender effect in the modulatory effects of ASA and zileuton on biomarkers of tobacco exposure.

3. SUMMARY OF STUDY PLAN

We propose to conduct a randomized, placebo-controlled study of 3-month ASA and zileuton in current smokers. We plan to accrue 66 eligible participants (33 per study arm, 18 men and 15 women per arm). With an estimated attrition rate of 38.5% for men and 26.7% for women (with respect to endpoint data), we expect to have at least 20 participants per group with evaluable endpoint data. Women and men will each comprise half of the study population in order to randomize women and men in equal numbers to the study arms. Planned accrual is 3-4 participants per month.

Participants will be healthy men and women age ≥18 years who are current smokers with ≥ 20 pack years smoking history and an average use of ≥10 cigarettes/day. Participants will be excluded if they have an allergy to aspirin or similar agents, including NSAIDs; have gastric intolerance attributable to ASA or NSAIDs; have a history of gastric ulcer within the last 5 years; have used ASA or NSAIDs for more than 5 days per month within 3 months of enrollment; are unwilling or unable to refrain from use of any non-study ASA or NSAID during the study period; have adult asthma; are currently using or have recently or chronically used (within the past 3 months) leukotriene antagonists; require chronic anticoagulation or anti-

platelet therapy; have a history of a bleeding disorder or hemorrhagic stroke; have asthma or other condition requiring use of a leukotriene antagonist; are currently using or have recently or chronically used glucocorticoids (systemic, topical and/or nasal sprays); have a history of chronic sinusitis or recent nasal polyps; are unwilling or unable to limit alcohol consumption during the study period; are pregnant or lactating women; are receiving other investigational agents; have uncontrolled intercurrent illness; have a known history of inability to absorb an oral agent; have invasive cancer within the past five years except non-melanoma skin cancer; have abnormal hematologic, renal and hepatic function; or are taking drugs known to interact with zileuton, including theophylline, warfarin, and propranolol. Compliance will be assessed by pill count, intake diary, and plasma salicylate, ASA and zileuton levels. Current smoker status will be assessed by urinary cotinine levels.

Screening Visit
The IRB-approved consent form will be reviewed and participants will sign informed consent. Participants will undergo a screening physical assessment (vital signs, brief physical exam), concurrent medication and supplement use, tobacco use history, recording of past medical history, and laboratory analysis with complete blood count (CBC), comprehensive metabolic panel (CMP) and PT/PTT. Women of childbearing capacity will have a urine pregnancy test. Urine may be collected at the screening visit for cotinine level to establish smoking status, at the discretion of the Principal Investigator.

12-Week Intervention Period
Eligible participants will be asked to refrain from use of any non-study ASA or NSAIDs during the intervention period. Participants who have taken ASA or NSAIDs within the preceding 2 weeks will have a 4-week washout period before receiving study treatment. Participants will undergo baseline specimen collection for the collection of nasal brushing (for gene expression analysis), urine (for PGE-M, LTE(4), and cotinine levels), blood (for plasma salicylate, ASA and zileuton levels and for AA oxylipin profiling), and buccal cells (as a reserved specimen for gene expression analysis and karyometric analysis). If urine cotinine level was collected at the screening visit, it will serve as the baseline and will not be repeated at baseline visit. Following baseline specimen collection, participants will be randomized to receive the combined treatment of ASA (81 mg QD) and zileuton (Zyflo CR) two 600 mg extended release tablets BID or placebo pills for 12 weeks. Two interim study visits will be conducted, at 4 and 8 weeks, for hepatic function testing, compliance check, AE review and a current tobacco use assessment. Following agent intervention, an end-of-intervention visit will be conducted for safety labs (CBC and CMP), compliance check, a current tobacco use assessment, and collection of nasal brushing, urine, blood, and buccal cells.

Post-intervention Follow-up Period
Study subjects will be instructed to continue to record adverse events and concomitant meds and will return for a post-intervention visit in 10-14 days where the AE and concomitant meds diaries will be collected, AEs will be assessed and study participants will have a hepatic panel done. A collection of nasal brushing, urine, blood, and buccal cells for evaluation of the persistence of the biomarker changes will be done. Participants will be provided with information on how to contact the Arizona Smokers' Helpline (toll free telephone number and URL to access the ASHLine website), which provides comprehensive services for tobacco cessation.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Male or female current tobacco smokers with ≥ 20 pack years of self-reported smoking exposure and an average use of ≥ 10 cigarettes/day.

4.1.2 Age ≥ 18 years.

4.1.3 Karnofsky ≥70%; (see Appendix A)

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

Leukocytes	≥3,000/microliter
Absolute neutrophil count	≥1,500/microliter
Hematocrit	≥ the lower institutional limit
Platelets	≥ the lower institutional limit
Total bilirubin	within normal institutional limits
AST (SGOT)/ALT (SGPT)	within normal institutional limits
Creatinine	≤ the upper institutional limits
PT/PTT	within normal institutional limits

4.1.5 Fertile subjects must use adequate contraception (abstinence, barrier methods, or birth control pills) prior to study entry and for the duration of study participation. The effects of aspirin and zileuton on the developing human fetus at the recommended therapeutic dose are unknown. For this reason women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.1.6 Participants may have a history of indeterminate pulmonary nodule(s) by chest imaging if nodule follow-up has been completed or the study procedures would not interfere with nodule follow-up.

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

4.2 Exclusion Criteria

4.2.1 History of allergic reaction to aspirin or attributed to compounds of similar chemical or biologic composition to aspirin, including other NSAIDs.

4.2.2 Gastric intolerance attributable to ASA or NSAIDs.

4.2.3 History of gastric ulcer within the past 5 years (with or without bleeding).

4.2.4 Use of ASA or NSAIDs for more than 5 days per month within 3 months of enrollment.

4.2.5 Not willing or are unable to refrain from use of any non-study ASA, NSAIDs and leukotriene antagonists during the study period.

4.2.6 Adult asthma

4.2.7 Chronic, current or recent (within the past three months) use of leukotriene antagonists.

4.2.8 Require chronic anticoagulation or anti-platelet therapy.

4.2.9 History of bleeding disorder or hemorrhagic stroke.

4.2.10 Chronic, current or recent (within the past three months) use of leukotriene antagonists.

4.2.11 Chronic, current or recent (within the past three months) use of glucocorticoids (systemic, topical and/or nasal sprays or steroid topical creams to large body surface area). Use

of steroid topical creams for small body areas (≤10% body surface) during study intervention is allowed.

4.2.12 History of chronic sinusitis or recent nasal polyps.

4.2.13 History of, or current, active or chronic liver disease even if transaminases have normalized.

4.2.14 History of allergic reaction to zileuton or attributed to compounds of similar chemical or biologic composition to zileuton.

4.2.15 Are taking drugs known to interact with zileuton, including theophylline, warfarin, and propranolol.

4.2.16 Not willing or are unable to limit alcohol consumption to ≤ 2 alcoholic beverages a day during the study period.

4.2.17 Pregnant or lactating women. Pregnant women are excluded from this study because aspirin and zileuton have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with aspirin, breastfeeding should be discontinued if the mother is treated with aspirin. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.2.18 Participants may not be receiving any other investigational agents.

4.2.19 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.20 Have a known history of inability to absorb an oral agent.

4.2.21 Invasive cancer within the past five years except non-melanoma skin cancer.

4.2.22 Urine cotinine level, if collected at screening, does not confirm active smoking status.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial. The University of Arizona Cancer Center has faculty and staff who are dedicated to conduct outreach into minority populations. There resources will be available to this recruitment effort.

4.4 Recruitment and Retention Plan

Study participants will be recruited from the greater Tucson area by word-of-mouth, advertisement in local newspapers and flyers posted around campus and in gathering places of minority populations, and through use of the local Craigslist and social media. Study personnel will promote retention/adherence with regular contact with study subjects. The study team is committed to provide a friendly and comfortable study setting for participants from initial contact through the completion of their activities. Wherever possible, flexibility will be built into the study schedule to promote compliance. Recruitment and retention efforts will be evaluated routinely by the study personnel and modified as necessary to promote rapid accrual and to assure 100% retention of participants.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported adverse events (AEs) and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

- The study agents are aspirin and zileuton.
- Participants will be randomly assigned to one of the following treatment groups:
    - Aspirin 81 mg QD + zileuton (Zyflo CR) 2 x 600 mg BID
    - Double placebo (Aspirin placebo 1 cap QD + Zileuton placebo 2 tabs BID)
- Duration of treatment is 12 weeks.

5.2 Study Agent Administration

- Participants will receive a supply of ASA + zileuton or double placebo from the study coordinator.
- Participants will be instructed to take one capsule of ASA/placebo QD and 2 tablets Zileuton/placebo BID.
- Participants will be instructed to take the study agents at the same time each day.
- Participants will be instructed to take the ASA/placebo in the morning with 8 oz. of water and with food to lessen stomach irritation.
- Participants will be instructed to take the zileuton/placebo within one hour after the morning and evening meals. The ASA/placebo and morning dose of zileuton/placebo may be taken simultaneously.

5.3 Wash-out Procedures

Participants who are using ASA or other NSAIDs within the preceding 2 weeks will undergo a minimum of 4 weeks washout period prior to receiving study treatment.

5.4 Contraindications

Participants should refrain from using more than 2 alcoholic beverages per day for the duration of the trial.

5.5 Concomitant Medications

Participants are required to refrain from taking non-study aspirin or other NSAIDs for the duration of the trial. Participants are required to refrain from taking non-study zileuton or other leukotriene antagonists. Participants are required to refrain from the use of anti-coagulant or anti-platelet medications for the duration of the trial. Participants are required to refrain from the use of medications that are known to interact with zileuton, including theophylline, warfarin, and propranolol. Participants should refrain from the regular use of glucocorticoids (systemic, topical or nasal) or OTC nasal sprays.

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included.

5.6 Dose Modification

For participants who demonstrate Grade 1 dyspepsia, a proton pump inhibitor (PPI) will be instituted promptly for relief of discomfort and subject may continue taking study drugs. For Grade 2 dyspepsia lasting for more than three days despite use of PPI, both study drugs will be held. If symptoms resolve within 2 weeks, the subject may resume study drugs at the same dose while using the PPI (PPI can be used at BID). For a second Grade 2 dyspepsia despite use of PPI, both study drugs will be permanently withdrawn and the participant followed for resolution of the adverse event(s). For any evidence of GI bleeding, the study drugs will be stopped promptly and the participant followed for resolution of the adverse event(s).

For other Grade 1 adverse events, no dose modification will be made. For other Grade 2 adverse events definitely, probably or possibly related to study drugs persisting for more than three days, both study drugs will be held. Both study drugs will be reintroduced at the same dose if the adverse event resolves to Grade 1 or below within 2 weeks. If the Grade 2 event does not resolve to Grade 1 or below within 2 weeks, both study drugs will be permanently withdrawn and the participant followed for resolution of the adverse event. If the Grade 2 adverse event recurs following reinstitution of the study agents and is considered definitely, probably or possibly related to study drugs, both study drugs will be permanently withdrawn and the participant followed for resolution of the adverse event(s). If the Grade 2 adverse event is not considered related to study drugs, both study drugs may be held and reintroduced within two weeks at the discretion of the study physician.

For any Grade 3 adverse events regardless of relationship to study drugs, both study drugs will be held. For Grade 3 events unrelated to study drugs, both study drugs may be resumed within two weeks at the discretion of the study physician. Grade 3 events possibly or probably related to study drugs, and Grade 4 events regardless of relationship to study drugs, will result in permanent withdrawal of both study drugs. Participants will be followed for resolution of adverse events. Where possible and appropriate, participants withdrawn early will be encouraged to return for sample collection and clinical labs for safety.

5.7 Adherence/Compliance

5.7.1 Participants will be considered compliant for statistical analysis if they have taken ≥ 80% of their assigned study doses based on count of return pills.

5.7.2 The primary measure of compliance includes pill count. The secondary measure of compliance will be the Intake Calendar. Plasma levels of study agents and metabolites can also be used to confirm compliance.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agents

6.1.1 Aspirin

A marketed 81 mg aspirin capsule will be used in this study. Aspirin is an odorless, white, needle-like crystalline or powdery substance. When exposed to moisture, aspirin hydrolyzes into salicylic and acetic acids, and gives off a vinegary odor. It is highly lipid soluble and slightly soluble in water. The capsule contains 81 mg aspirin and the following excipients: carnauba wax, cellulose, D&C Yellow #10 Aluminum Lake, FD&C Yellow #6 Aluminum Lake, hypromellose, iron oxides, methacrylic acid copolymer, polysorbate 80, propylene glycol, shellac, sodium lauryl sulfate, starch, titanium dioxide, triacetin.

6.1.2 Zileuton (IND #64362, IND Sponsor: NCI, DCP)

Zileuton (±-1-(1-benzo[b]thien-2-ylethyl-1-hydroxyurea) is an FDA-approved orally active drug indicated for the prophylaxis and long-term treatment of asthma in adults and children aged 12 years or older. It inhibits 5-lipoxygenase (5-LOX) and production of pro-inflammatory leukotrienes LTB_4, LTC_4, LTD_4, and LTE_4, producing moderate airway function improvement. Both the *R*(+) and *S*(–) enantiomers in the racemic mixture are pharmacologically active inhibitors *in vitro* and *in vivo*. The zileuton immediate-release tablet formulation (Zyflo R Filmtabs) was discontinued in March 2008. An improved extended-release formulation of zileuton (Zyflo CR) was approved by FDA in 2007 [Cornerstone Therapeutics, Inc., 2011]. The recommended dose in adults is 2 x 600 mg bid, taken one hour after morning and evening meals.

Zileuton (Zyflo CR™) 600 mg extended-release tablets are triple-layered tablets comprised of an immediate-release layer, a middle (barrier) layer, and an extended-release layer. Each tablet contains 600 mg zileuton and the following inactive ingredients: crospovidone, ferric oxide, glyceryl behenate, hydroxypropyl cellulose, hypromellose, magnesium stearate, mannitol, microcrystalline cellulose, povidone, pregelatinized starch, propylene glycol, sodium starch glycolate, and talc.

6.1.3 Combination of Aspirin and Zileuton

No IND is required for this combination of marketed drugs.

6.2 Reported Adverse Events and Potential Risks

6.2.1 Aspirin

Aspirin has been associated with gastrointestinal (GI) side effects. GI side effects include stomach pain, heartburn, nausea, vomiting, and gross GI bleeding. Minor upper GI symptoms, such as dyspepsia, are common and can occur anytime during therapy. Aspirin has also been associated with elevated hepatic enzymes, blood urea nitrogen and serum creatinine, hyperkalemia, proteinuria, and prolonged bleeding time.

6.2.2 Zileuton

Zyflo CR™ has been evaluated in two safety studies. In a 12-week study in patients with asthma receiving 1200 mg bid (n=199) or placebo (n=198), the most frequently reported adverse effects (AEs) were sinusitis (6.5%), nausea (5%), and pharyngolaryngeal pain (5%).[37, 38] Less common side effects in <1% of treated patients included GI disorders (upper abdominal pain, diarrhea, dyspepsia, vomiting), rash, hypersensitivity, and hepatotoxicity. In the zileuton group, elevated alanine aminotransferase (ALT) levels three times the normal limits occurred in 2.5% (5 of 199) of patients compared to 0.5% (1 of 198) in the placebo group. Most (60%) ALT elevations were reported in the first month of treatment; however, increased ALT was detected 14 days after completing three-months of zileuton treatment in two of the five patients.

Comparable AEs were reported in over 900 patients given 1200 mg Zyflo CR™ bid (n=619) or placebo (n=307) for six months.[37, 39] Other reported side effects included headache, upper respiratory tract infection, myalgia, and diarrhea. Incidences of low white blood cell (WBC) counts were reported in 2.6% (15 of 619) of zileuton-treated patients and in 1.7% (5 of 307) of placebo-treated patients; all WBC levels returned to normal or baseline upon discontinuation of treatment. Increased liver ALT levels were observed in 1.8% of zileuton-treated patients *versus* 0.7% in placebo. As in the 12-week study, the majority (82%) of ALT elevations occurred within the first three months of treatment and resolved within 21 days of zileuton discontinuation. No cases of jaundice, chronic liver disease, or death were reported due to hepatotoxicity from Zyflo CR™. In

contrast, several cases of death and life-threatening hepatic injury, hyperbilirubinemia, jaundice, and increases in ALT greater than eight times the upper limit of normal were reported in patients taking the discontinued zileuton immediate-release tablet formulation (Zyflo R) during the post-marketing period. [37] Since zileuton is contraindicated in subjects with active or acute liver disease, and in those who consume large quantities of alcohol, liver enzyme function tests will be performed prior to randomization in this study.

Zileuton has been shown to decrease the clearance of theophylline and antipyrine, possibly by inhibiting hepatic CYP1A2.[37] Likewise, concomitant administration of zileuton and warfarin in normal male adults increased warfarin plasma concentrations and increased prothrombin times; therefore, careful monitoring of prothrombin times is recommended with concurrent therapy. Co-administration of zileuton with propranolol significantly increased propranolol concentrations and increased β-blockade, producing a reduction in heart rate. Such patients require careful monitoring; a dose reduction of propranolol may also be required. Whether zileuton interacts with other β-adrenergic blocking drugs is unknown; no formal studies have been carried out to date.

No significant drug interactions occurred in healthy subjects given zileuton immediate-release tablets (Zyflo R) and naproxen, digoxin, phenytoin, or sulfasalazine.[37] Likewise, no drug interactions were observed in studies of healthy volunteers administered zileuton immediate-release tablets concurrently with ethinyl estradiol or prednisone, drugs known to be metabolized by the CYP3A4 isozyme.

6.2.3 Combination of Aspirin and Zileuton

No combination studies of aspirin and zileuton have been reported. Co-administration of aspirin (294 mg/kg-diet) with A-79175 (75 mg/kg diet), a 5-LOX inhibitor structurally related to zileuton, did not produce any gross pathological changes in the liver, kidney, stomach, intestines, or lungs of mice following 25 weeks of treatment.[11]

There are no published human safety data concerning the concomitant use of aspirin and zileuton in healthy individuals, although no pharmacokinetic or pharmacodynamic interactions were observed in a published study of 24 healthy volunteers co-administered the mixed COX-1/COX-2 inhibitor naproxen (500 mg) and zileuton (800 mg).[40] The drug combination had no effect on plasma concentration-time curves when compared with either agent given alone. Moreover, there was no evidence the drug combination altered serum LTB_4 or thromboxane B_2 levels beyond that observed with either naproxen or zileuton alone. Blockade of 5-LOX did not produce additive GI AEs typically associated with naproxen administration. This is in agreement with reports of reduced GI toxicity associated with dual COX/5-LOX inhibitors such as licofelone.[41, 42] Given these data, the risk of GI toxicity resulting from the combined administration of aspirin and zileuton appears to be low. The possibility of additive hepatic toxicity from the combined use of aspirin and zileuton cannot be completely ruled out since both drugs are metabolized by the liver.

6.3 Availability

Aspirin 81 mg and placebos for both aspirin and zileuton will be supplied to investigators by NCI, DCP. Zileuton will be ordered by the institutional pharmacy.

6.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or their authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form (NIH-986) (to include complete shipping contact information) and faxing or mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street

North Kansas City, MO 64116
Phone: (816) 360-3805
FAX: (816) 753-5359
Email: NCI.DCP@mriglobal.org
Emergency Telephone: (816) 360-3800

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF). The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to the University of Arizona Cancer Center (UACC) Research Pharmacy Staff. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.

6.6 Packaging and Labeling

Aspirin, aspirin placebo and zileuton placebo will be packaged and shipped by the NCI, DCP drug repository to the UACC research pharmacy. Zileuton will be ordered by the UACC research pharmacy who will re-package the zileuton in empty bottles supplied by DCP drug repository. Lot numbers will be captured in the research pharmacy's repackaging records with the participant ID and randomization number for traceability.

6.7 Storage

Study agents will be stored in a secure location at temperatures between 15°C and 30°C (59°F and 86°F).

6.8 Registration/Randomization

Participants will be randomized to receive ASA 81 mg QD + zileuton (Zyflo CR™) 2 x 600 mg BID or double placebo for three months.

Participants will be considered registered on the date they sign the approved informed consent document with a member of the study staff. The study coordinator will contact the UAZ Consortium Office for a subject number when the subject has been consented. See section 13.2 for details of the study randomization procedures.

6.9 Blinding and Unblinding Methods

The blinding and unblinding methods will follow a double-blind study design although this is a single-blind study because the zileuton placebo is not completely matched to zileuton. The study products will not be identified by product names but by a unique randomization number. The randomization number is assigned to the subject upon completion of eligibility evaluation.

A list of non-sequential randomization numbers linked to each study arm will be created and forwarded to the DCP drug repository and the UACC research pharmacy. The DCP repository will ship the packaged aspirin, aspirin placebo, zileuton placebo, and bottles for repackaging zileuton to the UACC research pharmacy by randomization number. The UACC research pharmacy will order and repackage zileuton, as needed. The study products will be identified with the subject randomization number but with no product information on the label. Participant ID stickers will be supplied by the DCP drug repository to the UACC research pharmacy who will apply the clinical labels when a participant is randomized.

The UACC research pharmacy will dispense the product to the study staff for distribution to participants based on the assigned randomization number. Study staff will submit the returned bottles to the UACC research pharmacy for pill count. None of the staff interacting with participants will know the link between randomization number and actual product. This process will allow the study to remain blinded to all study personnel and participants. The code that identifies the product will be kept by the UAZ Consortium Biometry Director, the study statistician or the data manager.

Unblinding is not expected to occur until all participants complete the intervention and data entry is complete. Study agents may be unblinded by the Principal Investigator or, in her absence, a Co-Investigator after discussion with the NCI medical monitor, if possible, in the event of a serious adverse event if deemed medically necessary. If the NCI medical monitor is not available and unblinding is deemed necessary, it should be done and the medical monitor can be notified subsequently. The investigator will notify the UAZ Consortium Biometry Director, the study statistician or the data manager that the blind is to be broken.

The NCI, DCP Medical Monitor and/or Scientific Monitor must be notified that the blind has been broken.

DCP Medical Monitor:
Malgorzata (Margaret) Wojtowicz, M.D.
Lung & Upper Aerodigestive Cancer Research Group
Division of Cancer Prevention, NCI, NIH
9609 Medical Center Drive, Rm 5E-104, MSC 9781
Bethesda, MD 20892 (For FedEx, use Rockville, MD 20850
Phone: (240) 276-7012
Fax: (240) 276-7848
email: wojtowim@mail.nih.gov

6.10 Agent Destruction/Disposal

At the completion of investigation, the UACC research pharmacy will destroy and dispose of the study agent per standard operating procedures.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Procedure/ Evaluation	Pre Intervention		Intervention[2]				
	Visit 1 Screening	Washout[1]	Visit 2 Baseline Specimen Collection/ Randomization /Intervention[7]	Visit 3 Wk 4 Interim Study Visit	Visit 4 Wk 8 Interim Study Visit	Visit 5 Wk 12 End-of-Intervention	Visit 6 Wk 14 Follow-Up
Informed Consent	X						
Eligibility assessment	X						
Medical history	X						
CBC-diff, CMP[3], PT/PTT[6]	X					X	
Hepatic panel[8]				X	X		X
Vitals (height, weight, temp, BP, heart rate)	X[4]		X	X	X	X	
Physical Exam	X						
Karnofsky performance status	X						
Nasal brushing			X			X	X
Buccal sampling			X			X	X
Smoking history	X						
Current tobacco assessment	X		X	X	X	X	
Cessation of non-study ASA/NSAIDs		X	X	X	X	X	
Urine pregnancy test[5]	X		X	X	X	X	
Blood for plasma salicylate/ASA/zileuton/AA oxylipins			X			X	X[10]
Urine for PGE-M, LTE(4) & cotinine level[9]			X			X	X[11]
Concomitant Medications	X		X	X	X	X	X
Dispense Study Agent			X	X	X		
Collect Study Agent				X	X	X	
Review Agent Diary/Record				X	X	X	
Adverse Events			X	X	X	X	X
Telephone/email Contact		X					

[1] Wash out period will be a minimum of 4 weeks if subject has taken ASA, NSAIDs or leukotriene antagonists within the preceding 2 weeks.
[2]Agent intervention to be 12 (± 1) weeks.
[3]CMP includes serum glucose, urea nitrogen, creatinine, sodium, potassium, chloride, bicarbonate, total protein, albumin, calcium, alkaline phosphatase, ALT, AST, total bilirubin. Calculations included with the CMP are: BUN/creatinine ratio, anion, gap, globulin, alb/glob ratio.
[4] Height required at Visit 1 only
[5]For women of childbearing potential
[6]Screening visit only.
[7]Randomization to occur within 30 days of consenting unless significant scheduling problems arise.
[8]Hepatic panel includes total protein, albumin, globulin, alb/glob ratio, alkaline phosphatase, ALT, AST, total bilirubin, direct bilirubin.
[9]Urine may be collected for a cotinine level at the screening visit to establish smoking status at the discretion of the Principal Investigator. If collected at the screening visit, it will serve as the baseline and will not be repeated at baseline visit.
[10] AA oxylipins only.
[11]PGE-M and LTE(4) only.
[12] Week 14 follow-up visit may be performed 10-14 days following end of intervention.

7.2 Prestudy Evaluation/Screening

Potential participants will undergo a brief interview to determine initial eligibility. During this interview, the study procedures and risks will be reviewed. Those who are interested in participating in the study and have been determined initially eligible will be scheduled for a clinic visit.

At the initial clinic visit (Visit 1, Screening Visit), the IRB-approved consent form will be reviewed with each potential study subject. All participants will be required to read and sign the consent form prior to enrollment. Once informed consent is obtained from provisionally eligible participants, they will be entered into the study and subjected to the following procedures:

A complete medical history of each subject including prior major illnesses, ongoing medical conditions, and ongoing medications will be obtained at baseline. Participants who are found to have any of the illnesses or use medications listed in the Exclusion Criteria (section 4.2) will be ineligible for the study.

All participants will undergo a brief physical exam at baseline to obtain height, weight, blood pressure, pulse, temperature measurements, a brief physical exam consisting of an examination of the heart, lungs and abdomen, and Karnofsky performance status assessment.

Blood samples will be collected for complete blood count (CBC) with differential, comprehensive metabolic panel (CMP), and PT/PTT. These blood tests are required to determine subject eligibility and for safety monitoring. Women of childbearing potential will have a urine pregnancy test. Concurrent medications, supplement use, and tobacco use will also be collected. Urine may be collected at the screening visit for cotinine level to establish smoking status, at the discretion of the Principal Investigator.

Once determined eligible, study participants who have taken ASA or NSAIDs within the preceding 2 weeks will undergo a washout period (minimum of 4 weeks) where no further ASA, NSAIDs or leukotriene antagonists will be allowed. Study participants will be provided with a daily diary for recording any adverse events and an intake calendar for recording medication usage.

7.3 Evaluation During Study Intervention

Study participants will return to the clinic for baseline specimen collection (Visit 2). During the visit, weight, blood pressure, pulse, temperature will be measured. A blood sample will be collected for plasma salicylate, ASA, zileuton levels and AA oxylipin analysis. Urine will be collected for PGE-M, LTE(4), and cotinine levels. If urine cotinine level was collected at the screening visit, it will serve as the baseline and will not be repeated at baseline visit. Participants will undergo a baseline nasal epithelium collection via cytology brushing of the nasal turbinate (see Section 7.6) for gene expression analysis. Buccal cells will also be collected (see Section 7.6) and archived as a reserved specimen for gene expression analysis and karyometric analysis. Women of childbearing potential will have urine pregnancy tests prior to receiving study agent at Visits 1 and 2 and also at Visits 3, 4, and 5.

Post baseline specimen collection, participants will be randomized to receive ASA 81 mg daily + Zileuton (Zyflo) 2 x 600 mg BID or placebos for 3 months. Participants will be supplied with a 6-week supply of study agents (to ensure sufficient agent supply until the interim visit), a study agents intake diary and AE diary.

Participants will return for two interim study visits (Visit 3, after 4±1 weeks of agent intervention and Visit 4, after 8±1 weeks of agent intervention). These visits will include vital signs, blood for hepatic panel, check of study agent intake, and a current tobacco use assessment. Concomitant medications and AEs will be

reviewed. Participants will return any unused study agents and receive the next 6-week supply of study agents.

7.4 Evaluation at Completion of Study Intervention

Participants will take study agents for 12 (± 1) weeks and return for the end-of-intervention visit (Visit 5). Participants will take the last dose of the study agents the morning of the end-of-intervention visit. When feasible, this visit will be scheduled between 4-6 hours after the last morning dose of ASA to allow for assessment of plasma ASA concentrations. At this visit, participants will return unused study agents. Evaluations will include vital signs, CBC and CMP panel, check of study agents intake, a current tobacco use assessment, and collection of nasal brushing (for gene expression), urine (for PGE-M, LTE(4), cotinine), blood (for plasma salicylate, ASA, zileuton and AA oxylipin analysis), and buccal cells (for banking and karyometric analysis). Concomitant medications and AEs will be assessed. The study agent diary will be collected.

7.5 Post-intervention Follow-up Period

Study participants will be instructed to continue to record adverse events and concomitant meds and return for a post-intervention follow-up visit (Visit 6) 10-14 days after the end of intervention visit. The AE and concomitant meds diaries will be collected, AEs will be assessed and study participants will have a hepatic panel done. A collection of nasal brushing (for gene expression), urine (for PGE-M, LTE(4)), blood (for plasma AA oxylipin analysis), and buccal cells (for banking and karyometric analysis) will be done.

7.6 Methods for Clinical Procedures

Nasal epithelium collection: A nasal speculum will be used to spread one of the nares while a standard cytology brush will be inserted underneath the inferior nasal turbinate. The brush will be rotated in place for 3 seconds, removed, and immediately placed in 1 ml of RNAProtect Cell solution. The same nare will be brushed again with another cytology brush as described above and immediately placed in RNAProtect Cell solution.

Buccal cell collection: The subject's mouth will be inspected for oral lesions so these areas can be avoided during the collection of the buccal cells. Participants will be instructed to rinse their mouths with tap water for 10 seconds before collection. First, a cytology brush will be used on one cheek using a twirling motion while moving the brush downward while applying counter pressure with their fingers against the external cheek; this will be done only once. Additionally, a cheek scraper will be rubbed in a firm fashion 5-10 times while applying counter pressure on the external cheek; this will be done 5 times using 5 individual scrapers on alternating cheeks as possible. The scrapers will be gently rinsed in RNAProtect Cell solution with the final scraper tip left in the RNAProtect Cell solution vial.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary endpoint is to compare changes in a smoking-related gene expression signature of nasal epithelium after ASA + Zileuton versus placebo.

8.2 Secondary Endpoints

The secondary endpoints are:

- To assess the impact of ASA and zileuton on three lung cancer gene signatures (an 80-gene bronchial signature, a PI3K pathway gene signature and a nasal diagnostic gene signature) and to compare this to placebo control.
- To determine whether the change in the smoking-related gene expression signature and the three lung cancer gene signatures of nasal epithelium persists 10-14 days off agent intervention.
- To measure urinary PGE-M and LTE(4) levels in current smokers after ASA and zileuton.
- To assess the safety in current smokers of 12 week exposure to ASA and zileuton.
- To evaluate a gender effect in the modulatory effects of ASA and zileuton on smoking-related gene expression signature.
- To explore the effect of ASA and zileuton on the metabolomics profile of the arachidonic acid pathway.
- To explore, in a discovery-driven fashion, the effect of ASA and zileuton on whole-genome gene expression.
- To analyze the impact of ASA and Zileuton on karyometric analysis of buccal cells.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, or medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure), pregnancy.

8.5 Study Termination

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

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

In this proposal we have chosen to measure nasal gene expression using Affymetrix Human Gene 1.0 ST microarrays. The microarray technology is ideal for this proposal because of its cost, ability to estimate gene expression for over ~19,000 genes, previously demonstrated successful use in measuring nasal gene expression.[22] and ease of data analysis. The ability to measure additional genes not included in the smoking biomarker is advantageous because it will allow us to look at gene expression alterations associated with ASA and zileuton that are not part of the biomarker. Thus, we have chosen to use microarrays because they are cheaper than next-generation transcriptome sequencing, provide more information that qPCR, and allow us to assess the smoking biomarker in the proposed study using the same platform in which it was developed.

Urinary PGE-M and LTE(4) will be measured by sensitive and specific liquid chromatography-tandem mass spectrometry assays.[43,29] These assays have been applied to measure urinary PGE-M and LTE(4) levels in smokers.

Urinary cotinine levels will be determined using a commercially available ELISA (GenWay Biotech). This assay kit is a competitive immunoassay designed for the measurement of cotinine in human urine samples.

Plasma concentration of salicylate and ASA will be analyzed by a published high performance liquid chromatography assay.[44] This assay has been applied to determine the concentration time course of ASA and salicylate following oral administration of 100-500 mg ASA.

A comprehensive panel of AA metabolites will be analyzed by a LC/MS/MS based method developed in Dr. Bruce Hammock's laboratory.[33] Currently, about 87 metabolites of arachidonate and linoleate metabolism emanating from COX, LOX, and CYP dependent metabolism can be routinely measured in serum, BALF and urine samples.[30, 28,34,45]

9.2 Comparable Methods

The methods proposed to collect nasal epithelial cells and to measure nasal gene expression have been published previously.[22] The methods proposed for microarray data analysis are widely published and used, and we expect the data generated in this study will be able to be compared to existing microarray data. Comparing data and establishing relationships between microarray datasets is very common and there are hundreds of manuscripts in Pubmed demonstrating this.

The methods for urinary PGE-M, LTE(4), cotinine and plasma salicylate and zileuton are standard methodologies used in other research studies. The method for AA oxylipins provides the most comprehensive profiling of the AA metabolites. The resulting data will be able to be compared to existing data.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

Clinical chemistry and hematology panels will be outsourced to a contracted commercial diagnostic laboratory service (i.e., Sonora Quest). If urine is collected for a cotinine level at the screening visit, it will also be outsourced to Sonora Quest laboratory.

The urine and plasma biomarker analysis will be conducted in the laboratory of Dr. Chow which is located in the University of Arizona Cancer Center. All specimens will be labeled with the participant study number. Dr. Chow and laboratory staff will be blinded to the study intervention. Buccal cell collections will be stored in the laboratory of Dr. Chow.

Nasal epithelium gene expression analysis will be conducted in the Division of Computational Biomedicine at Boston University and The Boston University Microarray Core overseen by Dr. Avrum Spira. All specimens will be labeled with the participant study number. Dr. Spira and laboratory staff will be blinded to the study intervention.

AA oxylipin analysis will be conducted in the laboratory of Dr. Chow located in the University of Arizona Cancer Center. The analysis will be overseen by Dr. Jessica Martinez. All specimens will be labeled with the participant study number. Dr. Martinez and laboratory staff will be blinded to the study intervention.

10.2 Collection and Handling Procedures

Blood for clinical chemistry, hematology and coagulation panels
Ten milliliters of blood (1 x 7 ml SST tube; 1 x 3 ml Lavender-EDTA tube) will be collected at screening and at the end of intervention for CBC with diff and CMP. A 2.4 milliliter Blue top tube will be collected at screening for PT and PTT. The SST tube will be held at room temperature for 30 min and then centrifuged. The lavender EDTA tube and blue top tube will be gently inverted to mix for anticoagulation. Blood tubes will be prepped, labeled, and packaged according to the recommendation from the diagnostic laboratory. All samples will be refrigerated prior to transfer to the commercial laboratory and sent for immediate analysis.

Urine for pregnancy test
A single void urine will be collected and tested for pregnancy in the clinic according to package instructions.

Urine for cotinine, PGE-M and LTE(4)
A single void urine will be collected and labeled with the participant study number, type of specimen and visit number. Urine will be aliquoted into 4 x 5 ml cryotubes and 4 x 2 ml cryovials and stored at (-80)°C until analysis.

Blood for salicylate, ASA and zileuton level
Ten milliliters of blood will be collected in heparinized vacutainer tubes for plasma salicylate/ASA and zileuton assay. Blood will be immediately chilled, and the plasma will be separated by centrifugation at 4°C within 15 min. The plasma will be aliquoted into 6 x 2 ml cryovials and stored at (-80)°C until analysis.

Blood for AA oxylipin analysis
Five milliliters of blood is collected into blue cap EDTA tubes and then plasma is separated into 300 uL aliquots. A stabilizing solution will be spiked into the plasma to provide 0.2% final concentration of triphenylphosphine (TPP) and butylated hydroxytoluene (BHT). The plasma aliquots will be stored at (-80)°C until analysis.

Nasal epithelium brushings
All nasal brushing samples will be preserved in RNAprotect Cell Reagent (Qiagen) upon collection and stored at (-80)°C. Specimens will be labeled with the participant study number, type of specimen and visit number. Specimens will be shipped on dry ice to Dr. Spira's laboratory for analysis.

Buccal cell collection
Buccal cells from 5 cheek scraping collections will be preserved in RNAprotect Cell Reagent (Qiagen) upon collection and stored at (-80)°C. Buccal cells from one cytology brushing will be transferred to glass slides by firmly rolling the brush onto two collection slides. Cells will be allowed to adhere to slide for two minutes. Then place slides in 10 percent neutral buffered formalin for 10 minutes. Slides will then be stained using a standard eosin and hematoxalin method designed to enhance nuclear contrast.

10.3 Shipping Instructions

All shipments must be in compliance with International Air Transport Association (IATA) Dangerous Goods Regulations and institutional policies and procedures. Current shipper and institutional procedures must be followed. Biologic specimens (Category B, UN3373) will be in leak-proof primary and secondary receptacles with puncture resistant packaging and absorbent material. Shipments are to be preceded with phone contact to the receiving lab or alternate (see phone numbers below) to assure the shipment will be met and processed promptly.
Nasal brushing samples will be shipped to:

Avrum Spira, MD Laboratory
Attn: Gang Liu
Boston University School of Medicine
E-635
72 East Concord St
Boston, MA 02118
Tel: 617-414-3387

Urine, plasma, and buccal cell collections will be placed on dry ice and hand delivered or shipped to Dr. Sherry Chow's laboratory for final storage and analysis.

10.4 Tissue Banking

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

11. REPORTING ADVERSE EVENTS

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

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

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

11.1 Adverse Events

11.1.1 Reportable AEs

Subjects must be carefully monitored for adverse events. The ADVERSE EVENT-REPORTING PERIOD for this study begins upon signing the informed consent form and ends 30 days after the study period. All AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

IN ADDITION, any known untoward serious event that occurs subsequent to the adverse event reporting period that the Investigator assesses as possibly related to the investigational medication/product should also be reported if the incident meets the definition of an adverse event.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

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

11.1.3 Severity of AEs

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

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

CTCAE v4.0 general severity guidelines:

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

ADL

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

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

11.1.4 Assessment of relationship of AE to treatment

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

11.1.5 Follow-up of AEs

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

11.2 Serious Adverse Events

11.2.1 DEFINITION: Fed. Reg. 75, Sept. 29, 2010 defines SAEs as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality/birth defect
- Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious 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.

11.2.2 Reporting SAEs

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

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

NCI/Division of Cancer Prevention
Malgorzata (Margaret) Wojtowicz, M.D.
Lung & Upper Aerodigestive Cancer Research Group
Division of Cancer Prevention, NCI, NIH
9609 Medical Center Drive, Rm 5E-104, MSC9781
Bethesda, MD 20892 (For FedEx, use Rockville, MD 20850)
Phone: (240) 276-7012
Fax: (240) 276-7848
email: wojtowim@mail.nih.gov

Include the following information when calling the Medical Monitor:

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

11.2.2.3 The Lead Organization and all Participating Organizations will FAX written SAE reports to the DCP Medical Monitor (240-276-7848) within 48 hours of learning of the event using the paper SAE form. The written SAE reports will also be FAX'ed (650-691-4410) or emailed (safety@ccsainc.com) to DCP's Regulatory Contractor, CCS Associates (phone: 650-691-4400).

11.2.2.4 The DCP Medical Monitor and regulatory staff will determine which SAEs require FDA submission.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.3 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAE will be followed until resolved, or deemed unlikely to further resolve by the Protocol Chair, or until the subject withdraws consent for further follow-up. SAE unrelated or unlikely to be related to study agent will be followed for at least 30 days after the last dose of study agent.

12. STUDY MONITORING

12.1 Data Management

This study will report clinical data using the OnCore application from Forte Research Systems, Inc., as stated in the Master Data Management Plan. All users of the database will have appropriate education, training and experience to perform assigned tasks. The data collection and management will be done according to the Consortia 2012 DMP.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDE). The approved CRFs will be used to create the electronic CRF (e-CRF) screens in the OnCore application. Site staff will enter data into the e-CRF for transmission to DCP according to pre-established DCP standards and procedures. Amended CRF will be submitted to the DCP Protocol Information Office for review and approval. Approved changes will be programmed into the OnCore database by the Consortium Data Management staff.

12.3 Source Documents

Source documentation for this trial will consist of protocol-specific source documents as well as clinical and research laboratory reports. In the event of a Serious Adverse Event, medical records related to the event will be sought for source documentation of the event and its treatment, if any.

12.4 Data and Safety Monitoring Plan

The University of Arizona Cancer Center (UACC) Data and Safety Monitoring Board (DSMB) will ensure subject safety by coordinating, monitoring, and providing oversight for study data and subject safety for all UA Consortium clinical trials consistent with the National Institutes of Health Policy for Data and Safety Monitoring dated June 10, 1998; further guidance statement issued by the NIH on June 5, 2000, and the policy for Data and Safety Monitoring by Data and Safety Monitoring Boards. Data from this study will be

monitored by the UACC DSMB every six months.

Regular monthly meetings of the UA Consortium, are used as a forum to review accrual rates, problematic issues relating to accrual and protocol implementation, adverse events occurrence, follow-up, and reporting; submission of all required study reports; and progress and outcomes of laboratory analyses.

12.5 Sponsor or FDA Monitoring

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

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

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

Not applicable.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This study utilizes a Phase II, randomized, placebo-controlled design in order to assess the effect of the combination of ASA and zileuton to decrease biases (participant and investigator-derived) that would potentially influence the reporting and interpretation of the data. Each eligible participant will be randomly assigned to receive either ASA and zileuton or placebo.

The primary endpoint analysis will use a previously developed nasal gene expression signature for tobacco smoke exposure as reported by Zhang et al.[34] The signature is composed of 119 genes whose expression is altered in both the bronchial and nasal epithelium of current versus never smokers. The first principal component from a principal component analysis across the 119 genes and nasal data from Zhang et al. was used to compute a smoking signature score for each of the samples in Zhang et al. and in an independent cohort of 130 nasal samples from current and former smokers. The nasal smoking signature was able to significantly separate never or former smokers from current smoker ($p<0.01$, see Figure 1).

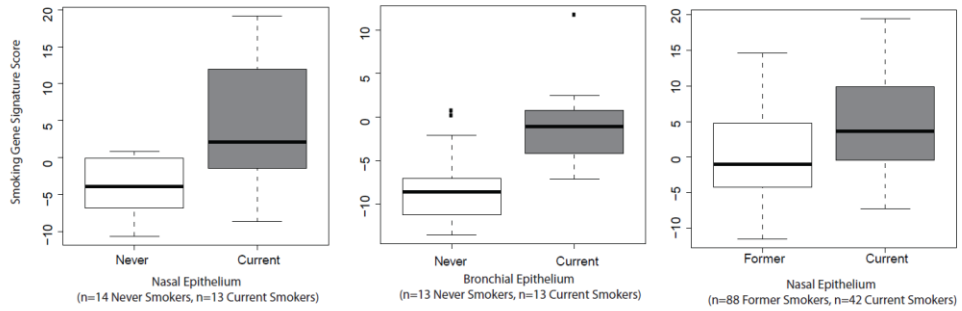

Figure 1. The smoking gene signature score separates never or former smokers from current smokers. The score is significantly different between the two groups in all three analyses by the Wilcoxon rank sum test ($p<0.01$).

13.2 Randomization/Stratification

Each eligible participant will be randomly assigned to receive ASA + zileuton or placebo based on a random allocation rule. In addition, the randomization will be stratified on gender to assure that equal number of males and females assigned to receive either ASA + zileuton or placebo. No blocking technique will be used.

13.3 Accrual and Feasibility

We plan to randomize 66 eligible participants to receive either ASA + zileuton or placebo (33 per group). With an estimated attrition rate of 38.5% for men and 26.7% for women (with respect to endpoint data), we expect to have at least 20 participants (10 males and 10 females) per group with evaluable endpoint data. We anticipate to enroll 3-4 participants/per month and estimate it will take approximately 18 months to complete accrual.

13.4 Primary Objective, Endpoint(s), Analysis Plan

The primary endpoint of this study is to compare changes in a smoking-related gene expression signature score in the nasal epithelium of current smokers after ASA and zileuton intervention. We propose to use the smoking gene signature score to assess the impact of ASA and zileuton on smoking-related nasal gene expression. A two-sided two-sample t test will be used to test whether or not there are significant differences in changes in smoking gene signature score (changes from baseline) between the treatment and placebo groups. Based on a sample size of 20 per group, the power will be at least 85% to detect an effect size of ≥ 1 (i.e. $\geq$ one standard deviation difference in the mean changes between the two groups) at a significance level of 5%. Exploratory analyses will be performed to evaluate whether the effect of ASA and zileuton on smoking-related gene expression signature is modulated by gender and whether gene expression changes are associated with ASA and zileuton exposure and changes in PGE-M and LTE(4) levels.

13.5 Secondary Objectives, Endpoints, Analysis Plans

The secondary endpoints are 1) To assess the impact of ASA and zileuton on three lung cancer gene signatures (an 80-gene bronchial signature, a PI3K pathway gene signature and a nasal diagnostic gene signature) and to compare this to placebo control; (2) To measure urinary PGE-M and LTE(4) levels in current smokers after ASA and zileuton; (3)To assess the safety in current smokers of 12 week exposure to ASA and zileuton; (4) To evaluate a gender effect in the modulatory effects of ASA and zileuton on smoking-related gene expression signature; (5) To explore the effect of ASA and zileuton on the metabolomics profile of the arachidonic acid pathway; and (6) To explore, in a discovery-driven fashion, the effect of ASA and

zileuton on whole-genome gene expression.

For the secondary endpoints, ANOVA will be performed to evaluate whether ASA and zileuton has significantly different impact on changes in the three lung cancer gene signatures and the changes are significantly different from the placebo group. Two sample t tests will be performed to evaluate whether or not there are significant differences in changes in PGE-M, LTE(4) and oxylipin metabolome, respectively, between the treatment and placebo groups. In addition, system biology methods will also be used to analyze the oxylipin metabolome data. Pair-wise comparisons based on two-sample t tests will be performed to whole-genome gene expression data to identify the genes for which ASA and zileuton has a significantly different expression level from the placebo group. Multivariate statistical techniques such as principle component analysis (PCA) will be used to reduce complexity of the whole-genome expression data. PCA analysis will help to identify the major analytes driving a process. For all of the secondary and exploratory analyses, adjustment for multiple comparisons will not be performed. However, the number of comparisons will be reported and we will also cautiously interpret the findings. For both primary and secondary endpoints, if the normality assumption is violated, potential transformation will be sought or nonparametric methods such as Wilcoxon rank sum and Kruskal Wallis tests will be performed.

Descriptive statistics of the type and frequency of all adverse events will be generated, including 95% confidence intervals. For each type of the adverse events, a Fisher's exact test will be performed to compare the frequency of the adverse event between the two groups.

13.6 Reporting and Exclusions

We plan to randomize 66 eligible participants to receive either ASA and zileuton or placebo (33 per group). With an estimated attrition rate of 38.5% for men and 26.7% for women (with respect to endpoint data), we expect to have at least 20 participants per group with evaluable endpoint data. Women and men will each comprise half of the study population in order to randomize women and men in equal numbers to the study arms.

Explanatory analyses will evaluate endpoints in those participants who achieve 80% or better compliance. The primary measure of compliance includes pill count. Participants will be considered compliant for statistical analysis if they have taken ≥ 80% of their assigned study doses based on count of return pills. The secondary measure of compliance will be the Intake Calendar. Serum levels of study agent and metabolite will also be used to confirm compliance.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of study agent.

13.8 Evaluation of Response

All subjects with endpoint data will be assessed for response to intervention, based on the endpoints described above in Sections 13.4 and 13.5.

Subanalyses may be performed on the subsets of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of intervention, major protocol violations, etc.). However, subanalyses may not serve as the basis for drawing conclusions concerning efficacy, and the reasons for excluding participants from the analysis should be clearly reported.

13.9 Interim Analysis

No formal interim statistical analyses are planned for this Phase II trial. Accrual, data collection, and any adverse events will be monitored on a regular basis.

13.10 Ancillary Studies

None

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

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

14.2 Other Required Documents

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

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

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

14.2.4 Documentation of training in "Protection of Human Research Subjects" for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

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

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form

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

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

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO

according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation.

14.4 Informed Consent

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

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

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

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

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates
2001 Gateway Place, Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400
Fax: 650-691-4410
E-mail Submissions:
regulatory@ccsainc.com

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

14.6 Other

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

15. FINANCING, EXPENSES, AND/OR INSURANCE

Study procedures performed during study visits will be covered by the study budget. Research tests, including serum and tissue biomarker evaluations, will not be billed to the subject. Subjects may incur minimal out-of-pocket expenses for transportation but will not be charged for study agent or any study-related activities. Subjects will receive monetary compensation which they may use at their discretion for out of pocket cost such as transportation. If injury occurs, medical care will be provided and charged to the subject's insurer.

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APPENDIX A

Performance Status Criteria

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