The following protocol information is provided solely to describe how the authors conducted the research underlying the published report associated with the following article:

A Randomized, Double Blind, Placebo-Controlled, Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non Small Cell Lung Cancer (ECOG 5597)

Karp, et al

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EASTERN COOPERATIVE ONCOLOGY GROUP
Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

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1.0 INTRODUCTION

1.1 Lung Cancer

Lung cancer continues to be difficult to detect early and difficult to treat when advanced. An estimated 171,500 new cases of lung cancer are expected to be diagnosed in 1998 (1). The incidence of lung cancer has just begun to decline in men from a peak rate of 87 per 100,000 in 1984 to 77 per 100,000 in 1993. Lung cancer incidence continues to increase among women, although the rate of increase has begun to decline. In 1993 the incidence rate in women was 42 per 100,000. There will be an estimated 160,400 deaths in 1997 due to lung cancer, accounting for 29% of all cancer deaths. Twenty-five percent of cancer deaths in women are now due to lung cancer, which surpassed breast cancer as leading cause of cancer death in women in 1987. Five year survival rates remain low at 12% in 1973 and 14% today. Only 15% of lung cancers are diagnosed while the disease is still localized based on data reported to the Surveillance Epidemiology and End Results program (1).

The reported incidence of early stage lung cancer (T_1N_0 or T_2N_0) varies in the literature. Mountain, *et al*., reported Lung Cancer Study Group data on 1,121 patients of whom 398 (35%) were T_1N_0 and 152 T_2N_0 (13.5%) (2). The University of Texas MD Anderson Cancer Center and National Cancer Institute Cooperative Lung Cancer Study Group, reported on 3,753 patients of whom 591 (16%) were T_1N_0 and 1,012 (27%) T_2N_0, for a total of 43% of the total data base under clinical stage I (3). Of that data reported 1,426 patients were surgically staged of whom 429 (30%) were T_1N_0 and 436 (30%) were T_2N_0. Flehinger, *et al*. reported the results of the Lung Cancer Early Detection Study at Memorial Sloan-Kettering Cancer Center which described 40% of all lung cancers diagnosed in that program as stage I under the old staging system (4). Survival data among early stage patients is still disappointing with 5-year survival of 68% for T_1N_0 and 60% for T_2N_0, 54% for T_1N_1, and 40% for T_2N_1. Even in the face of control of the primary disease the risk of recurrence and metastatic disease is significant. In addition, the risk of second primary disease results in significant morbidity and mortality in this population (5). Second primary tumors (SPTs) have been well studied in patients with squamous cell carcinoma of the head and neck. In the population of head and neck cancer patients, the rate of development of second primary tumors remains constant and therefore surviving patients cannot expect the risk to lessen over time (6-7). This paradigm of field cancerization in tobacco related aerodigestive epithelial cancers has also been observed in patients with primary lung cancer. Since patients who present with early stage malignancies have a longer survival, they are at the greatest risk for development of second primary tumors. As therapeutic methods continue to improve the survival of these early stage patients, we will have larger numbers of patients at risk for second primary tumors.

A Mayo Clinic Study reported the development of second primary lung cancer in patients with resected lung cancer at a rate of 2.6 patients per 100 patient years from the first through the fifth year following surgery, then decreasing to 0.8 patients per 100 patient years in the sixth and subsequent years (8). The Lung Cancer Study Group reported from 0.9% to 1.6% second primary tumors in patients with T_1 tumors who were followed up to five years (9).

The recently completed Intergroup Study (MDACC ID91-025), Phase III Double-Blinded Randomized Trial of 13-Cis-Retinoic Acid to Prevent Second Primary Tumors in Stage I Non-Small Cell Lung Cancer, had observed 73 confirmed second primary tumors as of August 1998, with 1,204 patients randomized (personal communication, R. Winn, D. Karp). This figure represents a greater than 2% per year occurrence of second primary tumors in this population. Because of the exposure of the entire aerodigestive tract to carcinogens present in cigarette smoke, it is believed that patients who have a first smoking related malignancy are at a significantly increased risk of developing second primary malignancies in that field. The risk for second primary tumors in non-smokers who develop lung cancer has not been determined. Once a cancer is diagnosed, the impact of smoking cessation on the risk of second primary tumors is also unknown. Most studies would suggest a reduction in risk in individuals who quit smoking. Because of the multi-stage nature of carcinogenesis, smoking cessation may have a greater impact when it occurs early in carcinogenesis.

1.2 Selenium

The nature of trying to prevent the occurrence of cancer requires the use of an agent or means with little to no toxicity. Selenium is an essential trace element in many species including humans. Although there are toxic forms, selenium supplementation decreases the frequency of chemically induced cancers in animals (10-19) and spontaneous mammary tumors (20) as well as inhibiting the growth of transplanted tumors in mice (21). Selenium decreases the mutagenic activity of several carcinogens (22-25) and in tissue culture it reduces the metabolic activation of certain carcinogens (26-28). Selenium also has been shown to enhance immune stimulatory properties in humans (29). Biologically, selenium is an essential component of the antioxidant enzyme glutathione peroxidase which protects tissue against oxidative damage and has also been implicated in the stimulation of apoptosis.

Epidemiologic studies have reported associations between low serum selenium concentrations and the risk of cancer (30-31). Geographical observations also support an inverse relationship between selenium levels in the soil and cancer incidence (32). The report of recent nutrition intervention trials has supported that supplementation with selenium may effect a reduction in cancer risk in certain populations. Blot, *et al*. (33) reported a two by two factorial design intervention trial in Linxian, China, in which one arm included supplementation with beta carotene, vitamin E, and selenium in doses one to two times the U.S. recommended daily allowances. They found significantly lower mortality (p=0.03) among those receiving supplementation with beta carotene, vitamin E, and selenium, primarily due to lower cancer rates (RR=0.87; 95% CI= 0.75 – 1.00). No significant effects on mortality rates from all causes or cancer incidence were observed among subjects receiving supplementation on other arms of this study (33). A recently published study by Clark, *et al*. (34) reported a nutritional supplementation among patients who had previously been treated for a basal cell or squamous cell carcinoma of the skin. This study administered 200 µg of selenium per day as a 0.5 gram high selenium yeast tablet vs. placebo for a mean of 4.5 $\pm$ 2.8 years and had a total follow-up of 6.4 $\pm$ 2.0 years. A total of 1,312 patients were randomized from 1983 through 1991. After a total follow-up of 8,271 person years there was no difference in the occurrence of subsequent basal cell or

squamous cell skin cancers. However, secondary endpoints were analyzed and revealed significant reductions in total cancer mortality (RR=0.50; 95% CI= 0.31 – 0.80), total cancer incidence (RR=0.63; 95% CI= 0.47 – 0.85), and incidence of lung, colorectal, and prostate cancers. In particular the relative risk of lung cancer in the treatment group was 0.54 (95% CI = 0.30 to 0.98) with a significance of 0.04. Mortality secondary to lung cancer was also reduced with a relative risk of 0.47 (95% CI= 0.22 – 0.98, p= 0.03) (34).

Rev. 10/01

1.3 Laboratory Studies

Recent studies by Belinsky and colleagues have targeted genes inactivated by aberrant cytosine-guanosine (CpG) island methylation as candidate biomarkers for early detection of lung cancer (43). Studying two genes involved in lung cancer, they demonstrated that aberrant methylation of the p16 and O^6-methylguanine-DNA methyltransferase (MGMT) promoters can be detected in DNA from sputum in 100% (n = 21) of patients with squamous cell carcinoma (SCC) up to 3 years before clinical diagnosis (44). Moreover, the prevalence of these markers in sputum from cancer-free, high-risk subjects (n = 300) approximates lifetime risk for lung cancer (44, unpublished). Additional studies indicate that prevalent cases of adenocarcinoma can also be detected through analysis of these and other genes in sputum and/or plasma (Belinsky, unpublished and Ref. 45). Added support for using these markers for population-based screening stems from our ability to reproducibly detect gene-specific methylation in sequential sputum samples obtained over a period of 3 years. Thus, the use of aberrant gene methylation as a molecular marker system represents a potentially powerful approach to population-based screening for detection of subjects at high risk of developing lung cancer and to evaluate the efficacy of chemopreventive interventions.

Persons who have undergone resection for stage I NSCLC are an ideal population for assessing efficacy of chemopreventive agents and validating potential surrogate endpoint biomarkers. Second primary tumors occur in this population at rates of 2 - 2.8% per year, a response most likely resulting from the extensive field cancerization that develops from exposure of the entire aerodigestive tract to carcinogens within cigarette smoke. The correlative laboratory studies being proposed would use specimens obtained from participants of ECOG5597, an intergroup study designed to evaluate the effectiveness of selenium supplementation in preventing second primary tumors in persons with resected stage I NSCLC.

Selenium may act as a chemopreventive agent through several different mechanisms: protecting tissue from oxidative damage through interactions with the antioxidant enzyme glutathione peroxidase and inhibition of 5-lipoxygenase (LO; anti-inflammatory effect). Two recent studies found that LO inhibitors have chemopreventive activity in animal lung carcinogenesis models (46,47). Moody *et al.* (46) and Rioux and Castonguay (47) showed that the general LO inhibitor NDGA (0.1% in drinking water), the 5-LO activating protein inhibitor MK 886 (25 mg/kg diet), and the 5-LO inhibitor A79175 (75 mg/kg diet) significantly reduced the multiplicity of NNK-induced tumors in strain A/J mice. A79175 also reduced tumor incidence. In one of these studies, aspirin (294 mg/kg diet) reduced tumor multiplicity; the combination of aspirin and A79175 (*i.e.,* inhibiting both the COX and LO pathways) synergistically lowered tumor incidence and multiplicity. These results strongly suggest that 5-LO pathway inhibitors may have chemopreventive activity in lung. Supporting evidence also links 5-LO metabolites with lung cancer cell growth. Studies conducted by Avis *et al.* in

several human lung cancer cell lines (small cell and non-small cell) found that 5-LO is stimulated by two autocrine growth factors, gastrin-releasing peptide and insulin growth factor, both of which stimulate production of 5-HETE (48). 5-HETE stimulated the growth of lung cancer cells, whereas cells treated with 5-LO inhibitors NDGA, AA-861, and MK-886 showed decreased proliferation; the COX inhibitor aspirin had little effect. Expression of 5-LO and 5-LO activating protein mRNA by lung cancer cell lines was confirmed using RT-PCR, and the presence of 5-LO mRNA was identified in samples of primary lung cancer tissue, including both small-cell and non-small cell lung carcinomas. Also relevant to lung cancer development are studies demonstrating that LOs mediate oxidation of potent carcinogens such as benzidine, *o*-dianisidine and others; this activation can be blocked by adding the LO inhibitors NDGA and esculetin (49). Rat lung LO also oxidizes the carcinogen benzo(*a*)pyrene (50). Multiple investigators have documented expression of LOs in human lung tissue (51,52). Reactive oxygen species stimulate 5-LO activity (53), and selenium-dependant peroxidases inhibit 5-LO activity (54, 55).

One of the oxidative stress markers, 5-OHmdU has been examined in a pilot study by Dr. Kucuk for monitoring effects of soy protein as a chemopreventive for prostate cancer. Protein-associated soy isoflavones have antioxidant properties . To validate 5-OHmdU as a marker for soy-mediated inhibition of oxidative stress, six healthy women participated in a study that involved consumption of one soy supplement pill each day (containing 50 mg isoflavones) with dinner for 3 weeks. Blood was obtained at baseline and weekly thereafter. DNA was isolated from the blood nuclei and analyzed for levels of 5-OHmdU. Levels decreased in four of the six women. The ones who decreased tended to have higher baseline levels of 5-OHmdU. Average levels of 5-OHmdU were 93 $\pm$ 36 at baseline and 48 $\pm$ 15 after 3 weeks of intervention (given as mean $\pm$ SD). This study demonstrates that 5-OHmdU can be used to detect changes in oxidative stress following ingestion of agents known to affect oxidative pathways and thus, should allow us to monitor the effect of selenium on these pathways.

Selenium also acts as a demethylating agent through the inhibition of cytosine DNA-methyltransferase (56), a family of proteins responsible for maintenance and *de novo* methylation of CpG dinucleotides. Additional preliminary results from our laboratory indicate that treatment of a lung tumor-derived cell line with seleno-L-methionine can lead to demethylation and reexpression of the p16 gene (Belinsky, unpublished). The p16 gene is one of the most frequently altered genes in NSCLC. The role of the p16 gene in maintaining the phosphorylation-state of the retinoblastoma gene is critical for cell cycle control. Moreover, the fact that p16 is inactivated in up to 70% of SCCs and that it is inactivated early in the development of this tumor type as well as adenocarcinoma may suggest a “gate-keeper” function for this gene. Adenoviral-mediated p16 gene therapy significantly decreased the growth of established tumors xenografted to nude mice (57). Thus, if selenium causes reexpression of the endogenous p16 gene in premalignant lung lesions through effects on cytosine-DNA methyltransferase, this could impede the development of secondary primary tumors. A similar scenario could also occur for the DAP-kinase gene.

Death-associated protein kinase (DAP-kinase) is a serine/threonine, microfilament-bound kinase involved in g-interferon-induced apoptosis (58). Ectopic expression of wild-type DAP-kinase induced the death of target cells through a mechanism that involves autophosphorylation and regulation through Ca^{2+}/calmodulin binding downstream of the kinase domain (58). Additional studies (59,60) demonstrated that DAP-kinase is involved in tumor necrosis factor (TNF-a) and Fas-induced apoptosis. The expression of DAP-kinase antisense RNA protected cells from killing by anti-Fas/APO-1 agonist antibodies, whereas deletion of the death domain abolished the apoptotic functions of this kinase. Furthermore, these studies showed that DAP-kinase apoptotic function could be blocked by bcl-2, crmA, and p35 inhibitors of caspases, but not by the dominant negative mutants of FADD/MORT1 or caspase 8. Thus, DAP-kinase appears to function downstream of the receptor complex and upstream of other caspases. The apparent involvement of this kinase in cell death, induced by several different activators, places DAP-kinase within one of the central molecular pathways leading to apoptosis. Loss of function of this kinase could significantly affect *de novo* and induced cellular apoptosis. A recent study (61) focused on stage I NSCLC reported methylation of the DAP-kinase promoter in 48% (34/71) and 31% (16/51) of adenocarcinomas and SCCs, respectively. Consistent with a central role for this gene in apoptosis, Tang *et al.* (61) reported that the probability for 5-year, disease-specific survival of patients with stage I NSCLC was 0.56 for those with DAP-kinase hypermethylation versus 0.92 for those without methyation.

The MGMT gene codes for a repair protein that removes mutagenic adducts from O^6-guanine in DNA. The failure to repair these adducts can lead to base mispairing whereby the O^6-methylguanine pairs with thymine during replication, leading to the conversion of guanine-cytosine to adenine-thymine pairs in DNA. These GC to AT transition mutations are most readily seen in activation of the K-ras oncogene and inactivation of the p53 tumor suppressor gene, two genes most likely involve in the initation and progression, respectively of lung cancer. Thus, the progression of field cancerization in the lungs of persons already resected for Stage I NSCLC could be greater in premalignant lesions where the MGMT gene is inactivated through methylation.

The goal of the proposed laboratory studies is to test the hypothesis that selenium supplementation reduces global oxidative damage, cytosine-DNA methyltransferase activity, and the prevalence of aberrant gene-specific promoter hypermethylation detected in sputum and/or serum prior to treatment. A nested, case-control study will be conducted with this population to determine whether changes in oxidative damage, cytosine-DNA methyltransferase activity, and aberrant promoter hypermethylation predict second primary lung cancer. These laboratory studies will address the mechanisms by which selenium may modulate lung cancer development. In addition, these studies will determine whether the methylation biomarkers predict who will develop a second primary lung tumor.

Rev. 6/03

1.4 Biomarker Analysis

A correlative molecular study to identify markers that predict patient response to selenium could greatly increase the value of this study. One powerful approach to help identify these markers is through serial testing of candidate markers by immunohistochemistry (IHC) for relationships to overall survival and disease-free survival in treated and control groups.

Patients who have undergone resection for stage I NSCLC are an ideal population for assessing molecular markers that predict response to a chemoprevention agent. Because of the large number of patient samples that will be needed to be tested for this clinical trial, we propose to construct tissue microarrays of tissue samples that will allow an efficient

platform for testing multiple IHC markers. The tissue microarray consists of multiple cylindrical paraffin embedded tissue cores that are acquired from multiple different patient samples and precisely positioned at high density into a tissue array block. Sections from this block that are cut with a microtome are placed onto standard glass slides and used for immunohistochemical analysis. Depending on the overall depth of tissue remaining in the donor blocks, tissue arrays can generate between 100 and 500 sections. Since relatively small areas of tissue (1mm diameter) are obtained from the donor blocks, this method can help to expand the usefulness of existing archival paraffin blocks by facilitating the construction of multiple "duplicate" blocks. Furthermore, use of tissue microarrays helps to economize on reagents, which can be significant for a study of this magnitude.

The aims of this correlative study are to: 1) construct tissue microarrays for all lung cancers of the E5597 clinical trial, and 2) test approximately 20 markers (including EGF-R, Her2/neu, p53, e-cadherin), which have been previously proposed as lung cancer prognostic markers.

Rev. 7/04

1.5 Smoking History and Diet Studies

The goal of these studies are to determine whether specific dietary variables alone or as a group are associated with detecting methylation in sputum and plasma and the subsequent ability of selenium to modulate this methylation profile as a function of diet. Comparisons will emphasize the effect of diets shown to be protective for lung cancer (high in vegetable and fruit intake) vs. diets associated with modest increase in risk for lung cancer (high in fat and red meat). In addition, the influence of vitamin supplements on methylation will be determined noting the already established protective effects of folate and vitamin C for lung cancer. The influence of antioxidants such as vitamin E, retinol, lycopene, and carotenoids on methylation will also be determined. These analyses will be initially conducted for the 400 participants selected and again for those followed for methylation changes as described for the main correlative laboratory study. However, all persons enrolled onto the laboratory study will complete basic lifestyle and food frequency questionnaires described below, in section 1.51, for secondary analyses described in section 1.52.

1.51 Questionnaires

All participants of the laboratory study will be asked to complete two questionnaires. These questionnaires will be competed at either the 6-month (for newly enrolled persons) or the next visit (for persons previously enrolled). Our strategy for not administering the questionnaire at the initial time of enrollment is based on several issues. First, we want to capture people who remain committed to the prevention trial and assume that some will drop out, usually within the first 6 months. Second, if people change their lifestyle and diet associated with either a diagnosis of lung cancer and/or entering this prevention trial, we want to capture these changes through the questionnaires. They will be sent as part of the sputum and blood kit to the participating site and will contain only the patient's study identification number. The questionnaires also may be downloaded by accessing the ECOG World Wide Web Home Page (http://www.ecog.org). They will also be available on the ECOG website. The site coordinator will review the questionnaires with the patient. The patient will complete the questionnaires at home. Based on previous experience, completion of the questionnaires will take approximately 30 minutes. A self-addressed envelope with postage will be provided for the patient to return the questionnaire to our study coordinator, who will enter receipt into the repository database.

We are using this questionnaire for a population-based study in Albuquerque, New Mexico and have found virtually 100% compliance in completion and return by the initial 400 participants.

The first questionnaire will document basic demographics and clinical risk factors. This questionnaire will obtain information on gender, ethnicity, age, the presence or absence of chronic obstructive disease, and smoking. For smoking history we will determine smoking duration and amount. If a person is a former smoker, the years quit will be determined and whether cessation of smoking was influenced by a diagnosis of lung cancer. The second questionnaire is the Harvard Food Frequency Questionnaire, a validated questionnaire that details food and vitamin use over the past 1 year and 10 years, respectively. The last page of this questionnaire queries how use of food and beverage has changed over the past 10 years. This section has been modified to address change after diagnosis with lung cancer.

1.52 Impact of Diet and Smoking on the Prevalence of Methylation and Response to Selenium

Methylation analyses for the main laboratory correlative study will initially focus on 400 subjects; however, we anticipate enrolling approximately 1400 persons onto the laboratory correlative study. This is based on our 15 months of enrollment and the acknowledgment that some sites as well as subjects do not elect to participate in our correlative laboratory study. For the 1,400 persons enrolled, information from the two questionnaires will be used to address the effect of smoking status (current vs. former), COPD (yes vs. no), and specific dietary variables (e.g., cruciferous vegetables, vitamin E) to identify any interactions with selenium for the prevention of second primary tumors. Several hypotheses can be evaluated. For example, current smokers would continue to generate oxidative DNA damage in their lungs and be more likely to develop a second primary tumor thus, overriding the protective effect of selenium. In contrast, persons whose diet is high in cruciferous vegetables would show a stronger protective effect when also taking selenium.

Rev. 10/01, 7/04 1.6 Women and Minority Representation

The proposed study is open to both men and women, and to all racial/ethnic groups. Since there are no a priori reasons to expect different effects of therapy in male and female patients, and in different racial/ethnic groups, the study will not have separate accrual targets for these groups. We will conduct subgroup analyses to detect gender and race/ethnicity treatment effects and will document any interactions between treatment and these factors.

2.0 OBJECTIVES

2.1 To evaluate the efficacy of selenium supplementation in reducing the incidence of second primary lung tumors in patients who have been treated for Stage I non-small cell lung cancer with complete surgical resection.

2.2 To evaluate the qualitative and quantitative toxicity of a selenium supplementation in a daily administration schedule.

2.3 To compare the incidence of specific cancers and mortality from cancer as well as overall survival of patients treated with selenium supplementation vs. patients treated with placebo.

Rev. 10/01 2.4 Correlative Objectives

2.41 Determine the prevalence for methylation of p16, O^6-methylguanine-DNA methyltransferase, and death associated protein kinase in sputum and blood after tumor resection and follow persons with positive methylation markers longitudinally to determine how selenium alters their methylation profile.

2.42 Determine if selenium supplementation decreases DNA oxidation products (5-hydroxymethyldeoxyuridine) and 5-lipoxygenase metabolites (5-HETE, LTB4).

2.43 Determine whether the reduction of oxidative stress damage predicts loss of the methylation marker(s) during treatment with selenium.

2.44 In a nested, case-control study, determine whether changes in oxidative stress damage and aberrant promoter hypermethylation predict development of a second primary lung cancer.

Rev. 6/03 2.45 To construct tissue microarrays for all lung cancers of the E5597 clinical trial, and test approximately 20 markers (including EGF-R, Her2/neu, p53, e-cadherin) using immunohistochemical analysis.

Rev. 7/04 2.46 To determine whether specific dietary variables alone or as a group are associated with detecting methylation in sputum and plasma and the subsequent ability of selenium to modulate this mehtylation profile as a function of diet.

3.0 SELECTION OF PATIENTS

NOTE: All questions regarding eligibility should be directed to the ECOG Coordinating Center at (617) 632-3610.

Rev. 7/04 **NOTE:** Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration/randomization by the treating physician.

3.1 Run-In Period

3.11 Patients who have undergone complete resection of a histologically proven stage 1A (pT_1N_0) or stage 1B (pT_2N_0) non-small cell lung cancer (except carcinoid) who are currently free of disease are eligible. To be pathologic stage N_0, at least one mediastinal lymph node must have been sampled at resection.

Rev. 3/07

Rev. 10/01, 3/07 [The **NOTE** in this section has been removed via Addendum #4; SWOG and CALGB patients who have stage 1B NSCLC are now eligible for this study.]

3.12 Eligible patients are those between 6 months and 36 months from date of surgical resection. Pathology material from the initial diagnosis must be available for review if recurrence occurs.

Rev. 10/01, 6/03, 3/07, 3/08 3.13 Patients must not be currently receiving chemotherapy, radiation therapy, or biologic agent(s) for lung cancer. Chest x-ray or CT scan $\leq$ 8 weeks prior to registration must show no sign of new or recurrent lung cancer.

3.13.1 Patients with stage 1A non-small cell lung cancer must not have received any therapy other than surgery, as specified in section 3.12.

3.13.2 Patients with stage 1B non-small cell lung cancer are allowed to have received other primary therapy (adjuvant chemotherapy, radiation therapy, or biologic agent(s) in addition to surgery provided all of the following criteria are met:

3.13.2.1 Surgery meets section 3.12 criteria.

3.13.2.2 The other prior primary therapy must have been completed at least six months prior to registration onto study.

3.13.2.3 All treatment-related symptoms must have subsided prior to registration onto study.

3.14 Patients must be $\geq$ 18 years of age.

3.15 Patients must have normal hepatic function (total bilirubin and SGOT (AST) or SGPT (ALT), less than or equal to the institutional upper limit of normal). All laboratory values (including CBC) must be obtained within 8 weeks prior to registration.

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3.16 Patients must have an ECOG performance status of 0-1.

3.17 Patients not taking mineral, herbal, phytochemical, or vitamin supplements at the time of entry must agree to not begin taking such supplements (except for the study designated tablet) during the course of participation. Patients taking any supplement(s) prior to study entry must agree to one of the following in order to be eligible:

NOTE: Supplements are defined as any non-food compound taken by mouth or injection which are intended to provide dietary factors.

Rev. 3/07, 8/08

3.171 Patients who have been regularly (at least 3 times per week for more than 4 consecutive weeks during the year prior to consideration for the trial) taking a supplement that contains > 70 mcg selenium are eligible, provided they have discontinued its use at least one month prior to registration.

3.172 Patients who have been regularly (at least 3 times per week for more than 4 consecutive weeks during the year prior to consideration for the trial) taking
Rev. 8/08 a supplement that contains ≤ 70 µg selenium are eligible, provided (s)he agrees to continue taking the same supplement on the same schedule for the entire duration of study participation.

3.173 A) If the supplement does **NOT** contain selenium, the patient must discontinue supplement(s) for at least two weeks prior to study entry and agree to remain off supplement(s) for the duration of study participation;

OR

B) agree to continue taking the same supplement(s), on the same schedule for the entire duration of study participation.

Rev. 8/08 Any supplement(s) containing > 70 µg of selenium are absolutely disallowed in this study.

3.18 No concurrent cancers or any prior cancer history within the past 5 years except localized non-melanoma skin cancer.

3.19 No synchronous lesions (lung + non-lung) or metastasis, even if resectable. No history of greater than one lung cancer primary at any time.

3.2 Study Phase

3.21 Patients must be free of disease.

3.22 Patients must have consumed at least 75% of tablets during 4 week run-in period.

4.0 RANDOMIZATION PROCEDURES

Rev. 6/03 **Submitting Regulatory Documents**

Before an ECOG Institution may enter patients, protocol specific regulatory documents must be submitted to the CTSU Regulatory Office at the following address:

CTSU Regulatory Office
Coalition of National Cancer Cooperative Groups
1818 Market Street, Suite 1100
Philadelphia, PA 19102
FAX: (215) 569-0206

Required Protocol Specific Regulatory Documents

1. CTSU Regulatory Transmittal Form.

2. Copy of IRB Informed Consent Document.

NOTE: Any deletion or substantive modification of information concerning risks or alternative procedures contained in the sample informed consent document must be justified in writing by the investigator and approved by the IRB.

3. A. CTSU IRB Certification Form

Or

B. HHS 310 Form

Or

C. IRB Approval Letter

NOTE: The above submissions must include the following details:

- **Indicate all sites approved for the protocol under an assurance number.**
- **OHRP assurance number of reviewing IRB.**
- **Full protocol title and number.**
- **Version Date**
- **Type of review (full board vs. expedited).**
- **Date of review.**
- **Signature of IRB official.**

The CTSU encourages you to link to the following RSS2.0 webpage so that more information on RSS2.0 as well as the submission forms can be accessed http://www.ctsu.org/rss2_page.asp. If you have questions regarding regulatory document submission, please telephone the CTSU Help Desk at 1-888-823-5923 or E-mail CTSUContact@westat.com**. Monday through Friday, 9:00am - 6:00pm**.

Patients must not start protocol treatment prior to randomization.

Treatment should start within 14 working days after randomization.

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Institutions may register eligible patients to this study via the ECOG webpage 24 hours a day, 7 days a week, using the Web-based Patient Registration Program (https://webreg.ecog.org). If you need assistance or have questions, please telephone the Central Randomization Desk at the ECOG

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Coordinating Center at (617) 632-2022. Please note that a password is required to use this program.

4.1 Recruitment

In addition to screening patients at thoracic surgery and pulmonary clinics, a **suggested** recruitment strategy which could be adopted at each institution is as follows. Eligible patients and their primary physicians may be identified utilizing institutional and regional cancer registries. In collaboration with the primary care provider, a letter (which has been appropriately reviewed and approved by the institutional IRB) describing the study is mailed to the patient with instructions on how to arrange to be screened for the trial.

4.2 **Step 1: ECOG Run-In Period Patient Registration**

The run-in period is a 4-week trial period prior to beginning the study which will be used to assess patient compliance. At least 75% of the run-in tablets must be consumed in order for the patient to be considered eligible for randomization to the study phase portion of the protocol.

To register eligible patients on study, the investigator will telephone the Central Randomization Desk at the ECOG Coordinating Center at (617) 632-2022. The following information will be requested:

4.21 Protocol Number

4.22 Investigator Identification

4.221 Institution name and/or affiliate

4.222 Investigator's name

4.23 Patient Identification

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4.231 Patient's initials and chart number

4.232 Patient's Social Security number

4.233 Patient Demographics

4.2331 Sex

4.2332 Birthdate (MM/YYYY)

4.2333 Race

4.2334 Nine-digit zip code

4.2335 Method of payment

4.24 Eligibility Verification

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Patients must meet all of the eligibility requirements listed in Section 3.1. An eligibility checklist is appended to the protocol. A confirmation of registration will be forwarded by the ECOG Coordinating Center.

4.25 Additional Requirements

Patients must give signed and dated written informed consent.

4.3 CALGB Run-In Period Patient Registration

Registration will be accepted only through institutions with direct registration privileges. Registration must occur prior to initiation of therapy. Confirm eligibility criteria (Sec. 3.1) . Call
Rev. 3/07 the CALGB Registrar (919-668-9396, Monday - Friday, 9:00 AM - 4:30 PM Eastern Time) with the following information:

- Your Name
- Study #
- Institution #
- Treating Physician
- Patient's Social Security #, or hospital ID#
- Patient's Name, I.D. #
- Signed Informed Consent (Date)
- Race, Sex, Date of Birth (ECOG Race/Ethnicity Codes will be requested)
- Zip code of residence
- Method of Payment
- Diagnosis, Date of Diagnosis
- Eligibility Criteria met (Sec. 3.0) (yes, no)
- If patient staged T_1N_0, patient refused/ineligible for 9633 (no, yes)
- List of prior CALGB protocols
- Date of most recent Institutional Review Board approval (< 1 year)

The CALGB Registrar will then contact the Eastern Cooperative Oncology Group Coordinating Center to register the patient. The Eastern Cooperative Oncology Group Coordinating Center
Rev. 3/07 will forward a confirmation of registration to the CALGB Statistical Center, Data Operations, which will subsequently forward the confirmation of registration to the main member institution.
Rev. 3/07 Please check for errors. Submit corrections in writing to CALGB Statistical Center, Data Operations, Hock Plaza, Suite 802, 2424 Erwin Road, Durham, NC 27705.

4.4 SWOG Run-In Period Patient Registration

NOTE: Southwest Oncology Group institutions will follow their normal procedures for documentation of IRB approval.

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a. You may register patients from Member, CCOP and approved Affliate institutions to a therapeutics study using the SWOG Regisitration program. To access the Registration program, go to the SWOG Web site (http://swog.org) and click on the *Logon* link to go to the SWOG Members Area logon page (https://swog.org/visitors/logon.asp). This Web program is available at any time except for periods listed under *Down Times*. Log on as an Individual Use using your SWOG Roster ID Number and individual web user password. Help for the logon process may be founf at https://swog.org/visitors/logonhelp.asp. After you have logged on, click on the *Clinical Trials* link and then the *Patient Reg* link to go to the Entry Page for the Patient Registration program. If you are a Registrar at an institution with Internet access you are encouraged to register this way. For new users, the link to a "Starter Kit" of help files may be found by clicking on Starter Kit link at the logon page.

To register a patient the following must be done (in order):

1. You are entered into the Southwest Oncology Group Roster and issued a SWOG Roster ID Number.

2. You are associated as an investigatory or CRA/RN to the institution where a registration is occuring, and

3. You are granted permission to use the Patient Registration program at that institution.

   For assistance with points 1 and 2 call the SWOG Operations Office at 210/677-8808. For point 3 you must contact your Web User Administrator. Each SWOG institution has one or more Web User Administrators who may set up Web Users at their institution and assign permissions and passwords to these users. For other password problems, or problems with the Patient Registration program, please e-mail webreghelp@crab.org. Include your name, Roster ID Number, and telephone number, when the problem occurred, and exactly what you were doing.

b. If the Web Reg program is not used, the registration must be done by phone.

   Member, Affiliate, and CCOP Institutions

   Registration by phone of patients from member, affiliate, and CCOP Institutions, must be done through the Southwest Oncology Group Data Operations Center in Seattle by telephoneing 206/652-2267, 6:30 a.m. to 2:30 p.m. Pacific Time, Monday through Friday, excluding holidays.

c. For either method of registration, exceptions to Southwest Oncology Group registration policies will not be permitted.

   1. Patients must meet all eligibility requirements

   2. Institutions must be identified as approved for registration.

   3. Registrations may not be canceled.

   4. Late registrations (after initiation of treatment) will not be accepted.

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4.5 NCCTG Run-In Period Patient Registration

NOTE: A signed HHS 310 form is to be on file at the NCCTG Randomization Center before patient entry.

NCCTG institutions must FAX (507) 284-0885) a completed eligibility checklist to register a patient (8 AM - 4 PM, Central Time, Monday through Friday.) Patient eligibility and existence of a signed consent form will be checked by the NCCTG Randomization Center before a patient will be registered to this study. Upon confirmation of initial registration eligibility, the NCCTG Randomization Center will contact the ECOG Coordinating Center to register the patient. The NCCTG Coordinating Center will then contact the registering institution to confirm registration.

Rev. 6/03 4.6

4.7 NCIC CTG Run-In Period Patient Registration

The following documentation must be on file at the NCIC CTG central office prior to registration: documentation of full REB approval of the study and consent form, a copy of REB approved consent form (on institutional letterhead), a completed NCIC CTG participant's list and a current Cooperative Project Assurance number.

Registrations will be accepted on Monday to Friday between 8:00 AM and 6:00 PM Eastern Time. The eligibility checklist must be completed prior to registration (eligibility requirements are listed in Section 3.1). The registration may be done by telephone at (613) 533-6430 or by
Rev. 7/04,10/04 FAX at (613) 533-2941. As soon as eligibility is ascertained a call will be placed to the ECOG Registrar between 9:00 AM and 5:00 PM Eastern Time. NCIC CTG will then contact the registering institution to confirm registration.

Rev. 10/01 4.8 RTOG Run-In Period Patient Registration

RTOG Institutions must FAX (215) 574-0300 a completed eligibility checklist to register a patient (8:30 AM to 4:30 PM, Eastern Time, Monday through Friday). Patient eligibility and presence of a signed consent form will be checked by the RTOG Randomization Desk before a patient will be registered to this study. Upon confirmation of initial registration eligibility, RTOG will contact the ECOG Coordinating Center to register the patient. RTOG will then contact the registering institution to confirm registration.

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4.9 Step 2: ECOG Study Phase Randomization

After the initial 4-week treatment period, the patient will be evaluated for randomization by determining the number of tablets taken and the current disease status. (Patients must be randomized within 3 to 6 weeks of start date of run-in period with nutritional supplement. See Section 4.93.) All patients are required to bring both calendar and pill bottle to clinic at the time of their evaluation. If the patient has taken at least 75% of the prescribed number of pills (determined by reviewing the Patient Diary and performing an actual pill count) and is still NED (no evidence of disease), the patient is then eligible for randomization.

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If the patient is not eligible for randomization, the ECOG Coordinating Center needs to be notified by telephone by the appropriate institution.

4.91 Special Randomization Instructions for Double-Blinded Studies

Participating patients, medical staff and ancillary medical staff will remain blinded as to the assignment of selenium yeast or placebo yeast. In the event of an emergency or severe adverse reaction necessitating identification of the medication (selenium yeast or placebo yeast) for the welfare of the patient, please contact the Study Chair, Dan Karp, M.D.(713) 745-7398, first to ensure reasons for unblinding are valid, and then call the ECOG Coordinating Center and ask for someone who can unblind treatment assignments (617) 632-3610. Breaking the code will lead to discontinuation of protocol treatment for that patient.

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4.92

To randomize eligible patients on study, the investigator will telephone the Central Randomization Desk at the ECOG Coordinating Center at (617) 632-2022. The following information will be requested:

4.921 Protocol Number

4.922 Investigator Identification

4.9221 Institution name and/or affiliate

4.9222 Investigator's name

4.923 Patient Identification

4.9231 Patient's name or initials and chart number

4.9232 Patient's Social Security number

4.9233 Patient Demographics

4.92331	Sex
4.92332	Birth date (MM/YYYY)
4.92333	Race
4.92334	Nine-digit zip code
4.92335	Method of payment

4.924 Eligibility Verification

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Patients must meet all of the eligibility requirements listed in Section 3.2. An eligibility checklist has been appended to the protocol. A confirmation of registration will be forwarded by the ECOG Coordinating Center.

4.925 Stratification Factors (for Randomization)

4.9251 Smoking Status (based on registration date):

actively smoking or stopped < 1 year ago
vs.
stopped ≥ 1 year ago
vs.
never smoked or ≤ 100 cigarettes ever.

4.9252 Gender

Male vs. Female

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4.9253 Stage and Previous Therapy

Stage 1a
vs.
Stage 1b with previous therapy*
vs.
Stage 1b with no previous therapy*

* previous therapy = other primary therapy in addition to surgery (adjuvant chemotherapy, radiation therapy, or biologic agent)

4.93 Additional Requirements

- Patients must be randomized within 3-6 weeks of start date of run-in period with nutritional supplement.
- Samples for Correlative Studies and Pathology Submissions should be submitted as described in Sections 10.0 and 11.0.

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NOTE: THE FACILITY PERFORMING THE BLOOD SELENIUM LEVELS (SECTION 11.1) HAS CHANGED. SECTIONS 7.2, 11.1, APPENDIX V AND APPENDIX X, HAVE BEEN UPDATED TO REFLECT THIS CHANGE. PLEASE UPDATE YOUR RECORDS ACCORDINGLY.

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NOTE: Study participants from outside the United States and Canada are exempt from sending fresh specimens for correlative studies due to costs and problems associated with international shipping.

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4.10 CALGB Randomization

Randomization will be accepted only through institutions with direct registration privileges. Randomization must occur prior to initiation of therapy. Confirm eligibility criteria (Section 3.2).

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Call the CALGB Registrar at (919) 668-9396, Monday - Friday, 9:00 AM - 4:30 PM, eastern time with the following information:

Your name
Study #
Patient's name, I.D. # (Intergroup I.D. #)
Stratification factors

The CALGB Registrar will then contact the Eastern Cooperative Oncology Group Coordinating Center for treatment assignment. The Eastern Cooperative Oncology Group Coordinating Center will forward a confirmation of treatment assignment to the CALGB Registrar, which will subsequently forward the confirmation of treatment assignment to the main member institution.

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4.11 SWOG Randomization

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a. You may register patients from Member, CCOP and approved Affliate institutions to a therapeutics study using the SWOG Regisitration program. To access the Registration program, go to the SWOG Web site (http://swog.org) and click on the *Logon* link to go to the SWOG Members Area logon page (https://swog.org/visitors/logon.asp). This Web program is available at any time except for periods listed under *Down Times*. Log on as an Individual Use using your SWOG Roster ID Number and individual web user password. Help for the logon process may be founf at https://swog.org/visitors/logonhelp.asp. After you have logged on, click on the *Clinical Trials* link and then the *Patient Reg* link to go to the Entry Page for the Patient Registration program. If you are a Registrar at an institution with Internet access you are encouraged to register this way. For new users, the link to a "Starter Kit" of help files may be found by clicking on Starter Kit link at the logon page.

To register a patient the following must be done (in order):

1. You are entered into the Southwest Oncology Group Roster and issued a SWOG Roster ID Number.
2. You are associated as an investigatory or CRA/RN to the institution where a registration is occuring, and
3. You are granted permission to use the Patient Registration program at that institution.

For assistance with points 1 and 2 call the SWOG Operations Office at 210/677-8808. For point 3 you must contact your Web User Administrator. Each SWOG institution has one or more Web User Administrators who may set up Web Users at their institution and assign permissions and passwords to these users. For other password problems, or problems with the Patient Registration program, please e-mail webreghelp@crab.org. Include your name, Roster ID Number, and telephone number, when the problem occurred, and exactly what you were doing.

b. If the Web Reg program is not used, the registration must be done by phone.

Member, Affiliate, and CCOP Institutions

Registration by phone of patients from member, affiliate, and CCOP Institutions, must be done through the Southwest Oncology Group Data Operations Center in Seattle by telephoneing 206/652-2267, 6:30 a.m. to 2:30 p.m. Pacific Time, Monday through Friday, excluding holidays.

c. For either method of registration, exceptions to Southwest Oncology Group registration policies will not be permitted.

1. Patients must meet all eligibility requirements

2. Institutions must be identified as approved for registration.

3. Registrations may not be canceled.

4. Late registrations (after initiation of treatment) will not be accepted.

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4.12 NCCTG Randomization

NCCTG institutions must FAX (507) 284-0885 a completed eligibility checklist to randomize the patient 8 AM - 4 PM, Central Time, Monday through Friday. Upon confirmation of randomization eligibility, the NCCTG Randomization Center will contact the ECOG Coordinating Center to randomize the patient. The NCCTG Randomization Center will then contact the registering institution with the treatment assignment.

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Rev. 10/01 4.14 NCIC CTG Randomization

Rev. 7/04, 10/04 Randomizations will be accepted on Monday to Friday between 8:00 AM and 6:00 PM Eastern Time. The eligibility checklist must be completed prior to randomization. The randomization may be done by telephone at (613) 533-6430 or by FAX at (613) 533-2941. As soon as eligibility is ascertained a call will be placed to the ECOG Registrar between 9:00 AM and 5:00 PM Eastern Time. NCIC CTG will then relay the treatment assignment to the center and confirm it in writing.

Rev. 10/01 4.15 RTOG Randomization

RTOG Institutions must FAX (215) 574-0300 a completed eligibility checklist to randomize the patient (8:30 AM to 4 PM, Eastern Time, Monday through Friday). Upon confirmation of randomization eligibility, RTOG will contact ECOG Coordinating Center to randomize the patient. RTOG will then contact the registering institution with the treatment assignment.

Rev. 10/01 4.16 Instructions for Patients Who Do Not Start Assigned Protocol Treatment

Rev. 6/03 If a randomized patient does not receive any assigned protocol treatment, baseline and follow-up data will still be collected and must be submitted according to the E5597 Forms Rev. 6/03 Packet. Document the reason for not starting protocol treatment on one of the baseline forms. Patients who are not randomized, following the run-in phase, should be taken off study and do not require submission of follow-up data.

5.0 TREATMENT PLAN

This is a double-blind, placebo-controlled study. Patients, medical staff, and ancillary medical staff will be blinded as to the treatment assignment of each patient until the completion of the Rev. 7/04 **study. Selenium yeast and placebo yeast tablets appear identical. At no time will the patient** Rev. 10/01 **be told whether s/he is receiving selenium yeast or placebo yeast.**

NOTE: A smoking-cessation program should be made available for all participants who are smoking at study entry.

All questions regarding treatment or dose modifications should be directed to the ECOG Study Chair.

5.1 Placebo Run-In

Study subjects will be given a supply of placebo tablets and instructed to take them daily in the morning for a 4-week period. They will be informed that if a pill is forgotten it should be taken as soon as it is remembered, if within 12 hours of the scheduled dose, and the time taken should be noted on the drug diary. Otherwise, the dose should not be taken and should be recorded as missed on the drug diary. Compliance will be defined as consuming at least 75% of the expected pills during the 4 week run-in period, determined by reviewing the Patient Diary and performing an actual pill count of pills remaining at the end of the run-in.

5.2 Selenium Study Supplement Administration

5.21 Selenium Study Supplement

The supplement provided for this study will consist of either 200 µg of selenium in the form of selenized yeast or a placebo yeast. Patients will take one tablet daily in the morning for 8 cycles (1 cycle = 6 months), for a total of 4 years. If a pill is forgotten it should be taken as soon as it is remembered, if within 12 hours of the scheduled dose, and the time taken should be noted on the drug diary. Otherwise, the dose should not be taken and should be recorded as missed on the drug diary. See Section 5.4 for dose modifications for toxicity. Telephone interviews assessing toxicity and compliance will be performed starting month 3 and continuing every 6 months for the duration of the study.

Rev. 10/01, 6/03, 10/04, 3/07 **5.3** **Adverse Event Reporting Requirements**
Rev. 6/10

NOTE: As of April 14, 2010, adverse event reporting is no longer required for this protocol.

5.31 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (please refer to the E5597 Forms Packet for the list of forms with directions for routine adverse event reporting). Additionally, certain adverse events must be reported in an expedited manner for more timely monitoring of patient safety and care. The following sections provide information about expedited reporting.

5.32 Determination of reporting requirements

Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) the characteristics of the adverse event including the grade (severity), the relationship to the study therapy (attribution), and the prior experience (expectedness) of the adverse event; 3) the phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

An investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Steps to determine if an adverse event is to be reported in an expedited manner:

Step 1: Identify the type of event using the NCI Common Toxicity Criteria (CTC) Version 2.0. The CTC provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTC can be downloaded from the CTEP home page (http://ctep.cancer.gov). Additionally, if assistance is needed, the NCI has an Index to the CTC that provides help for classifying and locating terms. All appropriate treatment locations should have access to a copy of the CTC.

Step 2: Grade the event using the NCI CTCAE.

Step 3: Determine whether the adverse event is related to the protocol therapy (investigational or commercial). Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Step 4: Determine the prior experience of the adverse event. Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is **NOT** listed in:

- **Arm X -** the investigator's brochure, drug package insert or protocol.

Step 5: Review the "Additional instructions, requirements, and exceptions for protocol E5597" table in section 5.3.6 for protocol and/or ECOG specific requirements for expedited reporting of specific adverse events that require special monitoring.

NOTE: For general questions regarding expedited reporting requirements, please contact the NCI Medical Help Desk: 301-897-7497.

5.33 Reporting methods

- **Arm X -** This study requires that expedited adverse event reporting use the NCI's Adverse Expedited Reporting System (AdEERS). The NCI's guidelines for AdEERS can be found at http://ctep.cancer.gov. For questions regarding the use of the AdEERS application, please contact the NCI Technical Help Desk: 301-840-8202.

An AdEERS report must be submitted to ECOG and the appropriate regulatory agencies by one of the following methods:

- Electronically submit the report via the AdEERS Web-based application located at http://ctep.cancer.gov

or

- Fax the completed NCI Adverse Event Expedited Report - Single Agent or Multiple Agents paper template located at http://ctep.cancer.gov to ECOG (617 632 2990), Attention: AE.

NOTE: Paper copies of AdEERS reports will only be accepted if the AdEERS system is down. Once the system is restored, a report submitted on a paper template must be entered into the AdEERS system by the original submitter of the report at the site.

Any supporting or follow up documentation must be faxed to ECOG (617 632 2990), Attention: AE.

5.34 When to report an event in an expedited manner

Some adverse events require 24-hour notification (refer to Section 5.36). Please complete a 24-Hour Notification Report via the NCl AdEERS website (http://ctep.cancer.gov/reporting/adeers.html) within 24 hours of learning of the event. The full AdEERS report must be completed and submitted via AdEERS within 5 calendar days.

If the AdEERS system is down, a 24-hour notification call must be made to ECOG (617-632-3610). Once the system is restored, a 24-hour Notification Report must be entered into the AdEERS system by the original submitter of the report at the site.

When an adverse event requires expedited reporting, submit a full AdEERS report within the timeframes outlined in Section 5.36.

NOTE: Adverse events that meet the reporting requirements in Section 5.36 and occur within 30 days of the last dose of protocol treatment must be reported on an expedited adverse event report form (using AdEERS). For any adverse events that occur more than 30 days after the last dose of treatment, only those that have an attribution of possibly, probably, or definitely AND meet the reporting requirements in Section 5.36 must be reported on an expedited adverse event report form (using AdEERS).

5.35 Other recipients of adverse event reports

ECOG will forward AdEERS reports to the appropriate regulatory agencies and pharmaceutical company, if applicable.

Adverse events determined to be reportable must also be reported by the institution, according to the local policy and procedures, to the Institutional Review Board responsible for oversight of the patient.

The drug supporter is obliged to forward reported AEs to the FDA. A drug supporter representative may call a site for additional information regarding a serious adverse event. Any additional written AE information requested by the drug supporter MUST be submitted to BOTH ECOG and the drug supporter.

5.36 Expedited reporting for investigational agents

Phase 2 and 3 Trials Utilizing an Agent under a non-CTEP IND: AdEERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days1 of the Last Dose of Investigational Agent (Selenium Yeast/Placebo Yeast) in this Study (Arm X) OR Within 30 Days of the Last Dose of Any Protocol Treatment.

Attribution	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
				Unexpected		Expected			
	Unexpected and Expected	Unexpected	Expected	with Hospitali-zation	without Hospitali-zation	with Hospitali-zation	without Hospitali-zation	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

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

AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

AdEERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

Please see additional information below under section entitled "Additional instructions, requirements, and exceptions for protocol E5597"

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

- **Expedited AE reporting timelines:**
  - **24 Hours; 5 calendar days -** The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - **10 calendar days -** A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates **hospitalization* (or prolongation of existing hospitalization)** must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in **persistent or significant disability/incapacity, congenital anomaly, or birth defect** must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND**

- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

*** Hospitalizations are defined as lasting 24 hours or longer and these events must be reported via AdEERS.**

**** These events should also be reported via AdEERS for non-CTEP IND agents.**

Additional instructions, requirements and exceptions for protocol E5597

1. Additional Instructions:

- With respect to determining the specific day by which the event must be reported, the day the reporter learns of the adverse event constitutes "Day 0"

- For instructions on how to specifically report events that result in persistent or significant disability/incapacity, congenital anomaly, or birth defect events via AdEERS, please contact the NCI Medical Help Desk at 301-897-7497 or adeersmd@tech-res.com.

2. ECOG and Protocol Specific expedited reporting requirements:

The adverse events listed below also require expedited reporting for this trial:

ECOG specific expedited reporting requirements:

- Hospitalizations: Any grade 1 or 2 adverse event which precipitates a hospitalization lasting ≥ 24 hours (or prolongs hospitalization) must be reported via AdEERS within 10 calendar days of learning of the event regardless of the attribution and designation as expected or unexpected.

5.37 Reporting secondary AML/MDS/ALL

All cases of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and acute lymphocytic leukemia (ALL) that occur in patients on NCI-sponsored trials following their chemotherapy for cancer must be reported to ECOG. Submit the following information within 30 days of an AML/MDS/ALL diagnosis occurring after treatment for cancer on NCI-sponsored trials:

- a completed NCI/CTEP Secondary AML/MDS/ALL Report Form (do not use AdEERS);
- a copy of the pathology report confirming the AML/MDS/ALL; and
- a copy of the cytogenetics report (if available).

ECOG will forward copies to the Investigational Drug Branch (IDB) of the NCI Cancer Therapy Evaluation Program (CTEP).

NOTE: If a patient has been enrolled in more than one NCI-sponsored study, the NCI/CTEP Secondary AML/MDS/ALL Report Form must be submitted for the most recent trial. ECOG must be provided with a copy of the report even if ECOG was not the patient's most recent trial.

5.38 Reporting of recurrence or other second primary cancers

All cases of recurrence or new second primary tumor (SPT) of any histology that occur on ECOG protocols during or after protocol treatment must be reported to ECOG, according to the follow up schedule outlined in the E5597 Forms Packet, on the E5597 Recurrence Form (#1370) or the E5597 Second Primary Form (#1371) within 30 days of diagnosis, regardless of relationship to protocol treatment.

The pathology reports from both the original diagnosis and the new primary should be forwarded to the ECOG Coordinating Center, along with the E5597 Recurrence Form (#1370) or E5597 Second Primary Cancer Form (#1371), as appropriate.

All new primary cancers, including basal cell and squamous cell skin cancers, must be reported.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted, including the NCI AML/MDS/ALL form and E5597 Second Primary Form.

Submit AML/MDS/ALL and Second Primary information to:
ECOG Coordinating Center
FSTRF
900 Commonwealth Avenue
Boston, MA 02215

Rev. 3/07

5.4 Dose Modifications

All toxicities should be graded according to the Common Toxicity Criteria (version 2.0).

The CTEP Active Version of the CTCAE is identified and located on the CTEP website at (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. **All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.**

Rev. 3/07 For any grade 3 or 4 toxicity observed in any organ system and thought to be treatment-related, the agent will be withdrawn. Toxicity will be reassessed weekly by telephone or clinic visits. Once the toxicity subsides to ≤ grade 1, the agent may be restarted at 1 tablet per day.

Rev. 7/04 5.5 Lifestyle Questionnaire and Dietary Assessment

At either the 6-month (for newly enrolled persons) or the next visit (for persons previously enrolled) participants of the laboratory study will be asked to complete two questionnaires. Administration of the questionnaires will allow us to determine whether specific dietary variables alone or as a group are associated with detecting methylation in sputum and plasma and the subsequent ability of selenium to modulate this methylation profile as a function of diet.

The questionnaires will be sent as part of the sputum and blood kit to the participating site and will contain only the patient's study identification number. They will also be available on the ECOG website. The questionnaires may be administered to patients by staff during visits or distributed to the patients to complete at their leisure at home. If the patient takes the questionnaires home, they may submit the questionnaires with their sputum samples (see section 11.0) or the may return the questionnaires to the institution at the next visit.

- The questionnaires will be sent as part of the sputum and blood kit to the participating site. The questionnaires also may be downloaded by accessing the ECOG World Wide Web Home Page (http://www.ecog.org).
- The questionnaires are to be administered at the time point listed above unless the patient refuses.
- The patient should be asked to read the instructions at the beginning of each questionnaire and complete all the items. It is permissible to assist the patient with the completion of the questionnaires as long as the staff person does not influence the patient's responses.
- The questionnaires are to be reviewed by the protocol nurse or research coordinator upon completion to ensure all items were marked appropriately. If more than one answer was marked, the patient should be asked to choose the answer which best reflects how s/he is feeling. If a question was not answered, the patient should be asked if s/he would like to answer it. The patient should always have the option to refuse. If the patient refuses, it should be indicated on the questionnaire that s/he declined to answer the item.
- Completed questionnaires are to be sent to:

  Ms. Darlene Harbour
  Lovelace Respiratory Research Institute
  2441 Ridgecrest Drive S.E.
  Albuquerque, NM 87108
  Tel: (505) 348-9500
- If a patient misses an appointment on the scheduled date, the questionnaires may be distributed to the patient at the next visit.

Rev. 7/04 5.6 Supportive Therapy

All supportive measures consistent with optimal patient care will be given throughout the study.

Rev. 7/04 5.7 Duration of Therapy

The supplement will be administered for 4 years, with continued follow-up anticipated for the duration of the 10-year study.

Every effort will be made by the investigator to keep the patient on treatment, however, should the patient discontinue treatment after randomization, efforts will be made to complete and report the observation as thoroughly as possible for the duration of the study. All randomized patients will be followed for the duration of the study. Follow-up is not required
Rev. 6/03 on patients who are not randomized following the run-in period.

Rev. 3/07 Patients must discontinue treatment for any of the following reasons, but all randomized patients remaining alive will be followed until death.

Rev. 7/04 5.71 The development of unacceptable toxicity, defined as unpredictable, irreversible Grade 2, 3, or 4 toxicity.

Rev. 7/04 5.72 Patient refusal to continue treatment.

Rev. 7/04 5.73 Death.

Rev. 7/04 5.74 Intercurrent illness that necessitates premature termination.

Rev. 7/04, 3/07 5.75 Development of any second primary cancer.

Rev. 7/04 5.76 Patients who develop recurrence which is successfully treated by surgery +/- radiotherapy, or chemotherapy will continue on this trial and complete the selenium treatment. The study supplement should be stopped while the patient is receiving surgery, XRT, or chemotherapy. The nutritional supplement may be restarted at completion of therapy.

Rev. 6/03, 7/04 5.77 Patients **with new primaries other than lung or** who develop recurrence which is not successfully treated by surgery +/- radiotherapy, or chemotherapy will continue to be followed every 12 months for survival status and other parameters specified
Rev. 6/03 in Section 7.0. Patients with new **lung** primaries will continue to be followed for
Rev. 6/03 survival status every 12 months. Refer to the E5597 Forms Packet for the appropriate forms.

Rev. 7/04 5.8 Intergroup Steering Committee

A Steering Committee will be created for the purpose of establishing guidelines for quality control and the reporting of results of the trial such as toxicity, rate of development of second primary and recurrence, and early stopping of the trial. The committee will address specific issues pertaining to the development of companion protocols, and reporting of data for publication.

The current statistical results of the trial (SPT development) will be reported by the Study Statistician.

A representative of DCP or his/her signee and the Chief of CORB of DCP or his/her designee will serve as non-voting members.

The reporting of SPT development will be done regularly on a yearly basis until all of the required number of patients have been randomized and followed for the prescribed length of time indicated. Interim reports of accrual and toxicity will be prepared and appear in the agendas of the participating cooperative groups. Two formal interim significance tests plus one final analysis are planned.

Rev. 6/03,7/04 However, as stated in Section 9.10, this study is monitored by the ECOG Data Monitoring Committee (DMC). The steering committee will not have access to the results of outcome comparisons until these are released by the DMC.

Members of this committee will include the following:

	ECOG:	Study Chair: Daniel Karp, M.D.
Rev. 7/04		Study Statistician: Sandra Lee, Sc.D.
		Study Pathologist: Seena Aisner, M.D.
Rev. 7/04		Surgery Co-Chair: Steve Keller, M.D.
	SWOG:	Omer Kucuk, M.D.
Rev. 10/04		
	CALGB:	Gerald Clamon, M.D.
	NCCTG:	Randolph Marks, M.D.
Rev. 6/03		
	NCIC CTG:	Ciarin McNamee, M.D.
Rev. 10/01	RTOG:	Gordon Okawara, M.D.

Rev. 7/04 5.9 Endpoint Review Committee

Rev. 7/04 5.91 An Endpoint Review Committee function will be performed as part of the duties of the Intergroup Steering Committee. All new occurrences of cancer in study participants will be reviewed to determine whether they represent a recurrence of the original primary tumor or a second primary tumor.

Rev. 7/04 5.92 This review will include a review of the pathology of both the original tumor and the new tumor by the ECOG pathologist as described in Section 10.0, Pathology Review, for all cases considered to be unresolved by the Endpoint Review Committee.

Rev. 7/04 5.93 The committee as a whole will review the case history, the radiology and the pathology as reported by the study pathologist and reach a consensus ruling of whether the second event represents a SPT or a recurrence of the original primary lung cancer. All unresolved cases will have pathology review of the original primary tumor and the SPT or recurrence and will return for re-review to the Endpoint Review Committee following the ECOG pathology review. Criteria for the definition of a second primary lung tumor are detailed in Section 6.2.

6.0 MEASUREMENT OF EFFECT

6.1 Accurate determination of whether a cancer occurrence is recurrent disease or whether it is a second primary is critical in assessing the outcome of this study. All suspicious lesions identified clinically and/or radiographically will be verified histologically.

6.2 Definitions

6.21 Lung / New Primary (at least one of the following)

a. Different histologic type
b. Location in different lobe
c. Location in contralateral lung
d. Occurrence > 5 years after initial diagnosis

6.22 Other site / New Primary

a. Head and neck
b. Esophagus
c. Bladder
d. Miscellaneous

7.0 STUDY PARAMETERS

7.1 Therapeutic Parameters

1. All prestudy scans and x-rays should be done ≤ 8 weeks before registration.

2. Prestudy CBC (with platelet count) should be done ≤ 8 weeks before registration.

3. All required prestudy chemistries, as outlined in Section 3.0, should be done ≤ 8 weeks before registration.

Rev. 6/03

	Step 1 Run-In[5] Prior to Registration	Step 2 Study Phase Prior to Randomization	Month 3	Follow-up Every 6 Months for Duration of Treatment	Follow-up From End of Therapy Until Second Primary Lung Tumor [3]
Complete History and Physical (w/PS)	X[1]			X	q 12 mo
Rev. 10/01 CBC (with platelet count)	X				
Rev. 6/03, 1/08, 8/08 Supplement/Alcohol/Tobacco Usage Assessment	X			X	
Compliance Measurement		X		X	
CXR or Chest CT	X			X	q 12 mo
Bilirubin, SGOT (AST) or SGPT (ALT)	X				
Telephone Interview			X[2]	X	

Rev. 10/01, 6/03

1 A complete history, physical, and on-study questionnaire to include details of tobacco consumption, performance status, recent weight loss, usual weight, and concurrent nonmalignant disease and therapy must be done within 8 weeks prior to registration.

2 **NOTE:** Telephone interviews will be conducted by the study nurse or CRA at month 3 and will continue every 6 months for the duration of the study, so that the participant has in-person or phone contact every 3 months.

Rev. 10/01, 6/03 3 Patients who experience a recurrence but have not had a second primary lung tumor should be followed until the occurrence of a second primary lung tumor. Pathology reports pertaining to recurrence and occurrence of an SPT must be submitted to the ECOG Coordinating Center as indicated in section 10.
Rev. 3/07

Rev. 10/01, 6/03, 8/08 4 [Deleted in Addendum #7]

Rev. 10/01, 6/03 5 For patients that do not continue past the run-in period no follow-up is required.

Rev. 10/01, 6/03
Rev. 6/03

7.2 Biological Sample Submissions

Rev. 3/07

Pathology reports at development of recurrence or a second primary lung tumor are required for central review. Failure to submit reports may render the patient unevaluable. See Section 10.0 for description of submissions.

Samples for the correlative studies should be submitted as outlined in Sections 10.0 and 11.0. Submission of samples is to be limited to those patients who have agreed to participate in the correlative studies.

Rev. 7/04

NOTE: THE FACILITY PERFORMING THE BLOOD SELENIUM LEVELS (SECTION 11.1) HAS CHANGED. SECTIONS 7.2, 11.1, APPENDIX V AND APPENDIX X HAVE BEEN UPDATED TO REFLECT THIS CHANGE. PLEASE UPDATE YOUR RECORDS ACCORDINGLY.

Rev. 3/07

NOTE: Study participants from outside the United States and Canada are exempt from sending fresh specimens for correlative studies due to costs and problems associated with international shipping.

	Step 2 Study Phase Prior to Randomization	Months 6 and 12	Months 24 and 48	Follow-up at Recurrence or Second Primary Lung Tumor
Rev. 7/04 Serum (royal blue top tube)[3,5]	X		X	
Rev. 7/04 Plasma and Buffy Coat (blue top CPT)[3]	X	X	X	
Sputum[3]	X	X	X	
Rev. 7/04				
Rev. 7/04 Lifestyle Questionnaire and Dietary Assessment		X[6]		
Rev. 7/04 Surgical Resection[3, 4]	X			
Rev. 3/07 **Central Pathology Review**	**X[1]**			**X[1]**

Rev. 3/07 1 MANDATORY: Pathology reports must be submitted at baseline for all patients and upon development of a second primary tumor or recurrence as described in section 10.0. Pathological samples may be requested for review for questionable cases.

Rev. 3/07 2 [Footnote 2 was removed via Addendum #4]

3 Baseline samples for the correlative studies are to be submitted AFTER registration to the run-in period, just prior to randomization to Step 2.

Rev. 7/04 4 Blocks from surgical resection and Questionnaires are to be completed by the patients participating in the research laboratory studies (Section 11.2).

Rev. 7/04 5 COLLECT THIS BLOOD SAMPLE PRIOR TO PERFORMING ANY OTHER BLOOD DRAW IF ALL BLOOD DRAWS TO BE TAKEN FROM THE SAMPLE VENIPUNCTURE. Samples are requested to monitor compliance to protocol treatment. Kits for sample collection are to be requested 1 week prior to sample collection by faxing kit order form (Appendix V) to 507-266-0188.

Rev. 7/04 6 Questionnaires are to be completed at month 6 for newly enrolled patients, or at the next visit for previously enrolled patients. The questionnaires will be sent as part of the sputum and blood kit (section 11.2) to the participating site, or downloaded by accessing the ECOG website (http://www.ecog.org). See section 5.5 for details.

8.0 DRUG FORMULATION AND PROCUREMENT

8.1 Selenium Yeast/Placebo Yeast

8.11 Other Names

L-selenomethionine

8.12 Classification

Essential trace mineral

8.13 Mode of Action

Selenium is considered to be an essential trace mineral with a recommended daily allowance for adults ranging from 55 to 70 µg/day. Selenium is found in meat, poultry, seafood, and grains. Current research has focused on one organic (L-selenomethionine) and two inorganic (sodium selenate and sodium selenite) forms of selenium.

L-selenomethionine is the selenium-bound analog of the essential amino acid L-methionine. It is highly stable and not chemically modified by vitamin C or other nutrients. It enjoys relatively low toxicity in humans and has high tissue retention compared to other forms of selenium.

The exact mechanism by which supplemental selenium inhibits cancer development is unknown. However, proposed mechanisms include: 1) stimulation of the antioxidant properties of the enzyme glutathione peroxidase, which facilitates the lowering of tissue peroxide levels in the body by destroying hydrogen peroxide; 2) protective alterations in carcinogen metabolism; 3) alterations on the endocrine and immune systems; 4) production of cytotoxic selenium metabolites; 5) inhibition of protein synthesis; 6) inhibition of specific enzymes; and 7) stimulation of apoptosis. Selenium also helps regulate the utilization of vitamin E. It is stored mainly in red blood cells, liver, spleen, heart, nails, tooth enamel, testes, and sperm. Selenium is primarily eliminated in the urine.

8.14 Storage And Stability

Store at room temperature. The formulation of selenium used in this protocol has a shelf life of 3-5 years. The product should be protected from moisture.

8.15 Dose Specifics

Patients will take 1 tablet daily in the morning for 8 cycles (1 cycle = 6 months), for a total of 4 years. See Section 5.4 for dose modifications for toxicity.

200 µg selenium as a 0.5 g high selenium baker's yeast

The active intervention supplement, L-selenomethionine, will be supplied as a tablet containing 0.5 g high selenium yeast with a selenium content of 200 µg per tablet. The high selenium containing yeast will contain 1,200 ppm selenium per batch.

Placebo: 0.5 g low selenium baker's yeast tablet with < 1 µg selenium.

8.16 Preparation

None required.

8.17 Administration

Oral.

8.18 Incompatibilities

No information available.

8.19 Availability

The high-selenium yeast is being supplied free of charge for this study by Cypress Systems and distributed by Proclinical Pharmaceutical Services. Institutions will complete the E5597 Drug Request Form in Appendix II and FAX the ECOG Coordinating Center at 617-632-2063 to order Selenium Yeast/Placebo Yeast from the NCI. An identical appearing placebo will also be provided and should be handled in the same manner as that described for high-selenium yeast. The placebo yeast tablet utilizes the identical non-selenized nutritional yeast used to produce the active intervention agent.

Investigators and Investigator's staff must be blinded as to supplement identity.

The supplement is supplied in tablets containing 200 µg of selenium in the form of selenized yeast or placebo yeast (IND# 59,935). Each patient will receive 1 bottle containing 35 tablets for the run-in period. If the patient is compliant and is randomized to the protocol, the institutions can order 6 months supplies, which will be shipped after each order for the remainder of the study. Each bottle will contain 200 tablets.

Rev. 6/03

Rev. 6/03

NOTE: Run In and treatment phase supplies, once received by the institution, can be shipped to the patient. When the patient complies with the follow-up schedule, the institution can continue to ship the supplement for the remainder of the study.

After patient registration or randomization, a supply of supplement may be obtained. Investigators must submit the following to the ECOG Coordinating Center, ATTN: DRUG ORDERS:

1. Signed and completed E5597 Drug Request Form (Appendix II). Remember to include the drug ID number (this is not applicable for the initial request).

Rev. 7/04 The drug ID number is assigned when the patient is randomized to Step 2. Drug requests cannot be processed without the drug ID number.

2. The Coordinating Center will confirm a copy of the institution's current (< 365 days) IRB approval letter for this protocol (HHS 310) is on file.

Rev. 6/03 3. All institutions must submit a Study Specific 1572 (see Appendix VIII) including all relevant personnel to ECOG with their next drug order. For a 1572 - Go to ECOG members website/member/forms/investigator 1572 for a editable version or find at

Rev. 7/04 Study Specific 1572. The 1572 may be mailed or faxed to the drug team.

Rev. 6/03 4. Once the drug is received at the institution, it must be dispensed in the original container and cannot be repackaged.

Rev. 6/03 5. Drug inventory records must be carefully maintained. The inventory, disposition and mailing of all supplements must be clearly documented. Please use the NCI drug accountability form.

Rev. 6/03 6. Institutions must be able to track the shipment by having the patient return the attached patient letter (see Appendix VII). Shipments are at the expense of the institution. Express distributors or First Class US mail must be used.

Rev. 6/03, 7/04 7. Institutions must enclose in the shipment a reply paid self addressed envelope and the attached patient letter.

Rev. 6/03 8. Patients must agree to return the letter to the institution on receipt of the nutritional supplement.

Rev. 6/03 9. If the letter confirming receipt of the drug is not received by the institution within 7 days the institution must personally follow-up the patient by telephone and document that contact. Patients must still return the letter for drug accountability records.

Rev. 6/03 10. Unused drug must be returned to the institution when the patient completes or discontinues treatment.

Rev. 10/01 NCIC institutions do not need to submit a copy of their IRB approval. The NCIC Operations Office will provide this to the ECOG Coordinating Center on an ongoing basis.

Any supplement request received at the ECOG Coordinating Center between 9:00 AM and 4:00 PM EST will be approved and faxed to ProClinical for shipment the next business day by Federal Express, with delivery the following day. See table below for anticipated delivery date:

Day Drug Requested	Day Drug Received
Monday	Wednesday
Tuesday	Thursday
Wednesday	Friday
Thursday*	Tuesday
Friday*	Tuesday

* Thursday and Friday Drug Requests are shipped on Monday.

NOTE: There will be no weekend or holiday delivery of supplements. All deliveries must be made by express carrier for tracking purposes.

Drug Inventory Records: The Investigator, or a responsible party designated by the investigator, must maintain careful record of the inventory and disposition of all supplement received. Please use the NCI Drug Accountability Record Form. (See the NCI Investigators Handbook for Procedures for Drug Accountability and Storage.) The unused selenium yeast/placebo yeast must be accounted for. Immediately upon
Rev. 3/07 return by the patient, the pharmacist should complete drug accountability forms,
Rev. 3/07 destroy used drug, and certify destruction per institution's policy. Regarding unused drug, at the completion of the trial, the pharmacist should complete drug accountability forms, destroy unused drug, and certify destruction per institution's policy.

Rev. 6/03 **NOTE: Investigational agents are carefully monitored during audits. Additional auditing of these procedures may take place. It is very important to**
Rev. 3/07 **confirm the dispensing and destruction of drug at the site in order to comply with NCI and FDA guidelines.**

8.110 Side Effects

Toxicity is dose-related and appears most commonly at doses greater than 750 µg/day. The incidence of toxicity is low and treatment is supportive.

1. Dermatologic: Dermatitis, hair loss, itching, fingernail weakness.
2. Gastrointestinal: Diarrhea, nausea, vomiting, constipation, metallic taste, belching.
3. Neurology: Irritability.
4. Constitutional: Weakness.
5. Hepatic: Liver damage.
6. Other: Garlic odor of breath and sweat.

Rev. 7/04 8.111 Nursing/Patient Implications

Monitor subjects for possible gastrointestinal and cutaneous adverse effects. Teach subjects to read labels for selenium content of any over the counter medications they may take including substances such as multivitamins or herbal preparations. Instruct

subjects not to take any selenium containing preparations without first discussing with the study investigator.

8.112 References

Clark LC, Combs GF, Turnbull BW, *et al.* Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. JAMA 1996; 276:1957-1963.

Date/Reviewer: March 1999/Wayne Spath, R.Ph. (847) 570-2205.

9.0 STATISTICAL CONSIDERATIONS

9.1 This is a double-blind, placebo-controlled, randomized study designed to evaluate whether selenium supplementation will prevent second primary lung tumors in non-small cell lung cancer.

The accrual goal for this phase III trial is 1960 eligible, consenting patients to enter the compliance run-in period. Subjects will be randomly assigned in a 2:1 ratio to receive either selenium yeast or placebo yeast respectively. Based on the success of MDACC ID91-025 (91025) we expect that at least 90% of such patients will prove compliant resulting in a minimum of 1764 patients to be randomized. If we further assume an on study compliance rate of approximately 83% we would then effectively have the equivalent of 1200 compliant subjects by adopting Lachin and Foulkes (1986) correction for noncompliance (i.e., multiplication of 1764 by the square of the compliance rate) (35). Additional discussion of issues pertinent to compliance are provided in Section 9.3.

The study is planned to be ten years in length with patient entry occurring during the first five years. Every effort will be made through the recruitment plan to complete accrual within 4 years. Approximately 392 patients must then be accrued per year for a total of 1960 patients. This number of patients will give 80% power to detect a reduction in the annual incidence of second primary lung tumors from 2% to 1.2% using a two-tailed hypothesis test of the null hypothesis that there is no difference in the risk of second primary lung tumors between treatment arms. Hypothesis tests will be performed with a Type I error of 5% and assuming two interim analyses based on O'Brien and Fleming's (1979) plan for early rejection of the null hypothesis (36). Interim analyses will be conducted after 50% and after 75% of the events have occurred. Hypothesis tests will be declared statistically significant if the two-tailed P-values are less than or equal to 0.00305, 0.01822 and 0.04378 at each of the three respective planned analyses. Interim analyses will be supplemented with calculation of repeated 95% confidence intervals using the critical values specified by the O'Brien-Fleming bounds (37).

The power calculations are based on a total of 180 study subjects developing a second primary lung tumor. The first planned interim analysis is estimated to take place 6.1 years after the first patient is randomized. At this time, there must have been 90 patients who developed a second primary lung tumor (i.e., 50% of the events have occurred). The second interim analysis is estimated to take place 8 years after the first patient is randomized. At this time there must have been 135 patients who developed a second primary lung tumor (i.e.,

75% of the events have occurred). Note that Clark *et al.* (1996) reported a 50% reduction in both lung cancer incidence and mortality for subjects receiving selenium (34). Therefore, the proposed sample size may prove conservatively large if in fact selenium halves the annual expected 2% risk of second primary lung tumors. The incorporation of two interim analyses might still allow the study to be completed in a timely fashion should selenium live up to its early promise. For example, if the annual risk of second primary lung tumors is 2% among placebo treated subjects and 1% among selenium treated subjects we will have 80% power to detect this effect of treatment after 75% of the events have occurred, i.e., at the second, planned interim analysis.

9.2 Baseline Hazard Rate for Second Primary Lung Tumor (SPLT)

Although there is no body of literature which definitively and consistently describes the incidence of SPLTs in this population (Stage I), various assumptions must be made to allow calculations of an adequate sample size to answer the questions posed. The existing published data on SPLTs in lung cancer seems to be roughly equivalent to that for head and neck as described by Cooper *et al*. of approximately 2-3% per year (6).

Mayo Clinic reported an annual risk of 2.6% for developing a second primary lung tumor (SPLT) in a series of patients with post-surgical stage I lung cancer (8). Analysis of the T_1N_0 patients treated on the Lung Cancer Study Group's (LCSG) studies showed an annual SPLT rate of 2.5% (9). For estimating the sample size, the annual rate of 2.0% was used and assumed to be constant over time. Patients will be eligible up to three years post their surgery. The SPLT risk is assumed to be approximately the same regardless of the length of the interval between surgery and the protocol entry for sample size calculations.

Standard sample size calculations assume the entry of patients at a constant rate during the stated accrual period; whereas the previously treated patients will be enrolled as soon as possible when the study is activated. Interim and final analyses will be conducted when the required number of second primary lung tumors have occurred. Thus, follow-up time may be less than anticipated if there is an initial surge in accrual.

9.3 Noncompliance

The failure of study subjects to follow their assigned treatments can easily compromise the chance of finding a significant result for a comparative trial. With the planned chemoprevention study, it is possible that patients assigned to the placebo yeast arm will begin significant non-protocol dietary supplementation to mimic the selenium yeast arm. In addition, patients assigned to the selenium yeast arm may discontinue therapy because of refusal. This has been accomplished by allowing for a total rate of non-compliance across intervention groups of 17%.

All patients assigned to the selenium yeast regardless of the amount of supplement taken will be included in the analysis. Noncompliance can possibly dilute the observable treatment effect in such a way that only a smaller reduction in the SPLT rate can be detected. The difficult problem is how to quantify impact of incomplete protocol selenium treatment on the risk of developing SPLT. A compliance run-in period will be initially used in the chemoprevention study to minimize the number of noncompliant subjects. Candidates must consume at least 75% of the placebo tablets during the 4 week period to be eligible for the randomization. Greenberg, *et al*. (38), have recently published a summary of the design and the early results of their placebo-controlled trial of beta-carotene for the prevention of second skin cancers. They reported a disqualification rate of 8% for otherwise eligible and consenting subjects (163/1968) within a one-month run-in period and a criterion of 80% of

the tablets consumed. Still 12% of the randomized subjects stopped taking the tablets before two years. Experience from 91025 demonstrated a high compliance with only 30 of 1329 registered patients taking less than 75% of tablets during the run-in period. The sample size has been adjusted for an assumed 83% rate of compliance with patients assigned interventions using the approach described by Lachin and Foulkes (35).

The anticipated 83% rate of compliance can arise in several different ways. For instance if each year approximately 5% of subjects failed to follow their assigned intervention then after four years only 83% of patients would still be compliant (i.e., .83=.95x.95x.95x.95). Alternatively an 83% compliance result would occur if nearly all randomized subjects remain compliant for the first two years of intervention followed by a 9% annual rate of non-compliance for the last two years of intervention (i.e., .83=1x1x.91x.91).

Compliance may prove better than anticipated if the run-in period helps to screen out patients who will not be able to complete their assigned treatments. Compliance might also be better than anticipated since two-thirds of subjects will be assigned to selenium yeast. In any event, noncompliance by control subjects may not dilute the effect of intervention since not all available forms of selenium are believed to be equally effective in preventing second primary cancers (personal communication, Larry Clark). Similarly treated subjects who remain on study for some period of time may yet benefit from the intervention since there is little evidence suggesting that a full four year course of intervention is required. Note that the method used to correct for noncompliance makes the extreme assumption that noncompliant treated subjects are identical to control subjects while noncompliant control subjects are identical to treated subjects. Finally the use of regular pill counts might also ensure added compliance. In any event, the effects of noncompliance will be assessed after the study has been open for two years. These blinded analyses will also be used to determine if accrual is as anticipated.

9.4 Randomization Scheme

The treatment allocation will be done using a randomized permuted block within strata to balance patient factors other than institution. There will be a check on the balance of treatment assignment within each full member institution as described by Zelen (39). Patients will have a two-thirds (66.67%) probability of being assigned to receive selenium yeast and a one-third (33.33%) probability of being assigned to receive placebo yeast. Smoking status at randomization (current, previous, non/ never smoker), gender (male vs. female), and
Rev. 3/07 stage/previous therapy (Stage 1a vs. Stage 1b with previous therapy vs. Stage 1b with no previous therapy), will be used as the stratifying factors prior to randomization.

9.5 Statistical Analysis

Standard survival analysis methods such as the log rank test and the Cox regression model will be applied to analyze the treatment effect on time to the development of a second
Rev. 6/03 primary lung tumor. The time to second primary lung tumor is defined to be the time from randomization to date of diagnosis of a second primary lung tumor.

Rev. 6/03 Primary analyses will use a stratified log rank test to compare the randomized treatment groups, i.e., analysis by intention to treat (40). Smoking status and gender will be used as the
Rev. 6/03 stratification factors. To further explore the relationship between selenium supplementation and the time to development of a second primary lung tumor, a Cox regression model will be examined. We will include treatment, histology (squamous vs. non-squamous), stage of resected tumor (T_1N_0 vs. T_2N_0) and baseline selenium levels in the model. The Cox regression

analysis would also be stratified on the variables used to stratify the randomization (i.e., smoking status and gender). The proportional hazards assumption will be examined. Secondary, efficacy analyses will be used to assess the effect of selenium based on the actual degree of compliance to the study protocol estimated for each subject. There are, at present, a number of competing approaches developed to adjust for non-compliance and contamination in randomized clinical trials (41,42). Efficacy analyses will therefore explore the effect of the different assumptions required when adjusting for noncompliance as part of a sensitivity analysis. The degree of reliance which can be given to the study results will depend, in part, on how robust the statistical inferences about the effect of intervention are to the selected method of analysis. The trial will, of course, have the greatest impact if there is little or no difference in the inferences constructed using intention to treat or using one of the efficacy analyses.

Primary analyses will include all second primary lung tumors diagnosed after randomization.

In these analyses death due to recurrence of lung cancer or death due to other causes (e.g., heart disease) will be treated as censored observations.

Second primary lung tumors diagnosed within, perhaps, six months following randomization could likely not have been prevented by patients use of selenium. Therefore we will explore the effect of omitting all second primary lung tumors diagnosed within six months following randomization as part of a secondary analysis.

The effect of imbalance on baseline prognostic factors on the estimated effect of intervention will also be explored. Prognostic factors used in these analyses include histology (squamous vs. nonsquamous), stage of resected tumor (T_1N_0 vs. T_2N_0), and baseline selenium levels. Examination for effect modification by race and gender will be conducted as part of a secondary, exploratory analysis.

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9.6 Correlative Studies

9.61 Methylation of p16, O^6-methylguanine-DNA Methyltransferase, and DAP Kinase

The main objective for this aim is to first identify persons positive for methylation of at least one gene in sputum and/or plasma and then to follow those persons through analysis of their specimens collected at baseline, 6, 12, 24 and 48 month time points. This study design will compare the effect of selenium versus placebo on the persistence of these markers in sputum and/or plasma. The use of these markers in longitudinal studies is support by our published study where we demonstrate the ability to repeatedly detect either p16 or MGMT in sequential sputum samples over a period of 3 years. In addition to this study, our new investigation using subjects from the Colorado Cohort has contained some subjects where multiple sputum samples have been collected over a period of 4 years. While this study is just beginning, we have identified 9 of 10 subjects whose sequential sputum was positive if the prior specimen was positive. This finding together with the fact that we are prescreening to select persons whose first sputum is adequate should minimize our false negative rate. In addition, Dr. Bocklage will also evaluate all the longitudinally collected sputums selected for methylation analysis for adequacy and cytology from the persons whose baseline sputum contains one of the methylated genes. We have not had the opportunity to conduct sequential analyses for methylation in blood, but have no reason to suspect that shedding of DNA into blood from premalignant lesions or early lung cancer should differ over time.

Based on our studies in cancer-free current and former smokers, we hypothesize that at least 30% (this is a conservative estimate) of the participants will still be positive for a methylation biomarker in blood or sputum. Thus, from the initial 400 people (260 treated, 140 placebo) selected, approximately 80 treated (selenium) and 42 placebo participants will be followed for 2 years to determine changes in their methylation profile. Loss of a methylation marker will be noted if two sequential samples (e.g, 6- and 12- month points) are negative for the methylation change detected at entry onto the study. Because the initial study by Clark *et al.* (1) saw a 50% reduction in lung cancer rates, we also hypothesize that 50% of the treatment group will lose the methylation marker(s) during treatment. Therefore, if the proportion of placebo (n = 42) and treated patients (n = 80) who lose their biomarkers is < 0.25 and 0.5, respectively, we will have at least 80% power to detect a difference between the groups losing biomarkers in the two arms. This is based on a one-sided Fisher's exact test with a significance level of 0.05, and with 0.5 as the proportion corresponding to the alternative hypothesis. Even if prevalence for methylation in the placebo and treatment group at baseline is 20% less (64 treated, 34 placebo), the above statement is still valid when 0.25 is replaced by 0.22.

In addition to testing for association between biomarker loss and treatment as detailed above, logistic regression analyses will be performed to explore the relationships of methylation at baseline to covariates, including age and gender. These exploratory analyses will be performed for markers separately and in combination. Similar analyses will also be conducted for the subjects who lose markers before or at the 24-month assessment. The timing of sequential measurements makes the testing of the hypothesis that the rate of marker loss over time is constant in the treated and control group straightforward. The proportion of subjects who lose a marker by the 12-month measurement will be compared with the corresponding proportion for subjects positive at 12 months and negative at 24 months, for both the placebo and selenium groups, and for markers separately and in combination. If the rate of marker loss is sufficiently high (presumably for treated patients) such that few people remain positive at 12 months, then a similar analysis can be performed using the 6- and 12-month data. Exact binomial tests will be used with adjustments made for multiple comparisons as appropriate.

9.62 DNA Oxidation Products and 5-LO Metabolites

This specific aim will examine oxidative damage endpoints in the approximately 122 participants positive for a methylation biomarker at baseline, 6, 12, 24 and 48 months. The statistical analysis discussed in this section will be performed using data from the first three post-baseline sequential measurements. Oxidative damage measurements will be considered as ratios of a continuous response to baseline. Thus, a one-sided t-test will be used to compute the power against alternatives under a null hypothesis that this ratio is equal to one. If the standard deviations for oxidative damage ratios to baseline are each < 0.3, then we will have at least 95% power to detect a 20% decrease from baseline for both the treated and placebo groups. This calculation is based on independent evaluations made at the three time points for the treated and placebo groups, each test at the 0.05/6 significance level. The stated power is for the placebo subjects. Using the data in Table 2, the standard deviation of the ratio of week 1 measurements to baseline (n = 5) is 0.17; the corresponding result for week 3 to baseline (n = 6) is 0.37. If we assume that the true standard deviation is not > 0.39, then there will be at least 80% power for the above test for the placebo group.

Because data on between- and within-subject variability are not yet available, the t-tests discussed above will be used for the primary statistical test. Log transformation may also be used to improve the approximation to normality, if necessary. If the normality assumption appears to be inappropriate even after transformation, then the Wilcoxon test will be used. In addition, repeated measures models will be used to investigate how oxidative damage changes over time, both within individual patients, and on average for patients in the treatment and placebo groups. The hypothesis that the change in oxidative damage over time is the same for the placebo and treatment groups will be tested. Further statistical analyses will introduce loss of methylation markers as covariates, which will facilitate the comparison of oxidative damage among subgroups based on marker loss as well as treatment. Additional covariates will be considered, and adjustment for multiple comparisons will be made as appropriate.

9.63 Association of Oxidative Stress with Loss of Methylation Markers

The statistical analyses are based on 25% fewer placebo and 50% fewer treated subjects than baseline having markers at each followup. For each methylation marker, oxidative damage ratios to baseline will be made for four groups of patients: 1) placebo/lose marker, 2) placebo/do not lose marker, 3) treated, lose marker, and 4) treated, do not lose marker, with assumed sample sizes of 10, 32, 40, and 40, respectively. We also assume that the standard deviation is equal to 0.3 for all four groups. Using a two-sided, two-sample t-test with a significance level of 0.05/6 (treatment and placebo comparisons at each of the first three post-baseline timepoints), we will have 80% power to detect a difference in mean oxidative damage of 0.24 for each treatment comparison. The corresponding difference in means for 80% power for the placebo group is 0.40.

As discussed in the planned data analysis for objective 2.42, t-tests (possibly after log transformation) will be used for the primary determination unless severe non-normality indicates that the two-sample Wilcoxon test is appropriate. Also, loss or presence of a methylation marker will be included as a covariate in logistic regression models. Statistical comparisons will be made of changes in oxidative damage over time between groups of subjects who do or do not lose the markers.

9.64 Changes in Oxidative Stress and Hypermethylation and Development of Secondary Cancer

This case-control study will have at least 80% power to detect an odds ratio of 2.8 based on a one-sided Fisher exact test with a significance level of 0.05. This assumes that the proportion of the population with secondary tumors that have a biomarker (positive for a methylated gene in sputum or plasma) present at the end of the study will be > 0.51, while the corresponding population of matched cases who are positive for a marker, but do not have a second tumor will be < 0.27. Although 2.8 is a large odds ratio, the power of this study is not unreasonable since previous work (2) has shown the methylation changes to be a strong predictor of lung cancer. In addition, although univariate tests have been assumed in order to facilitate power calculations, the statistical analyses will include multivariate methods where appropriate.

A similar situation applies for the oxidative markers based on 120 patients and using a one-sided Fisher exact test with significance of 0.05. Power is calculated based on a single assessment that uses the maximum ratio of each oxidative marker to the baseline (study entry) value. We will classify the subjects into two equal groups according to whether they have ratios below the median ("low oxidative stress") or above the median ("high oxidative stress"). This will result in 80% power to detect differences if the probability of a second primary tumor given high oxidative stress is greater than 0.65, and if this probability for people with low oxidative stress is < 0.41. This corresponds to an odds ratio of 2.7

Fisher's exact test will be used for the primary test of significance. Regression models with the presence of a second tumor as a response, and with covariates including treatment status, presence or absence of biomarkers, and other subject-level covariates will be analyzed. In addition, oxidative damage will be included as a longitudinal covariate in these models.

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9.7 Correlative Molecular Study

For the correlative molecular study, tissue blocks will be requested from each randomized patient. It is anticipated that 70% of the patients will submit blocks than can be used in the correlative study. Therefore, we expect approximately of 1236 samples for this analysis, approximately 824 samples from the selenium arm and 412 from the placebo arm.

The power calculations presented below are for the EGFr marker. Similar calculations apply for the other markers examined in the study.

The main objective of this correlative study is to correlate different marker levels with overall survival. Disease-free survival is a secondary endpoint. For the power calculations for the EGRr marker, subjects will be divided into 2 groups based on their baseline EGFr levels, using the median value as the cut-off (EGFr+above the median vs. EGFr-below the median). To determine if differences in EGFr tumor expression affect overall survival and disease-free survival, two-sided 5%-level logrank tests will be conducted to compare overall survival between subjects who are EGFr+ versus those who are EGFr-, separately for each treatment arm. We assumed that patients will be accrued over a 5-year period and followed for at least 5 years.

With 412 samples in each EGFr grouping in the selenium arm, there is at least 81% power to detect a difference in overall survival of 6 years in the EGFr- group versus 7.9 years in the EGFr+ group. In the placebo arm, we expect 206 samples from each EGFr group. There is at least 82% power to detect a difference in overall survival of 6 years (EGFr- group) versus 9 years (EGFr+ group). In this population of Stage I (T1N0 and T2N0) non-small cell lung cancer patients, the expected median overall survival times in the T1N0 patients is 9 years and 6.8 years in the T2N0 patients (see section 1.1).

Similarly, with 412 samples in each EGFr grouping in the selenium arm, there is at least 81% power to detect a difference in disease-free survival of 5 years in the EGFr- group versus 6.5 years in the EGFr+ group. In the placebo arm, we expect 206 samples from each EGFr group. There is at least 82% power to detect a difference in overall survival of 5 years (EGFr- group) versus 7.3 years (EGFr+ group). In Stage I non-small cell lung cancer patients, the estimated median disease-free survival time is 5.4 years (estimated from ratio of median disease-free survival time to median overall survival time for K-ras- patients in paper by Graziano SL, et.al. in JCO vol. 17, p. 668, 1999).

To further explore the relationship between EGFr tumor expression and overall survival, as well as the relationship between EGFr tumor expression and disease-free survival, proportional hazards regression models will be examined. We will include EGFr expression level, treatment group, the interaction between EGFr expression and treatment and other possibly related covariates in the model, e.g. histology and tumor stage. Furthermore, as other marker levels become available, we will also consider proportional hazard models that include several different markers, as well as their interactions, as independent covariates in the model.

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9.8 Smoking History and Diet Studies

Summary information will be computed as mean, standard deviation, median, and range. Interquartile range will be computed for the continuous variables. Proportions will be computed for the discrete variables. Summarized variables will include demographics (age, gender, and ethnicity), clinical risk (presence/absence of COPD, duration, and pack years of cigarette smoking), and nutritional factors (average daily intake of micronutrients and vitamin and mineral supplements; average daily intake of specific food groups). Summary information will be examined by treatment status and a comparison made of the baseline data to check for similarity of the selenium and placebo groups.

Simple associations between the presence or absence of hypermethylation at study entry, clinical risk factors, and dietary factors will be assessed using two-way tables of frequencies or linear models. When the clinical risk factors or dietary variables are continuous, the methylation positive and negative groups will be compared using a two-sample t-test with more complex linear regression models adjusting for confounding factors such as age. If these variables are categorical, the groups will be compared using the Chi-square test or Fisher's exact test. In subsequent analyses, logistic regression models will be used to adjust for confounding factors. Hypermethylation (presence or absence) of p16, MGMT, and DAP-kinase will be examined individually. Hypermethylation of at least one of the three genes will also be considered as an outcome. To address the issue of multiple endpoints, we will expand our data analysis to polytomous logistic regression models using a categorical methylation index (presence of none, one, or multiple methylation changes in sputum or plasma). If the more restrictive assumptions of polytomous logistic regression are not met, log linear models will be used. We will also explore the use of latent class models to investigate clustering of the methylation outcomes.

Predictor variables will include clinical risk factors such as COPD, smoking, and nutritional intake indicators. Some predictor variables will necessarily be discrete, such as the presence or absence of COPD. Other variables may be either continuous or discrete. Smoking and nutritional intake measures may be treated as categorical, ordinal, or continuous variables. For example, smoking may be coded as current vs. former smoker, or >30 pack-years vs. <30 pack-years. Alternatively, smoking may be represented as a continuous variable, such as total pack years or duration of smoking. Nutritional intakes can be categorized by quantiles or treated as continuous variables. For example, the relative risks for the effect of nutritional variables on methylation will be calculated for quartiles of dietary exposure, and when appropriate, for a linear increase of one serving/day. Single dietary factors to be evaluated include fat (total, saturated, and the ratio of polyunsaturated to saturated), folate, carotenoids (e.g., lycopene, lutein, and zeaxanthin), vitamin E, vitamin C, and selenium at entry onto the prevention trial. Relationships will be examined based on food intake, supplemental intake, and combined intake. Several of these variables are proposed to affect the prevalence for gene promoter hypermethylation and, thus, our main rationale for their assessment. In addition, food groups related to cancer risk will be evaluated. These include cruciferous vegetables, total fruits and vegetables, and red meat. Similar analyses will be conducted for the 1400 person group where cancer rather than methylation status will be the outcome. Data will be collected over the period of the supplemental grant; however; due to the length of the prevention trial, analysis may continue until completion of the trial. To minimize residual confounding acknowledged between smoking and nutrition, models will be developed that include number of years smoked and cigarettes smoked per day, both as continuous variables, and using an indicator variable for current smoking.

Similar statistical methods outlined above will be used to determine whether diet affects the modulation of promoter methylation by selenium. Statistical methods to determine whether nutritional variables and clinical risk factors are associated with the development of second primary tumors and/or modify the effectiveness of selenium will also follow the strategy detailed above. In addition, the time to tumor and survival models (proportional hazards model) will be used in the analysis.

Statistical analyses will be conducted in SAS. If the sample sizes are small, which may occur when examining subsets of the entire population, and proportions with hypermethylation are small, then exact statistical procedures will be used (StatXact and LogXact, Cytel Software Corporation, Cambridge, MA).

Rev. 10/01,7/04 9.9 Anticipated Accrual

The proposed trial is a replacement for 91025, a trial of 13-cis-retinoic acid to prevent second primary lung tumors. Both trials include subjects from the same population. Based on data from 91025, the anticipated accrual in subgroups defined by race and gender is:

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	Total
Rev. 7/04 Female	2	4	54	7	744	-	811
Rev. 7/04 Male	3	6	75	10	1055	-	1149
Unknown	-	-	-	-	-	-	-
Rev. 7/04 Total	5	10	129	17	1799	-	1960

The accrual targets in individual cells are not large enough for definitive treatment comparisons to be made within these subgroups. Therefore, overall accrual to the study will not be extended to meet individual subgroup accrual targets.

Rev. 10/01,7/04 9.10 Study Monitoring

This study will be monitored by the ECOG Data Monitoring Committee (DMC). The DMC meets twice each year. For each meeting, all monitored studies are reviewed for safety and progress toward completion. When appropriate, the DMC will also review interim analyses of outcome data. Copies of the toxicity reports prepared for the DMC meetings are included in the reports prepared for the ECOG group meeting (except that for double blind studies, the DMC may review unblinded toxicity data, while only pooled or blinded data will be made public). These group meeting reports are made available to the local investigators. Any DMC recommendations for changes to this study will be circulated to the local investigators in the form of addenda to this protocol document. A complete copy of the ECOG DMC Policy can be obtained from the ECOG Coordinating Center.

Rev. 6/03, 7/04, 3/07 **10.0 PATHOLOGY REVIEW**

When a patient is registered to receive protcol therapy, the submitting pathologist and clinical research associate should refer to the Forms Submission Schedule and Appendix IX (Pathology Submission Guidelines).

MANDATORY for evaluability

Submit to the ECOG Coordinating Office: Copies of the pathology reports from the original diagnosis are to be submitted at randomization. If the patient develops a recurrence or a new second primary tumor (SPT) of any histology, the pathology reports from the recurrence or new primary must be forwarded with the Recurrence Form #1370 or the E5597 Second Primary Cancer Form #1371, as appropriate.

Representative diagnostic material from both the original diagnosis and the recurrence or new second primary may be requested for central review and classification. Slides will be requested ONLY on those cases considered unresolved or questionable by the Endpoint Review Committee. Investigators should be aware that they may be asked to submit these samples in the future. If requested, these materials are to be submitted to the ECOG Pathology Coordinating Office as outlined below and in Appendix IX.

NOTE: ECOG's diagnostic review project for secondary AML/MDS ended December 1, 2005. Submission of diagnostic slides upon diagnosis of secondary AML/MDS for central diagnostic review and classification is no longer required.

From patients whom have consented to participate in the correlative studies

Tissue from the original surgical resection is to be submitted to the ECOG Pathology Coordinating Office (PCO). The sample submission is described in this section, the correlative study is described in Section 11.5.

10.1 Submission Requirements

10.11 Forms and Reports

10.111 MANDATORY to the ECOG Coordinating Center:

- At Randomization - Original diagnostic pathology report
- Upon recurrence or new SPT
  1. Recurrence Form #1370 or the E5597 Second Primary Cancer Form #1371, as appropriate.
  2. Pathology Report

10.112 With Samples Submitted to the PCO

- A Copy of the Institutional Pathology Report
- Copies of immunologic studies, if performed
- ECOG Pathology Material Submission Form (#638), Parts A & B completed. Please identify the clinical status of the submitted material (i.e., pretreatment as opposed to remission and relapse).

10.12 Biological Materials: Submitted to the PCO

10.121 IF REQUESTED: For central review upon recurrence or the development of a new SPT

Slides from the original diagnosis and the recurrence or new primary will be requested ONLY on those cases considered unresolved or questionable by the Endpoint Review Committee.

NOTE: Submission of pathologic materials for central review, if requested, is required in order for the patient to be considered evaluable.

10.122 FROM PARTICIPATING PATIENTS: For correlative study described in Section 11.5:

Tissue block from the original surgical resection.

NOTE: If blocks will not be submitted, 20 unstained sterile sections non heat treated on plus slides are requested.

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10.2 Shipping guidelines

Sample submission schedule:

Rev. 3/07

- Upon recurrence or development of a new SPT, the required materials must be submitted within 30 days of diagnosis. If representative diagnostic samples are requested, samples are to be submitted within one month of the request.

Rev. 3/07

- [Deleted via Addendum #4]
- Materials for the correlative study are to be submitted within one month of randomization to Step 2.

Materials are to be at ambient temperature. During the summer months it is recommended that the biological materials be shipped with a frozen cool pack. Submit materials to:

ECOG Pathology Coordinating Office
Robert H. Lurie Comprehensive Cancer Center
of Northwestern University Medical School

Rev. 3/07

Olson Pavilion - Room 8421
710 North Fairbanks Court
Chicago, IL 60611
Tel: (312) 503-3384
FAX: (312) 503-3385

Rev. 7/04, 3/07 10.3 Central Processing and Routing

The PCO will process the materials and forwarded them to the appropriate individuals.

Rev. 7/04, 3/07 10.31 Central Review

Rev.3/07 Any materials associated with recurrence or new SPT will be routed to the appropriate reviewer.

Rev. 3/07 [Deleted via Addendum #4]

Rev. 7/04, 3/07 10.32 Correlative Samples

Rev. 7/04, 3/07 10.321 Generation of tissue microarrays (TMAs)

Rev. 7/04 From the blocks submitted, H&E stained slides will be generated for purposes of quality control and assessment. Two H & E slides will be forwarded to Dr. Seena Aisner who will mark the areas of interest. One slide will be returned to the PCO and the other retained for documentation.

Rev. 7/04 Duplicative TMAs will be generated by the ECOG Pathology Coordinating Office. Two core (1.00mm punch) will be taken from each tumor block submitted, then placed in duplicate TMA recipient blocks. A total of 60 cores will be placed into a TMA, representing 60 patients per TMA.

Rev. 7/04, 3/07 10.322 Routing

The following will be forwarded to Seena Aisner, M.D. for analysis by her laboratory and Dr. Edward Gabrielson's laboratory.

- Slides from each representative TMA
- Slides submitted from patients for which blocks were unavailable

Rev. 10/01, 6/03 10/04 **11.0 CORRELATIVE STUDIES FOR ECOG, CALGB, NCCTG, NCIC CTG, RTOG AND SWOG INSTITUTIONS**

Rev. 7/04 **NOTE:** THE FACILITY PERFORMING THE BLOOD SELENIUM LEVELS (SECTION 11.1) HAS CHANGED. SECTIONS 7.2, 11.1, APPENDIX V AND APPENDIX X, HAVE BEEN UPDATED TO REFLECT THIS CHANGE. PLEASE UPDATE YOUR RECORDS ACCORDINGLY.

Rev. 6/03 **NOTE:** Biological materials for correlative studies and/or banking are to be collected and submitted only from those patients who have given written signed consent for the use of their samples for these purposes.

Rev. 6/03 **NOTE:** There are three sets of samples described in this section. Serum is requested for the correlative described in subsection 11.1. Subsection 11.2 describes the samples requested for the correlatives described in subsections 11.2, 11.3, and 11.4. **Separate sample collection/shipping kits are available for each sample set from the respective central laboratories as described in subsections 11.11 and 11.21.** The submission guidelines for the samples used in section 11.5 are outlined in section 10.0.

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SAMPLE SUBMISSION SUMMARY

	Sample	Section with Submission Description	Timepoint: Prior to Randomization to Step 2	Timepoint: Months 6 and 12	Timepoint: Months 24 and 48	SUBMIT TO
Rev. 7/04, 1/08	Serum (royal blue top tube) [4,5]	11.1	X[1]		X	Beth Hodgman MAYO CTRL/CLIN TRIALS 3050 Superior Drive NW Rochester, MN 55901 Tel: (800) 533-1710
Rev. 7/04	Plasma (blue top CPT)[3,5]	11.2	X[1, 4]	X[5]	X	Ms. Darlene Harbour Lovelace Respiratory Research Inst 2441 Ridgecrest Drive S.E. Albuquerque, NM 87108 Tel: (505) 348-9500
Rev. 7/04	Buffy Coat (blue top CPT) [3, 5]					
Rev. 7/04	Sputum [3,5]					
Rev. 7/04						
Rev. 7/04	Questionnaires [6]	5.5				
	Surgical Resection	10	X[2]			See Section 10.3

1 Baseline blood samples for the correlative studies are to be collected and submitted AFTER registration to the run-in period, just prior to randomization to Step 2.

2 Surgical resection blocks or slides are to be submitted within 1 month after randomization to Step 2.

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3 **Kits are available** for the collection and shipping of samples (see section 11.2)

Rev. 7/04 4 Samples are for selenium level analysis. COLLECT THESE BLOOD SAMPLES PRIOR TO PERFORMING ANY OTHER BLOOD DRAWS. **Kits are to be ordered two weeks prior to collection** of the sample. Order kits by faxing the Mayo Central Laboratory for Clinical Trials Supply Order Form (Appendiz V) to 507-266-0188.

Rev, 7/04 5 The ECOG Material Submission Form (#709) must be submitted with each sample at all required time points. The submission of this form should be sample specfic.

Rev. 7/04 6 Questionnaires for the study assessments described in section 5.5 will be distributed with the kits or may be downloaded by accessing the ECOG website (http://www.ecog.org). Questionnaires should be completed at month 6 or the next scheduled visit if patient has been on study longer than 6 months.

Rev. 10/01, 3/07 **NOTE:** Study participants from outside the United States and Canada are exempt from sending fresh specimens for correlative studies due to costs and problems associated with international shipping.

Rev. 10/01, 6/03, 7/04 11.1 Blood Selenium Levels

Blood selenium levels will be used to assess compliance to the treatment protocol. Compliance will also be monitored by pill counts, patient diaries, etc.

These analyses will be performed by the Mayo Central Laboratory for Clinical

Trials (MCLCT).

Rev. 10/01, 6/03, and 7/04, 1/08

11.11 Sample Submission Schedule

Samples will be collected

- Baseline, prior to randomization to Step 2
- at Month 24 (year 2), and
- at Month 48 (year 4).

Selenium kits should be ordered two weeks prior to collection of the sample by faxing the Mayo Central Laboratory for Clinical Trials Supply Order Form found in Appendix V to (800) 441-1297. Kits should not be ordered by phone.

All questions concerning the preparation or shipping of the selenium levels should be directed to the MCLCT, Amber Bolles at (800) 826-5561 or bolles.amber@mayo.edu.

Rev. 10/01, 6/03, and 7/04

11.12 Sample Preparation Guidelines

NOTES:

- If blood is also being collected for other test(s) at this visit, be sure to fill a metal-free tube first.
- Use an alcohol swab to cleanse the venipuncture site. Avoid iodine-containing disinfectants.
- Only use a stainless steel phlebotomy needle.

1. Draw blood into the royal-blue "metal free" collections tube by direct venipuncture (i.e., no syringe or butterfly).
2. Allow the blood to clot for 30 minutes at room temperature. DO NOT remove the stopper.
3. Cetrifuge the tube for 10-15 minutes at 300 rpm or until there is a good separation of serum from cells.
4. Remove the stopper and carefully pour at least 1.0 mL of serum into the tube named "Selenium" DO NOT insert a pipette into the serum to accomplish transfer, and DO NOT ream the specimen with a wooden stick.
5. Camp the aliquot tube securely. Discard the collection tube.

Samples should be labeled with the following:

Patient Initials
ECOG Protocol/Patient Sequence #
Institution/ Affiliate
Date of Draw
Timepoint (Baseline, month 24 or month 48)

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11.13 Shipping Procedures

NOTE: Overnight courier air bills are included in kits.

Packaging:

1. Complete the shaded areas of the requisition form, and confirm that the specimen is correctly labeled. Be sure to indicate the appropriate visit on the form.
2. Put the aliquot tube into the bubble pack.
3. Place the bubble pack into the "Refrigerated specimen" transport bag.
4. Place a copy of the completed requisition form into the outer pocket of the bag. Seal the bag.
5. Place the bag into a refrigerator while you make transportation arrangements.

Preshipment Checklist

Please ensure that the following criteria are met prior to sending specimens to MCLCT:

- The serum specimen was collected and processed using the "metal-free" tubes in the kit.

- The specimen tube is completely and legibly labeled.
- The requisition form from the kit has been completed, and a copy is enclosed in the specimen bag.

Specimen Shipment

Speciments for this study will be shipped via Federal Express® (Fed Ex®). Care must be taken to ensure that specimens are properly shipped. Specimens should be sent to MCLCT on the same day they are collected if at all possible. Do not collect any speciment on the day before, the day of, or the observed day of a United States national holiday. Transportation services are frequently not available and the results may be compromised by delayed arrival at MCLCT. Contact MCLCT for specific instructions if specimens must be collected on the day before, the day of, or the observed day of a United States national holiday. Call your local Federal Express® office to determine service availability for pickups late in the day or on Saturday.

Ensure that specimens are packaged in MCLCT transport bags according to instructions.

1. Specimens must be completely packaged in shipping containers prior to Fed Ex® pickup.

   Check that all of the required labels are clearly visible on the shipping boxes. Boxes will be delayed enroute specimen quality may be jeopardized if any of the required labels are missing, covered, or not filled out properly.

2. Complete a pre-printed Fed Ex® airbill. Request "Priority Overnight" service. Indicate "Deliver Saturday" on the airbill if you ship on Friday.

3. Write your return address on the shipping container.

4. Fax a completed copy of the "Shipment Alert" (Appendix X) form to MCLCT, Transportation Department at 1-507-284-1790. No cover sheet is necessary. The following information should be provided.
   - Your name and location of your study site.
   - Fed Ex® tracking number (top center of airbill)

   The transportation department will use the tracking number to monitor the shipment.

   Samples should be shipped overnight courier to:

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Beth Hodgman
Mayo Central Laboratory for Clinical Trials (MCLCT)
3050 Superior Dr., NW
Rochester, MN 55901
Tel: (800) 533-1710

Saturday deliveries are accepted.

Patient Samples from more than one patient can be batched and sent on a weekly basis.

The ECOG Material Submission Form (#709) must be submitted with each sample.

Rev. 10/01, 7/04

11.14 Banking

The serum samples submitted will be used for the correlative study described in Section 11.1 only. No residuals will be stored for future studies.

Rev. 10/01

11.2 Analysis of Gene-Specific Promoter Hypermethylation

Methylation of genes such as p16, MGMT, and DAP-kinase is detected by the methylation-specific PCR (MSP) assay. DNA is first isolated from sputum or plasma by standard technology (43). This procedure (45) uses genomic DNA that is first modified by bisulfite. Bisulfite converts cytosines to uracil; however, if the cytosine is methylated, it is not converted. Following the bisulfite modification, the DNA template can be amplified for specific genes that may be methylated through the design of methylation-specific primers. These primers recognize only methylated alleles (another set of primers can be designed that recognize unmethylated alleles of the gene of interest). We have improved the MSP procedure for the p16, MGMT, and DAP-kinae genes by incorporating a nested PCR approach. This approach has given us more specificity and sensitivity (> 1 methylated allele in 50,000 unmethylated alleles). Thus, we have developed a very sensitive and objective (presence of the PCR product is scored as a positive) assay for detecting gene alterations indicative of NSCLC. All samples positive for methylation will be confirmed through a second PCR assay provided adequate DNA is available.

We plan to collect blood and sputum at the time of entry onto study, and at 6, 12, 24, and 48 months during the study from all participants willing to consent to the laboratory studies. As part of the current protocol, one tube of blood is already being drawn at study entry, 24 and 48 months to measure selenium levels. Study participants will return to their participating institution every 6 months to receive the selenium or placebo pills and a standardized physical examination. Thus, drawing blood at the 6- and 12-month time interval should be feasible. Therefore, at these five time-points, an additional 32 cc of blood will be drawn into Citrate Vacutainer CPT tubes (Becton Dickinson). These tubes are designed to separate plasma and mononuclear cells from whole blood. A detailed protocol for blood isolation is included.

As part of consenting to the laboratory study, each participant will also provide two consecutive spontaneous sputums collected at home at each time point. Many participants in the selenium trial may not have a productive cough since they will not have COPD or be current smokers. However, current statistics indicate that 30% of persons with lung cancer have COPD, and 50% will likely be current smokers. Thus, most people with COPD, and those who smoke will have a productive cough. In addition, some former smokers also have a productive cough. Therefore, being conservative, we estimate that 60% of the participants for this chemoprevention trial can provide sputum specimens. Our lab is currently collaborating with the University of Colorado Health Sciences Center on a study involving cancer-free subjects with COPD. To date, 75% of the participants have submitted samples and only 10% of the submitted samples have been deemed inadequate. Thus, a conservative estimate of 790 persons will submit adequate sputum samples at each time point for potential analysis.

These assays will be done by Dr. Steven Belinsky's laboratory.

The samples described here are sufficient for the correlatives described in sections

11.2 through 11.4.

11.21 Sample Submission Schedule

Peripheral blood and sputum samples will be collected

- prior to randomization at Step 2, and at
- Month 6
- Month 12 (year 1)
- Month 24 (year 2)
- Month 48 (year 4)

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Please ship samples within five days of the blood draw.

Special tubes, supplies for washing and collecting the plasma and mononuclear cells, and shipping labels will be provided by the Lovelace Respiratory Research Institute in the form of a blood isolation kit. Sputum collection cups will also be provided. Order the Blood Isolation Kits by contacting Ms. Darlene Harbour, Email: dharbour@LRRI.org, or Ms. Elia Casas, Email: ecasas@LRRI.org, or call 505-348-9500.

Ms. Darlene Harbour or Elia Casas will also answer any questions regarding sample collection.

11.22 Sample Preparation Guidelines

General Strategy:

The study coordinators, Darlene Harbour or Elia Casas will phone the institution's contact person to go over the laboratory component of the study. At that time, an introduction to the study and detailed protocol will be Emailed to the contact person so they can go over the protocols together. In addition, Ms. Harbour will obtain the name of the person who will be doing the blood separation and also go over the protocol with that person if the original contact person is unable or would prefer this approach.

11.221 Peripheral blood samples

Rev. 7/04 **NOTE:**Label the tubes with ECOG protocol E5597, ECOG patient sequence/case number, date of draw, time point, and sample type
Rev. 7/04 (plasma or cells).

Rev. 7/04 • At each time point, draw 32 mL of peripheral blood into the 4 blue top sodium citrate vacutainer CPT tubes provided in the blood isolation kit.

Rev. 7/04

Rev. 7/04 • Blue top tubes: Gently invert the collected blood 8 times before centrifugation at room temperature. Please centrifuge within 2 hours after blood collection.

Rev. 7/04

• The blood collection tubes are then centrifuged at 1900 x g RCG for 20 minutes in a swinging bucket centrifuge.

Rev. 7/04 • **Plasma:**: After centrifugation, use a disposable transfer pipet to draw the plasma (top layer) off into the two 15 mL polypropylene collection tubes, **leaving approximately 0.5 cm of the plasma at the interphase to the mononuclear cells** (this will prevent excess contamination with the mononuclear cell layer that is just below the plasma). Put the plasma from two blood collection tubes into one 15 mL polypropylene tube, thus ending up with 2
Rev. 7/04 tubes.

Rev. 7/04 Centrifuge the two plasma 15 mL tubes at 1500 rpms for 10 min to pellet contaminating mononuclear and other cells.

After repeat centrifugation, use a disposable transfer pipet to draw the clean plasma (top layer) off into two clean 15 mL polypropylene collection tubes that have been labeled with the ECOG protocol number, patient's ECOG sequence number, and collection date.

Rev. 7/04 Place the two 15 mL tubes in the freezer and hold in the -20°C freezer until shipping.

Rev. 7/04 • **Mononuclear cells** from the blue top tubes: Draw off the remaining 0.5 cm of plasma and discard.

• Then draw off the mononuclear cell fraction from each of the blood tubes with a new disposable transfer pipet into one 15 mL polypropylene collection tube. Bring the volume of mononuclear cells up to approximately 4 mL in addition to the provided phosphate buffered saline and mix gently by inversion.

Transfer the 4 mL of mononuclear cells into 4 separate screw top eppendorf tubes (1 mL of cells per tube). Spin in a table top microfuge at 2000 rpms for 2 minutes. Decant off the liquid, date

the tubes, and put the tubes with the mononuclear cell pellet into the -20°C freezer and hold along with the plasma for shipment.

11.222 Spontaneous sputum

Two spontaneous sputum samples are to be provided at each time point. Participants will be provided with a sterile specimen cups containing Saccomanno's fixative (50% ethyl alcohol and 2% carbowax). For three consecutive mornings each, the patient is to cough deeply and expectorate the resulting mucous into the cup. The patient can keep the cup at room temperature.

Detailed instructions for the patient are provided in Appendix VI.

11.23 Shipping Procedures

Note that the patients will send the sputum at ambient temperature directly to lab via a prepaid mailer. The address will be the same as for the plasma and mononuclear cell samples.

The participating institution should ship the aliquots of mononuclear cells and plasma in the provided styrofoam box surrounded by an outer card board box that contains a prepaid Federal Express form. In addition, ice packs which should be kept in the freezer along with the samples are provided. The samples are placed in the provided bubble wrap between the 4-6 ice packs prior to shipping.

Samples should be shipped within 5 days of collection. Notification of shipment can be by Email to Darlene Harbour or Elia Casas, Email address: dharbour@LRRI.org and ecasas@LRRI.org.

Use the pre-addressed Federal Express Tag and ship the samples to:

Ms. Darlene Harbour
Lovelace Respiratory Research Institute
2441 Ridgecrest Drive S.E.
Albuquerque, NM 87108
Tel: (505) 348-9500

Shipping should occur on Monday through Thursday. Do not ship on Friday.
The ECOG Material Submission Form (#709) must be submitted with each sample.

11.24 Banking

The residuals and/or derivatives of samples collected for this study will be retained at the sample repository at the Lovelace Respiratory Research Institute or the ECOG Central repository at the ECOG PCO for use in future ECOG approved studies. If future use is denied or withdrawn by the patient, the samples will be removed from consideration in any future study.

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11.3 Analysis of Lipoxygenase Metabolites.

Selenium's effect on 5-LO will be assessed by HPLC measurement (63) of LTB-4 and 5-HETE in plasma at baseline and during treatment on the randomized arms at 6, 12, and 24 months. The sensitivity and specificity of this method of analysis are superior to radioimmune assay (63). At each time point, plasma recovered from 10 mL of whole blood from each subject will be spiked with internal standards. Reference standards and internal standard will be purchased from Sigma Chemical Company to monitor HPLC performance and establish calibration curves. Standards containing increasing concentrations of LTB-4 and 5-HETE, as well as samples, will be extracted with the aid of acetonitrile to precipitate the protein and centrifuged. The supernatant will be diluted and acidified to pH 3.5 with 1.0 mM HCl immediately before addition to a C18 Sep-Pak cartridge. The cartridge will be washed with H_2O, acetonitrile/water (20% and 70%) with the eluate being dried and reconstituted with 66% MeOH/H_2O. The samples will be clarified by centrifugation and 100 microliters injected on a Rainin Microsorb C18 3 mm 10 x 0.46 cm or NovaPak C18 4 mm 150 x 4.6 mm column. The mobile phase will consist of MeOH/H_2O/trifluoroacetic acid/triethylamine (80:20:0.1:0.05) at a flow rate 1.0 mL/min for 20 min. Analytes will be detected by absorbance at 280 nm for LTB-4 and 235 nm for 5-HETE.

11.31 Sample Submission

The samples submitted for 11.2 are sufficient for studies described in sections 11.2 through 11.4.

Rev. 10/01

11.4 Analysis of 5-OHmdU

Of the various reactive oxygen species that can be generated, hydroxyl radical has been shown to damage DNA and has been the focus of many studies. Hydroxyl radicals are readily generated from hydrogen peroxide in the presence of metal ions (67,68). Approximately 80% of hydroxyl radical-mediated DNA damage is on the DNA bases, with 20% of the reaction on the deoxyribose (64). Each base in DNA can be oxidized by hydroxyl radicals; however, the thymidine is oxidized at relatively higher levels (70,71). The oxidized thymidine derivative to be quantified in this study will be 5-hydroxymethyl-2'-deoxyuridine (5-OHmdU). Unlike some oxidized nucleosides including thymidine glycol, 5-OHmdU is relatively stable (72), which facilitates its analysis. In DNA that is hydrolyzed enzymatically, the levels of 5-OHmdU are about 10-fold higher than those of 8-hydroxy-2'-deoxyguanosine (73). 5-OHmdU is incorporated into DNA and is toxic to cells (74), although it does not appear to block the initiation of transcription (75).

Oxidation of nucleosides during derivatization may be a problem. Using our method for determination of 5-OHmdU that includes enzymatic hydrolysis, 20-min derivatization at 120°C with TMCS as a catalyst, and simultaneous quantitation of thymidine, we have detected five-fold lower levels of 5-OHmdU in rat liver than others who used HPLC methodology that requires no derivatization (76,77). Therefore, under our conditions, oxidation of thymidine during derivatization is not expected to be a problem. We have successfully used this assay to examine the

effects of diet on levels of 5-OHmdU in several studies (78-80).

Frozen lymphocytes will be used for this assay. Nuclei will be prepared from the lymphocytes as described below and samples will be stored at -70°C as nuclei in 50-mM mannitol and 1% SDS. For quality control, nuclei will be prepared from 500 mL Red-Cross blood from one donor. This will yield 50 tubes of nuclei that can be stored frozen and processed with each batch of blood samples obtained from this proposed study. We expect about 750 samples from this study and they will be analyzed in batches of 30. To assure the equivalency of levels from batch-to-batch, two calf thymus DNA samples will be analyzed along with two aliquots of Red-Cross blood. The calf thymus DNA samples will be one unoxidized sample and one oxidized with iron and hydrogen peroxide. These samples will be aliquoted at the beginning of the study and stored frozen in water containing 50 mM mannitol.

Nuclei will be prepared by the method of Ciulla from frozen lymphocytes (81). The sucrose buffer will contain 50 mM mannitol. DNA will be isolated from nuclei using a modification of the procedure of Miller et al. (82) in order to avoid the possible deleterious effects of phenol exposure on the DNA (83,84). The nuclei in 1% SDS, 1 mM EDTA, and 50 mM mannitol will be treated with RNases A and proteinase K. The proteins will be precipitated by addition of 300 mL 6 M sodium chloride, shaking and centrifugation. One extraction with n-butanol will be used to remove residual protein. The DNA will be precipitated twice, dissolved in 200 mL water, and a UV scan obtained. Using this technique, we have obtained 100 mg DNA from 7-mL blood, and the DNA was judged equally as pure as that obtained with standard phenol and chloroform extractions based on the UV spectral characteristics of the DNA and HPLC and gas chromatogranphic-mass spectral (GC-MS) chromatograms.

The GC-MS method for detecting DNA damage has advantages over other methods, such as the immunoassay or ^{32}P-postlabeling. Not only can small quantities of damage be detected, but structural confirmation of the analyte also can be obtained. This is of utmost importance when monitoring human populations for DNA damage due to the large variety of damaged bases that can be formed upon environmental exposures. We can quantify as little as 5 pg 5-OHmdU by GC-MS with single-ion monitoring. Thus, if 1 mg DNA is injected onto the GC column, we can detect $1/10^5$ damaged bases (78).

The isolated DNA will be hydrolyzed, derivatized, and 5-OHmdU levels determined by GC-MS using isotopically labeled internal standards as described (84). The samples will be derivatized with BSTFA containing 1% trimethylchlorosilane and acetonitrile (2:1) by heating at 120°C for 20 min based on the procedure of Dizdaroglu (85).

Gas chromatographic separations will be performed with a 25-m Hewlett-Packard SE54 Ultra 2 column using helium as the carrier gas. The column head pressure will be 10 psi with a total flow of 23 mL/min. The injection temperature will be 250°C. The initial temperature of the column will be 50°C and, immediately after injection, the temperature will be increased to 150°C at a rate of 40°C/min. The temperature then will be increased to 190°C at a rate of 3°C/min, followed by heating to 290°C. Initial temperatures higher than 50°C resulted in poor peak shapes. Samples will be analyzed at a low electron multiplier setting to quantify thymidine and at the maximum electron multiplier setting to quantify 5-OHmdU. The peak area ratios of molecular ions for DNA bases and their respective isotopically labeled internal standards will be calculated using 358/362 for 5-OHmdU and 270/274 for thymidine. This procedure will correct for possible variations in the derivatization efficiency of each sample. Standard curves will be used to determine the levels of thymidine and 5-OHmdU in each sample. It will be important to quantify thymidine in order to control for DNA derivatized and the extent of DNA hydrolysis.

11.41 Sample Submission

The samples submitted for 11.2 are sufficient for studies described in 11.2 to 11.4.

Rev. 6/03 11.5 Evaluation of Predictive Markers

A correlative molecular study to identify markers that predict patient response to selenium could greatly increase the value of this study. One powerful approach to help identify these markers is through serial testing of candidate markers by immunohistochemistry (IHC) for relationships to overall survival and disease-free survival in treated and control groups.

Patients who have undergone resection for stage I NSCLC are an ideal population for assessing molecular markers that predict response to a chemoprevention agent.

The aims of this correlative study are to: test markers (including EGF-R, Her2/neu, p53, e-cadherin), which have been previously proposed as lung cancer prognostic markers as well as other significant candidate markers.

The study will be performed in the laboratories of Seena Aisner, M.D. (New Jersey Medical School) and Edward Gabrielson, M.D (Johns Hopkins University).

11.51 Sample Submission

The appropriate materials will be forwarded to the investigators by the ECOG PCO. Samples submissions are described in Section 10.0. No additional materials are requested for this correlative study.

11.6 Sample Inventory Submission Guidelines

Inventories of all samples collected and the respective aliquots made and used on the above mentioned laboratory correlative study(ies) will be submitted to the ECOG Coordinating Center on a monthly basis. Inventories will be submitted electronically or by diskette by any laboratory holding and/or using any specimens associated with this study. Electronic submissions should be submitted to 303.lab@jimmy.harvard.edu. All other correspondence should be addressed to the attention of the Correlative Science Team.

Rev. 10/01 11.7 Lab Data Transfer Guidelines

The data collected on the above mentioned lab correlative study will be submitted to the ECOG Coordinating Center by the Central Laboratory on a quarterly basis. The quarterly cut-off dates are March 31, June 30, September 30 and December 31. Data is due at the Coordinating Center 1 week after these cut-off dates. Electronic submissions should be submitted to 303.lab@jimmy.harvard.edu. All other correspondence should be addressed to the attention of the Correlative Science Team.

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12.0 RECORDS TO BE KEPT

Please refer to the E5597 Forms Packet for the forms submission schedule and copies of all forms. The E5597 Forms Packet may be downloaded by accessing the ECOG World Wide Web Home Page (http://www.ecog.org). Forms must be submitted to the ECOG Coordinating Center, FSTRF, 900 Commonwealth Avenue, Boston, MA 02215 (ATTN: DATA).

This study will be monitored by the CTEP Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly from the ECOG Coordinating Center to CTEP by electronic means.

12.1 Records Retention

FDA regulations (21 CFR 312.62) require clinical investigators to retain all trial-related documentation, including source documents, long enough to allow the sponsor to use the data to support marketing applications.

This study will be used in support of a US marketing application (New Drug Application), all records pertaining to the trial (including source documents) must be maintained for:

- two years after the FDA approves the marketing application, or
- two years after the FDA disapproves the application for the indication being studied, or
- two years after the FDA is notified by the sponsor of the discontinuation of trials and that an application will not be submitted Please contact the ECOG Coordinating Center prior to destroying any source documents.

12.2 CALGB Institutions

CALGB participants should submit data forms as listed in this section at the required intervals to:

Rev. 3/07

CALGB Statistical Center, Data Operations
Hock Plaza, Suite 802
2424 Erwin Road
Durham, North Carolina 27705
Phone: (919) 668-9350
FAX: (919) 668-9348

Include the ECOG protocol number and patient sequence number as well as the CALGB study number and patient number. The CALGB Data Management Center will forward the forms to the ECOG Coordinating Center.

12.3 SWOG Institutions

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Southwest Oncology Group members, CCOP and affiliate institutions must submit one copy of the original data forms as listed in the forms packet at the required intervals to:

ECOG Coordinating Center
FSTRF, ATTN:Data
900 Commonwealth Ave.
Boston, MA 02215
(ATTN:DATA)

Include the ECOG protocol number and patient ID number as well as the Southwest Oncology Group study number and patient number.

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12.4 NCCTG Institutions

Data forms are to be submitted to:

NCCTG Operations Office
200 First Street, SW
Rochester, ME 55905

NCCTG Operations Office will forward all materials to the ECOG Coordinating Center.

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12.6 NCIC CTG Institutions

A single set of case report forms (CRFs) will be sent to each center (for photocopying and use) following local activation. CRFs should be completed and submitted to the NCIC CTG central office according to the submission schedule. In addition to the required forms as listed, a copy of the signed consent form must be submitted for each patient. The ECOG and NCIC CTG patient number as well as patient initials must be recorded on each form. CRFs will be forwarded to ECOG by the NCIC CTG.

Rev. 10/01 12.7 RTOG Institutions

RTOG participants should submit data forms as listed in this section at the required intervals to:

RTOG Headquarters
1101 Market Street, 14th Floor
Philadelphia, PA 19107
Tel: (214) 574-3191

Include ECOG protocol and patient sequence number as well as the RTOG study number and patient number. RTOG will forward the forms to the ECOG Coordinating Center.

13.0 PATIENT CONSENT AND PEER JUDGEMENT

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

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Rev. 10/01

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Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

APPENDIX I

SUGGESTED PATIENT CONSENT FORM

Rev. 7/04, 10/04 Version Date: April 8, 2010

You are being asked to take part in this study because you had lung cancer which was surgically removed.

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part. Please take your time to make your decision and discuss it with your friends and family.

WHY IS THIS STUDY BEING DONE?

This research is being done because it is unknown if taking selenium can prevent the development of second lung cancers.

The purpose of this study is to find out if a high selenium yeast tablet can prevent new lung cancers in people with surgically removed non-small cell lung cancer. The study will compare how the high selenium yeast affects patients with surgically removed lung cancer when compared to a placebo yeast.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY

About 1,960 people will take part in this study.

WHAT IS INVOLVED IN THE STUDY?

Please see the **study plan** attached to this form.

You will be "randomized" into one of two study groups. One group will receive high selenium yeast and the other group will receive a placebo yeast. Randomization means that you are put into a group by chance. You are twice as likely to be assigned to the group receiving high selenium yeast as the group receiving the placebo yeast. You and your doctor will NOT be able to choose which group you will be assigned to. After you are randomized and start

receiving tablets you, your doctor, the study nurse, and the pharmacist will not know if you are receiving high selenium yeast or the placebo yeast. Both tablets, the high selenium yeast and the placebo yeast, will look the same. You will not know which tablet you received until after the study is complete. All patients will receive the placebo yeast for a short period of time at some point during the study.

Rev. 3/07 If your tumor comes back or you develop a new second primary tumor, the pathology reports
Rev. 3/07 from both the original diagnosis and the recurred or new second primary will be submitted for review. If necessary, your doctor may be asked to submit pathology slides for a central review.

If you take part in this study, you will have the following tests and procedures:
Some of these tests would be done even if you do not take part in the study.

Tests:

- Physical exam
- Medical history
- Blood tests
- Chest x-ray

Rev. 6/03, 3/07 • Central Review: As a part of this study, a sample of your tumor may be sent to a laboratory to be examined by a central reviewer. This review is to confirm the results of the local institutional review.

Procedures:

- "Run-In" period: This part of the study is to find out if you can remember to take the tablets on a regular basis. For the first 28 days of the study, you will
Rev. 3/07 be asked to take one tablet of placebo yeast by mouth every day in the morning. You will be given a calendar diary to record the days and times at which you take the tablet. If you are not able to take your tablets on a
Rev. 6/03 regular basis, you will not be allowed to continue in the study. If you do not continue past the run-in period, no follow-up is required.

- If you continue on the study, then you will take one tablet every morning for a maximum of 4 years. You will record the days you take the tablet on a calendar diary.

Rev. 8/08 • You will be asked to complete a questionnaire on which you will provide a detailed history of supplement, tobacco, and alcohol use every 6 months until the end of your treatment.

- You will be contacted by a nurse or research assistant by telephone on month 3 of your participation in this study and then every 6 months after that while you are taking tablets as a part of this study. During this telephone interview, the nurse or research assistant will ask you questions about how well you are following your assigned tablet regimen.

- There are restrictions on the type and amount of mineral, herbal, phytochemical, or vitamin supplements (any non-food compound taken by mouth or injection which is intended to be a dietary supplement) you may take while on this study. If you currently take supplements, ask your doctor if you may continue taking them while on this study.

- If you are a smoker when you enter the study, talk to your doctor about smoking-cessation programs that are available in your area.

HOW LONG WILL I BE IN THE STUDY?

If you proceed beyond the run-in period, we think you will be in the study for four years. We would like to keep track of your medical condition for the rest of your life to look at the long-term effects of the study.

Your doctor may decide to take you off this study if:

- You develop a new lung cancer.
- Your cancer comes back.
- You have serious side effects.
- Your health gets worse.
- New information on preventing second lung cancers becomes available.

You may stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to your doctor first.

WHAT ARE THE RISKS OF THE STUDY?

While on the study, you are at risk for the following side effects. The high selenium yeast or the placebo yeast may cause some, all or none of the side effects listed. You should discuss these with your doctor. There also may be other side effects that we cannot predict. Most people do not have any side effects or problems when taking selenium. However, in some cases side effects can be serious or long-lasting.

Risks and side effects related to selenium include:

<u>More Likely</u>

- Nausea
- Diarrhea
- Constipation

- Red itchy skin
- Garlic breath
- Garlic odor sweat
- Dry nails
- Hair loss
- Irritability
- Metallic taste
- Vomiting
- Tiredness
- General ill feeling

Less Likely

- Liver damage

Rev. 7/04

- In a prior study with selenium, patients with a history of basal or squamous cell cancers were found to have an increased incidence of occurrence of these cancers with selenium supplementation. Therefore, participants on this trial who are at high risk for non-melanoma skin cancer due to a) previous basal cell and/or squamous cell cancers of the skin or b) high sun exposure, may experience an increased risk of recurrent squamous cell and basal skin cancers.

Rev. 1/08

Selenium and Diabetes

A report has been published suggesting that selenium supplements may be associated with an increased risk of developing type 2 diabetes. The new finding comes from 1,202 participants in the Nutritional Prevention of Cancer Trial, a study different from E5597. The Nutrition Prevention of Cancer Trial was designed to learn if selenium might help in preventing skin cancer. In that study, patients received a daily selenium supplement of 200 micrograms or a placebo.

While we are still waiting to learn if selenium helps prevent melanoma a review of this study found that 58 out of 600 people in the selenium group developed type 2 diabetes compared to 39 of the 602 in the placebo group. Although this difference raises concern, we can not say with certainty that selenium does or does not cause diabetes. But we believe it is important that you are aware of this information.

Some of the early warning signs of diabetes include frequent urination, excessive thirst, increased hunger, unusual weight loss, increased fatigue, irritability and blurry vision.

Your physician will check you closely to see if any of these side effects are occurring.

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

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct medical benefit to you. We hope the information learned from this study will benefit other patients with lung cancer in the future.

The possible benefits of taking part in the study are the prevention of unrelated new lung cancers, increased length of time until unrelated new lung cancers develop, and increased survival.

WHAT OTHER OPTIONS ARE THERE?

Instead of being in this study, you have these options:

- No participation in clinical studies at this time.
- It is possible that you could receive selenium supplement elsewhere without participating in this study.

Please talk to your cancer doctor about these and other options.

WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as:

- National Cancer Institute
- Food and Drug Administration
- The Eastern Cooperative Oncology Group
- Drug manufacturers, distributors, and/or their designated representatives
- Other regulatory agencies and/or their designated representatives

Rev. 3/07
- *Add here the name of the Cooperative Group through which your institution is participating in this trial*

WHAT ARE THE COSTS?

Taking part in this study may lead to added costs to you or your insurance company. Please ask about any expected added costs or insurance problems.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury.

Rev. 10/01
Rev. 6/03
The sponsor Cypress Systems will provide the investigational drug selenium-yeast free of charge for this study.

You or your insurance company will be charged for continuing medical care and/or hospitalization.

You will receive no payment for taking part in this study.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study, or choosing not to take part, will not result in any penalty or loss of benefits to which you are entitled.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

A Data Safety and Monitoring Board, an independent group of experts, will be reviewing the

data from this research throughout the study. We will tell you about the new information from this or other studies that may affect your health, welfare, or willingness to stay in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, contact your cancer doctor *NAME(S)* at *TELEPHONE NUMBER*.

For questions about your rights as a research participant, contact the *NAME OF CENTER* Institutional Review Board (which is a group of people who review the research to protect your rights) at *TELEPHONE NUMBER*.

WHERE CAN I GET MORE INFORMATION?

You may call the NCI's **Cancer Information Service** at:

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

Visit the NCI's Web sites:

CancerTrials: comprehensive clinical trials information **http://cancertrials.nci.nih.gov.**

CancerNet™: accurate cancer information including PDQ **http://cancernet.nci.nih.gov.**

You will get a copy of this form.

SIGNATURE

I agree to take part in this study.

Participant: _____ Date: _____

SCIENTIFIC STUDIES

Rev. 10/01, 6/03 This study includes laboratory tests that will analyze small samples of tissue, blood, and sputum. The tissue sample will be from your original surgical resection. The blood and sputum will be collected before you start treatment, and at 6 months, 12 months, 24 months and 48 months after treatment begins. The blood will be collected using a needle to draw some blood out of a vein. The sputum will be collected by you, at home. The samples will be sent to central laboratories where tests will be performed. Researchers will be performing these tests in order to understand how selenium may or may not help prevent lung cancer. They hope this will help them better understand your type of cancer.

Rev. 7/04 At six months or later, you will be asked to complete two surveys about your diet and lifestyle. The information from these surveys will be compared with the results from the laboratory studies.

Rev. 10/01,7/04 Rev. 6/03 The results from the trial related tests and surveys will not be sent to you or your doctor, and will not be used in planning your care. You or your insurance company will NOT be billed for these tests. These tests are only for research purposes.

Making Your Choice

Please read the sentence below and think about your choice. After reading the sentence, circle "Yes" or "No." No matter what you decide to do, it will not affect your care. You can participate in the treatment part of the study without participating in the research studies. If you have any questions, please talk to your doctor or nurse, or call our research review board at *IRB's phone number*.

1. I agree to participate in the scientific laboratory tests that are being done as a part of this study.

Yes No

Please print and sign your name here after you circle your answer.

Your Name: _____

Your Signature: _____ Date: _____

Rev. 10/01

WILL ANY OF THE TISSUE, BLOOD, AND SPUTUM SAMPLES TAKEN FROM ME BE USED FOR OTHER RESEARCH STUDIES?

About Using Tissue for Research

Rev. 6/03, 3/07

As a part of this study, samples of your tumor may be sent to laboratories for review. If you participate in the additional laboratory studies that are a part of this clinical trial, samples of your tumor, blood and sputum will be submitted to central laboratories for analysis. The results of these tests will not be placed in your medical chart and will not be used to plan your care.

Rev. 6/03
Rev. 6/03

We would like to keep some of the tissue, blood, and sputum that is left over for future research. If you agree, this tissue, blood, and sputum will be kept and may be used in research to learn more about cancer and other diseases. This tissue, blood, and sputum will only be given to researchers approved by the Eastern Cooperative Oncology Group. Any research done on the tissue must also be approved by the researcher's Institutional Review Board.

Rev. 6/03
Rev. 6/03

Your tissue, blood, and sputum may be helpful for research whether you do or do not have cancer. The research that may be done with your tissue, blood, and sputum will probably not help you. It might help people who have cancer and other diseases in the future.

Rev. 6/03

Reports about research done with your left over tissue, blood, and sputum will not be given to you or your doctor. These reports will not be put in your health record. The research will not

have an effect on your care.

Things to Think About

Rev. 6/03 The choice to let us keep the left over tissue, blood, and sputum for future research is up to you. No matter what you decide to do, it will not affect your care and you may still take part in the Eastern Cooperative Oncology Group study.

Rev. 6/03 If you decide now that your tissue, blood, and sputum can be kept for research, you can change your mind at any time. Just contact your study doctor and let him or her know that Rev. 6/03 you do not want us to use your tissue, blood, and sputum. Then the tissue, blood, and sputum will no longer be used for research.

In the future, people who do research may need to know more about your health. When the Eastern Cooperative Oncology Group gives them reports about your health, it will not give them your name, address, or phone number.

Rev. 6/03 Sometimes tissue, blood, and sputum is used for genetic research (about diseases that are Rev. 6/03 passed on in families). Even if your tissue, blood, and sputum is used for this kind of research, the results will not be put in your health records.

Rev. 6/03 Your tissue, blood, and sputum will be used only for research and will not be sold. You will Rev. 6/03 not be paid for allowing your leftover tissue, blood, and sputum to be used in research even Rev. 6/03 though the research done with your tissue, blood, and sputum may help to develop new Rev. 6/03 products in the future. Similarly there will be no cost to you for any tissue, blood, and sputum collected and stored by the Eastern Cooperative Oncology Group.

OPTIONAL

It is possible that at some time in the future, that as part of deciding on what therapy to give Rev. 6/03 you, a new test might be available that could be done on some of the tissue, blood, and sputum that is now thought of as leftover. This situation is unusual, but it could happen. In Rev. 6/03 order to see that not all this leftover tissue, blood, and sputum is used up, the Eastern Rev. 6/03 Cooperative Oncology Group will take care to see that some of your cancer tissue, blood, and sputum is stored for 10 years so that it is available should it be needed by you or your Rev. 6/03 doctors. This will depend upon the amount of leftover tissue, blood, and sputum that is Rev. 6/03 submitted for this study, however, there may not be any left over tissue, blood, and sputum to store.

Benefits

Rev. 6/03 The benefits of research using tissue, blood, and sputum include learning more about what causes cancer and other diseases, how to prevent them, how to treat them, and how to cure

them.

Risks

There are very few risks to you. The greatest risk is the release of information from your health records. The Eastern Cooperative Oncology Group will protect your records so that your name, address, and phone number will be kept private. The chance that this information will be given to someone else is very small.

Making Your Choice

Please read each sentence below and think about your choice. After reading each sentence, circle "Yes" or "No." No matter what you decide to do, it will not affect your care. You can participate in the treatment part of the study without participating in all or part of the tissue, blood, and sputum research studies. If you have any questions, please talk to your doctor or nurse, or call our research review board at *IRB's phone number*.

Rev. 6/03

Rev. 6/03, 3/07

1 My tissue, blood, and sputum may be kept for use in future research to learn about, prevent, treat, or cure cancer.

Yes No

Rev. 6/03, 3/07

2 My tissue, blood, and sputum may be kept for future research about other health problems (for example: causes of diabetes, Alzheimer's disease, and heart disease).

Yes No

3 My doctor (or someone from the Eastern Cooperative Oncology Group) may contact me in the future to ask me to take part in more research.

Yes No

Please print and sign your name here after you circle your answers.

Your Name: _____

Your Signature: _____ Date: _____

STUDY PLAN

REGISTER

↓

"RUN-IN": FOR 4 WEEKS

(This is a practice session to see if you can remember to take the tablets on a regular basis. You will be receiving placebo during this 'Run-In' period.)

Rev. 3/07

↓

RANDOMIZE

↓

HIGH SELENIUM YEAST OR **PLACEBO YEAST**

You will not know whether you are taking high selenium yeast or a placebo yeast.

↓

You will take one tablet every day for four years.

E5597
CALGB 79803
SWOG E5597
NCCTG E5597
NCIC CTG BR16
RTOG L0127
REVISED
Revised 3/07, Update #4

Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

APPENDIX II

E5597 Drug Request Form

Requested By:

Dr. _____ Signature _____
Institution _____ Current IRB Approval Date _____
Principal Investigator _____
Institution Contact _____
Telephone _____ Fax _____

Ship To:

Name _____
Address:* _____
_____
_____
_____

*** Please do not use PO Box numbers**

			Specify one	
Drug ID #	Patient Sequence Number(s)	Patient Initials (Last, First)	Run-In **OR**	Selenium/Placebo

Date Drug Needed by:

_____

Return Completed, Signed and Dated form to:
ECOG Coordinating Center
FSTRF
900 Commonwealth Avenue
Boston, MA 02215
ATTN: Drug Orders
FAX: (617) 632-2063

Reminder: **See protocol Section 8.19 for required documentation that must be submitted with this form**.

Coordinating Center use only:

Inst/Affil#: _____
IRB: _____
_____
Signature Date

Rev. 6/03

Rev. 10/01

Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

APPENDIX III

Patient Diary

Directions for Study Supplement Administration

1. Take 1 tablet daily in the morning.

2. If a pill is forgotten, it should be taken as soon as it is remembered if within 12 hours of the scheduled dose, and the time taken should be noted on the drug diary. Otherwise, the dose should not be taken.

3 Complete one line of the patient diary each day. If a dose is missed, please write in the date on the diary and "Dose not taken" in the comments section.

4. Please bring the empty vial or any leftover pills and your Patient Diary pages to your next clinic visit.

Patient Diary

Dose No.	DATE Month	Day	Year	TIME Record Time of Dose (Circle AM or PM)				Use the space below to make notes about things you would like to tell t experience, other medicine you have taken and anything else you thin
1					:		AM PM	
2					:		AM PM	
3					:		AM PM	
4					:		AM PM	
5					:		AM PM	
6					:		AM PM	
7					:		AM PM	
8					:		AM PM	
9					:		AM PM	
10					:		AM PM	
11					:		AM PM	
12					:		AM PM	
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25					:		AM PM	
26					:		AM PM	
27					:		AM PM	
28					:		AM PM	
29					:		AM PM	
30					:		AM PM	

Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

APPENDIX IV

Patient Thank You Letter

We ask that the physician use the template contained in this appendix to prepare a letter thanking the patient enrolling in this trial. The template is intended as a guide and can be downloaded from the ECOG web site at
Rev. 6/03 http://www.ecog.org. As this is a personal letter, physicians may elect to further tailor the text to their situation.

This small gesture is a part of a broader program being undertaken by ECOG and the NCI to increase awareness of the importance of clinical trials and improve accrual and follow-through. We appreciate your help in this effort.

* * * * * * * * * * * * * * *

[PATIENT NAME] [DATE]
[PATIENT ADDRESS]

Dear [PATIENT SALUTATION],

Thank you for agreeing to take part in this important clinical program. Programs like this offer a chance to get the best care while helping us make better care available for all patients. Many questions remain unanswered in cancer. With the help of people like you who participate in these programs, we will achieve our goal of effectively treating and ultimately curing cancer.

We believe this program will provide you with high quality, thorough care. Your physician and research staff will maintain very close contact with you. This is important to allow your physician to provide you with the best care while learning as much as possible to help you and other patients.

On behalf of [INSTITUTION] and the Eastern Cooperative Oncology Group, we thank you again and look forward to helping you.

Sincerely,

[PHYSICIAN NAME]

Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

APPENDIX V

Selenium Kit Order Form

Mayo Central Laboratory For Clinical Trials

Fax Supply Order Form- No Cover Sheet Necessary

Rev. 1/08

Fax to Amber Bolles at 800-441-1297

Eastern Cooperative Oncology Group (ECOG)

PROTOCOL: E5597

Account # CT204441

Investigator:_____ Phone #: (____)_____
Order placed by:_____ Fax #: (____)_____

Address (kits sent to):

_____
_____
_____

Today's Date:

Rev. 3/08

YOU WILL RECEIVE YOUR SUPPLIES WITHIN 2 WEEKS OF TODAY'S DATE

Please call 1-800-826-5561 if you have questions about your order

Number of "Selenium Testing kits" _____

Number of Other Supplies:
Supplies needed for Federal Express service only:
5 lb. Refrigerated Mailer (Refrigerated; non-infectious)_____
(Federal Express Airbill is provided for each mailer)
Note: The number of mailers should equal the number of kits requested.

Rev. 1/08

Questions? Call Amber Bolles at 800-826-5561

Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

APPENDIX VI

HOME SPUTUM COLLECTION PROCEDURE

You will be collecting two sputum specimens, each over a 3-day period. Thank you very much for participating in this laboratory portion of the selenium prevention trial.

You should have received two sputum cups in a self-addressed mailer.

WHAT IS SPUTUM?

The mucoid material that is expelled from the respiratory passages or the "breathing tubes" is called sputum. A specimen is obtained by coughing up sputum from deep in the lungs and placing it in a bottle containing fixative. This preserves the cells until they can be prepared for evaluation in the laboratory.

Collection of the specimen requires you to produce a morning sputum specimen for three consecutive days. It is important that you do not eat food or drink liquid prior to collecting your sputum.

Please remember that sputum comes from deep coughing. Please try to avoid contaminating this specimen with saliva or sinus drainage.

To collect your sputum specimen:

1. Upon going to bed, place the first sputum collection container at your bedside.

2. Upon awakening in the morning, brush and rinse your mouth well with water (DO NOT USE TOOTHPASTE).

3. Carefully remove the cap from the first sputum collection container.

4. Sit at the bedside and take 3 slow deep breaths and exhale through pursed-lips.

5. Breathe deeply a fourth time and forcefully cough. Coughing should be from deep down in your chest and cough directly in the container. You may find that bending forward slightly during the cough may help you raise the sputum.

6. Be sure to clear the sputum from the back of your mouth and throat. Repeat the process three to four times until you feel that there is no more sputum coming up.

7. Replace the cap tightly on the container.

8. Repeat steps 1-7 for three consecutive mornings for the first sputum collection container. Do not worry if you miss a day, just do it the following morning.

9. After finishing the third collection day, please write the date on the affixed label and place the first sputum cup in the mailer.

10. Remove the second sputum cup and repeat the process over again for the 3-day collection. Again do not worry if you miss a day, just do it the following morning.

11. After finishing the third collection into the second sputum cup, please again write the date on the affixed label and place it on top of the first cup inside the mailer. Seal the mailer and place it in your mailbox for pick up.

NOTE: Please check to see that the container is sealed tightly. If you have difficulty securing the lid, please have someone tighten it for you.

NOTE: If you are having difficulty producing a sputum sample upon awakening, you may find your sputum production is increased after inhaling the misty air in the shower. If this is the case, feel free to first take a shower and then collect your sputum sample.

Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

Appendix VII

Patient Selenium Tracking Form

Patients must complete the information below
And
Return in the reply paid self addressed envelope provided.

Re Study Drug for E5597

Phase III Chemoprevention Trial of Selenium Supplementation
In Persons with Resected Stage I Non-Small Lung Cancer

I received the study drug that was shipped to me from

_____

(Institution must insert name of institution where patients enrolled)

on_____.
(Patient to insert date they received the drug)

	Yes	No
Had the package been opened prior to you receiving it?	☐	☐
Was the package damaged?	☐	☐

_____ _____
Patient Name ***Patient Signature***

Please send this back immediately in the envelope provided or to this address

_____

_____
(Institution to insert a contact name and address)

Rev. 7/04, 3/07

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION STATEMENT OF INVESTIGATOR ***(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)***	Form Approved: OMB No. 0910-0014. Expiration Date: May 31, 2009. *See OMB Statement on Reverse.* NOTE: No investigator may participate in an investigation until he/she provides the sponsor with a completed, signed Statement of Investigator, Form FDA 1572 (21 CFR 312.53(c)).

1. NAME AND ADDRESS OF INVESTIGATOR

2. EDUCATION, TRAINING, AND EXPERIENCE THAT QUALIFIES THE INVESTIGATOR AS AN EXPERT IN THE CLINICAL INVESTIGATION OF THE DRUG FOR THE USE UNDER INVESTIGATION. ONE OF THE FOLLOWING IS ATTACHED.

■ CURRICULUM VITAE ☐ OTHER STATEMENT OF QUALIFICATIONS

3. NAME AND ADDRESS OF ANY MEDICAL SCHOOL, HOSPITAL OR OTHER RESEARCH FACILITY WHERE THE CLINICAL INVESTIGATION(S) WILL BE CONDUCTED.

4. NAME AND ADDRESS OF ANY CLINICAL LABORATORY FACILITIES TO BE USED IN THE STUDY.

5. NAME AND ADDRESS OF THE INSTITUTIONAL REVIEW BOARD (IRB) THAT IS RESPONSIBLE FOR REVIEW AND APPROVAL OF THE STUDY(IES).

6. NAMES OF THE SUBINVESTIGATORS *(e.g., research fellows, residents, associates)* WHO WILL BE ASSISTING THE INVESTIGATOR IN THE CONDUCT OF THE INVESTIGATION(S).

7. NAME AND CODE NUMBER, IF ANY, OF THE PROTOCOL(S) IN THE IND FOR THE STUDY(IES) TO BE CONDUCTED BY THE INVESTIGATOR.

E5597, A Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

8. ATTACH THE FOLLOWING CLINICAL PROTOCOL INFORMATION:

☐ FOR PHASE 1 INVESTIGATIONS, A GENERAL OUTLINE OF THE PLANNED INVESTIGATION INCLUDING THE ESTIMATED DURATION OF THE STUDY AND THE MAXIMUM NUMBER OF SUBJECTS THAT WILL BE INVOLVED.

■ FOR PHASE 2 OR 3 INVESTIGATIONS, AN OUTLINE OF THE STUDY PROTOCOL INCLUDING AN APPROXIMATION OF THE NUMBER OF SUBJECTS TO BE TREATED WITH THE DRUG AND THE NUMBER TO BE EMPLOYED AS CONTROLS, IF ANY; THE CLINICAL USES TO BE INVESTIGATED; CHARACTERISTICS OF SUBJECTS BY AGE, SEX, AND CONDITION; THE KIND OF CLINICAL OBSERVATIONS AND LABORATORY TESTS TO BE CONDUCTED; THE ESTIMATED DURATION OF THE STUDY; AND COPIES OR A DESCRIPTION OF CASE REPORT FORMS TO BE USED.

9. COMMITMENTS:

I agree to conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.

I agree to personally conduct or supervise the described investigation(s).

I agree to inform any patients, or any persons used as controls, that the drugs are being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.

I agree to report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with 21 CFR 312.64.

I have read and understand the information in the investigator's brochure, including the potential risks and side effects of the drug.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

I agree to maintain adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.

I will ensure that an IRB that complies with the requirements of 21 CFR Part 56 will be responsible for the initial and continuing review and approval of the clinical investigation. I also agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

I agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 21 CFR Part 312.

INSTRUCTIONS FOR COMPLETING FORM FDA 1572
STATEMENT OF INVESTIGATOR:

1. Complete all sections. Attach a separate page if additional space is needed.
2. Attach curriculum vitae or other statement of qualifications as described in Section 2.
3. Attach protocol outline as described in Section 8.
4. Sign and date below.
5. FORWARD THE COMPLETED FORM AND ATTACHMENTS TO THE SPONSOR. The sponsor will incorporate this information along with other technical data into an Investigational New Drug Application (IND).

10. SIGNATURE OF INVESTIGATOR 11. DATE

(**WARNING:** A willfully false statement is a criminal offense. U.S.C. Title 18, Sec. 1001.)

Public reporting burden for this collection of information is estimated to average 100 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research (HFM-99)
1401 Rockville Pike
Rockville, MD 20852-1448

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Please **DO NOT RETURN** this application to this address.

FORM FDA 1572 (5/06) **PREVIOUS EDITION IS OBSOLETE** **Page 2 of 2**

Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

Appendix IX

Pathology Submission Guidelines

The following items are included in Appendix IX:

1. Guidelines for Submission of Pathology Materials
   (instructional sheet for Clinical Research Associates (CRAs))
2. Instructional memo to submitting pathologists
3. List of Required Materials for E5597
4. ECOG Pathology Material Submission Form (# 638)

GUIDELINES FOR SUBMISSION OF PATHOLOGY MATERIALS

The following items should always be included when submitting pathology materials to the ECOG Pathology Coordinating Office:

- Institutional Surgical Pathology Report
- Pathology materials (see attached List of Required Material)
- ECOG Pathology Material Submission Form (# 638)

Instructions:

1. Place the Patient ID label provided by the ECOG Coordinating Center in **Part A** of the ECOG Pathology Material Submission Form.

   If a label is not available **TYPE or PRINT** the following information in Part A of the form:

   - Patient's name (last, first)
   - Protocol number
   - Protocol case number (the patient's ECOG sequence number; for intergroup studies, include both the ECOG and other group's sequence numbers)
   - Patient's hospital number
   - Institution
   - Affiliate (if appropriate)

2. Complete blank areas of the pathologist's instructional memo, and forward it, along with the List of Required Material and the ECOG Pathology Material Submission Form, to the appropriate pathologist.

   The pathologist should return to you the required pathologic samples and surgical pathology reports, along with the completed ECOG Pathology Material Submission Form (# 638) (Part B completed). If any other reports are required, they should be obtained from the appropriate department at this time.

3. Keep a copy of the ECOG Pathology Material Submission Form (# 638) for your records (the original should be sent to the PCO).

4. Double check that **ALL** required forms, reports, and pathology samples are included in the package to send to the Pathology Coordinating Office (see appropriate List of Required Material).

   Pathology specimens submitted for a patient WILL NOT be processed by the Pathology Coordinating Office until all necessary items are received.

5. Mail pathology materials to:

   ECOG Pathology Coordinating Office
   Robert H. Lauria Comprehensive Cancer Center of Northwestern University Medical School
   Olson Pavilion - Room 8421
   710 North Fairbanks Court
   Chicago, IL 60611

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If you have any questions concerning the above instructions, or if you anticipate any problems in meeting the pathology material submission deadline of one month, contact the Pathology Coordinator at the ECOG Pathology Coordinating Office - Tel: (312) 503-3384 or Fax: (312) 503-3385.

Eastern Cooperative Oncology Group

Coordinating Center
Frontier Science
900 Commonwealth Ave•Boston, MA 02215
(617) 632-3610•Fax: (617) 632-2990
Randomization: (617) 632-2022

Jean MacDonald, Director of Research Operations
Mary Steele, Director of Group Administration

Group Chair: Robert L. Comis, M.D.
Group Statistician: Robert Gray, Ph.D.

MEMORANDUM

TO: _____
(Submitting Pathologist)

FROM: Stanley Hamilton, M.D.
ECOG Pathology Committee

DATE: _____

SUBJECT: Submission of Pathology Materials for E5997: *Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer*

The patient named on the attached ECOG Pathology Material Submission Form (# 638) has been entered onto an ECOG protocol by _____ *(ECOG Investigator).* This protocol requires the submission of pathology materials for pathology review and correlative studies.

Please complete **PART B** of the Submission Form. Keep a copy for your own records, and return the completed Submission Form, the surgical pathology report(s), tissue blocks or slides, the bone marrow smears, and any other required material (see attached List of Required Material) to the Clinical Research Associate (CRA). The CRA will forward all required pathology material to the ECOG Pathology Coordinating Office.

Pathological materials submitted for this study will be retained at the ECOG Central Repository for possible use in future ECOG approved studies. If patient denies or withdraws consent for banking, the materials will be withdrawn from consideration in any future
Rev. 3/07 study. Blocks will be returned to the institution upon written request for purposes of patient management.

Rev. 3/07 [Deleted via Addendum #4]

If you have any questions regarding this request, please feel free to contact the Pathology Coordinating Office at (312) 503-3384.

The ECOG CRA at your institution is:

Name: _____

Address: _____

_____

Phone: _____

Thank you.

LIST OF REQUIRED MATERIAL

E5597	Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

Rev. 3/07 Upon development of a recurrence or new second primary tumor (SPT) of any histology:

Rev. 3/07 **NOTE:** The pathology reports from both the original diagnosis and, upon development, the recurrence or new primary must be forwarded to the ECOG Coordinating Center. Representative diagnostic material from both the original diagnosis and the recurrence or new second primary may be requested for central review and classification on those cases considered unresolved or questionable by the Endpoint Review Committee. **If these samples are requested, samples must be submitted for the patient to be considered evaluable**. Materials to be submitted upon request of diagnostic tissue request:

1. ECOG Pathology Material Submission Form (# 638) – Parts A & B completed.

Rev. 3/07 2. A copy of the pathology reports from the original diagnosis and the recurrence or new SPT
In addition to the pathology reports, if immunologic studies have been performed at the home institution, it is necessary that these be forwarded as well.

Rev. 3/07 3. [Section deleted via Addendum #4]

Rev. 3/07 4. Slides from the original diagnosis and recurrence or the new primary.

Rev. 3/07

Rev. 3/07 [Upon Development of AML or MDS section deleted via Addendum #4]

Materials for Correlative Study

1. ECOG Pathology Material Submission Form (# 638) – Parts A & B completed.

2. A copy of the pathology report from the surgical resection
In addition to the pathology report, if immunologic studies have been performed at the home institution, it is necessary that these be forwarded as well.

3. Tissue block from the original surgical resection. If blocks will not be submitted, 20 unstained sterile sections non heat treated on plus slides are requested

NOTE: A copy of the completed submission form will be sent to the ECOG Coordinating Center by the ECOG Pathology Coordinating Office.

NOTE: Blocks are being submitted for correlative studies thus materials may be depleted. Upon written request, remaining blocks will be returned to the institution.

INSTRUCTIONS: Please complete and submit this form **along with all pathology material and corresponding pathology reports** requested by the protocol. **See list of required materials specific to EACH protocol.**

PART A: TO BE COMPLETED BY DATA MANAGER

Date sample sent to ECOG ____ / ____ / ____ **(M,D,Y)**

Data Manager ____

Telephone No. ( ) ____ ____

FAX No. ( ) ____ ____

ECOG Parent Prot. No. ____ **Seq. No.** ____

PLACE ID LABEL HERE

Patient's Name ____

ECOG Prot. No. ____ **ECOG Patient Seq. No.** ____

Participating Group Prot. No. ____ **Participating Group Patient ID No.** ____

Group ____ **Institution** ____

Step No. ____ **Affiliate** ____

PART B: TO BE COMPLETED BY THE SUBMITTING PATHOLOGIST

	STATUS * (see Below)	DATE SPECIMEN COLLECTED (M,D,Y)	DISEASE SITE	NUMBER OF SLIDES	TYPE OF STAIN	SPECIMEN ID NUMBERS
COMPLETE FOR SLIDES		/ /				
		/ /				
		/ /				
	STATUS * (See Below)	**DATE SPECIMEN COLLECTED (M,D,Y)**	**DISEASE SITE**	**NUMBER OF BLOCKS**	**TYPE OF FIXATIVE**	**SPECIMEN ID NUMBERS**
COMPLETE FOR BLOCKS		/ /				
		/ /				

* **STATUS:** Please **identify** the clinical status of the sample. **List ALL that apply.**

1. Original Diagnostic Material
2. Pre-Protocol Treatment Biopsy
3. Post-Protocol Treatment Biopsy
4. Post-Surgery Biopsy
5. Relapse/Recurrence
6. Remission/Response
7. Other, Specify ____

Submitting Pathologist ____

Telephone No. ( ) ____ ____

Address ____

INSTITUTION COMMENTS ____

Can this sample be retained by the ECOG Central Tissue Repository? _____ **Yes** _____ **No**

NOTE: Samples submitted for protocols requiring submission for tissue banking **will not be** returned except for purposes of individual patient management. For this reason, the **submitting pathologist should retain at least one paraffin block** at their institution.

[A block has been retained at the submitting institution: _____ **Yes** _____ **No]**

Please **CIRCLE THE REASON** for non-submission and **INCLUDE** a formal letter of explanation:
State Regulations, Institutional Policy, Insufficient Tissue, Patient Refusal, Other

specify other ____ Pathologist or Investigator's Signature____.

PART C: ECOG COORDINATING CENTER USE ONLY

Date Sample Received by ECOG ____ / ____ / ____ (M / D / Y)

Date Sent to Central Lab ____ / ____ / ____ (M / D / Y)

Date Sample Sent to Reviewer ____ / ____ / ____ (M / D / Y)

Items Received **(if different from above)** ____

Name of Reviewer ____

NOTES: ____

INVESTIGATOR: Keep a copy for your files and submit original form to destination specified in protocol. 2/96

Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer

APPENDIX X

Mayo Central Laboratory for Clinical Trials

Shipment Alert Form

Eastern Cooperative Oncology Group (ECOG)

Protocol: E5597

NOTE: This information will help MCLCT track your shipment. Please make additional copies of this form as necessary. FAX a completed form each time you make a Federal Express shipment to MCLCT for this protocol.

TO: FAX Number: 507-284-1790

Name: Transportation Department

Shipment is from:

Investigator name: _____

Site Location (city, state): _____

Name of person making shipment: _____

Telephone number: _____

Federal Express airbill number(s): _____

_____

Quantity/number of boxes in shipment: _____

Date of shipment: _____

CAUTION:

****When shipping on FRIDAYS, indicate "SATURDAY DELIVERY" on airbill.****

(If you fail to do this, the shipment will not be delivered until the following MONDAY, which may jeopardize specimen integrity)

Does the shipment contain dry ice? NO