A Randomized Phase II Trial of Low Dose Aspirin versus Placebo in High-Risk Individuals with CT-Detected Subsolid Lung Nodules

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SCHEMA

A Randomized Phase II Trial of Low Dose Aspirin versus Placebo in High-Risk Individuals with CT-Detected Subsolid Lung Nodules

Asymptomatic volunteers (128) current/former smokers (≥20 pack-years), age ≥50 years, with persistent subsolid lung nodules confirmed by CT scan

↓

CT scan and phone contact (days -61 - 0)

CT scan lung lesion measurement for nodule eligibility and phone contact for additional eligibility assessment

↓

Baseline visit (days -61 - 0)

Informed consent, medical history, physical exam, tobacco assessment, concomitant medication, baseline symptoms, clinical lab tests (pregnancy test, if applicable), blood and urine collection

↓

Randomization (Month 0)

Confirm eligibility, randomize (stratify by screening vs non screening CT scan, gender, smoking status (current vs former), nodule characteristics (non solid vs partially solid)

↓

Intervention

Aspirin 100 mg/day or placebo qd for 12 months

↓

Telephone contact (Month 1 ± 1 W)

Toxicity assessment, concomitant medications, review of self reported compliance

↓

Telephone contact (Month 3 ± 2 W)

Toxicity assessment, concomitant medications, review of self reported compliance

↓

Clinic Visit (Month 6 ± 2 W)

Physical exam, toxicity assessment, tobacco assessment, concomitant medication, clinical lab tests (pregnancy test, if applicable), blood and urine collection, compliance/review pill diary

↓

Telephone contact (Month 9 ± 2 W)

Toxicity assessment, concomitant medications, review of self reported compliance

↓

Clinic Visit (Month 12 ± 2 W)

Physical exam, toxicity assessment, tobacco assessment, concomitant medication, clinical lab tests (pregnancy test, if applicable), blood and urine collection, drug compliance/review pill diary, CT scan-detected lesion measurement

↓

Telephone contact (Month 13 ± 1 W)

Toxicity assessment, concomitant medications

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1. OBJECTIVES

1.1 Primary Objectives – The primary objective is the evaluation of the effect of Aspirin as a chemopreventive agent for lung cancer. Particularly, the efficacy of Aspirin in reduction of size and number of CT-detected lung subsolid nodules will be evaluated in high-risk asymptomatic subjects currently undergoing CT screening programs at the European Institute of Oncology (EIO) and at MD Anderson Cancer Center (MDACC).
In addition, we will include subjects undergoing CT outside the context of a screening program with a subsolid nodule confirmed by pulmonary CT scan (qualifying scan).
The efficacy will be evaluated in a person-specific analysis.

1.2 Secondary Objectives – Secondary objective of the study will be the modulation of biological markers after treatment and the correlation of these findings with modification of lung nodules diameters. In particular we will evaluate the effect of Aspirin on a signature of serum microRNA correlated to subsolid nodules. Other secondary endpoints will be the per-lesion analysis including the evaluation of lung nodule density before and after treatment, the number and size of non target lesions including solid nodules and evaluation of response according to modified RECIST criteria. Additional biomarkers will include the modulation of ultrasensitive circulating hs-CRP, the evaluation of urinary cotinine as marker of tobacco exposure and investigation of the potential effect of aspirin according to its concentration, the measurement of urinary prostaglandin metabolites (PGEM) and leukotriene (LTE4), both normalized normalized to urinary creatinine concentration. Serum concentration of thromboxane B2 (TXB2) will be determined as a measure of compliance. We will also evaluate the tolerability of Aspirin at dose of 100 mg/day.

2. BACKGROUND

2.1 Lung cancer

Lung cancer is one of the most common cancers in humans and the annual incidence continues to grow. The burden caused by lung cancer is enormous worldwide and largely due to tobacco use. In Western countries smoke cessation campaigns and stricter regulations are beginning to multiply. This strategy will surely achieve good results in the future. However, there are still age and gender groups (the younger and women) who are at present more reluctant to avoid smoking, while the smoking epidemic is hitting the underdeveloped countries more and more alarmingly. In the meanwhile, new screening modalities are under evaluation: the most promising is currently spiral low-dose CT scan (ld-CT).

2.2 Aspirin

Aspirin, the first non steroidal anti-inflammatory drug (NSAID) identified, may have anti-cancer properties, especially for diseases whose etiology implicates the role of chronic inflammation, such as colorectal and lung cancer. The anti-inflammatory effect of NSAIDs operates through inhibition of prostaglandins via suppression of cyclooxygenase-1 (COX-1) and COX-2. The most convincing evidence of this effect is derived from several recently published meta-analyses of aspirin in the prevention of cardiovascular events which showed that daily aspirin reduced the incidence of colorectal cancer and several other cancers and reduced metastases[1-3]. In 2011, Rothwell et al.[4] pooled data from 8 double-blind randomized controlled trials of daily aspirin and analyzed the effect of aspirin on cancer mortality as secondary endpoints. The 20-year risk of death due to all cancers was consistently lower in the aspirin groups than in the control groups and the benefit increased with duration of treatment.

Lung cancer-specific mortality rates were reduced by 29% (95% CI, 11-42) in the aspirin group in the 20-year period after the trial commenced[4]. No trend with dose (above 75 mg/day) was observed, but the effect on all cancers was more evident in adenocarcinomas and was present in both smokers and nonsmokers[4]. Several further observational studies have been published. In a prospective cohort study, total NSAID use was associated with a small reduction in risk of lung cancer, which was strongest for adenocarcinoma in men, and in long-term former smokers[5]. A suggestion of a protective effect of low-dose aspirin was seen in the Women's Health Study[6]. A hospital-base case-control study found that lung cancer risk was significantly lower for aspirin users compared to non-users and prolonged duration of use was associated with reduced lung cancer risk. Risk reductions were observed in both sexes, but significant dose-response relationship were only seen among male participants[7].
Controversy remains regarding the most appropriate long-term daily dose of aspirin in the prevention setting. The mechanisms by which aspirin reduces the risk of cancer are not clear. COX-2 is thought to be the isoform most commonly implicated in carcinogenesis, but at the low doses (80-325 mg), aspirin has little activity against this isoform[8]. Aspirin is, in fact, 170 fold less effective at inhibiting COX-2 than COX-1[9]. Nonetheless, aspirin may suppress the expression of COX-2[10], and it may also have antineoplastic effects that are unrelated to cyclooxygenase[11,12]. Since the 81-mg dose and the 325-mg dose appear to suppress colorectal prostaglandin levels to a similar extent[13-15], we could postulate that the two doses may have equivalent potency through COX-related mechanisms. The major risk of aspirin, as with other NSAIDs, is the risk of bleeding. Although the antiplatelet effects of aspirin likely contribute to an increase in the risk of bleeding, as highlighted by an increased risk of hemorrhagic stroke of 0.2 events per 1000 patient-years[16], the majority of the increased bleeding has a gastrointestinal tract etiology. The influence of aspirin on gastric prostaglandin levels is dosage-dependent, with almost 50% inhibition at just 30 mg/d, but maximal inhibition requires approximately 1300 mg/d[17]. Consistent with these data, all conventional dosages of aspirin are associated with an increased bleeding risk. In a clinical trial in which 1,121 patients with a recent history of histologically documented adenomas were randomly assigned to receive placebo (372 patients), 81 mg of aspirin (377 patients), or 325 mg of aspirin (372 patients) daily, the risk of bleeding was not a substantial problem and the risk of death and serious bleeding were similar among the groups[18]. In a case-control study of patients admitted with gastrointestinal bleeding, the odds ratio (OR) for admission with a bleeding ulcer was increased among aspirin users regardless of dosage[19]. Treatment with a 75-mg/d dosage of aspirin was associated with an odds ratio of 2.3 for a bleeding ulcer (95% CI, 1.2-4.4), whereas 300 mg/d increased the OR to 3.9 (95% CI, 2.5-6.3).

2.3 Rationale

With the evolution of helical CT technology, CT screening for early detection of lung cancers is under evaluation in high risk individuals (former and current heavy smokers) with encouraging results[20-22]. At the EIO, a single center screening trial recruited 5,202 high-risk volunteers (both current or former smokers) to undergo an annual multidetector low dose CT (ld-CT) for 5 or more years, beginning in 2004[21,23]. The screening ld-CT, a non invasive test with low radiation exposure and no contrast medium, affords the opportunity to serially examine the peripheral lung for the first time, albeit with the limitation that small lesions cannot be biopsied and thus their identity remains unknown.

In September 2012, a new multicenter screening program has been launched at the EIO (Cosmos 2) with the aim of enrolling 10,000 high-risk individuals in 6 centers in Italy in a one-year period, with the goal of recruiting 5000 at the EIO. The Cosmos protocol requires once yearly low-dose CT for 5 years. One of the main objective of the study is the validation of a serum signature of 34 circulating microRNA able to predict with high sensitivity the presence of an asymptomatic lung cancer and to validate the Cosmos risk model[24] on an independent cohort of high risk individuals to better select the target population for screening and the optimal screening interval. The population enrolled in the Cosmos 2 will represent the major source of potentially eligible subjects for the chemoprevention study.

In the meantime, two other screening programs are still ongoing at the EIO with annual ld-CT scans (Cosmos 1 and a pilot study) and they represent an additional source of potential participants.

Unfortunately, the high rate of benign nodules and issues of making a differential diagnosis are critical factors that currently hamper introduction of large-scale screening programs. Small pulmonary nodules are, in fact, a frequent finding in the subjects submitted to ld-CT for early detection of lung cancer and have no clinical implications. Our data have demonstrated an incidence of undetermined lung nodules in more than 50% of high risk individuals[21]. The nature of these nodules remained uncertain but it is reasonable that some of them could represent precancerous lesions. In particular, literature suggests that ground glass opacities (GGO), as compared with solid nodules, can represent either localized bronchioloalveolar carcinoma without foci of active fibroblastic proliferation or atypical adenomatous hyperplasia[20] which is a known putative adenocarcinoma precursor lesion[25]. The possibility to measure the nodule density in GGO lesions is an additional tool to monitor these preneoplastic lesions[26].
The actual identity of ld-CT detected GGO cannot be ascertained without histologic analysis, but this is the category of nodule that is most likely to represent atypical alveolar hyperplasia, the putative precursor of pulmonary adenocarcinoma[27]. Kim et al. reported that of 53 persistent GGO in 49 patients who underwent resection, 68% proved to be bronchoalveolar carcinoma, 7.5% were adenocarcinoma with predominant brochoalveolar components, 6% were atypical adenomatous hyperplasia, and 19% were nonspecific fibrosis or organizing pneumonia[28]. Similarly, Ohtsuka et al. reported that of 26 patients who underwent resection, bronchoalveolar carcinoma was diagnosed in 10 patients (38%), atypical adenomatous hyperplasia was diagnosed in 15 patients (58%), and focal scar was seen in 1 patient (4%)[29]. Although criteria such as size, the presence of air bronchograms, and nodule sphericity on CT scan have been used to differentiate carcinomas from atypical alveolar hyperplasia, histological analysis remains the gold standard for definitive categorization of nodules and there continues to be debate regarding the overlap between small bronchoalveolar carcinomas and atypical alveolar hyperplasia[30,31]. Since resected nodules represent lesions that are suspicious enough to merit surgery, it is possible and even likely that the smaller nodules identified in the context of our ld-CT screening study represent less advanced neoplasia than described in the above cited studies as well as non-neoplastic etiologies.

In 2010 we published the results of the Budesonide trial (MDA05-5-01), a randomized phase IIb study that evaluated the role of inhaled Budesonide in the shrinkage of CT detected undetermined lung nodules. The study showed an effect of the treatment only on non solid nodules and a trend in partially solid nodules[32]. A 5-year follow up of the nodules with annual low dose CTs showed that the effect of treatment was maintained years after treatment and was significant for non solid and partially solid nodules (unpublished data). Specifically, after 60 months the mean reduction of maximum diameter in the treated arm were 2.65 mm and 0.69 mm in the non-solid and partially solid lesions, respectively.

We concluded that screen-detected non solid and partially solid nodules represent a good target to test chemopreventive agents and that the role of Budesonide deserves further investigation.

The University of Texas MD Anderson Cancer Center launched its Lung Cancer Screening Program in 2011. To date, nearly 800 patients have been screened averaging approximately 20-30 screened patients per month. An additional resource for screening CTs is the active protocol entitled "*Biospecimen Banking and Biomarker Validation for Lung Cancer Early Detection in Cohort Receiving Low Dose Helical Computed Tomography Screening*" which has 194 patients currently enrolled. Although literature indicates the frequency of eligible lesions is approximately 30%, MDACC's experience is slightly less at 10% (2-3 patients per month) identified by low dose screening CT scan. From this population, it is anticipated the Lung Cancer Screening program may enroll less than 1 eligible patient per month after evaluation of other eligibility criteria. However, with the inclusion of lesions identified by diagnostic CT scan, MDACC's large cancer survivor population offers a larger pool of potentially eligible patients undergoing CTs for surveillance.

Based on the recent knowledge on the chemopreventive effect of Aspirin on lung cancer, we intend to evaluate the effect of low dose Aspirin (100 mg/day) on non solid and partially solid nodules identified in high risk volunteers submitted to annual low dose CTs among the ongoing screening programs at EIO and MDACC.
In addition, we will include subjects undergoing CT outside the context of a screening program with a subsolid nodule confirmed by pulmonary CT scan.

Rationale for Biomarkers selection
microRNA: Modulation of biological markers after treatment and the correlation of these findings with modification of lung nodules diameters will be investigated as secondary endpoints. In particular, we have recently identified serum circulating microRNA (miRNA) able to accurately predict the presence of lung cancer during early stages disease[33]. The test was accurate enough to capture the onset of the disease in patients of whom serum samples were available before and after diagnosis of lung cancer by ld-CT. The same signature was also able to identify the premalignant GGO lesions among Cosmos 1 participants (unpublished data). In brief, we screened a group of 27 individuals with GGO lung nodules and applied the miRNA test. Twenty-four out of 27 were positives to the test (~89%). Furthermore, the average miRNA-test risk score of the GGO group was 12.5 while the average score of two other groups of individuals with solid nodules (N=25), or without nodules (N=25), were -2.2 and -4.4 respectively ($p < 0.0001$; Student's t-test). This confirmed the validity of the miRNA-test to predict the presence of GGO lesions as well as early stages lung cancer, and suggest that active or passive release in the blood of specific miRNA species is already present in lung premalignant lesions.
The advantages of such a test compared to ld-CT are the potential for an easy applicability on a population-wide scale, the high potential for implementation of screening on a nation-wide scale, the absence of risks related to ionizing radiations, and the reduced costs
As regards the technical aspects we have developed a fully automatized procedure based on low-density qRT-PCR array that allows the screening of expression of ~800 different miRNA species and using less than 300µl of serum. This will give us the opportunity to test every known miRNA present in the serum and to perform an accurate analysis of their quantities.
We intend to apply the very same technology to identify serum miRNAs predictive of the effect of aspirin treatment in patients with GGO lung nodules.

C-reactive Protein: Inflammation has been hypothesized to increase the risk of cancer[34] although data from epidemiologic study are sparse. Many inflammatory conditions are well-known cancer precursors (Barrett's esophagus, chronic gastritis, hepatitis, pancreatitis, and ulcerative colitis). So, early correction of inflammatory conditions by chemoprevention may be theoretically very useful. C-reactive protein (CRP), a member of the pentraxin family of proteins involved with pattern recognition in innate immunity, is the principal downstream mediator of the acute-phase response and is primarily derived via the interleukin 6-dependent hepatic biosynthesis[35]. Moreover, ultrasensitive CRP (hs-CRP) is an known index of subclinical systemic inflammation associated with increased risk for cardiovascular events and diabetes. Elevated levels of hs-CRP have been linked to colon cancer development and a recent meta-analysis[36] including 1,918 lung cancer cases points to its implication also with lung cancer risk.
Given our previous experience in measuring hs-CRP in subjects at higher risk for breast cancer[37], we are interested in investigating change in hs-CRP also in subjects at higher risk of lung cancer.

Cotinine: Measurement of cotinine, the nicotine metabolite, provides an accurate estimate of recent tobacco smoke exposure and are able to account for individual differences in smoking practices, such as depth of inhalation and how much each cigarette is smoked[38,39].

Prostaglandin (PGEM): Prostaglandin synthesis is driven by the two COX. Prostaglandin E2 is the most abundant prostanoid in the human body and it has been implicated in various tumorigenic processes and

induces proliferation by activating at least two signalling pathways: Ras–Erk and glycogen synthase kinase-3β (GSK3β)–β-catenin in colon and lung cancer cells[40]. Circulating prostaglandin E2 is rapidly cleared by a single passage through the lungs and measurement of the metabolites is necessary to provide a reliable estimate for its actual production.

Leukotriene E4 (LTE4): Leukotriene E4 is an urinary metabolite indicating activation of the lipoxygenase pathway[41]. To perform urine analysis for LTE4, we will show relationship between acetyl-salicylic acid (ASA) and the LTE4 shunt in healthy smokers.

Thromboxane B2 (TXB2): Thromboxane B2 is a biologically inactive metabolite of Thromboxane A2, an arachidionate metabolite with potent platelet aggregation activity and prothrombotic properties, that is rapidly hydrolyzed to TXB2. TXB2 is thus a suitable markers for monitoring platelet function and assessment of aspirin compliance.

3. SUMMARY OF STUDY PLAN

We will conduct a double-blind placebo-controlled phase IIb study in which participants will be randomized to receive either Aspirin (100 mg/day) or placebo for 12 months.

A total of 128 subjects will be accrued (randomized to drug or placebo); 64 per arm in a 3.5 year time period.

Participants will be men and women, current or former smokers, with undetermined subsolid lung nodules detected at low dose screening CT scan (without constrast medium) running at the EIO and MDACC. In addition, we will include subjects undergoing CT outside the context of a screening program, providing a subsolid nodule confirmation by a CT scan. Participants will be given 100 mg Aspirin daily or placebo for 12 months.

The primary endpoint will be the shrinkage of GGO and partially solid lung nodules after one-year treatment per-subject analysis.

Secondary endpoints will be the modulation of biological markers after treatment and the correlation of these findings with modification of lung nodules diameters. In particular we will evaluate the effect of Aspirin on a signature of serum miRNA correlated to subsolid nodules. Other secondary endpoints will be the per-lesion analysis including the evaluation of lung nodule density before and after treatment and the number and size of non target lesions. Additional biomarkers will include the modulation of hs-CRP, the evaluation of urinary cotinine as marker of tobacco exposure and investigation of the potential effect of aspirin according to its concentration, the measurement of urinary prostaglandin metabolites (PGEM) and urine leukotriene E4 (LTE4) both normalized to urinary creatinine concentration. Serum concentration of thromboxane B2 (TXB2) will be determined as a measure of compliance. We will also evaluate the tolerability of Aspirin at dose of 100 mg/day.

Complete physical exam and safety lab tests will be performed at 0, 6 and 12 months. Phone contact will occur at month 1, 3, 9 and 13 (i.e, one month after treatment completion).

Biomarkers: blood and urine will be collected at 0, 6 and 12 months

Lung lesions will be measured with a CT scan at 0 and 12 months. Both standard dose and low dose CT scans are allowed provided that the same procedure is used at both time points.

Duration of study will be five years: 3.5 years of accrual, one year of treatment and 0.5 year for biomarkers measurement.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Asymptomatic current or former smokers (having stopped within the last 20 years). See Appendix D

4.1.2 Age ≥ 50 years (risk of lung cancer in subjects under 50 is low thus screening is not indicated by actual international guidelines).

4.1.3 Smoking history ≥ 20 pack/years; subjects must be included in an ongoing annual screening with low dose CT scan **or**

must have two consecutive CT outside the context of a screening program confirming subsolid nodules as per inclusion criteria number 4.1.4.

4.1.4 Subjects must have subsolid (non solid or partially solid) nodules with size between 4 and 10 mm with any Volume Doubling Time (VDT) not candidate to surgical excision **and/or**

subsolid (non solid or partially solid) nodule larger than 10 mm with VDT higher than 400 days and not candidate to surgical excision.

4.1.5 All nodules should be persistent at least after three months follow up with CT; a reduction up to 15% of the diameter of the largest target nodule from the previous CT scan is allowed

4.1.6 All current smokers should accept to receive support for smoking cessation

4.1.7 ECOG performance status ≤1 (Karnofsky ≥70%; see Appendix A)

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

Leukocytes	≥3,000/microliter
Absolute neutrophil count	≥1,500/microliter
Platelets	≥100,000/microliter
Total bilirubin	≤2 × institutional ULN and/or history of Gilbert's syndrome
AST (SGOT)/ALT (SGPT)	≤1.5 × institutional ULN
Serum creatinine	≤ institutional ULN

4.1.9 The effects of Aspirin on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because acetyl salicylic acid (ASA) is known to be teratogenic, women of child-bearing potential (from first menstruation to 1 year after last menstruation) must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.
Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

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

4.1.11 Signed informed consent.

4.2 Exclusion Criteria

4.2.1 Subjects with chronic treatment (at least twice/week for more than 3 months) with Aspirin or other NSAIDs.

4.2.2 History of allergic reactions attributed to compounds of similar chemical or biological composition to aspirin, NSAIDs, COX2 inhibitors.

4.2.3 Invasive malignancy (with the exclusion of basal cell carcinoma or skin squamous cell carcinoma) diagnosed during the last 2 years before randomization; stage I-II invasive malignancies that were diagnosed more than 2 years prior to randomization and have been treated curatively are allowed as long as all treatment is finished at least 18 months prior to randomization.

4.2.4 History of therapeutic doses of anticoagulants including warfarin and low molecular weight Heparin (e.g. for prior deep venous thrombosis and pulmonary embolisms) in the preceding year.

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

4.2.6 Pregnant women are excluded from this study because Aspirin is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Aspirin, breastfeeding should be discontinued if the mother is treated with Aspirin.

4.2.7 Individual may not be receiving any other investigational agents, antiplatelet agents (e.g. aspirin, clopidogrel (plavix or others)), anticoagulants (e.g. heparin or heparinoids, coumadin, or others), metotrexate, lithium.

4.2.8 Participants with bleeding diathesis, history of gastric/duodenal ulcers in the last 5 years, NSAID-precipitated bronchospasm, patients unwilling or unable to limit alcohol consumption to i.e. ≤ 3 alcohol drinks a day.

4.2.9 Participants who in the opinion of the PI will be at higher risk of ASA-related complications.

4.2.10 Participants with known inability to adequately absorb oral medication.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups will be eligible for this trial. However, given that the distribution of the Italian population mainly consists of white Caucasians (about 93%), we expect very few subjects of different ethnic background to be enrolled in the screening. The gender distribution among the Cosmos 2 screening population is about 65% and 35% for men and women, respectively and we expect a similar distribution in the chemoprevention study. Detailed information about distribution is reported in the DCP Protocol Submission Worksheet (PSW).

4.4 Recruitment and Retention Plan

The projected number of eligible subjects in the entire cohort of 8,000 individuals undergoing annual screening at EIO will be close to 600. Considering that 20% of subjects will be excluded because of ongoing treatment with Aspirin, the eligible pool will include about 480 subjects. Accrual rate is expected to be no less than 32% of the predicted pool. This percentage is higher than the average recruitment rate in chemoprevention trials in healthy subjects (average 10%), but is reasonable given that the population is represented by highly motivated volunteers and previous EIO screening compliance has been higher than 97%. This would allow to consent about 150 people, whose slides will be re-reviewed and re-measured by a study dedicated radiologist and pulmonologist. It is expected that approximately 128, or 80% of consented individuals, will meet all eligibility criteria for the protocol and will be randomized. Since the Cosmos 2 trial is a five year protocol with annual low-dose CT scans, we will have the opportunity to pre-screen the individuals participating in the Cosmos trial for eligibility and to approach them with the present protocol at the time of their next scheduled Cosmos trial-directed CT scan that falls within the window of our trial accrual period. Additionally, there will be individulas who develop new small nodules for the first time while participating in the Cosmos protocol. Such individuals will return per Cosmos protocol for a repeat CT scan in 3 months, and if they meet the inclusion criteria of the present trial after the repeat CT scan, they will also be captured.

MDACC launched its Lung Cancer Screening Program in 2011. To date, nearly 800 participants have been screened averaging approximately 20-30 screened participants per month. An additional resource for screening CTs is the active protocol entitled "*Biospecimen Banking and Biomarker Validation for Lung Cancer Early Detection in Cohort Receiving Low Dose Helical Computed Tomography Screening*" which has 194 participants currently enrolled. Although literature indicates the frequency of eligible lesions is approximately 30%, MDACC's experience is slightly less at 10% (2-3 participants per month) identified by low dose screening CT scan. From this population, it is anticipated the Lung Cancer Screening program may enroll approx. 1 eligible participant per month after evaluation of other eligibility criteria. However, with the inclusion of lesions identified by diagnostic CT scan, MDACC's large cancer survivor population offers a larger pool of potentially eligible participants undergoing CTs for surveillance.

Pre-Initiation phase

Both at IEO and MDACC, subjects with persistent subsolid lung nodules detected during any of the screening programs are potentially eligible for the trial and will be identified as potential candidates for the study by the local Lung Cancer Screening program coordinator. In addition, subjects with subsolid lesions identified outside the context of a screening program will be indicated to the research staff by the collaborating radiologists creating a future recruitment resource. While waiting for the subsequent CT scan, the potential candidate will be given a leaflet about the possibility of being contacted for taking part in a prevention trial.

Active Recruitment phase
At EIO, after the evaluation of the CT scan by the radiologist and the thoracic surgeon (Co-PI), and the verification of all inclusion criteria, the potential candidate will be contacted by the staff of the Division of Cancer Prevention and Genetics for the first phone interview. Each subject will be given a unique and consecutive ID number (four digits). The trial design will be explained and availability to participate will be asked. Candidates accepting to participate or interested in the study will be invited for a visit at EIO for informed consent and baseline visit.

At MDACC, after the evaluation of the CT scan by the thoracic radiologist (Co-PI), and the verification of all inclusion criteria, the potential participant will be contacted by the MDACC staff for the first phone

interview. The study design, treatment plan, procedures, follow-up schedule, risks, side effects and potential benefits will be discussed with the potential participants. Interested participants will be scheduled for informed consent and baseline visits in the Cancer Prevention Center. Each subject will be given a unique and consecutive ID number (four digits).

Retention phase (which include adherence strategies)
The retention phase will be identical between EIO and MD Anderson. Participants will be scheduled for periodic clinic visits (at 0, 6 and 12 months) during which a complete physical exam and lab tests will be performed. At months 1, 3 and 9 subjects will be contacted by phone to evaluate self-reported compliance/adherence to the treatment and toxicity. In addition they will be reminded about the visit, CT scan and blood and urine samples at month 6 and 12.

Recruitment and retention effort will be evaluated routinely by the site coordinator and the study staff. It can be modified as necessary to promote rapid accrual and to assure maximally high retention rate of participants.

5. AGENT ADMINISTRATION

Drug will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

Group 1
Agent: Aspirin
Daily doses: one dose of 100 mg once a day
Duration: one year

Group 2
Agent: placebo
Daily doses: one dose once a day
Duration: one year

5.2 Aspirin Administration

At EIO, Dr Bonanni (PI) will be responsible for drug administration. He can delegate the colleagues of the Division of Cancer Prevention and Genetics (MDs and research nurses) to administer the agent on his behalf.

At MD Anderson, Dr Bevers (MD Anderson Site PI) will be responsible for drug administration. She can delegate to MDACC site collaborators to administer agent on her behalf as noted in the Delegation of Authority log.

Subjects will receive a 7-month supply of the drug/placebo as outlined in the clinical procedure schema (see paragraph 7.1. for a detailed description). Each kit contains of 210 tablets (7 bottles of 30 tables each) of aspirin 100 mg or placebo.

Subjects will be advised to take aspirin after food.

5.3 Run-in Procedures

No run-in procedure will take place in the present protocol.

5.4 Contraindications

Aspirin should not be taken by people who are allergic to ibuprofen or naproxen, or who have salicylate intolerance or a more generalized drug intolerance to non-steroidal anti-inflammatory drugs (NSAIDs), and caution should be exercised in those with asthma or NSAID-precipitated bronchospasm. Owing to its effect on the stomach lining, manufacturers recommend people with peptic ulcers, mild diabetes, or gastritis seek medical advice before using aspirin. Even if none of these conditions is present, the risk of stomach bleeding is still increased when aspirin is taken with alcohol or warfarin. Patients with hemophilia or other bleeding tendencies should not take aspirin or other salicylates. Aspirin is known to cause haemolytic anemia in people who have the genetic disease glucose-6-phosphate dehydrogenase deficiency, particularly in large doses and depending on the severity of the disease. People with kidney disease, hyperuricemia, or gout should not take aspirin because it inhibits the kidneys' ability to excrete uric acid, and thus may exacerbate these conditions.

5.5 Concomitant Medications

Other NSAIDs or glucocorticosteroids should be avoided or limited. Any use of systemic or topic NSAIDs or glucocorticosteroids should be clearly documented (time, doses, routes, indications) and followed regularly.

If a participant requires pain relievers or antipyretics, the use of non-NSAIDs, e.g., acetaminophen, should be encouraged. It is not necessary to break the blind in most situations. A protonic pump inhibitor (PPI, omeprazole 20 mg/day tablet) will be added during the period of any NSAIDs or glucocorticosteroids treatment and will be stopped once NSAIDs or glucocorticosteroids are withdrawn.

Short courses of systemic NSAIDs or glucocorticosteroids (defined as up to 2 weeks at a time) are allowed. No more than 1 month total of off-protocol NSAIDs or glucocorticosteroidsis allowed in a 6 months period. If it is anticipated that continuous NSAIDs or glucocorticosteroids will be needed or if more than 1 month of NSAIDS or glucocorticosteroids occurs during a 6 months period, the participant will stop study drug. However, since the participant will still undergo his/her yearly scan, they can still be assessed in an intent-to-treat analysis and thus do not come off the study. Non systemic glucocorticosteroids use is allowed without time restrictions.

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

5.6 Dose Modification

Subjects will be asked to maintain the full dose throughout the treatment period. However, should some toxicity appears, dose modification will be applied and recorded.

Toxicity will be evaluated at each visit using the most recent version of the NCI terminology criteria (CTCAE version 4.0.3, published 06/14/2010).

If grade 1 toxicity occurs, the patient will be maintained on full dose. Toxicity will be checked depending on clinical relevance.

In case of grade 2 toxicity or higher, the dose will be changed according to the potential relationship to study drug as listed in the table below.

Toxicity	Attribution to study drug				
Grade	Unrelated	Unlikely	Possible	Probable	Definite
1	C	C	C	C	C
2	C	C	S-R^0	S-R^0	S-R^1
3	S-R^2	S-R^2	S-R^1	W	W
4	S-R^2	S-R^2	W	W	W

C = Continue drug

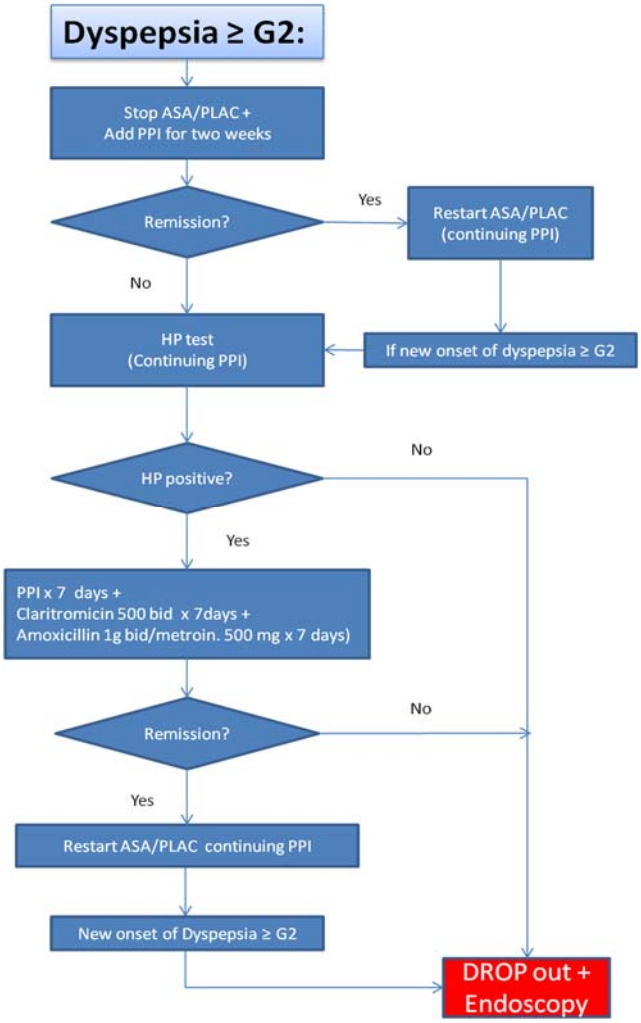
S-R^0 = Stop drug until toxicity reaches grade 1 or lower, then restart drug at full dose (100 mg/day). If toxicity resumes, stop drug again until toxicity reaches grade 1 or lower, and then restart at alternate day and maintain the reduced dose.

S-R^1 = Stop drug until toxicity reaches grade 1 or lower, then reduce drug by 50% (100 mg at alternate day) and maintain the reduced dose.

S-R^2 = Stop drug until toxicity reaches grade 1 or lower, then reduce drug by 50% (100 mg at alternate day) for 1 month. If toxicity is no more than grade 1, restart drug at full dose (100 mg/day). If toxicity resumes after reintroduction of full dose, stop drug again until toxicity reaches grade 1 or lower and then reduce drug by 50% (100 mg at alternate day) and maintain this dose. No further reductions will be permitted and patients with returning toxicities will be removed from the study.

W = Withdrawal

For dyspepsia and GERD management only, a PPI,) will be added.
In case of dyspepsia grade 1 (G1), PPI will be added for two weeks. If remission, PPI will be stopped. If symptom persists or recurs, it will be defined as $\geq$ grade 2 (G2).
In case of dyspepsia $\geq$ G2, the symptom will be managed as follows:



The clinical investigators of the study may be helped in their decisions by consultation of other specialists (Gastroenterologist).

5.7 Adherence/Compliance

Estimates for patient non-adherence to various medical regimens range from 20% to 100%, and some authorities have come to view the difficulty of guaranteeing patient adherence as one of the most serious problems facing medical practice today. Recent data from ongoing chemoprevention studies have estimated adherence to be between 60% and 100%. The disparity between the highest and lowest estimates may largely be attributed to methodology, given that the assessment of adherence remains problematic - there is as of yet no 'gold standard' to measure patient adherence. Because no such gauge exists, we propose the use of multiple methods of adherence monitoring: subject self-reporting, calendar completion and dose count.

Although drug concentration will not be measured in the blood, compliance will be monitored in the following ways:

Patient Self-Report: The patient's history, the most direct source of information, is the most widely employed measure of a patient's adherence to their medical regimen. However, patient self-reporting has

been criticized as being too subjective, with patients tending to over-report their adherence by as much as two to fourfold. Positive information is helpful, but false negatives are common.

Calendar completion: Each subject will be provided a 7-month calendar as a reminder of drug intake (Appendix B). Each subject will be asked to cross the corresponding day of the calendar (1 cross for each tablet, i.e. 1 cross per day). Subjects are asked to fill the calendar and some additional space will be left for patient's notes. Each calendar will be returned at the next scheduled visit.

Dose count: Each subject will receive a 7-month supply of the drug or placebo and will be asked to return all full and empty bottles. Compliance will be measured as follows:
number of tablets taken (i.e., number of tablets given-number of tablets returned)/number of tablets that should have been taken during that period of time.
In cases where the tablets are not returned, the compliance has to be calculated from the calendar.

Serum Thromboxane (TXB2): As an additional measure of compliance, we will evaluate the concentration of serum TXB2, a reliable marker of aspirin use[42].

The chemoprevention research nurse and the physicians will provide education for subjects, indicating when and how should the tablets be administered (general measures to reduce risk of GI adverse effects).

In case of concomitant medications, subjects are asked to contact the chemoprevention staff in order to check whether any potential interference is expected. Data on drug suspension or interruption will be reported into the CRFs.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agent (IND #, IND Sponsor)

FDA has determined that the study is exempt from the IND requirement

6.2 Reported Adverse Events and Potential Risks

Potential risks and rare side effects related to the treatment can be summarized in the following four major categories:

- Upset stomach and heartburn.

- Easy bruising/bleeding, difficulty hearing, ringing in the ears, change in the amount of urine, persistent or severe nausea/vomiting, unexplained tiredness, dizziness, dark urine, yellowing eyes/skin.

- Black/tarry stools, persistent or severe stomach/abdominal pain, vomit that looks like coffee grounds, slurred speech, weakness on one side of the body, sudden vision changes or severe headache.

- Rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

6.3 Availability

Bulk aspirin and matching placebo will be manufactured and supplied by Bayer Europe, using a dose currently available on the European market. Packaging and labeling will be performed by Monteresearch srl, Bollate, Italy and labeling performed by MRIGlobal for US supplies.

Aspirin is provided to the EIO under a Clinical Trials Agreement (CTA) between Bayer Europe and the EIO (see §12.7).

6.4 Agent Distribution

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

EIO
Bayer Europe will release active drug and matching placebo to the EIO only after documentation of IRB approval and a specific contract has been signed by the EIO representative. The distributor will be Bayer Europe.

Bayer will ship the unmasked drug to the external Pharmacy service appointed by the EIO (Monteresearch srl, Bollate, Italy) that will be responsible for re-labeling and packaging the bottles according to protocol requirements. Each bottle contains 30 tablets. The pharmacy service will provide blinded study agent to investigators with a current FDA form 1572 filed with DCP. Drug will not be used or distributed out of the present protocol.

MD Anderson
For US clinical supplies, the packaged drug will be labeled in US English by the NCI DCP repository, MRIGlobal. The NCI DCP repository will distribute the clinical material to MDACC.

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

John Cookinham
MRIGlobal
DCP Repository
425 Volker Blvd.
Kansas City, MO 64110
Phone: (816) 360-5369
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF) or an institutionally-approved accountability system. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant. At EIO, this responsibility has been delegated to EIO Pharmacy. At MD Anderson, this responsibility has been delegated to MDACC Investigational Pharmacy.

6.6 Packaging and Labeling

Aspirin and matching placebo will be provided to EIO by Bayer Europe as a bulk shipment, but labeled and packaged by an external Pharmacy where they will be stored until delivery. An undisclosed randomization list will be provided by the statistician to the dedicated Pharmacist. Each package consists of a 7-month supply of the drug or placebo. Each box contains seven bottles with 30 tablets each. Each box will be labeled with the following information: EIO study number (S-XXX), randomization number (3 digits), PI's name and phone number, number of total doses, daily dose and schedule, lot numbers and expiring dates, specific instruction such as storage temperature, indication to keep the bottle out of reach of children. Subject's identification number will not be reported on drug packages. The same instructions will be stuck in the external part of the box package. Half of the label will be removed from the box and stuck into the drug delivery CRF to confirm appropriate drug has been administered to the subject.

For clinical supplies in the US, the NCI, DCP repository (MRIGlobal) will perform the product labeling, using US English labels, and will store the product for distribution to MDACC.

6.7 Storage

Study drug will be stored in a secure location, at controlled room temperature (59°F and 86°F) under low humidity.

6.8 Registration/Randomization

Baseline/ Registration into the DMI Database:
Participants will be screened, consented and, once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's PID upon completion of the registration process.

Randomization:
Participants will be assigned a randomization number once the following has been accomplished: eligibility has been verified at the site level, eligibility has been confirmed by the site PI, and eligibility CRF has been entered into the DMI web application. The randomization number will be generated by the database and assigned to the participant. Refer to Section 13.2 for details of randomization.

Baseline/Registration/Randomization into site-specific databases:
The DMI is the database of record for the study. Registration and randomization should occur per the procedures outlined above. If the site staff need to enter study data into site-specific electronic databases per their institutional requirements, they should do so in accordance with their institutional policies and procedures.

Appropriate CRFs must be completed for any participant who signs an informed consent. If a consented participant is a screen failure and deemed ineligible, the following CRFs must be completed: 1) the Registration CRF with the eligibility box checked "no", 2) the Inclusion and Exclusion CRFs showing why the participant is ineligible, 3) the Off-Study CRF, 4) the Adverse Event CRF, 5) the Concomitant Medication CRF and 6) the Verification CRF. If no Adverse Event and/or Concomitant Medications were assessed by the time the participant is deemed ineligible, the "NONE" box will be checked to complete both CRFs. All participants who sign an informed consent must formally go off study. All participant registration information will be entered into DMI. If a participant experiences a serious adverse event during the screening process, an SAE form must be completed.

6.9 Blinding and Unblinding Methods

- Participants will be blinded to aspirin or placebo.
- The Statistician and the Study Pharmacist will not be blinded to Aspirin or placebo.
- All other Investigators will be blinded to Aspirin or placebo.
- The MDACC Data and Safety Monitoring Board will also be blinded unless unblinding is warranted (the reasons for unblinding are outlined below).
- All participants will take 1 tablet per day of aspirin/matching placebo.
- Study assignments will be unblinded to the Study Investigators and Site Coordinators after all of the data are collected and the study database has been locked. Unblinding will also occur if the participant's physician deems that unblinding is necessary, such as in the case of unacceptable toxicity thought to be related to the study agent or progressive disease, or if the participant becomes pregnant.
- Unblinding will only take place after consultation with the NCI, DCP Task Order Monitor Eva Szabo, MD.
- If unblinding is indicated prior to the completion of the study, it will be conducted as follows:

    - The Site PI contacts the Protocol Chairman (the Protocol Principal Investigator) and requests the participant's treatment status be unblinded.
    - The Protocol Chairman (the Protocol Principal Investigator) contacts the NCI, DCP Task Order Monitor (Medical Monitor) and requests the participant's treatment status be unblinded. The Protocol Chairman then conveys the Task Order Monitor's decision to the Site PI. The Site PI then proceeds with unblinding as written out below.
    - If the NCI Task Order Monitor cannot be reached and the participant requires emergency care, the Protocol Chairman (the Protocol Principal Investigator) may authorize the site PI to break the blind.
    - If the Site PI is unable to reach the Protocol Chairman and the participant requires emergency care, then the Site PI must proceed with unblinding as written out below.
    - The Site PI requests the participant's treatment status be unblinded by the research pharmacist (or designated individual responsible for dispensing drug).
    - The Site PI officially takes the participant off-study.
    - The date and reason for breaking the blind must be submitted by the Site PI to the Protocol Chairman, Bernardo Bonanni, MD, as soon as possible.
    - It is the responsibility of the Study Chairman to report the date and reason for breaking the blind to the **NCI Task Order Monitor,** Eva Szabo, MD**,** as soon as possible.
    - The date and reason for breaking the blind must be submitted by the Study Chairman to the MD Anderson Consortium Principal Investigator, Powel H. Brown, MD, PhD, or

designee as soon as possible via email to phbrown@mdanderson.org and lavornik@mdanderson.org or phone at (713) 792-4509.

- The date and the reason for breaking the blind will be reported by the MD Anderson Consortium Principal Investigator or designee to the MD Anderson DSMB as soon as possible.

6.10 Agent Destruction/Disposal

EIO
At the completion of investigation at EIO, all unused study agent together with all returned tablets will be returned to the Pharmacy Service and kept till destruction. Documentation of disposal and destruction will be kept until the end of the trial.

MDACC
At the completion of investigation at MD Anderson, all unused study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using the DCP form "Return Drug List". The guidelines and the form are available on the DCP website.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

On a weekly basis, all subjects participating in the ongoing IEO screening programs COSMOS 1 and COSMOS2 presenting lung nodules are discussed during multidisciplinary meetings involving radiologist and clinicians in order to assess the most appropriate monitoring schedule.
In addition, subjects with eligible subsolid lesions identified outside the context of a screening program will be discussed to select potential participants.

Subjects presenting one or more persistent subsolid lung nodules according to the inclusion criteria (see par 4.1) are eligible for this chemoprevention trial. At EIO, potential candidates will be contacted by phone by trained personnel illustrating the possibility of taking part in a chemoprevention trial and scheduling an appointment for an outpatient visit at the Institute. Randomization should be performed within 2 months (i.e., 61 days) from the CT scan. An exception may be done for those subjects with nodules who are eligible at the CT scan performed 3 months apart: in order to maintain 12 months treatment period, these subjects will be randomized within 6 weeks (i.e. 42 days) and will repeat the CT scan 12 months after.

At MDACC, subjects with persistent subsolid lung nodules detected during any of the screening programs are potentially eligible for the trial and will be identified as potential candidates for the study by the Lung Cancer Screening program coordinator. In addition, subjects with subsolid lesions identified outside the context of a screening program will be provided to the research staff by the collaborating radiologists creating a future recruitment resource upon subsequent CT for stability. While waiting for subsequent CT scan, the potential candidate will be given a leaflet about the possibility of being contacted for taking part in a prevention trial. Both standard dose and low dose CT scans are allowed provided that the same procedure is used at both time points.

Baseline and Randomization:

- Signed informed consent.

- Collection of a blood samples (safety including β-HCG if applicable, biomarkers including TXB2 and biorepository), urine samples (biomarkers), physical exam and medical history including baseline symptoms; concomitant medications; confirmation of eligibility criteria
- Participants are randomized to receive either study agent or placebo and will be given a 7-month supply of drug or placebo (a kit containing 7 bottles of drug or placebo, with 30 tablets in each bottle, 1 tablet per day) together with a daily calendar.

Month 1 (+/- 1 weeks from randomization):
Phone contact in order to:
- record concomitant medications and record and evaluate adverse events;
- record self-reported compliance.

Month 3 (+/- 2 weeks from randomization):
Phone contact in order to:
- record concomitant medications and record and evaluate adverse events;
- record self-reported compliance.
- confirm date of the next schedule clinic visit and blood/urine tests
- CT scan if determined to be needed during multidisciplinary meetings

Participant will be asked to return used and unused boxes at the month 6 visit.

Month 6 (+/- 2 weeks from randomization):
- Participants will undergo physical exam, vital signs, weight, lab test (including β-HCG, if applicable) and biological sample collection (blood, urine) for biomarker measurement including TXB2.
- Concomitant medications will be recorded and adverse events will be recorded and evaluated.
- Compliance will be measured (subjects will be asked to return both used and unused bottles as well as calendar).
- CT scan if determined to be needed during multidisciplinary meetings

A 7-month supply of drug or placebo will be provided (a kit containing 7 bottles of drug or placebo, with 30 tablets in each bottle, 1 tablet per day).

Month 9 (+/- 2 weeks from randomization):
Phone contact in order to:
- confirm date of the next CT scan and month 12 clinic visit and ;
- record concomitant medications and record and evaluate adverse events;
- record self-reported compliance.

Month 12 (+/- 2 weeks from randomization):
- Participants will undergo physical exam, vital signs, weight, lab test and biological sample collection (blood and urine) for biomarker measurement including TXB2 and biorepository.
- Concomitant medications will be recorded and toxicity will be evaluated.
- Compliance will be measured (subjects will be asked to return both used and unused bottles as well as the calendar).
- CT scan will be performed.

Month 13: Phone contact; adverse events and concomitant medication will be recorded and evaluated.

SCHEDULE OF EVENTS

Evaluation/ Procedure	Pre-study evaluation	Baseline Visit/Registration	Randomization	Month 1	Month 3	Month 6 visit	Month 9	Month 12 visit	Month 13	Early Termination
Informed Consent		X								
Assess pre study Eligibility (CT scan)	X									
Assess Eligibility		X								
Medical History		X								
Physical Exam/ height/weight/ vital signs		X				X		X		X
Interim medical history						X		X		X
Tobacco use assessment		X				X		X		X
Laboratory Tests		X				X		X		X
Biomarkers*§ (blood, urine)		X				X		X		
Biorepository (blood)		X						X		
CT scan					(X)#	(X)#		X		
Concomitant Medications		X		X	X	X	X	X	X	X
Baseline symptoms		X								
Dispense Study Agent			X			X				
Collect study agent						X		X		X
Compliance				X°	X°	X	X°	X		X
Review Agent Diary/Record			X			X		X		X
Adverse Events				X	X	X	X	X	X	X
Phone contact				X	X		X		X	

§Biomarkers collection at baseline, 6 and 12 month visit requires the subiect to have fasted for at least 6 hours and includes TXB2 measurement; *Blood for miRNA and urine for LTE4 and PGEM will be collected and measured at baseline and month 12 only; ° review of self reported compliance; Serum pregnancy test will be done only on potentially fertile women by β-HCG measurement; # if determined to be needed during COSMOS multidisciplinary meetings

7.2 Prestudy Evaluation/Baseline Visit/Registration

Pre study evaluation is:

- Measurement of subsolid nodules detected at any qualifying CT scan (for details see section 7.6)

Baseline exams are:

- Fasting blood lab tests (hemogram, AST, ALT, Na, K, glucose, BUN, total bilirubin, Cl, Ca, alkaline phosphatase, creatinine, β-HCG, if applicable)
- Physical exam and medical history; for the purpose of the present study, minimal requirements for the physical exam visit include the following body/system sites: appearance, chest, lungs, heart and abdomen.
- Fasting (at least 6 hours) blood sample collection for biomarkers, biorepository and baseline level of serum TXB2
- Urine sample collection for urinary creatinine and for biomarkers
- At baseline clinic visit, participants will be instructed on the importance of drug compliance. Participants will be provided and instructed on the use of a pill diary to be completed daily, signed and dated by the participant. Participants will be informed that a pill count will be conducted on their study medication use so that their study medication bottle with remaining tablets must accompany them in the subsequent clinic visit.

7.3 Evaluation During Study Intervention

During study intervention, participants will undergo physical exam, interim history, vital signs, weight, tobacco use assessment, in a dedicated outpatient clinic at month 6. Phone contact will occur on month 1, 3, and 9.

Safety blood lab tests (hemogram, AST, ALT, Na, K, glucose, BUN, total bilirubin, Cl, Ca, alkaline phosphatase, creatinine, β-HCG, if applicable) will be performed at month 6.

Fasting (at least 6 hours) biological samples (blood and urine) will be collected at month 6 for biomarker measurement including TXB2. Samples will be stored until measurement.

7.4 Evaluation at Completion of Study Intervention

Participants will undergo CT scan, physical exam, interim history, vital signs, weight, tobacco use assessment, in a dedicated outpatient clinic at month 12.

Safety lab tests (hemogram, AST, ALT, Na, K, glucose, BUN, Total Bilirubin, Cl, Ca, Alkaline phosphatase, creatinine, β-HCG, if applicable) will be performed at month 12.

Fasting (at least 6 hours) blood samples will be collected at month 12 for biomarkers measurement including TXB2 and biorepository. Samples will be stored till measurement.

Urine sample will be collected for urinary creatinine and for biomarkers. Samples will be stored till measurement.

7.5 Post-intervention Follow-up Period

Participants will be contacted with a phone call at month 13, i.e. 1 month after the final CT scan, to check about adverse events and concomitant medication.

After that, subjects will continue the CT scan evaluation as needed

7.6 Methods for Clinical Procedures

Assessment of lung nodules with low dose CT scan at IEO

Investigations will be performed with low-dose CT scans (average effective dose <1.5 mSv, without contrast medium), with a multidetector (64 slices) CT scanner (CT750 HD/Optima 660, General Electric Healthcare, USA) with the following parameters:

- 120 Kvp, 30 mA
- Matrix 512x 512
- pitch 1.75
- 2.5 mm thickness
- Soft tissue algorithm
- single breath, in respiratory apnoea
- retro-reconstruction at 1.25 mm interval
- at the end of a session of 15 - 20 subjects the investigations will be sent to the workstation and archived both on hard-disk and on CD in DICOM format.

• multiplannar reconstruction and 3-dimensional volume reconstruction will be performed on the workstation (Advantage Windows 4.2, General Electric medical system)

Nodule volumes and volume doubling time (VDT) will be automatically calculated by Lung VCAR specific software (GE healthcare). In absence of available volumetry by the software, VDT will be calculated by the following formula using the maximum diameter[43].

$$VDT = \frac{\log_{10} 2 \times (\text{difference in days between first CT and second CT})}{3 \times \log_{10} (\text{difference in nodule size [mm] between first CT and second CT})}$$

Lung nodule maximum and mean density will be measured by the software lung VCAR (GE) before and after treatment.

Within 6 days an expert radiologist will read the images on the workstation through the windows for lung parenchyma (WW 1500, WL -650) and with MPVR (maximum projection visual resolution) reconstruction and mediastinum windows (WW 400, WL 40). A comparison between first year and baseline CT scan will be performed by Cosmos radiologists. All exams which show persistence of previous identified subdolid lung nodules will be discussed in a specific meeting with a senior radiologist (chemoprevention trial-responsible) and a senior thoracic surgeon (chemoprevention trial-responsible) within 10 days of the CT investigation. Eligible cases for the chemoprevention trial according to the criteria in section 4.1 and 4.2 are scheduled for the preliminary phone contact.

Assessment of lung nodules with low dose CT scan at MDACC

Investigations will be performed with low-dose CT scans (average effective dose <1.5 mSv, without contrast medium), with a multidetector (64 slices) CT scanner (CT750 HD, General Electric Healthcare, USA) with the following parameters:

• 120 Kvp, noise modulation, Radiation dose < 3mGy for standard size patient.

Matrix 512x 512

• 2.5 mm, 1.25 mm and 0.625 mm thickness

Soft tissue and lung algorithm

• single breath, in respiratory apnea
• retro-reconstruction at 50% interval
• multiplannar reconstruction at scanner.3-dimensional volume reconstruction performed with dedicated software .

Assessment of lung nodules with standard chest CT

Investigations will be performed with standard CT scans (with or without contrast medium), with multidetector scanner(16 or 64 slices):

• 120 Kvp.

Matrix 512x 512

• 2.5 mm thickness

Soft tissue and lung algorithm

• single breath, in respiratory apnea
• retro-reconstruction at 1.25 slice interval
• multiplannar reconstruction at scanner.3-dimensional volume reconstruction performed with dedicated software .

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

For the purposes of the primary endpoint, only sub-solid (non-solid or partially solid) nodules will be assessed. Target lesions are defined as non solid or partially solid nodules persistent at least after 3 months follow up with standard or low dose CT scan, at least 4 mm in size. Non target lesions include solid nodules (larger than 3.5 mm), new lesions at baseline (e.g., that were not present on prior CT) or non-solid or partially solid nodules < 4mm in size.

The primary end-point will be the difference in sum of longest diameters of baseline target lesions in a person-specific analysis. New subsolid lesions identified during follow-up satisfying the same criteria for the definition of target lesion will also be included in the analysis.

It has been assumed a -0.2 mm average reduction of the sum of the longest diameter of all non-solid and partially-solid lesions at 12 months with respect to baseline in the placebo arm and an expected average reduction of -1.0 mm in the aspirin group.

8.2 Secondary Endpoints

We will investigate the effects of aspirin on the following secondary end points: (see paragraph 13.5 for a detailed statistical analysis):

Evaluation of response according to modified RECIST criteria:

The per-lesion analysis will be done according to the Modified RECIST criteria:

Complete Response (CR)= disappearance of the lesion
Partial Response (PR) = shrinkage of ≥30% of the longest diameter
Stable Disease (SD) = anything other than CR, PR or PD
Progressive Disease (PD) = growth ≥20%, with a minimum increase of 2 mm

The per-subject analysis will be done according to Modified RECIST criteria:

For single nodules a clinical meaningful shrinkage of 30% or more of the longest diameter will be considered as a treatment success after one year of treatment as long as there are no new non-solid or partially solid nodules or nodules highly suspicious for malignancy or shown to be malignant (modification to RECIST criteria). Growth of 20% or more with a minimum of 2 mm, will be considered progression.

For multiple lesions the sum of the maximum diameters of all target nodules will be considered, using the same parameters to define success and progression (30% or more decrease and 20% or more increase, respectively).
In case of multiple lesions, treatment will be considered successful when a complete response (CR) or partial response (PR) occurs according to modified RECIST criteria, while treatment will be considered a failure when progression of disease (PD) or stable disease (SD) occurs according to the same criteria. Specifically:

CR: disappearance of all target lesions, + no progression of non-target lesions + no appearance of new non-solid or partially solid lesions, and no appearance of new nodules highly suspicious for malignancy

or shown to be malignant (even if solid);

PR: CR for some target lesions + PR/SD for other target lesions + no PD or new lesions as described in CR above; or, PR (i.e., ≥ 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter) for target lesions + no PD for non target lesions + no appeareance of new lesions as described in CR above;

PD: PD (A ≥20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the start of treatment) for target lesions irrespective of response of non target lesions; or PD for non target lesions irrespective of response for target lesions; or appearance of new non-solid or partially solid lesions or appearance of new nodules highly suspicious for malignancy or shown to be malignant irrespective of response of target or non target lesions;

SD: neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum longest diameter since the start of treatment.

All solid nodules will be considered in a separate secondary analyses, unless they are highly suspicious for malignancy or are actually shown to be malignant, in which case they will represent progressive disease (PD). Non-suspicious (stable solid nodules with clearly benign features) will not be considered in the analysis according to RECIST criteria[44]

Analysis of non target lesions
Evaluation of number and size of non target lesions before and after treatment including :
-Subsolid nodules less than 4 mm .
-Solid nodules: evaluation of number and size of preexisting or new solid nodules will be performed. The analysis will be analogous to the analysis of non-solid and partially solid nodules described above, but substituting solid lesions for subsolid lesions. In addition, a combined analysis of nonsolid and solid lesions (with solid lesions considered to be non-target lesions) will be performed.
- New nodules: A separate analysis examining new nodule appearance at 0 and 12 months
- Cancer: the rate of cancer in each arm will be assessed.

Nodule density
We will evaluate nodule density measured by quantitative changes in mean and maximum Hounfields Unit (HU) before and after treatment.

miRNA
The effectiveness of the miRNA serum protocol will be evaluated by comparing the sensitivity, specificity and accuracy of miRNA to identify patients with GGO or with partially solid nodules and to predict aspirin treatment response considering the LDCT screening protocol as the reference procedure.
The ultimate goal is to evaluate if miRNA analysis in the serum can represent a new non-invasive test to identify patients with GGO or with partially solid nodules and to predict treatment response to low-dose aspirin.

Additional biomarkers
Additional biomarkers will include the modulation of hs-CRP as marker of inflammation, the evaluation of urinary cotinine as marker of tobacco exposure and investigation of the potential effect of aspirin according to its concentration, the measurement of urinary PGEM and LTE4 normalized to urinary creatinine concentration. Serum TXB2 will be determined as a measure of compliance. Results will be compared between the two treatment arms (aspirin and placebo).

Tolerability
Aspirin tolerability will be evaluated.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. Subjects will not be replaced.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention is completed, protocol violation, adverse event/serious adverse event, lost to follow-up, physician decision (i.e., non-compliance, concomitant medication, medical contraindication), participant withdrawal (i.e., withdraw consent), death, ineligibility or screening failure, disease progression or other reason.

8.5 Study Termination

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

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

Blood
miRNA analysis
miRNA extraction and purification from serum (4.5 ml of blood) will be done accordingly to standard procedure[33]. We will analyze approximately ~850 miRNAs using the TLDA qRT-PCR microfluidic cards (Applied Biosystems) and we will determine serum miRNA relative quantities using the ΔΔCt-method. Importantly, we will check the presence of hemolysis in all serum samples collected for miRNA screening. This will be done before the miRNA extraction by visual inspection and by measuring the absorbance at 414nm[45], and after analysis of the expression ratios of couples of miRNAs found by us and by others to be sensitive enough to reveal hidden hemolysis[45]. In addition, we will introduce spike-in synthetic miRNAs to be used as standard references in every sample screened. This will allow us to control either the efficiency of the extraction procedure and of all the qRT-PCR reactions.
Data analysis will be performed using R software (http://www.r-project.org/) and SAS software (http://www.sas.com/) for correlation analysis with all other clinical, pathological and radiological parameters.

hs-CRP
Serum concentrations of C-reactive protein as marker of inflammation will be measured by means of commercially available assays. Serum samples from the same patient obtained at different time-points will be run in batches in order to reduce analytical variability. Standards for good laboratory practice are applied. Monitoring of precision and reproducibility will be performed by processing commercially available control samples and internal in-house prepared pool of samples in each run.

Urine
Urine concentrations of cotinine, prostaglandin E metabolite and leukotriene E4 will be measured by

means of commercially available assays. Urine samples from the same patient obtained at different time-points will be run in batches in order to reduce analytical variability. Standards for good laboratory practice are applied. Monitoring of precision and reproducibility will be performed by processing commercially available control samples and internal in-house prepared pool of samples in each run.

Cotinine

We will use NicAlert® test (Nymox Pharmaceutical Corporation, Quebec, Canada) to measure tobacco product exposure. The test is able to detect exposed to tobacco products such as cigarettes, pipes, or chewing tobacco within the past 48 hours. Second hand smoke exposure may cause a positive result in a non-user of tobacco products.

NicAlert® is a semi-quantitative immunochromatographic assay for urinary cotinine measurements, that uses monoclonal antibody-coated gold particles and a series of avidity traps that allow quantification. The sample collection end of the strip contains gold particles coated with monoclonal antibodies to cotinine, a relatively long-lived metabolite of nicotine. The distance the gold migrates on the strip is shown by a clear color change and provides an accurate measure of the amount of cotinine in the sample. The cutoff concentration for the NicAlert® test is 100 ng/mL. The NicAlert™ Negative Controls consist of cotinine-free (NicAlert® Level "0") human preserved pathogen-free urine. The NicAlert® Positive Controls consists of cotinine-free urine spiked with cotinine to concentrations of 400 ng/mL cotinine (Low Positive Control, NicAlert® level "4") and 2000 ng/mL cotinine (High Positive Control, NicAlert® level "6").

PGEM

Because of the rapid metabolism of prostaglandin E_2 (PGE_2), the determination of the *in vivo* biosynthesis is often best accomplished by the measurement of the concentration of PGE_2 metabolites. We will measure urinary concentrations of prostaglandin E metabolites (PGEM) by a competitive enzyme immunoassay (EIA) kit purchased from Cayman (Cayman Chemical Co., Ann Arbor, Michigan, United States) that converts all major metabolites into a single stable derivative which is easily measurable by EIA. The assay is based on the competition between PGE_2 and PGE_2-acetylcholinesterase (AChE) conjugate (PGE_2 tracer) for a limited amount of PGE_2 monoclonal antibody. Because the concentration of the PGE_2 tracer is held constant while the concentration of PGE_2 varies, the amount of PGE_2 tracer that is able to bind the PGE_2 monoclonal antibody is inversely proportional to the concentration of PGE_2 in the well. This antibody complex PGE_2 binds to goat polyclonal anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellmans reagent (which contains the substrate to AChE) added to the well. The product of this enzymatic reaction has a distinct yellow color that absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PGE_2 tracer bound to the well, which is inversely proportional to the amount of free PGE_2.

We will check for interference by contaminants present in urine by 1:10 and 1:30 dilution of crude samples with phosphate-buffered saline. If the two different dilutions of the samples show good correlation (differ by 20% or less) in the final calculated PGE_2 concentration, purification is not required. If we do not see good correlation of serial dilutions, purification according to the purification protocol provided with the assay method will be applied.

LTE4

Urinary leukotriene E_4 ($uLTE_4$) will be determined by a competitive enzyme immnoassay kit (Cayman Chemical Co., Ann Arbor, Michigan, United States). The assay is based on the competition between $uLTE_4$ and $uLTE_4$-acetylcholinesterase (AChE) conjugate ($uLTE_4$ tracer) for a limited amount of $uLTE_4$ Antiserum. Because the concentration of the $uLTE_4$ tracer is held constant while the concentration of $uLTE_4$ varies, the amount of $uLTE_4$ tracer that is able to bind the $uLTE_4$ antiserum will be inversely proportional to the concentration of $uLTE_4$ in the well. This antibody $uLTE_4$ complex binds to a mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent (which contains the substrate to AChE) added to the

well. The product of this enzymatic reaction has a distinct yellow color that absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of $uLTE_4$ tracer bound to the well, which is inversely proportional to the amount of free $uLTE_4$.
We will check for interference by contaminants present in urine by 1:10 and 1:30 dilution of crude samples with phosphate-buffered saline. If the two different dilutions of the samples show good correlation (differ by 20% or less) in the final calculated $uLTE_4$ concentration, purification is not required. If we do not see good correlation of serial dilutions, purification according to the purification protocol provided with the assay method will be applied.

TXB2
Serum concentration of thromboxane B2 (TXB2) will be determined by a competitive enzyme linked immunosorbent assay (Neogen TM, Lexingotn, KY, USA). The assay is based on the competition between the enzyme conjugate and the TXB2 in the sample for a limited amount of binding sites on the antibody coated plate. The detection limit for the TXB2 assay is 4 pg/ml.

BIOREPOSITORY
DNA and RNA will be prepared and stored for future unspecified analyses for specific markers for whole genome DNA and RNA profiling. Samples will be stored at IEO Biobank and Biomolecular Resource Infrastructure (IBBRI). A specific institutional informed consent will be used.

9.2 Comparable Methods

We will use established and validated assays for determination of changes in biomarkers' expression in this study. We plan to evaluate biomarkers in both serum, and urine specimens for secondary endpoints evaluation. Specifically, for miRNA signature, we will use protocols developed and validated by our group[33] within the Cosmos 1 screening program.

For serum hs-CRP and urine biomarkers (PGEM, leukotriene and cotinine) we will utilize standard protocols of commercially available kits already described in peer-reviewed publications. As to hs-CRP, our group has already published several papers involving this biomarker[37,46-48]. Two different methods have been applied. The former was a chemiluminescent enzyme immunometric assay (Diagnostic Products Corp, Los Angeles CA) designed for the immulite automated analyzer. more recently a high sensitivity turbidimetric method for cobas integra 800 was adopted (Roche Diagnostics, Mannheim, Germany). The two methods showed an excellent correlation (r^2=0.99).

10. SPECIMEN MANAGEMENT

10.1 Laboratories

The analysis of serum microRNA will be perfomed at the EIO laboratories of the Molecular Medicine Division (Chief Pierpaolo di Fiore) under the supervision of Fabrizio Bianchi, PhD.

The analysis of serum ultrasensitive hs-CRP, and urinary levels of cotinine, PGEM and LTE4 will be performed in the dedicated EIO lab of the Division of Lab Medicine under the supervision of Harriet Johansson, MSc, PhD.

10.2 Collection and Handling Procedures

All blood and urine samples will be withdrawn under fasting condition (at least 6 hours) preferably between 8 a.m. and 10 a.m. at each time point. Instructions about specimen handling and storage will be provided in a dedicated Manual of Procedures.

A total of **8 ml** of blood will be withdrawn for safety labs (at baseline, 6 and 12 months)

A total of **4.5 ml** of blood will be collected for **miRNA** (at baseline and 12 months) with the following recommendation for each blood withdrawal:
1) Patients should be fasting at least 6 hours before blood collection

2) Information about oral therapy with anti-inflammatory, anticoagulants, steroids or other drugs will be collected the same day.

3) Patients must have been free of any infection (including flu) and inflammatory diseases for a week prior to the collection of blood samples.

4) Before any instrumental or physical examination has been performed

Fasting (at least 6 hours) blood samples will be collected in all participants free of any infection (including flu) and inflammatory diseases for a week prior to the collection of blood samples at baseline and after 12 months of treatment. In order to avoid skin contamination, the first 3 mL of blood should not be used for serum preparation. Blood samples (4.5 ml) will be collected in tubes without anti-coagulant (white cap), left at room temperature for 30-60 minutes to clot, then spun at 3000 rpm (1000 g, Megafuge 2.0 – Heraeus) for 10 minutes at room temperature. The serum will be immediately removed slowly by using a plastic transfer pipette, leaving 0.5 cm remaining to avoid disturbing the serum–clot interface, dispensed in 0.6 ml aliquots (for a total of 4 aliquots) into 1.5 ml cryotubes (Thermo Fisher Scientific), specifically labeled with a barcode, and snap frozen in dry ice. Aliquots will then be transferred in a dedicated -80°C freezer.

A total of **7.5 ml** of blood will be collected into tubes containing clot activator for **hs-CRP** (at baseline, 6 and 12 months):
Morning fasting blood samples are centrifuged at 1850xg and one ml serum aliquots will be stored into 1.5 ml cryotubes (Thermo Fisher Scientific), specifically labeled with a barcode and transferred in a dedicated -80°C freezer.

A total of **7.5 ml** of blood will be collected into tubes containing clot activator for **TXB2** (at baseline, 6 and 12 months):
Morning fasting blood samples will be collected and the time of blood draw will be reported. Samples will be incubated at 25°-37°C (77-98.6°F) for 1 hour and then centrifuged at 1000xg for 7 minutes. One ml serum aliquots will be stored into 1.5 ml cryotubes (Thermo Fisher Scientific), specifically labeled with a barcode and transferred in a dedicated -80°C freezer.

First morning **urine** samples **(15 ml)** should be collected under fasting conditions following the standard procedure for appropriate urine collection (may be provided) and samples should be kept refrigerated until arrival to hospital. Alternatively, samples should be collected under fasting conditions (15 ml) at the hospital in concomitance with blood draw. Urinary creatine will be determined on fresh specimen to normalize subsequent urinary biomarker results. The remaining sample will be aliquoted into 2.0 ml cryotubes, specifically labeled with a barcode and transferred in a dedicated -80°C freezer.

A total **2.5 ml** whole blood will be collected at baseline and 12 months of treatment in tubes containing

anti-coagulant CBC - Na-EDTA (red cap) and stored at -80°C in order to collect DNA and RNA for future unspecified analysis.
DNA will be purified using the QIAamp DNA Blood Mini Kit (QIAGEN) that is designed for processing up to 200 µl fresh or frozen human whole blood with an expected yield of 4-12ug of DNA, which is sufficient for all downstream sequencing and PCR analyses. RNA will be purified using the QIAamp RNA Blood Mini Kit (QIAGEN) specifically designed for extracting high-quality RNA (1-5ug yeald) for all low- and high-throughput screening technologies (qRT-PCR, microarray, RNA-seq).
All DNA/RNA samples will be stored at -80°C in the IEO biobank (IBBRI).

10.3 Shipping Instructions

Samples will be collected and stored locally till shipping to EIO after study completion. Instructions about specimen handling and storage will be provided in a dedicated Manual of Procedures.

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

10.4 Tissue Banking

Aliquots will be transferred in a dedicated -80°C freezer at the IBBRI and kept till measurement.

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

11. REPORTING ADVERSE EVENTS/ADVERSE DRUG REACTIONS

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

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

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

DEFINITION ADVERSE DRUG REACTION (ADR): all untoward and unintended responses to an investigational medical product related to any dose administered (Directive 2011/20/EC).

Comment: all AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medical product qualify as ADR. The expression of reasonable causal relationship means

to convey in general that there is evidence or argument to suggest a causal relationship (ENTR/CT/ 3 April 2006).

Unexpected ADR: an ADR, the nature and severity of which is not consistent with the applicable product information (Directive 2001/20/EC-art. 2 §p; art 16§1)

11.1 Adverse Events

11.1.1 Reportable AEs/ADRs

All AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) must be recorded on the AE CRC (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

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

11.1.3 Severity of AEs/ADRs

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

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

CTCAE v4.0 general severity guidelines:

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

5	Fatal	Death related to AE.

ADL

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

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

11.1.4 Assessment of relationship of AE/ADR to treatment

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

11.1.5 Follow-up of AEs/ADRs

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

11.2 Serious Adverse Events

11.2.1 DEFINITION: According the European Directive 2001/20/EC and Fed. Reg. 75, Sept. 29, 2010 defines SAEs as those events, occurring at any dose, which meet any of the following criteria:

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

11.2.2 Reporting Serious Adverse Events

The Sponsor is responsible for AE/ADR reporting to Regulatory Authority and Ethic Committee according to local regulation.
The Principal Investigator will notify the Sponsor of any SAE within 24 hours of knowledge of the event.
All SAEs (irrespective of suspected causation) which occur while the patient is on study must be reported by the investigator to the local ethics committee regulatory office and to Servizio Farmacia - European Institute of Oncology (fax: 0257489361)
All relevant information about serious, unexpected adverse events suspected to be related to the investigational product that are fatal or life-threatening must be reported by the Sponsor to the Health Authority as soon as possible and in any case no later than seven days after knowledge of such a case. Relevant follow-up information for these cases will be subsequentely submitted within an additional eight days. All other serious unexpected events suspected to be related to the investigational product must be reported as soon as possible, but within a maximum of 15 days of first knowledge by investigator.

The organization that experiences the serious adverse event (SAE) should report the SAE to the following 3 entities: 1) NCI DCP, 2) DCP's regulatory contractor CCSA, and 3) MDACC, the CLO. Detailed reporting instructions are provided below.

11.2.2.1The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE form found at http://prevention.cancer.gov/sites/default/files/uploads/clinical_trial/SAE-form.pdf.

11.2.2.2 Reporting within 24 hours of knowledge of the event.

11.2.2.2(A) **Report to the NCI DCP Medical Monitor within 24 hours:**
Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

Eva Szabo, MD
NCI/Division of Cancer Prevention
9609 Medical Center Drive, Rm 5E-102
Bethesda, MD 20892 (For FEdEX, Rockville, MD 20850)
Phone: (240) 276-7011
Fax: (240) 276-7848
Email: szaboe@mail.nih.gov

Include the following information when calling the Medical Monitor:

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

11.2.2.2(B) **Report to the Consortium Lead Organization (CLO) PI (Dr. Powel Brown) within 24 hours of knowledge of the event:**
Report all SAEs to the Consortium Lead Organization PI (Dr. Powel Brown) within 24 hours of knowledge of the event. The same information reported to the DCP Medical Monitor should be provided to the CLO via email, phone or fax within 24 hours of knowledge of the event.

11.2.2.2(C) **Report to the drug supplier (Bayer) within 24 hours of knowledge of the event:**
Report all SAEs to Bayer (Fax: +49 30 468 96765, email: GPV.CaseProcessing@bayerhealthcare.com) within 24 hours of knowledge of the event. The same information reported to the DCP Medical Monitor should be provided to Bayer via fax or email, within 24 hours of knowledge of the event (Fax: +49 30 468 96765, email: GPV.CaseProcessing@bayerhealthcare.com).

11.2.2.3 Reporting within 48 hours of knowledge of the event:

11.2.2.3 (A) FAX or email the written SAE reports to the DCP Medical Monitor within 48 hours of learning of the event using the paper SAE form. The SAE forms should be obtained at http://prevention.cancer.gov/sites/default/files/uploads/clinical_trial/SAE-form.pdf.

11.2.2.3 (B) The written SAE reports will also be FAX'ed to DCP's Regulatory Contractor, CCS Associates, at (650)691-4410 (phone: (650)691-4400), email: safety@ccsainc.com

11.2.2.3 (C) The written SAE report will also be faxed or emailed to the Consortium Lead Organization PI (Dr. Powel Brown), at (713) 792-4003 or emailed to PHBrown@mdanderson.org.

It is the responsibility of the CLO to inform the Lead Protocol PI upon receipt of the report from the organization experiencing the event.

11.2.2.3 (D) The written SAE report will also be faxed or emailed to Bayer Fax: +49 30 468 96765, email: GPV.CaseProcessing@bayerhealthcare.com

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

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

11.2.2.6 Follow-up of SAE
Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAEs related to the study agent will be followed until resolved. The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the local IRB/IEC, and to the DSMB.

12. STUDY MONITORING

12.1 Data Management

The study PI will identify a dedicated data manager who will be responsible for the correct management of the trial conduct. In particular, he/she will periodically verify subject eligibility, informed consent signatures, accuracy and completeness of CRFs and congruency with all source documents (including laboratory print outs, drug inventory and distruction logs). All source documents will be collected in individual binders, stored in locked cupboards and accessible for any audit or inspection by authorized personnel. Should the PI change, custody of the records may be transferred to another competent person who will accept responsibility for those records.
Written notice of such event has to be given to the local Ethics Committee at least one month in advance.
This study will report clinical data using the Data Management Initiative (DMI) web-based application managed by the Consortium Biostatistics and Data Management Core. Data Management Initiative (DMI) infrastructure has been developed in the Division of Quantitative Sciences (DQS), MD Anderson Cancer Center. This infrastructure supplies integrated database and software services for web-based data collection, randomized treatment assignment, reporting, query, data download, and data quality management. The DMI will be the database of record for the protocol and subject to NCI and FDA audit. All DMI users will be trained to use the DMI system and will comply with the instructions in the protocol-specific "DMI User Manual" as well as applicable regulatory requirements such as 21 CFR; Part 11. Data management procedures for this protocol adheres to the Data Management Plan (DMP) contract HH2620100034I for Consortia 2012.

12.2 Case Report Forms

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

12.3 Source Documents

Source documentation will include only those documents containing original forms of data, including clinic charts, shadow files, hospital charts, and physician notes. Data recorded directly on the CRFs designated as source documents (i.e., no prior written or electronic record of data) will be considered source data. All other data recorded on the CRFs will not be considered source documentation.

12.4 Data and Safety Monitoring Plan

The Data and Safety Monitoring Plan for the MD Anderson Consortium is on file at the DCP. This study will be monitored by the MDACC Data and Safety Monitoring Board, the data and safety monitoring board of record for this study. The Data Safety and Monitoring Board (DSMB) reports to the President, or his designee, as the on-campus representative of The University of Texas Board of Regents. It oversees the data and patient safety issues for randomized clinical trials that originate at MD Anderson; that are coordinated or analyzed by MD Anderson and are not being monitored by any other DSMB; or have been designated as requiring DSMB monitoring at the request of the IRB, the CRC, or institution. The primary objectives of the DSMB are to ensure that patients' rights pertaining to participation in a research study are protected, and that patients' interests are prioritized over the interests of the scientific investigation. Responsibilities include:

(a) Review interim analyses of outcome data (prepared by the study statistician or other responsible person at the time points defined in the study) approved by the IRB and additional time points as determined by the DSMB, and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;
(b) Determine whether, and to whom, outcome results should be released prior to the reporting of study results;
(c) Review interim toxicity data and efficacy of treatment;
(d) Review major research modifications proposed by the investigator or appropriate study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results from the study or results of other studies, increasing target sample size).

Refer to the Data and Safety Monitoring Plan for the MD Anderson Consortium on file at the DCP for further details.

12.5 Sponsor or FDA Monitoring

The Sponsor, members of the Ethics Committee, representatives of the Italian Ministry of Health or representatives of the national Competent Authority, the NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

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

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

The agent(s) supplied by DCP, NCI, used in this protocol, is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between the Pharmaceutical Company(ies) (hereinafter referred to as Collaborator(s)) and the NCI Division of Cancer Prevention. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator@ contained within the terms of award, apply to the use of Agent(s) in this study:

12.7.1 Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a patient participating on the study or participant's family member requests a copy of this protocol, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from the DCP website.

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

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

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

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

12.7.6 Clinical Trial Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate. All data made available will comply with HIPAA regulations.

12.7.7 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators of Collaborator's wish to contact them.

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

Head, DCP Protocol Information Office
9609 Medical Center Drive, Room 5E450
Rockville, MD 20850
E-mail: NCI_DCP_PIO@mail.nih.gov

The Protocol Information Office will forward manuscripts to the DCP Task Order Monitor for distribution to the Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

Participant data will be collected using protocol-specific case report forms (CRFs) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs).

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

The trial will be a randomized double-blind placebo controlled phase IIb study. Patients will be randomized to receive either aspirin 100 mg/day or matched placebo for 1 year. Analysis will be performed according to intention to treat principle and then according to efficacy.

The primary endpoint will be the difference of the sum of longest diameters of subsolid (non-solid or partially solid) lung nodules in a person-specific analysis.

The sample size has been calculated assuming a -0.2 mm average reduction of the sum of the longest diameter of all non-solid and partially-solid lesions at 12 months with respect to baseline in the placebo arm vs. an expected average reduction of -1.0 mm in the aspirin group.

13.2 Randomization/Stratification

In order to keep at minimum the imbalance in treatments a stratified blocked randomization strategy will be used considering only the relevant prognostic factors. Subjects will be stratified according to screening vs non screening CT scan, gender, smoking habits (current versus former smokers) and nodule type (non solid versus partially solid nodules). A blocked randomization will be done within each stratum.
Other possible prognostic factors are age, the number of lesions, their volume doubling time and their size but since there is no evidence that they affect the response probability they will be included just in a stratified analysis, not during the randomization process.

Justification of stratification:
Stratification by nodule type and smoking status is planned because both non solid type and active smoking are associated with the risk of developing lung malignancies and their natural history, while stratification by screening vs non screening CT scan, and gender are conservative. The effect of aspirin has been variable in men and women for the prevention of certain conditions[49].

13.3 Accrual and Feasibility

A sample size of 128 patients (64 per arm) achieve 80% power to detect a difference of -0.8 mm between the null hypothesis that both groups average reduction is –0.2 mm and the alternative hypothesis that the average reduction of target nodules in the aspirin group is –1.0 mm, with estimated effect size of 0.5 (common standard deviations of 1.6 mm) and with a significance level (alpha) of 0.05 using a two-sided two-sample t-test. The average shrinkage in the placebo arm is derived from the Budesonide trial.

13.4 Primary Objective, Endpoint(s), Analysis Plan

All randomized patients who received at least one dose will be included in the study analysis with an intent-to-treat approach. A secondary analysis will also be carried out after excluding non-compliers and subjects who will drop-out and who will refuse treatment after randomization.
Subject will be considered compliant if at least 80% of the drug has been taken.
Using a more conservative approach, patients who drop-out or have missing final CT scans will be considered in the failure group. Both the intent-to-treat and efficacy analyses will be conducted on a per subject basis A two-sided paired t-test will be used to compare the average change in the dimension of sub-solid nodules in the aspirin gorup compared to the placebo group at 1 year.

13.5 Secondary Objectives, Endpoints, Analysis Plans

Generalized linear mixed models for longitudinal data will be used to compare the study groups using the standard general model $\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}$, with $\mathbf{y}$ the response variable (e.g. secondary endopoint variable), $\mathbf{X}$ and $\boldsymbol{\beta}$ the design matrix and unknown parameters for the fixed effects and $\mathbf{Z}$ the design matrix for the random effects and $\mathbf{u}$ the random effects. Treatment arm (aspirin or placebo) and time visit (baseline, 6 and 12 months) will be considered fixed effects, smoking history and age at study entry random effects. Secondary endpoints include a per-lesion analysis by nodule type. Identity and logit link functions will be used for normal and binary data respectively All tests will be two-sided and considered significant at the 5% level.

13.6 Reporting and Exclusions

Patients will be considered compliant with treatment if at least 80% of the drug has been taken. Non-compliers and patients who switch the treatment assigned will be excluded from the efficacy analysis.

Patterns of observations will be classified as complete cases (no missingness), terminal (monotone) dropouts, intermittent (non-monotone) dropouts, mixed and no-data. A MCAR (Missing Completely At Random) vs. MAR (Missing At Random) statistical test will be conducted as described by Little[50]. Kendall's τ_b will be computed in order to test the correlation between a dichotomous indicator of missing observations and treatment assigned as well as any possible baseline covariates. Multiple imputation procedures will be adopted only for those variables whose missing data proportion will exceed the 5% value.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of Aspirin/placebo.

13.8 Evaluation of Response

All participants included in the study who have taken at least one dose of study agent will be assessed for response to study treatment even if there are major protocol deviations or if they are ineligible. All of the participants who met the eligibility criteria (with the possible exception of those who did not receive study agent) will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants.
No subgroup analyses are foreseen.

13.9 Interim Analysis

There is no interim analysis planned since repeat CT is planned only at baseline and 1 year.

13.10 Ancillary Studies

None planned.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

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

14.2 Other Required Documents

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

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

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

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

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

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form

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

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

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation
Locally, the study will be submitted for authorization and approval to the national competent authority and competent ethics committee in compliance with the European and national regulations. All relevant communication as well as adverse event reporting to the regulatory authorities will be performed according to the national regulation.

14.4 Informed Consent

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

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. Statement of this option may be included within the informed consent document or may be provided as an addendum to the consent.

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

14.5 Submission of Regulatory Documents

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

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates
1923 Landings Drive
Mountain View, CA 94043
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions:
regulatory@ccsainc.com

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

14.6 Other

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

15. FINANCING, EXPENSES, AND/OR INSURANCE

Participants will not be responsible for the costs of this study. Study agent will be provided at no cost to the subject. If, as a result of participation in this study, an individual experiences injury from known or unknown risks of the research procedures as described in the informed consent, immediate medical care and treatment, including hospitalization, if necessary, will be available. Insurance coverage for participants is provided in accordance with all relevant legal requirements by the Sponsor (N. A1201436399 Sindacato Lloyd's 1218 New Line).

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Informed Consent Form

A RANDOMISED PHASE II TRIAL OF LOW DOSE ASPIRIN VERSUS PLACEBO IN HIGH RISK INDIVIDUALS WITH CT-DETECTED SUBSOLID LUNG NODULES

(Testing aspirin in high risk lung cancer subjects with lung nodules)

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part in it. Please take your time to make your decision. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.
You are being asked to take part in this study because you are at increased risk for lung cancer, you are already participating in a screening program in this Institute and/or you have a CT (Computer Tomography) scan recently performed having confirmed one or more small subsolid lung nodules.

What is the usual approach to my early detected lung nodules?

Currently no effective drug to prevent lung cancer in people at increased risk has been discovered. In your case these nodules are probably not cancerous but it is not possible to exclude that they may become cancers, so they should be monitored with periodic exams to evaluate their growth rate. At the moment the growth of your nodules does not have clinical relevance, but requires a yearly follow up with a CT scan.

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

If you decide not to take part in this study, you have other choices. For example:
you may choose to have the usual approach described above, annual CT scan
or you may choose to do nothing.

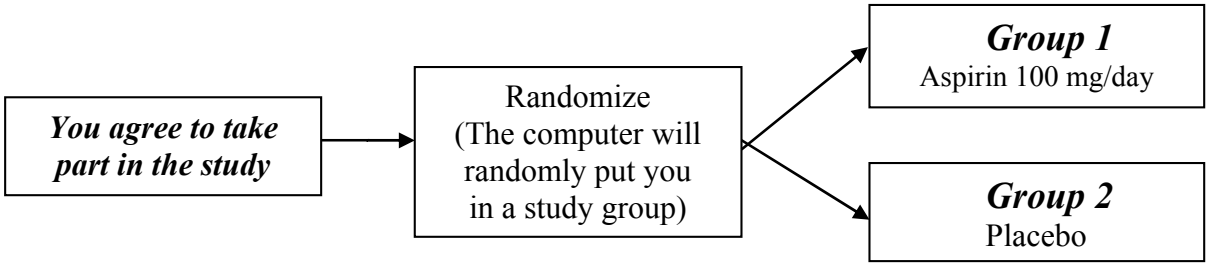
Why is this study being done?

The purpose of this randomized phase II study is to compare the safety and effects of low dose aspirin (100 mg/day) with placebo on subjects with subsolid nodules. In this study, you will get either aspirin 100mg/day or placebo, a pill that looks like the study drug but contains no medication. There will be 128 subjects taking part in this study. The study will be conducted at the European Institute of Oncology in Milan, Italy and at the University of Texas MD Anderson Cancer Center in Houston, TX, US.

What are the study groups?

This study will enroll current and former asymptomatic smokers, enrolled in lung cancer annual screening with low dose CT or having a confirming CT scan, presenting no clinically relevant nodules.
The study population will be divided in two study groups. Group 1 will receive the study drug, aspirin 100 mg/day, Group 2 will receive a placebo, a pill that looks like the study drug but contains no medication.

A computer will randomly put you in a study group—like a coin toss—to decide what group you get placed into. This is done because no one knows if one study group is better, the same, or worse than the other group. Once you are put in a group, you cannot switch to the other group. Neither you nor your doctor will know if you are receiving the study drug or placebo. Your doctor cannot choose which group you will be in.



How long will I be in this study?

You will receive the study drugs for 12 months. Even if you do not finish the study, your doctor will continue to watch you for side effects and follow your condition for 1 month after the discontinuation of the study drug.

What extra tests and procedures will I have if I take part in this study?

At the moment the presence of pulmonary nodules requires only a yearly follow up with CT scan. However, there are some extra exams that you will need to have if you take part in this study (e.g., fasting state (at least 6 hours) for blood and urine exam, including serum pregnancy test in women of childbearing potential).

Before you begin the study

You will need to have the following extra tests and procedures *to* verify your eligibility (to find out if you can be in the study):
Signed informed consent.
Collection of fasting blood sample (about 30 ml) for safety routine tests and biological markers and urine sample for biological markers, including thromboxane B2 (TXB2), an indicator of aspirin use.
Physical exam and record of any other medication that you are taking.
If fully eligible you will be randomized to receive either aspirin or placebo and will be given a 7-month supply of aspirin or placebo.

The collection of these samples (blood and urine) is required in order for you to take part in this study because the research on the samples is an important part of the study.

Month 1 (+/- 1 week from randomization)

Through a telephone call, side effects and any other medication that you are taking will be recorded.

Month 3 (+/-2 weeks from randomization)

Through a telephone call, side effects and any other medication that you are taking will be recorded.
Confirmation of Month 6 clinical visit.

Month 6 (+/-2 weeks from randomization)

Collection of fasting blood (about 28 ml) and urine sample for safety routine tests and biological markers including TXB2, an indicator of aspirin use.
Physical exam, record of any side effects and of any other medication that you are taking.

All the unused tablets will be returned.
A 7-month supply of the study drug will be given.

Month 9 (+/-2 weeks from randomization)

Through a telephone call, side effects and any other medication that you are taking will be recorded.
Confirmation of Month 12 clinical visit.

Month 12 (+/-2 weeks from randomization)

Collection of fasting blood (about 30 ml) and urine sample for safety routine tests and biological markers including TXB2, an indicator of aspirin use.
Physical exam, record of any side effects and of any other medication that you are taking.
All the unused tablets will be returned together with the calendar.

As part of the usual approach for your condition at month 12 you will have the annual CT scan .

Month 13 (+/-1 week from Month 12)

Through a telephone call, the side effects and the concomitant medications will be recorded.

The most common risks of all these procedures are related to drawing blood from your arm: a brief pain and possibly a bruise.

After Month 13 your participation to the aspirin study will be completed. You will continue the CT scan evaluation as needed.

What possible risks can I expect from taking part in this study?

If you choose to take part in this study, there is a risk that you may:
Lose time at work or home and spend more time in the hospital or doctor's office than usual.
Be asked sensitive or private questions which you normally do not discuss. There is a risk someone could get access to the personal information in your medical records or other information researchers have kept about you. Someone might be able to trace this information back to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information. In some cases, this information could be used to make it harder for you to get or keep a job.
There can also be a risk in finding out new genetic information about you. New health information about inherited traits that might affect you or your blood relatives could be found during a study.
There is also a risk that you could have side effects.

Here are important points about side effects:
The study doctors do not know who will or will not have side effects.
Some side effects may go away soon, some may last a long time, or some may never go away.
Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:
Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
The study doctor may be able to treat some side effects.
The study doctor may adjust the study drugs to try to reduce side effects.

The tables below show the most common side effects that we know about aspirin, some of which may be serious, most of them very rare. There might be other side effects that we do not yet know about. If important new side effects are found, the study doctor will discuss these with you.

Possible Side Effects of Aspirin:

POSSIBLE, SOME MAY BE SERIOUS **Many adverse reactions due to aspirin ingestion are dose-related. The frequency of some individual side effects has not been determined**		
Fever Low body temperature Thirst Irregular or rapid heartbeat Low blood pressure Agitation Swelling of the brain Coma Confusion Dizziness Headache Bleeding in the brain Tiredness Seizures Dehydration Increased blood potassium level More acid than normal in the blood Low blood carbon dioxide	Heartburn Gastrointestinal bleeding Nausea Vomiting Temporary elevations of liver enzymes Hepatitis Reye's syndrome (symptoms include rash, vomiting, and liver damage) Pain in stomach Impaired blood clotting Formation of small blood clots inside the blood vessels Decreased blood platelets Allergic reaction Swelling of the lips and eyes Asthma Blockage of the airways	Hives Damage of muscle tissue Low blood sugar level (in children) High blood sugar level Prolonged pregnancy and labor Stillbirths Lower birth weight infants Bleeding associated with child birth Abnormally deep or rapid breathing Abnormal buildup of fluid in the lungs Hearing loss Ringing in the ears Swelling of the kidneys Kidney disease or failure

Females must be postmenopausal (i.e, at least 1 year passed after the last menstruation), surgically sterile, or using acceptable birth control measures as judged by the Investigator. (A fertile woman is defined as being of child-bearing potential, from first menstruation to 1 year after last menstruation). Negative serum beta-HCG for women of childbearing potential will be required at baseline and during study. It is important you understand that you need to use birth control while on this study. Check with your study doctor about what kind of birth control methods to use and how long to use them. Some methods might not be approved for use in this study. For more information about risks and side effects, ask your study doctor.

What possible benefits can I expect from taking part in this study?

This study may or may not help you because we do not know how the study drug will compare to the usual approach for your condition. This study may help us to learn things that could help people in the future. You have 50% chance to take Aspirin. Aspirin may be protective against lung cancer.

Can I stop taking part in this study?

Yes. You can decide to stop at any time. If you decide to stop for any reason, it is important to let the

study doctor know as soon as possible so you can stop safely. If you stop, you can decide whether or not to let the study doctor continue to provide your medical information to the organization running the study.

The study doctor will tell you about any new information or changes in the study that could affect your health or your willingness to continue in the study.

The study doctor may take you out of the study:
If your health changes.
If the study is no longer in your best interest.
If new information becomes available.
If you do not follow the study rules.
If the study is stopped early for any reason by the sponsor, IRB or FDA.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights while in this study, call the _____(insert name of center) Institutional Review Board at _____(insert telephone number). (Note to Local Investigator: Contact information for patient representatives or other individuals at a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can also be listed here.)

What are the costs of taking part in this study?

The study drug will be supplied at no charge while you take part in this study. The cost of study-specific exams and tests, and any other procedures will be paid for by the study.
For the Italian site only
Some costs associated with your care may be considered standard of care, and billed to the national health system.

For US site(s) only
Some costs associated with your care may be considered standard of care, and will be billed to you or your insurance company. You will have to pay for any costs (including deductibles and co-payments) not covered by your health insurer. Before you decide to be in the study, you should check with your health plan or insurance company to find out exactly what they will pay for.

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

If you feel you have been injured or hurt as a result of taking part in the study, it is important that you tell the study doctor immediately. You will get medical treatment if you are injured or hurt as a result of taking part in this study.

The study sponsors will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance coverage, you would be responsible for any costs. Even though you are in a study, you keep all of your legal rights to receive

payment for injury caused by medical errors.

Who will see my medical information?

Your privacy is very important to us and we will make every effort to protect it. Your information may be given out if required by law. However, we will do our best to make sure that any information that is released will not be able to identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.
There are organizations that may inspect your records. These organizations are required to make sure your information is kept private. Some of these organizations are:
The study sponsor and any drug company supporting the study
The Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
The Food and Drug Administration and the National Cancer Institute in the US, and similar organizations if other countries are involved in the study (i.e. MDACC)
The Italian Ministero della Salute
The National Cancer Institute will obtain information from this clinical trial under data collection authority Title 42 U.S.C. 285.

Where can I get more information?

For more information about this study, call your study doctor's office (*insert Local Investigator's contact information)*.

The National Cancer Institute will obtain information from this clinical trial under data collection authority Title 42 U.S.C. 285.

You may visit the NCI website at http://cancer.gov/ for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor _____ *(insert name of study doctor[s])* at _____ *(insert telephone number).*

Additional study section

This section is about optional studies you can choose to take part in

This part of the consent form is about optional studies that you can choose to take part in. You will not

get health benefits from any of these studies. The researchers leading this optional study hope the results will help other people with cancer in the future.

The results will not be added to your medical records, and you or your study doctor may not know the results.

You will not be billed for these optional studies.

You can still take part in the main study even if you say 'no' to any or all of these studies. If you sign up for but cannot complete any of the studies for any reason, you can still take part in the main study.

Optional Sample Collections for Biobanking for Possible Future Studies

Researchers are trying to learn more about cancer, and other health problems. Some of this research is done using samples from your blood and urine. Through these studies, researchers hope to find new ways to prevent, detect, treat, or cure health problems.

Some of these studies may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment.

If you choose to take part, a sample of blood and urine will be collected on the scheduled day of the main study. The researchers ask your permission to store and use your samples and health information for medical research. Both DNA and RNA will be prepared and stored for future unspecified analyses for markers of whole genome RNA and DNA profiling. The research that may be done is unknown at this time.
Storing samples for future studies is called "biobanking" The Biobank is being run by Istituto Europeo di Oncologia (IBBRI) and the section regarding this study is supported by the National Cancer Institute.
If you need information about the biological samples or results obtained from the research, you can contact the coordinator of IBBRI, Dr Giuseppina Bonizzi (email Giuseppina.bonizzi@ieo.it or phone: +390294375155)

What is involved?
If you agree to take part, here is what will happen next:
Your sample and some related information may be stored in the Biobank, along with samples and information from other people who take part. The samples will be kept until they are used up.
Qualified researchers can submit a request to use the materials stored in the Biobank. A research committee at the clinical trials organization, and/or the National Cancer Institute, will review each request. There will also be an ethics review to ensure that the request is necessary and proper. Researchers will not be given your name or any other information that could directly identify you.
Neither you nor your study doctor will be notified if/when research is conducted using your samples.
Some of your genetic and health information may be placed in central databases that may be public, along with information from many other people. Information that could directly identify you will not be included.

What are the possible risks?

The most common risks related to drawing blood from your arm are brief pain and possibly a bruise.
There is a risk that someone could get access to the personal information in your medical records or other

information we have stored about you.
There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.

4) There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

For US site(s) only
A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways
Health insurance companies and group health plans may not request your genetic information that we get from this research.

Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.

Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment. All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

How will information about me be kept private?
Your privacy is very important to the researchers and they will make every effort to protect it. Here are just a few of the steps they will take:
When your sample(s) is sent to the researchers, no information identifying you (such as your name or social security number) will be sent. Samples will be identified by a unique study code only. The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and related staff with access to the list must sign an agreement to keep your identity confidential.
Researchers to whom Istituto Europeo di Oncologia sends your sample and information will not know who you are. They must also sign an agreement that they will not try to find out who you are.
Information that identifies you will not be given to anyone, unless required by law.
If research results are published, your name and other personal information will not be used.

What are the possible benefits?
You will not benefit from taking part but the researchers, using the samples from you and others, might make discoveries that could help people in the future.

Are there any costs or payments?
There are no costs to you or your insurance. You will not be paid for taking part. If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

What if I change my mind?
If you decide you no longer want your samples to be used, you can call the study doctor, _____, *(insert name of study doctor for main trial)* at _____ *(insert telephone number of study doctor for main trial)* who will let the researchers know. Then, any sample that remains in the bank will no longer be used. Samples or related information that have already been given to or used by researchers will not be returned.

What if I have more questions?
If you have questions about the use of your samples for research, contact the study doctor, _____, *(insert name of study doctor for main trial)*, at _____ *(insert telephone number of study doctor for main trial)*.

Please circle your answer to show whether or not you would like to take part in each option:

SAMPLES FOR FUTURE RESEARCH STUDIES:
My samples and related information may be kept in Biobanks for use in future health research.

YES NO

I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to participate in other research in the future.

YES NO

My Signature Agreeing to Take Part in the Main Study

I have read this consent form. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study and any additional studies I circled "YES"

Participant's signature _____

Date of signature_____

Signature of person(s) conducting the informed consent discussion

_____

Date of signature_____

APPENDIX A

Performance Status Criteria

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

APPENDIX B CLINIC DIARY/CALENDAR

Patient ID: _____ Initials: _____/_____ Semester ____ (specify 1st or 2nd)

This calendar will be provided to you every six months in order to record the drug daily intake. Please do not forget to take 1 tablet a day, every day, after food.
Should you forget to take the drug, do not take any extra dose during the following day. Should you experience any symptom during the study, please write it down in the space provided and should you need any medical assistance, do not hesitate to contact the Division of Cancer Prevention and Genetics (02-94372657) during working hours. Please do not trash any empty bottle and do not forget to bring with you all the drug boxes (empty o full) together with the present calendar at the next clinic visit.

Month: _____

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Month:_____

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Month:_____

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Please fill also the back page

Month:______________________________

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Month:______________________________

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Month:______________________________

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Month:______________________________

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Participant signature: ____ Date ____/____/_____

APPENDIX C

Diagnostic algorithm for subsequent years CT detected nodules (WP4-2-2)

New detected nodules will be treated according to WP4-2-1 algorithm (not applicable to the MDA2013-01-01 trial)

Solid or partially solid nodules in progression will be managed according to VDT:

Nodules 5-8 mm and VDT >600 days CT after 1 year

Nodules 5-8 mm and VDT 400-600 days CT after 6 months

Nodules 5-8 mm and VDT < 400days CT after 3 months

Nodules >8 mm and VDT >600 days CT after 6 months

Nodules ≥8 mm and VDT < 600 days CT after 3 months

Non solid nodules in progression either in dimension (longest diameter) or density

Nodules <8 mm: CT after 6 months
Nodules >8 mm: CT/PET +biopsy

APPENDIX D

Definition of smoking status

As per Italian guidelines for epidemiological surveys, the definition of smoking status is below (http://www.epicentro.iss.it/passi/rapporto2011/IndicatoriFumo.asp). The definition below will be used by all sites participating in the protocol:

Non smoker: a person declaring having smoked less than 100 cigarettes in his/her life (5 packs of 20 cig/each) and not being smoker at the time of survey

Smoker (as per WHO definition): a person declaring having smoked more than 100 cigarettes in his/her life (5 packs of 20 cig/each), currently smoker or having interrupted smoking in the last 6 months

Occasional smoker: A smoker declaring of not smoking every day

Daily smoker: A person declaring smoking at least 1 cigarette/day

Former smoker: A person declaring having smoked more than 100 cigarettes in his/her life (5 packs of 20 cig/each), currently non smoker and having interrupted smoking more than 6 months ago