COVER PAGE

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Clinical Study of Avmacol® for Detoxification of Tobacco Carcinogens in Heavy Smokers

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SCHEMA

Clinical Study of Avmacol® for Detoxification of Tobacco Carcinogens in Heavy Smokers

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

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Visit 1: Screening
Informed consent, clinical evaluation (medical history, vital signs, brief physical exam), clinical labs, conmeds and supplement use, Karnofsky performance status, baseline tobacco/alcohol assessment, pregnancy test (if applicable)

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Randomization
High-Low Arm or Low-High Arm

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Visit 2: Baseline Specimen Collection for Intervention Period 1
Collection of buccal cells, nasal brushing and blood, AEs, conmeds, current tobacco use, pregnancy test (if applicable), ensure overnight urine collection, dispense study agent

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Intervention Period 1 (10-14 days)
High-Low Arm: 8 tablets Avmacol® per day or Low-High Arm: 4 tablets of Avmacol® per day

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Visit 3: Interim Visit (Intervention Period 1, (may occur on Day 4 to Day 8)
Collection of buccal cells, AEs, conmeds, current tobacco use

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Visit 4: End-of-Intervention Period 1Visit
Collection of buccal cells, nasal brushing and blood, AEs, conmeds, current tobacco use, ensure overnight urine collection, study agent return, compliance

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Washout Period (10-14 days)

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Visit 5: Baseline Specimen Collection for Intervention Period 2
Collection of buccal cells, nasal brushing and blood, AEs, conmeds, current tobacco use, pregnancy test (if applicable), ensure overnight urine collection, dispense study agent

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Intervention Period 2 (10-14 days)
High-Low Arm: 4 tablets Avmacol® per day or Low-High Arm: 8 tablets of Avmacol® per day

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Visit 6: Interim Visit (Intervention Period 2, (may occur on Day 4 to Day 8))
Collection of buccal cells, AEs, conmeds, current tobacco use

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Visit 7: End-of-Intervention Period 2 Visit
Collection of buccal cells, nasal brushing and blood, AEs, conmeds, follow-up tobacco/alcohol assessment, ensure overnight urine collection, study agent return, compliance, clinical labs

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Endpoints
Urinary tobacco carcinogens
Buccal cell NRF target gene transcripts and nuclear morphometry
GSTM1 and GSTT1 genotyping

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1. OBJECTIVES

1.1 Primary Objective

To determine whether Avmacol® increases the urinary excretion of the mercapturic acid of the tobacco carcinogen, benzene, in healthy volunteers who are current heavy smokers.

1.2 Secondary Objectives

- To determine whether Avmacol® increases the urinary excretion of the mercapturic acids of other tobacco carcinogens, including acrolein and crotonaldehyde;
- To determine whether Avmacol® increases the urinary excretion of the mercapturic acids of tobacco carcinogens, normalized by bio-measurement of tobacco exposure;
- To determine whether Avmacol® upregulates the NRF2 target gene transcripts in the buccal cells of current smokers;
- To evaluate for a dose-response relationship between Avmacol® and the detoxification of tobacco carcinogens and the expression of NRF2 target gene transcripts.
- To determine the relationship between systemic study agent exposure and biomarker modulation.

1.3 Exploratory Objectives

- To determine whether the *GSTM1* and *GSTT1* genotypes are important genetic modulators of detoxification of tobacco carcinogens with Avmacol® treatment;
- To bank specimens for future research including evaluation of tobacco gene signatures in buccal and nasal epithelium and buccal cell nuclear morphometry.

2. BACKGROUND

2.1 Tobacco-related Head and Neck Cancer

Long-term success in the treatment of tobacco-related head and neck squamous cell carcinoma (HNSCC) is hindered by an alarming rate of second primary tumor (SPT) formation following curative treatment. Patients with human papillomavirus (HPV)-negative HNSCC develop a SPT of the upper aerodigestive tract at the rate of 3-6% per year, and are most likely to succumb to these secondary cancers (1-4). Although smoking cessation reduces the occurrence of SPTs, moderation of risk is not observed for 5 years, and is insufficient to return risk to baseline (3, 5). Moreover, although 70% of smokers attempt to quit annually, tobacco cessation is unsuccessful in the majority due to the power of nicotine addiction, and recidivism occurs in approximately 90% (6). Thus, most tobacco users are unable or unwilling to quit, and those who do succeed still face chronic exposure to the endogenous carcinogens of metabolism or the exogenous carcinogens of industrialization.

The concept of “condemned mucosa”, or epithelial field cancerization from chronic exposure to environmental carcinogens, first raised the possibility of chemoprevention for HNSCC in 1953 (7); however, no nontoxic, effective chemopreventive agent has been identified. When epidemiologic studies found that reduced risk for both HNSCC and SPTs is associated with diets rich in fruits and vegetables (8-12), the first chemoprevention strategies focused on pharmacologic doses of derivative micronutrients. Preclinical models supported retinoids, synthetic vitamin A analogues, as modulators of oral environmental carcinogenesis (13, 14). Although high dose isotretinoin reversed oral premalignant lesions (OPLs) (15) and prevented SPTs in a landmark Phase III HNSCC chemoprevention study (16), substantial toxicity precluded chronic administration and SPT incidence reverted to baseline within 3 years. A subsequent trial of low dose isotretinoin was tolerable but ineffective (17). Molecular targeting of EGFR or COX-2 has shown preclinical chemopreventive efficacy against oral cancer (18-22), but clinical application has been hampered by poor efficacy and tolerability in humans (23-27). Thus, a tremendous unmet need remains for an effective and well-tolerated chemopreventive agent against tobacco-related HNSCC and SPTs.

2.2 Avmacol®

Epidemiologic studies report that reduced risk for both HNSCC and SPTs is associated with diets rich in the Brassica family of cruciferous vegetables (8-12). Broccoli extract potently induces cytoprotective enzymes (28), which mitigate the effects of environmental carcinogens including benzene, aldehydes and polycyclic aromatic hydrocarbons found in tobacco smoke by enhancing their detoxication (29, 30). More than 80% of inducer activity is driven by the phytochemical sulforaphane (SF), produced when its precursor glucoraphanin (GR) is hydrolyzed by myrosinase during food preparation or chewing (31). Mechanistically, SF disrupts the polyubiquitination of the NRF2 transcription factor mediated by its inhibitory protein, KEAP1. This frees NRF2 to translocate to the nucleus and bind to anti-oxidant response elements (AREs) in the promoter regions of target genes (32, 33). As GR is 20-50 times more concentrated in broccoli seeds relative to mature plants (31, 34), we are developing broccoli seed preparations (BSPs) as a chemopreventive agent against carcinogen-induced cancers, and have determined the safety and pharmacokinetics (PK) of various BSPs in humans (33, 35-39).

Two types of highly standardized, lyophilized boiling water extracts of 3-day-old broccoli sprouts have been developed by our collaborators (J. Fahey, P. Talalay) at the Johns Hopkins Cullman Chemoprotection Institute under GMP conditions, and used extensively under clinical protocols – including our own pilot study evaluating the oral bioactivity of broccoli sprout extracts (BSE) in the oral mucosa of healthy volunteers (NCT02023931) (39). However, there are three main problems in achieving consistent delivery and bioavailability of active SF by means of lyophilized BSE: (1) SF is only moderately storage- and heat-stable over time, especially in aqueous solution. (2) SF-rich BSE powders are extremely hygroscopic and the preparation of capsules containing accurate doses by weight is challenging and very expensive. (3) Administration of GR as an oral precursor of SF results in highly variable conversions of GR to SF metabolites (dithiocarbamates) among individuals, ranging from 1 – 40% (35, 38, 40-42). Moreover, the curated GMP manufacturing process for BSEs is laborious and expensive, and consequently BSE is no longer available for clinical investigation.

Avmacol® is a commercially available dietary supplement that contains GR plus the fully active enzyme myrosinase, thus yielding a higher and more consistent dose of SF upon ingestion. Avmacol® tablets contain only GR-rich broccoli seed extract, freeze-dried broccoli sprouts for the myrosinase source, and the inert excipients required to form a tablet. Avmacol® is manufactured by Nutramax Laboratories, Inc., has been sold as a nutritional supplement in the United States since 2013, and is manufactured under GMP standards. The coated tablets contain 12.5 mg of GR, and have a minimum shelf life of 30 months at ambient temperature. We have completed a healthy volunteer study confirming the bioavailability of SF when delivered as Avmacol® (NCT02800265), and this dietary supplement is now under investigation as a chemoprevention agent in multiple disease contexts, including the detoxication of air pollutants in Qidong, China (Kensler).

2.3 Rationale

Proof-of-concept clinical trials in healthy volunteers have shown that BSPs rich in GR and/or SF are well-tolerated and promote rapid, sustained detoxication of the airborne pollutants acrolein and benzene (33, 35-38). In preclinical studies, SF has exhibited chemopreventive activity against carcinogen-induced stomach, skin and breast cancers (43-47). Studies in *Nrf2*$^{-/-}$ mice have shown that the chemopreventive effect of sulforaphane against benzo[a]pyrene-induced gastric cancer and 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced skin cancer depends on the NRF2 signaling pathway (43, 44, 47). The relevance of NRF2 to oral cancer chemoprevention is highlighted by the enhanced susceptibility of *Nrf2*$^{-/-}$ mice to oral cancer induced by the carcinogen 4-nitroquinoline-1-oxide (4NQO), and the reduced susceptibility of *Keap1*$^{-/-}$ mice (48). We have shown that SF induces NRF2 and NRF2 target gene expression in normal oral keratinocytes and in HNSCC cell lines *in vitro*. In a preclinical chemoprevention study, we have determined that SF reduces both the incidence and total burden of oral tumors in 4NQO-treated mice. Finally, we have observed mucosal bioactivity of two well-tolerated BSPs, sulforaphane-rich BSE and Avmacol® ((39); unpublished data NCT02800265), which both upregulated transcripts for the NRF2 target gene *NQO1* in the buccal mucosa of healthy volunteers.

2-Phenethyl isothiocyanate (PEITC), another glucosinolate present in certain cruciferous vegetables including watercress and broccoli sprouts, has been demonstrated to have multiple chemoprotective effects on xenobiotic metabolizing enzymes and molecular pathways related to cancer (1, 2). In a randomized, crossover study, PEITC promoted detoxication of benzene and acrolein in a healthy smoking population (49). A much stronger effect was observed among subjects with the null genotype of both *GSTM1* and *GSTT1*. Due to established mechanisms of action, SF-rich BSPs are likely to be more effective inducers of NRF2 target genes and consequent detoxication of tobacco carcinogens in this population. Moreover, in a 300-patient study in Qidong, China, the upregulation of the urinary excretion of the mercapturic acid of benzene by broccoli sprout extracts was independent of *GSTM1* and *GSTT1* genotypes, suggesting broader applicability of this green chemoprevention strategy across a general population (38).

In the proposed trial, we aim to evaluate the pharmacodynamic activity of Avmacol® in healthy current heavy smokers. The primary objective is to determine whether Avmacol® increases the urinary excretion of the mercapturic acid of the tobacco carcinogen, benzene in healthy volunteers who currently smoke. Secondary objectives include: 1) to determine whether Avmacol® increases the urinary excretion of the mercapturic acids the tobacco carcinogens, acrolein, and crotonaldehyde; 2) To evaluate for a dose-response relationship between Avmacol® and the detoxification of tobacco carcinogens and the expression of NRF2 target gene transcripts; 3) to determine whether NRF2 target genes are upregulated in the mucosa of current smokers. Exploratory objectives include: 1) to determine whether the *GSTM1* and *GSTT1* genotypes are important genetic modulators of detoxication of tobacco carcinogens during Avmacol® treatment; 2) to bank biospecimens for future study, including the evaluation of tobacco gene signatures in buccal and nasal epithelium, as well as buccal cell nuclear morphometry.

Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is including assessment of tobacco and alcohol use at baseline and at the End of Intervention visit, to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

3. SUMMARY OF STUDY PLAN

This is an open-label, randomized, cross-over study, evaluating whether two different doses of Avmacol® (4 tablets per day for 10-14 days; 8 tablets per day for 10-14 days) increase the detoxification of tobacco carcinogens in otherwise healthy, current heavy smokers. Participants will serve as their own controls. We chose the open-label design because the study endpoints are not likely to be affected by the knowledge of the dosage assignment. We believe that the proposed randomized cross-over design would minimize the potential bias from the open-label design. We plan to accrue 61

eligible participants to initiate agent intervention. With an anticipated attrition rate of 33% or less (with respect to completing the intervention and having complete endpoint data), we expect to have at least 41 participants with evaluable endpoint data. Projected accrual is 2 participants per month.

Participants will undergo a screening evaluation (Visit 1) in which the informed consent form will be signed. Participants will be assessed for study eligibility. Detailed inclusion and exclusion criteria are listed in sections 4.1 and 4.2. All participants will undergo evaluation to obtain height, weight, blood pressure, pulse, temperature measurements, a brief physical exam consisting of an examination of the heart, lungs and abdomen, and Karnofsky performance status assessment. They will also be evaluated for concurrent medication and supplement use, baseline tobacco/alcohol assessment, medical history, and laboratory analysis with complete blood count with differential (CBC-diff), comprehensive metabolic panel (CMP). Women of childbearing capacity will have a urine pregnancy test. Study participants will be provided with a daily diary for recording any adverse events (AEs) and medication usage and a urine collection kit (an overnight urine collection container, a collection hat, and instructions for collecting overnight urine) to be used the night prior to Visit 2.

Once determined eligible, participants will be randomized (1:1) to one of the following arms: High-Low Arm (8 tablets Avmacol® per day during Intervention Period 1 and 4 tablets Avmacol® per day during Intervention Period 2) and Low-High Arm (4 tablets Avmacol® per day during Intervention period 1 and 8 tablets Avmacol® per day during Intervention Period 2).

Participants will return to the clinic for Baseline Specimen Collection for Intervention Period 1 (Visit 2). Participants will be contacted prior to the visit to remind them to collect overnight urine the night prior to Visit 2. Participants will bring the overnight urine to the clinic and undergo baseline specimen collection for the collection of buccal cells (for NRF target gene transcripts), nasal brushing (banked for gene expression analysis) and blood (for genotyping and banking). Participants will be assessed for baseline symptoms, concomitant medications, and be queried on current tobacco use. Urine pregnancy test will be repeated, if applicable. Those who miss the overnight urine collection prior to Visit 2 will be asked to collect overnight urine the night of the visit and return the urine to the clinic the following day. Participants will be provided with Avmacol® pills for Intervention Period 1 after confirmation of overnight urine collection. Participants will be provided with an Intake Calendar for recording agent intake.

During Intervention Period 1 (10-14 days), participants in the High-Low Arm will take 8 tablets of Avmacol® each day and participants in the Low-High Arm will take 4 tablets of Avmacol each day. Participants will be instructed to take the Avmacol® pills with the evening meal. Day 1 of the Intervention Period 1 begins the day the first dose is taken. During Intervention Period 1, participants will return to the clinic on Day 4, 5, 6, 7, or 8 for an Interim Visit (Visit 3). Participants will undergo the collection of buccal cells and be assessed for AEs, concomitant medications and be queried on current tobacco use. Participants will be provided with a new urine collection kit to be used the night prior to Visit 4.

Participants will return to the clinic at the end of Intervention Period 1 (Visit 4). Participants will be contacted prior to this visit to remind them to collect overnight urine the night prior to Visit 4. Participants will bring the overnight urine to clinic, return unused pills, undergo specimen collection for the collection of buccal cells, nasal brushing and blood. Participants will also be assessed for AEs, concomitant medications, compliance, and be queried on current tobacco use. Those who miss the overnight urine collection prior to Visit 4 will be provided with additional Avmacol® pills and be asked to take the pills the evening of Visit 4 and collect overnight urine the night of the visit and return the urine to the clinic the following day. Participants will be provided with a new urine collection kit to be used the night prior to Visit 5.

Participants will undergo a Washout Period (10-14 days) after completing Intervention Period 1. After the Washout Period, participants will return to the clinic (Visit 5) to undergo baseline specimen collection for Intervention Period 2. Participants will be contacted prior to the visit to remind them to collect overnight urine the night prior to Visit 5. Participants will bring the overnight urine to the clinic and undergo baseline specimen collection for the collection of buccal cells, nasal brushing and blood. Participants will also be assessed for AEs, concomitant medications, and be queried on current tobacco use. Urine pregnancy test will be repeated, if applicable. Those who miss the overnight urine

collection prior to Visit 5 will be asked to collect overnight urine the night of the visit and return the urine to the clinic the following day. Participants will receive Avmacol® pills for Intervention Period 2 after the overnight urine collection is confirmed.

During Intervention Period 2 (10-14 days), participants in the High-Low Arm will take 4 tablets of Avmacol® each day and participants in the Low-High Arm will take 8 tablets of Avmacol® each day. Participants will be instructed to take the Avmacol® pills with the evening meal. Participants will be provided with an Intake Calendar for recording agent intake. Day 1 of the Intervention Period 2 begins the day the first dose is taken. During Intervention Period 2, participants will return to the clinic on Day 4, 5, 6, 7, or 8 for an Interim Visit (Visit 6). Participants will undergo the collection of buccal cells and be assessed for AEs, concomitant medications and be queried on current tobacco use. Participants will be provided with a new urine collection kit to be used the night before Visit 7.

Participants will return to the clinic at the end of Intervention Period 2 (Visit 7). Participants will be contacted prior to the visit to remind them to collect overnight urine the night prior to Visit 7. Participants will bring the overnight urine to clinic, return unused pills, undergo specimen collection for the collection of buccal cells, nasal brushing and blood. Participants will also be assessed for AEs, concomitant medications, compliance, and follow-up tobacco/alcohol assessment. Additional blood samples will be collected for safety labs (CBC-diff and CMP). Those who miss the overnight urine collection prior to Visit 7 will be provided with additional Avmacol® pills and be asked to take the pills the evening of Visit 7 and collect overnight urine the night of the visit and return the urine to the clinic the following day.

After completion of Intervention Period 2, participants will be provided with information on how to contact the Arizona Smokers' Helpline (toll free telephone number and URL to access the ASHLine website), which provides comprehensive services for tobacco cessation.

Participants will be contacted by telephone (or email) 10-14 days after completing Intervention Period 2 for a final AE assessment and be reminded to return the AE Diary to the study office.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

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

4.1.2 Age ≥18 years.

4.1.3 Karnofsky performance scale ≥ 70% (see Appendix A).

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

Leukocytes	≥3,000/microliter
Absolute neutrophil count	≥1,500/microliter
Platelets	≥100,000/microliter
Total bilirubin	≤ 2 × institutional upper limit of normal (ULN)
AST (SGOT)/ALT (SGPT)	≤ 1.5 × ULN
Creatinine	≤ ULN

4.1.5 Fertile subjects must use adequate contraception (abstinence, barrier methods, or birth control pills) prior to study entry and for the duration of study participation. The effects of Avmacol® on the developing human fetus are unknown. For this reason women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study

participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

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

4.2 Exclusion Criteria

4.2.1 History of invasive cancer within the past 2 years, with the exception of excised and cured non-melanoma skin cancer or carcinoma in situ of the cervix.

4.2.2 Chronic, current or recent (within the past 2 weeks) use of systemic steroid doses equivalent to prednisone > 5 mg daily for continued use > 14 days. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.

4.2.3 Participants may not be receiving any other investigational agents.

4.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Avmacol®.

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 or lactating women.

4.3 Inclusion of Women and Minorities

Both men and women (as applicable) and members of all races and ethnic groups are eligible for this trial.

4.4 Recruitment and Retention Plan

Study participants will be recruited from the greater Tucson and Phoenix areas by word-of-mouth; advertisement in local newspapers; posting/distributing study information around campus, through accessible listservs, and in community gathering places; engaging collaborating clinics; and through use of the local Craigslist and social media. Study personnel will promote retention/adherence with regular contact with study subjects. The study team is committed to provide a friendly and comfortable study setting for participants from initial contact through the completion of their activities. Wherever possible, flexibility will be built into the study schedule to promote compliance. Recruitment and retention efforts will be evaluated routinely by the study personnel and modified as necessary to promote rapid accrual and to assure high retention of participants.

5. AGENT ADMINISTRATION

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

5.1 Dose Regimen and Dose Groups

- The study agent is Avmacol®
- Participants will be randomly assigned to one of two treatment arms:
    - High-Low Arm: 8 tablets Avmacol® per day during Intervention Period 1 and 4 tablets Avmacol® per day during Intervention Period 2
    - Low-High Arm: 4 tablets Avmacol® per day during Intervention Period 1 and 8 tablets Avmacol® per day during Intervention Period 2
- Each Intervention Period is 10-14 days.

5.2 Avmacol® Administration

- Participants will receive a supply of Avmacol® for each Intervention Period upon confirmation of overnight baseline urine collection.
- Participants will be instructed to take the assigned Avamacol pills (4 or 8) with the evening meal.

5.3 Run-in Procedures

Not applicable.

5.4 Contraindications

Use of systemic steroid doses equivalent to prednisone > 5 mg daily is to be avoided during study participation.

5.5 Concomitant Medications

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

If a participant misses an Avmacol® dose, the dose is considered skipped and should not be replaced. If a participant vomits following an Avmacol® dose, the dose should not be retaken.

If a participant experiences a Grade 1 AE, no dose modification will be made unless the AE is unacceptable to the physician or participant. Doses may be held for unacceptable grade 1 AEs for up to 3 days. If the AE has not resolved within the 3 days to a level acceptable by the participant or investigator, Avmacol® will be permanently withdrawn.

For Grade 2 or Grade 3 AEs not related or unlikely to be related to study drug, the dose may be held for up to 3 days at the discretion of the investigator. If the AE has not resolved within the 3 days to a level acceptable by the participant or investigator, Avmacol® will be permanently withdrawn.

If a participant experiences a Grade 2 AE possibly/probably/definitely attributable to Avmacol®, Avmacol® may be held for up to 3 days until the AE is resolved to Grade 1 or less. If the AE is not resolved to Grade 1 or less within 3 days, Avmacol® will be permanently withdrawn and the participant followed for resolution of AEs. When possible and appropriate, participants withdrawn early will be encouraged to return for sample collection and clinical labs for safety.

Grade 3 events possibly/probably/definitely attributable to Avmacol®, and Grade 4 events regardless of relationship to study agent, will result in permanent withdrawal of study agent. Participants will be followed for resolution of AEs. When possible and appropriate, participants withdrawn early will be encouraged to return for sample collection and clinical labs for safety.

5.7 Adherence/Compliance

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

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

6. PHARMACEUTICAL INFORMATION

6.1 Avmacol® (IND information pending)

Avmacol® is a commercially available dietary supplement that contains glucoraphanin (GR) plus the fully active enzyme myrosinase, thus yielding a higher and more consistent dose of sulforaphane (SF) upon ingestion. Avmacol® tablets contain only GR-rich broccoli seed extract, freeze-dried broccoli sprouts for the myrosinase source, and the inert excipients required to form a tablet. Avmacol® is manufactured by Nutramax Laboratories, Inc., has been sold as a nutritional supplement in the United States since 2013, and is manufactured under GMP standards. The coated tablets contain 12.5 mg of GR, and have a minimum shelf life of 30 months at ambient temperature.

6.2 Reported Adverse Events and Potential Risks

Broccoli seed preparations (BSP) have been studied extensively in more than 500 healthy volunteers in multiple Phase I and Phase II clinical trials without Grade 2 or higher adverse effects. Broccoli seeds contain high concentrations of glucoraphanin (GR), the glucosinolate precursor of sulforaphane (SF), which is converted to SF by the release of intrinsic seed myrosinase during chewing or by thioglucosidaes in the microbiota in the human gut (40, 50, 51). The isothiocyanate SF (1-isothiocyanato-4R-(methylsulfinyl)butane) was originally isolated from commercial broccoli, and is one of the most potent naturally-occurring inducers of cytoprotective enzymes identified to date (52). SF activates the KEAP1-NRF2 signaling pathway, thereby increasing transcription of antioxidative and detoxication genes. For example, SF has been shown to induce cytoprotective enzymes in a variety of animal and human cell lines and tissues, including rat and human mammary and prostate cell lines (45).

In our initial clinical trials, we investigated BSP in the form of re-hydrated, previously lyophilized broccoli sprout powders rich in either GR or SF produced by the Cullman Chemoprotection Center at Johns Hopkins under the supervision of Drs. Jed Fahey and Paul Talalay. Broccoli sprouts (Brassica oleracea) were grown from specially selected BroccoSprouts™ seeds to provide a consistent yield of glucoraphanin or sulforaphane. In our initial trial, the GR-rich powder was considered as a food by the Johns Hopkins IRB. It is simply the freeze-dried powder derived from a hot water extract of broccoli sprouts. In 2010, the US FDA made a determination that beverages containing SF-rich broccoli sprout powders (but not GR-rich broccoli sprout powders) must be considered as drugs because the SF is formed ex vivo from the plant by the addition of myrosinase-containing daikon seeds during the preparation of the SF-rich powder. Thus, subsequent trials in Qidong, China as well as our pilot study at the University of Pittsburgh (NCT 02023931, "Broccoli Sprout Extracts in Healthy Volunteers: A Pilot Study of NRF2 Pathway Activation in Oral Mucosa") have been conducted with an IND from the FDA (#112038 "Broccoli Sprout Extract for Protection Against Environmental Toxins") held by Thomas Kensler, PhD.

There are three main problems in achieving consistent delivery and bioavailability of active SF by means of lyophilized broccoli sprout extracts: (1) SF is only moderately storage- and heat-stable over time, especially in aqueous solution. (2) SF-rich broccoli sprout extract powders are extremely hygroscopic and the preparation of capsules containing accurate doses by weight is challenging and very expensive. (3) Administration of GR as an oral precursor of SF results in highly variable conversions of GR to SF metabolites (dithiocarbamates) among individuals, ranging from 1 – 40%, but fairly consistent between individuals. Oral delivery of SF-rich broccoli sprout extract, on the other hand, results in consistent conversion of 60-80% of it to urinary dithiocarbamate metabolites.

In this clinical study, BSP will be provided in the form of the commercially available dietary supplement, Avmacol®. Although a number of dietary supplements containing GR have been available in the U.S. market for several years now, Avmacol® is the first commercial product to contain GR plus the fully active enzyme myrosinase, thus yielding a higher and much more consistent SF dose upon ingestion. Avmacol® tablets contain only GR-rich broccoli seed extract, freeze-dried broccoli sprouts for the myrosinase source, and the inert excipients required to form a tablet. They are manufactured

by Nutramax Laboratories, Inc. Avmacol® has been sold as a nutritional supplement in the United States since 2013, and is manufactured under GMP standards. The coated tablets contain 12.5 mg of glucoraphanin, which has a shelf life of at least 30 months at ambient temperature (ongoing stability testing). Based on previous human studies with both dietary and topical broccoli sprout extracts, and related broccoli seed preparations, and the absence of severe or serious risks (no Grade II toxicities have been observed in > 500 patients), we do not anticipate serious AEs to be associated with the administration of Avmacol® to study participants in this proposed trial. Consistent with that expectation, in 2016 Bauman completed a healthy volunteer study (N=10) where Avmacol® was dosed at 8 tablets daily for 3 days. No grade 2 or higher AEs were observed (Bauman, unpublished data).

6.2.1 Non-clinical Toxicology

A number of animal studies have investigated the biological effects and toxicology of oral SF-containing broccoli seeds and SF itself. While these investigations have examined a wide variety of endpoints and effects, they have demonstrated the overall in vivo safety and beneficial biological effects of broccoli seeds and SF. Animal studies are summarized below.

Myzak et al. treated C57BL mice with 6 μmol SF/Day for 10 weeks with no adverse effect on body weight, hematocrit, or spleen weight. Taking into account species scaling factors, human consumption of 106 g of broccoli seeds daily would achieve daily SF intake similar to this murine study (53).

Jones et al. treated male F344 rats with SF 50 mg/kg/Day (280 μmol /kg/Day) by gavage for five Days. The dose was selected based on reports of non-toxicity and efficacy in inducing cytoprotective enzymes in other model systems. During the study, 2 of 10 animals in the SF treatment group died after feeding due to aspiration of the dose. On the sixth Day, after five Days of SF feeding, rats were sacrificed and organs examined. The SF was well tolerated by the animals and there was no apparent toxicity over the duration of the study. The SF feeding did not affect the relative weights of the prostate, kidneys, liver or bladder. Body weight was not affected compared to control animals, though there was an 8% decrease in body weight in the SF treated group compared to initial weight. The SF dose used in this study is >10-times greater than that achieved by the proposed BSP dosing for our protocol (54).

Zhang et al. treated female Sprague-Dawley rats with BSP to study the effect on tissue GST and NQO1 and the urinary excretion levels of isothiocyanate (ITC) metabolites. These experiments included administration of BSP providing 40, 80, and 160 μmol isothiocyanate/kg body weight daily for 14 Days. None of the extract doses were associated with any sign of toxicity, with all rats in good health and body weight gain not significantly different among treatment and control groups. No gross abnormalities were detected at necropsy. No pathological changes were visible in rat bladder tissues when examined microscopically. BSP in the doses administered effectively induced GST and NQO1 enzyme expression in rat bladder, duodenum, and stomach in a dose-dependent fashion. Significant enzyme expression was induced in rat colon, kidney, and lungs at the higher dose of broccoli seed extract. Measurement of urinary ITC and metabolites by cyclocondensation assay showed extensive (70-78%) elimination of the ITC doses within 24 hours. ITCs are known to be metabolized in vivo mainly through the mercapturic acid pathway and to be excreted in the urine as NAC conjugates (55).

6.2.2 Mutagenicity

In a study in rats by Yoxall et al. conversion of 2-amino-3-methylimidazolo-[4,5-f] quinoline to mutagenic intermediates (Ames test) was reduced by treatment with SF at both 3 and 12 mg/kg/Day (17 and 68 μmol /kg/Day) doses (56).

6.2.3 Clinical Safety and Efficacy

Cruciferous vegetables, including broccoli sprouts, are generally regarded as safe and are regular dietary components in many regions of the world. Previous estimates of the daily dietary intake of cruciferous vegetables vary regionally, averaging 40 g/day in Singapore (57), 11 g/day in the United States (58), 16 g/day in Canada (59), 30 g/day in the UK (60), and 112 g/day in Japan (61).

Shapiro et al. conducted a double-blind, placebo controlled, randomized, Phase I study of two BSPs, beverages containing SF-rich or GR-rich powder made from sprouted broccoli seeds, in healthy individuals to determine the safety and tolerance of repeated oral administration. Twelve healthy human volunteers received doses of BSP every 8 hours for 7 Days (total 21 doses) while undergoing clinical evaluation and a battery of laboratory tests. Doses used in 3 cohorts of 3 subjects and 1 control were 25 μmol glucosinolates, 100 μmol glucosinolates, and 25 μmol isothiocyanates, respectively. Thus subjects received 75 – 300 μmol glucosinolates daily, equivalent to 12 - 50 g of fresh broccoli seeds, or 75 μmol isothiocyanate. No clinical AEs were reported. With regard to laboratory testing, samples were obtained 6 times during the 19 Day study and included evaluation of the blood for the following: CBC with differential, reticulocyte count, PT, PTT, BUN, creatinine, Na, K, CO2, Cl, glucose, albumin, direct/total bilirubin, alkaline phosphatase, AST, ALT, GGT, T3, T4, TSH. Urinalysis was performed for urine creatinine, and urine dithiocarbamates (DTC). Two of 12 individuals (both receiving active preparations) showed an increase in plasma ALT exceeding the upper limit of normal with one meeting criteria for a Grade 1 toxicity. Notably, ALT levels rose for all subjects during the course of the study including placebo-treated subjects. Plasma AST levels rose above normal on Day 19 for 2 of 12 subjects. They were released from the inpatient portion of the study on Day 17 and their post-discharge activities (i.e., possible alcohol intake) could not be ascertained. Monitoring of TSH levels demonstrated that in 3 of 12 subjects (2 active treatment, 1 placebo), TSH levels exceeded the upper limit of normal during or after the dosing period. Notably, TSH levels rose for 11 of 12 subjects during the first 6 Days of hospitalization before broccoli seed administration was begun. TSH increases were not associated with any clinical symptoms or abnormalities of T3 or T4. Evaluation by 2 independent endocrinologists determined that the changes in TSH were mild and reversible and did not pose an obstacle to further studies with administration of BSPs. No other significant laboratory abnormalities occurred. Thus, this Phase I safety study in healthy volunteers revealed no evidence of systematic, clinically significant adverse effects that could be attributed to the administration of repeated doses of broccoli sprout extracts containing SF or GR (41).

Cornblatt et al. conducted a study to assess the bioavailability of SF-rich broccoli sprout extract in human breast tissue. In this proof-of-principle study, 8 women undergoing elective mammoplasty consumed a preparation containing 200 μmol of SF approximately 50 minutes prior to surgery. The extract was well-tolerated without any AEs or complications. SF metabolite levels measured by cyclocondensation reaction as dithiocarbamates were used to determine SF distribution. Mean post-dose plasma dithiocarbamate (DTC) level was 0.92 ± 0.72 μM, and mean epithelial/stromal enriched breast tissue DTC concentration was 1.45 ± 1.12 and 2.00 ± 1.95 picomol/mg tissue for right and left breast respectively. In addition, the investigators were able to measure NQO1 and HO-1 transcripts in the human breast tissue, demonstrating the feasibility of assessing a pharmacodynamic action of SF in these tissues (45).

In an ongoing randomized clinical trial at Johns Hopkins, 21 women completed a 10 day intervention of broccoli sprout extract or placebo (mango juice). There were only three grade 1 mild gastrointestinal AEs reported and no significant changes (1.5 times or greater) in bloods (comprehensive metabolic panel, full blood count, coagulation panel and thyroid tests), taken pre and post intervention (personal communication K. Visvanathan).

In the cross over trial conducted by Egner et al (36), 2 of the fifty participants randomized to receive the SR-rich beverage (150 μmol/day) during either the first or second wave complained of nausea or bitter taste and dropped out of the study. Serum chemistry studies conducted on samples obtained after the last of seven daily doses of SF did not present any abnormal values. This study suggests that 150 μmol SF/day, approximates the maximum tolerated dose.

A number of small pilot clinical studies have evaluated the effect of broccoli sprouts on antioxidant endpoints:

Murashima and colleagues reported in 2004 on the elevation of multiple biomarkers of oxidative stress following a one-week course (6 young male and 6 young female smokers) of 100 g/d of fresh broccoli sprouts (62). Plasma markers were measured before and after treatment. Treatment produced decreases in serum total cholesterol, LDL cholesterol, coenzyme Q10, plasma cystine and phosphatidylcholine hydroperoxide, and urinary 8-isoprostane, and 8-OHdG. Increases in CoQ10H2/CoQ10 ratio, and HDL cholesterol were observed. Blood lymphocyte markers, natural killer cell

activity, triacylglycerol, urea nitrogen, uric acid, AST, ALT, γ-GPT, and plasma amino acids were also measured and there were no before- to after-treatment differences in any of these biomarkers.

In 2009, a group at UCLA reported on the ability of orally administered broccoli sprout homogenates (BSH) to increase phase 2 antioxidant enzymes in the upper airway (63). After feeding 57 subjects doses of BSH ranging from 25 to 200 g on 3 separate days, followed on the 4th day by blood and nasal lavage collection, there was a dramatic and dose-dependent increase in phase 2 enzyme expression (mRNA for GSTM1, GSTP1, HO-1, and NQO1). Induction of individual phase 2 enzymes in nasal lavage cells was strongly correlated. No serious AEs were reported, and dose intolerance or side effects of broccoli sprouts were not observed, and mild, digestive effects are presented in the report.

Christiansen et al (Denmark) report that ingestion of broccoli sprouts does not improve endothelial function in human beings with hypertension (n = 40 hypertensive, non-diabetic subjects with cholesterol in the normal range) (64). Subjects were fed 10 g of dried broccoli sprouts for 4 weeks, and their blood pressure, endothelial function (measured by flow-mediated dilation), and blood samples were obtained every other week. Glucoraphanin content of the sprouts was measured, and equated to a dose of 259 μmol GR/d – a reasonable level, but in light of our findings perhaps not high enough to expect a measurable effect due to low conversion of GR to SF by the intestinal microflora of individuals.

A recent report from Iran, describes the effect of oral broccoli sprouts on a variety of oxidative stress biomarkers in a double-blind, placebo-controlled, randomized controlled trial, in type 2 diabetes patients. Eighty-one patients were randomly assigned to one of three treatment groups for 4 weeks. They received either 10 g/d, or 5 g/d broccoli sprout powder (BSP), or a placebo of cornstarch and chlorophyll (65). The authors report that consumption of BSP resulted in a significant decrease in malondialdehyde, oxidized LDL cholesterol, and "oxidative stress index", and a significant increase in total serum antioxidant capacity. They found no significant effect on "total antioxidant status" or fasting blood glucose. Since this study employed an over-the-counter broccoli sprout supplement and the authors did not make independent determinations of its SF content, and since the fasting blood glucose values for the three groups were not closely matched, there is substantial concern about the robustness of reported results, although there do appear to be clear trends for difference.

In a recent phase II study of men with recurrent Prostate Cancer at the Knight Cancer Institute in Portland Oregon, treatment with 200 μmol of sulforaphane per day for 20 weeks produced no major side effects and AEs were mostly grade 1 gastrointestinal (66).

Another intervention was conducted October 2011 to January 2012, in which 267 healthy volunteers in Qidong Province, China (a region with extremely high liver cancer prevalence) received 84 consecutive daily doses of a broccoli sprout beverage containing 600 μmol of glucoraphanin and 40 μmol of sulforaphane, or an indistinguishable placebo (38). The study participants comprised 136 treated with broccoli sprout beverage and 131 placebo controls. Of the 267 participants completing the study, 50% consumed all assigned doses, and the remaining participants consumed at least 80 of the 84 doses. Extensive blood chemistries at the termination of the study showed that the means of 13 analytes were identical between treated and control groups, and that specifically no abnormalities were detected in BUN, creatinine, and transaminases (ALT and AST). Three individuals, 2 in the placebo arm and 1 in the treated arm, had slightly elevated total bilirubin (1.1, 1.6, 1.8 mg/dL) and two individuals, 1 each in placebo and treated arms, had slightly elevated direct bilirubin (0.5, 0.6 mg/dL levels).

In summary, no grade 2 or higher AEs have been observed in multiple phase I and II clinical trials evaluating broccoli seed preparations in more than 500 participants. Reported grade I AEs have included: the poor taste of the study agent, most common during ingestion of SF-rich broccoli sprout extract beverages, eructation, and flatulence. During the healthy volunteer study with Avmacol®, the taste of the study agent was not a source of toxicity – likely because the taste is masked by encapsulation.

6.3 Availability

Avmacol® is a commercially available dietary supplement manufactured under GMP conditions by Nutramax, and will be provided to investigators at the University of Arizona without cost. Avmacol® will be shipped in bottles containing 500 tablets, and will be repackaged by the University of Arizona Investigational Drug Pharmacy. A total of 16,000 tablets (32 bottles of #500) from the same lot number will be shipped.

6.4 Agent Distribution

Nutramax will ship Avmacol® at ambient temperature to the Investigational Drug Pharmacy at the address below:

Jennifer Hoiles, PharmD
Investigational Drug Pharmacy
Room 0626 (basement)
Banner - University Medical Center Tucson
1501 N. Campbell Ave.
Tucson, Arizona 85724

The IDP will repackage Avmacol® into individually dispensed bottles of 84 tablets, which will then be delivered to the UACC Chemoprevention Clinic. Bottles will be dispensed from the Chemoprevention Clinic according to the Intervention Treatment Period (two bottles for the high dose treatment period; one bottle for the low dose treatment period).

6.5 Agent Accountability

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

6.6 Packaging and Labeling

Avmacol® bottles will packaged in bottles of 84 tablets and will be labeled by the IDP, The label will contain, but not be limited to, the following information:

- Protocol number
- Patient study ID number (blank, to be filled in at dispensal)
- Dosing information (take 4 tablets every evening; OR take 8 tablets every evening)
- Expiration date (provided as testing due date, as stability testing is ongoing and 36 month data will be provided during the course of the study)
- Number of tablets per container

6.7 Storage

Study agents will be stored in a secure investigational agent storage room in the UACC Chemoprevention Clinic at temperatures between 15°C and 30°C (59°F and 86°F).

6.8 Registration/Randomization

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

6.9 Blinding and Unblinding Methods

Not applicable.

6.10 Agent Destruction/Disposal

All unused study agents will be disposed according to the institutional standards. Per the University of Arizona Cancer Center pharmacy's investigational drug destruction policy, returned study agents and de-identified returned drug containers are disposed of in a chemo-safety biohazard waste container.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Procedure/ Evaluation	Pre-Intervention		Study Intervention[1]							Follow-Up[10]
			Intervention Period[2] 1 (10-14 days)			Washout Period[3] (10-14 days)	Intervention Period 2 (10-14 days)			
	Visit 1 Screening	Randomization	Visit 2 Baseline	Visit 3 Intervention Period Day 4,5,6,7 or 8	Visit 4 End of Intervention		Visit 5 Baseline	Visit 6 Intervention Period Day 4,5,6,7 or 8	Visit 7 End of Intervention	
Informed consent	X									
Eligibility assessment	X									
Medical history	X									
Concomitant medications	X	X	X	X	X	X	X	X	X	
Blood for CBC-diff, CMP[4]	X								X	
Vitals (height, weight, temp, BP, heart rate)[5]	X		X		X		X		X	
Brief physical exam	X									
Karnofsky performance status	X									
Tobacco Baseline Assessment Questionnaire	X									
Tobacco Follow up Assessment Questionnaire									X	
Alcohol Baseline Assessment Questionnaire	X									
Alcohol Follow up Assessment Questionnaire									X	
Query current tobacco use[11]	X		X	X	X		X	X	X	
Urine pregnancy test[6]	X		X				X			
Overnight urine[7]			X		X		X		X	
Buccal cell collection			X	X	X		X	X	X	
Nasal brushing collection			X		X		X		X	
Research blood collection			X		X		X		X	
Dispense Study Agent[8]			X				X			
Collect Study Agent					X				X	
Review Agent Diary/Record				X	X			X	X	
AEs		X	X	X	X	X	X	X	X	X
Telephone/email Contact[9]		X	X	X	X	X	X	X	X	X

[1] Study intervention to initiate within 30 days of screening unless significant scheduling problems arise.
[2] Intervention Period Day 1 is defined as the first day the dose is taken; each Intervention period is to last 10-14 days. For scheduling problems, agent intervention can continue beyond 14 days but no more than 21 days.
[3] Washout period for 10-14 days. For scheduling problems, the washout period can continue beyond 14 days but no more than 28 days.
[4] CMP includes serum glucose, urea nitrogen, creatinine, sodium, potassium, chloride, bicarbonate, total protein, albumin, calcium, alkaline phosphatase, ALT, AST, total bilirubin. Calculations included with the CMP are: BUN/creatinine ratio, anion, gap, globulin, alb/glob ratio.
[5] Height required at Visit 1 only.
[6] For women of childbearing potential.
[7] Overnight urine to be collected the night prior to the visit. If urine collection was missed the night prior to the visit, overnight urine may be collected the night of the visit.
[8] Avmacol® pills may not be dispensed before confirmation of overnight urine collection.
[9] Participants will be contacted prior to each clinic visit to remind them about the study visit and the overnight urine collection.
[10] Participants will be contacted by telephone (or email) 10-14 days after completing Intervention Period 2 for a final AE assessment.
[11] Participants will be asked at each visit whether they have smoked ≥ 10 cigarettes per day since the previous visit. This information will be captured on the PE CRF.

7.2 Screening Evaluation

Potential participants will undergo a brief interview to determine initial eligibility. During this interview, potential participants will be queried on the inclusion/exclusion criteria that could be addressed by phone such as smoking history, age, and general state of health. The study procedures and risks will be reviewed. Those who are interested in participating in the study and have been determined initially eligible will be scheduled for a clinic visit.

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

- Height, weight, blood pressure, pulse, temperature measurements
- A brief physical exam consisting of an examination of the heart, lungs and abdomen
- Karnofsky performance status assessment
- Evaluation of concurrent medication and supplement use, tobacco use history, medical history
- Baseline tobacco/alcohol use assessment
- Collection of blood for clinical labs (complete blood count with differential (CBC-diff), comprehensive metabolic panel (CMP)
- Women of childbearing capacity will have a urine pregnancy test
- Participants provided with a daily diary for recording any AEs and medication usage
- Participants provided with a urine collection kit (an overnight urine collection container, a collection hat, and instructions for collecting overnight urine) to be used the night before Visit 2

Once determined eligible, participants will be randomized (1:1) to one of the following arms: High-Low Arm (8 tablets Avmacol® per day during Intervention Period 1 and 4 tablets Avmacol® per day during Intervention Period 2) and Low-High Arm (4 tablets Avmacol® per day during Intervention Period 1 and 8 tablets Avmacol® per day during Intervention Period 2).

7.3 Evaluation During Study Intervention

Participants will return to the clinic for Baseline Specimen Collection for Intervention Period 1 (Visit 2). Participants will be contacted prior to the visit to remind them to collect overnight urine the night prior to Visit 2. Participants will bring the overnight urine (for urinary tobacco carcinogens, systemic study agent exposure, and bio-measurement of tobacco exposure) to the clinic and undergo the following procedures:

- Collection of buccal cells (for NRF target gene transcripts and nuclear morphometry)
- Collection of nasal brushing (banked for gene expression analysis)
- Collection of blood for research (for genotyping and banking)
- Assessment of AEs, concomitant medications, and current tobacco use
- Urine pregnancy test, if applicable
- Participants provided with Avmacol® pills for Intervention Period 1 after confirmation of overnight urine collection.
- Participants provided with an Intake Calendar for recording agent intake

Those who miss the overnight urine collection prior to this visit will be asked to collect overnight urine the night of the visit and return the urine to the clinic the following day.

During Intervention Period 1 (10-14 days), participants in the High-Low Arm will take 8 tablets of Avmacol® each day and participants in the Low-High Arm will take 4 tablets of Avmacol® each day. Participants will be instructed to take the Avmacol® pills with the evening meal. Day 1 of the Intervention Period 1 begins the day the first dose is taken.

During Intervention Period 1, participants will return to the clinic on Day 4, 5, 6, 7 or 8 for an Interim Visit (Visit 3) to

undergo the following procedures:

- Collection of buccal cells
- Assessment of AEs, concomitant medications and current tobacco use
- Participants provided with a urine collection kit to be used the night before Visit 4.

Participants will return to the clinic at the end of Intervention Period 1 (Visit 4). Participants will be contacted prior to the visit to remind them to collect overnight urine the night prior to Visit 4. Participants will bring the overnight urine to the clinic and undergo the following procedures:

- Return unused pills and Intake Calendar
- Collection of buccal cells
- Collection of nasal brushing
- Collection of blood for research
- Assessment of AEs, concomitant medications, compliance, and current tobacco use
- Participants provided with a urine collection kit to be used the night before Visit 5

Those who miss the overnight urine collection prior to this visit will be provided with additional study pills and be asked to take the study pills in the evening of Visit 4 and collect overnight urine the night of the visit and return the urine to the clinic the following day.

Participants will undergo a Washout Period (10-14 days) after completing Intervention Period 1. After the Washout Period, participants will return to the clinic (Visit 5). Participants will be contacted prior to the visit to remind them to collect overnight urine the night prior to Visit 5. Participants will bring the overnight urine to the clinic and undergo the following procedures:

- Collection of buccal cells
- Collection of nasal brushing
- Collection of blood for research
- Assessment of AEs, concomitant medications, and current tobacco use
- Urine pregnancy test, if applicable
- Participants provided with Avmacol® pills for Intervention Period 2 after confirmation of overnight urine collection.
- Participants provided with an Intake Calendar for recording agent intake

Those who miss the overnight urine collection prior to this visit will be asked to collect overnight urine the night of the visit and return the urine to the clinic the following day.

During Intervention Period 2 (10-14 days), participants in the High-Low Arm will take 4 tablets of Avmacol® each day and participants in the Low-High Arm will take 8 tablets of Avmacol® each day. Participants will be instructed to take the Avmacol® pills with the evening meal. Participants will be provided with an Intake Calendar for recording agent intake. Day 1 of the Intervention Period 2 begins the day the first dose is taken.

During Intervention Period 2, participants will return to the clinic on Day 4, 5, 6, 7 or 8 for an Interim Visit (Visit 6) to undergo the following procedures:

- Collection of buccal cells
- Assessment of AEs, concomitant medications and current tobacco use
- Participants provided with a urine collection kit to be used the night before Visit 7.

Participants will return to the clinic at the end of Intervention Period 2 (Visit 7). Participants will be contacted prior to the visit to remind them to collect overnight urine the night prior to Visit 7. Participants will bring the overnight urine to the clinic and undergo the following procedures:

- Return unused pills and Intake Calendar
- Collection of buccal cells
- Collection of nasal brushing

- Collection of blood for research and for safety labs (CBC-diff and CMP)
- Assessment of AEs, concomitant medications, compliance, and current tobacco use
- Follow-up tobacco/alcohol use assessment

Those who miss the overnight urine collection prior to this visit will be provided with additional study pills and be asked to take the study pills in the evening of Visit 7 and collect overnight urine the night of the visit and return the urine to the clinic.

7.4 Evaluation at Completion of Study Intervention

See section 7.3 for evaluation at the end of intervention for each Intervention Period. In addition, participants will be provided with information on how to contact the Arizona Smokers' Helpline (toll free telephone number and URL to access the ASHLine website), which provides comprehensive services for tobacco cessation.

7.5 Post-intervention Follow-up Period

Participants will be contacted by telephone (or email) 10-14 days after completing Intervention Period 2 for a final AE assessment and will be reminded to return the AE Diary to the study office using a stamped, addressed envelope provided by the study office.

7.6 Methods for Clinical Procedures

Buccal cell collection: Participants will be instructed to rinse their mouths with plain water for approximately 10 seconds prior to collection. Buccal (inner cheek) cells will be collected with 4 buccal cytobrushes per collection (2 on the right, 2 on the left). The first cytobrush will be vigorously brushed across the buccal surface of the selected side for a total of 10 strokes, approximately 15 seconds, taking care to cover the entire surface area. Upon completion, the cytobrush will be immediately swirled into a cryovial with RNALater to deposit the cells then discarded. The same process will be repeated with a second cytobrush on the same inner cheek. A third cytobrush will then be used to collect cells from the opposite buccal surface, and swirled into the same cryovial. At the completion of the first three buccal cell collections, the cryovial will contain the buccal cells deposited from a total of 3 brushes. The cryovial will be frozen for later gene expression analysis. A fourth and final cytobrush will be used to collect cells from the most recently collected buccal surface (the side that has only been collected once). This fourth brush will be rolled across the length of three glass slides, then fixed for nuclear morphometry.

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

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary endpoint is to determine the change (from baseline to end-of-intervention for each Intervention Period) in the urinary excretion of the mercapturic acid of benzene after Avmacol® intervention. The urinary levels of mercapturic acid of benzene will be normalized by urinary creatinine levels to account for varying water content of individual urine samples. The normalized mercapturic acid of benzene at end-of-intervention will be compared with that at baseline. The changes following 4 tablets of Avmacol® per day and after 8 tablets of Avmacol® per day will be determined separately.

8.2 Secondary Endpoints

The secondary endpoints are:

- To determine the changes (from baseline to end-of-intervention for each Intervention Period) in the urinary excretion of the mercapturic acids of acrolein and crotonaldehyde after Avmacol® intervention. Similarly, the urinary biomarkers will be normalized by the urinary creatinine levels. The changes following 4 tablets of Avmacol® per day and after 8 tablets of Avmacol® per day will be calculated separately for each tobacco carcinogen.
- To determine whether Avmacol® increases the urinary excretion of the mercapturic acids of tobacco carcinogens, normalized by bio-measurement of tobacco exposure. Bio-measurement of tobacco exposure will be assessed by the total urinary levels of nicotine and major nicotine metabolites (i.e., the sum of the molar concentrations of nicotine and major nicotine metabolites in urine). The changes following 4 tablets of Avmacol® per day and after 8 tablets of Avmacol® per day will be calculated separately for each tobacco carcinogen.
- To determine the changes (from baseline to day 4-8 and to end-of-intervention) in the NRF2 target gene transcripts, assessed by the expression of *NQO1*, in the buccal cells after Avmacol® intervention. The changes following 4 tablets of Avmacol® per day and after 8 tablets of Avmacol® per day will be determined separately.
- To determine whether there is a dose-response relationship between the Avmacol® dose and the detoxification of tobacco carcinogens and the change in the NRF2 target gene transcripts.
- To determine the relationship between systemic study agent exposure, assessed by the total urinary levels of sulforaphane and its glutathione-derived metabolites (i.e., the sum of the molar concentrations of sulforaphane and its glutathione-derived metabolites in urine), and changes in detoxification of tobacco carcinogens and NRF2 target gene transcripts.

The study also includes the following explorative endpoints:

- To determine whether the *GSTM1* and *GSTT1* genotypes are important genetic modulators of detoxification of tobacco carcinogens. The change in the urinary excretion of the mercaptoruinc acids of tobacco carcinogens will be compared between *GSTM1*-null and *GSTM1*-positive subjects and between *GSTT1*-null and *GSTT1*-positive subjects.
- To bank specimens for future research including tobacco gene signature in nasal brushing, and fixed buccal cells for nuclear morphometry.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, AE or serious adverse event (SAE), 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.

8.4 Off-Study Criteria

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

8.5 Study Termination

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

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

Urinary metabolites of the tobacco carcinogens, including benzene, acrolein and crotonaldehyde will be quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS), as previously described by Hecht and colleagues (67, 68). This method is sensitive and specific for quantification of the urinary levels of these tobacco carcinogens.

Urinary levels of sulforaphane and its metabolites will be quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS), as previously described (69). This method is sensitive and specific for quantification of the urinary levels of sulforaphane and its metabolites.

Urinary levels of nicotine and its major metabolites will be quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS), as previously described (70). This method is sensitive and specific for quantification of the urinary levels of nicotine and its major metabolites.

Buccal cell NRF2 target gene transcripts will be determined using quantitative real-time PCR as previously described (39).

GSTM1 and *GSTT1* will be genotyped using standard methods as previously described (71).

Nuclear morphometry, a quantitative mathematical characterization of the spatial and statistical distribution of nuclear chromatin across the spectrum of transformation, will be conducted according to methods previously described (72, 73).

9.2 Comparable Methods

The methods proposed are standard methodologies used in other research studies. The resulting data will be able to be compared to existing data.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

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

The urinary biomarker analysis will be conducted in the University of Arizona Cancer Center Analytical Chemistry Shared Resource directed by Dr. Sherry Chow.

The NRF2 target gene transcripts and GSTM1/P1 genotying will be performed at a University of Arizona research laboratory under the supervision of Dr. Donna Zhang.

The nuclear morphometry will be performed in the University of Arizona Cancer Center Karyometry Laboratory directed by Dr. David Alberts under the supervision of Dr. Julie Bauman.

10.2 Collection and Handling Procedures

Blood for clinical chemistry, hematology and coagulation panels
Twelve and a half milliliters of blood (1 x 8.5 ml SST tube; 1 x 4 ml Lavender-EDTA tube) will be collected at screening and at the end of intervention for CBC with diff and CMP. The SST tube will be held at room temperature for 30 min and then centrifuged. The lavender EDTA tube will be gently inverted to mix for anticoagulation. Blood tubes will be prepped, labeled, and packaged according to the recommendation from the diagnostic laboratory. All samples will be refrigerated prior to transfer to the commercial laboratory and sent for immediate analysis.

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

Overnight urine collection
Overnight urine will be collected the night prior to Visits 2, 4, 5, 7. Participants will record the start and stop time of urine collection on a designated collection form and will bring overnight urine collections to research visits. If urine collection was not done the night prior to the visit, overnight urine may be collected the night of the visit. The volume of urine collected from each overnight void will be recorded by study staff to the nearest 10 mL. Five 5 mL aliquots of urine will be retained for testing and the remaining urine discarded. The sample tubes will be labeled with the study ID, participant ID, and visit number/date and stored at -70° or below until analysis.

Buccal cell collection
Buccal cells will be collected at Visits 2, 3, 4, 5, 6, 7. As described, the buccal cells will be collected in two forms: 1) into a cryovial preserved in RNALater upon collection (the first 3 brushes); 2) fixed onto 3 glass slides for nuclear morphometry (the fourth brush). Cryovial specimens will be labeled with the study ID, participant ID, and visit number/date and stored at -70° or below until analysis. Buccal cell glass slides will also be labeled with the study ID, participant ID, and visit number/date. After the buccal cell glass slides are collected, the slides are allowed to dry at room temperature for about 30 minutes. Buccal cell slides are then fixed for 10 minutes in room temperature 10% neutral buffered formalin (using a coplin jar). Slides can then be dried for as long as overnight at room temperature in a slide rack. Slides will then be stored in a labeled slide box stored at room temperature.

Blood for genotyping and banking
Twelve and a half milliliters of blood (1 x 8.5 ml SST tube; 1 x 4 ml Lavender EDTA tube) will be collected at Visit 2. SST tube will be held at room temperature for 30 min followed by centrifugation. Serum will be aliquoted evenly into 5 x 2 ml cryovials. The lavender-EDTA tube will be inverted gently 5 times and the whole blood will be aliquoted evenly into 4 x 2 ml cryovials. Cryovials will be labeled with the study ID, participant ID, and visit number/date and stored at -70° or below until analysis.

Eight and a half milliliters of blood (1 x 8.5 ml SST tube) will be collected at Visits 4, 5, 7. SST tube will be held at room temperature for 30 min followed by centrifugation. Serum will be aliquoted evenly into 4 x 2 ml cryovials. Cryovials will be labeled with the study ID, participant ID, and visit number/date and stored at -70° or below until analysis

Nasal epithelium brushings
Nasal epithelium brushing samples will be collected at Visits 2, 4, 5, 7. All nasal brushing samples will be preserved in RNAprotect Cell Reagent (Qiagen) upon collection and stored at -70° or below. Specimens will be labeled with the study ID, participant ID, and visit number/date and stored at -70° or below until analysis.

10.3 Shipping Instructions

Collected frozen specimens will be placed on ice packs and hand delivered to the research laboratory for analysis.

10.4 Tissue Banking

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

11. REPORTING ADVERSE EVENTS

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

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 can be found in §6.2 Reported Adverse Event AEs and Potential Risks, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

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

11.1.2 AE Data Elements:

The following data elements are required for AE reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a SAE
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE 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 grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

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

ADL

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

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

11.1.4 Assessment of relationship of AE to treatment

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

11.1.5 Follow-up of AEs

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

11.2 Serious Adverse Events

11.2.1 DEFINITION: Regulations at 21 CFR §312.32 (revised April 1, 2014) defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to perform normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE Report Form found at http://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

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

DCP Medical Monitor:
Eva Szabo, MD
Chief
Lung & Upper Aerodigestive Cancer Research Group
Division of Cancer Prevention, NCI, NIH
9609 Medical Center Drive, Room 5E-102, MSC 9781
Bethesda, MD 20892-9781 (For FedEx, use 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

11.2.2.3 The Lead Organization and all Participating Organizations will email written SAE reports to the DCP medical monitor and DCP's Regulatory Contractor CCS Associates, Inc. (CCSA; phone: 650-691-4400) at safety@ccsainc.com within 48 hours of learning of the event using the fillable PDF SAE Report Form.

11.2.2.4 The DCP Medical Monitor and CCSA regulatory and safety staff will determine which SAEs require FDA submission as IND safety reports.

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

11.2.3 Follow-up of SAE

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

12. STUDY MONITORING

12.1 Data Management

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

12.2 Case Report Forms

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

12.3 Source Documents

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

12.4 Data and Safety Monitoring Plan

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

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

12.5 Sponsor or FDA Monitoring

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

12.6 Record Retention

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

outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

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

Not applicable.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is an open-label, randomized cross-over study to determine whether two different doses of Avmacol® (4 tablets per day for 10-14 days; 8 tablets per day for 10-14 days) increase the detoxification of tobacco carcinogens in otherwise healthy, current smokers. Participants will serve as their own controls based on a 2 x 2 cross-over design. Specifically, each participant will have two Intervention Periods (one period taking 4 tablets per day; the other taking 8 tablets per day) with a 10-14 day washout period in between to eliminate the potential carry-over effect.

13.2 Randomization/Stratification

Each participant will be randomly assigned, based on random allocation rule, to either receiving 4 tablets per day first and then 8 tablets per day or receiving 8 tablets per day first and then 4 tablets per day to eliminate the dose sequence effect. No stratification or blocking will be performed.

13.3 Accrual and Feasibility

We plan to accrue 61 eligible participants to initiate agent intervention. With an anticipated attrition rate of 33% or less (with respect to completing the intervention and having complete endpoint data), we expect to have at least 41 participants with evaluable endpoint data. Projected accrual is 2 participants per month.

13.4 Primary Objective, Endpoint(s), Analysis Plan

The primary objective of this study is to determine whether Avmacol® increases the urinary excretion of the mercapturic acid of the tobacco carcinogen, benzene, in healthy current smokers. The primary endpoint is the change (from baseline to end-of-intervention for each Intervention Period) in the mercapturic acid of benzene after Avmacol® intervention. The changes following 4 tablets of Avmacol® per day and after 8 tablets of Avmacol® per day will be determined separately. Two-sided paired t tests will be performed to evaluate whether the changes are significantly. Bonferroni correction will be used to correct for multiple comparisons (two dose levels). With a sample size of 41 and an overall significance level of 5% (based on Bonferroni correction, i.e. a 2.5% significance level for each test), there will be at least 80% power to detect an effect size of 0.50 standard deviations. This is in line with an effect size of 0.45 standard deviations for a similar study (49).

13.5 Secondary Objectives, Endpoints, Analysis Plans

The secondary objectives include to determine whether Avmacol® increases the mercapturic acids of acrolein and crotonaldehyde and the mercapturc acids of tobacco carcinogens normalized by bio-measurement of tobacco exposure and upregulates the NRF2 target gene transcripts in the buccal cells of current smokers and to evaluate for a dose-response relationship between Avmacol® and the detoxification of tobacco carcinogens and the expression of NRF2 target gene transcripts and the relationship between systemic study agent exposure and biomarker modulation. The secondary endpoints are the changes in mercapturic acids of acrolein and crotonaldehyde levels (from baseline to end-of-intervention for each Intervention Period), the changes in mercapturic acids of tobacco carcinogens, normalized by bio-measurement

of tobacco exposure (from baseline to end-of-intervention for each Intervention Period) and in NRF2 target gene transcripts (from baseline to day 4-8 and to end-of-intervention for each Intervention Period) assessed by the expression of *NQO1* after Avmacol® intervention. Similar to the primary endpoint, each of the changes following 4 tablets of Avmacol® per day and after 8 tablets of Avmacol® per day will be evaluated separately. For changes in tobacco carcinogens, two-sided paired t tests will be performed to evaluate the significance of changes. For changes in NRF2 target gene transcripts, linear mixed effects models will be fitted to evaluate the significance of changes since each participant will have two changes in each Intervention Period (from baseline to day 4-8 and to end-of-intervention). Linear mixed effects models will be also fitted to determine whether there is a dose-response relationship between the Avmacol® dose and the change in carcinogen metabolites and the change in the NRF2 target gene transcripts since each participant will have more than one change for each endpoint. Spearman correlation coefficient will be calculated to evaluate the correlation between systemic study agent exposure and biomarker modulation.

In addition, we will also explore whether the *GSTM1* and *GSTT1* genotypes are important genetic modulators of detoxification of tobacco carcinogens. This will be evaluated by comparing the change in benzene for each dose level between *GSTM1*-null and *GSTM1*-positive subjects and between *GSTT1*-null and *GSTT1*-positive subjects, respectively, using two-sided two-sample t tests. For all of the secondary and exploratory analyses, adjustment for multiple comparisons will not be performed. However, the number of comparisons will be reported and we will also cautiously interpret the findings. For both primary and secondary endpoints, two-sample t tests will be performed to evaluate whether there is a significant sequence effect (e.g. receiving 4 tablets per day in the first Intervention Period vs. receiving 4 tablets per day in the second Intervention Period and receiving 8 tablets per day in the first Intervention Period vs. receiving 8 tablets per day in the second Intervention Period). Regression techniques will be used to control for baseline levels and potential confounders if necessary. Also, if the normality assumption is violated, potential transformation will be sought or nonparametric methods such as signed rank test will be performed. For any of the endpoints, if a missing rate of >15% is observed, multiple imputation by chained equations will be performed.

13.6 Reporting and Exclusions

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

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of Avmacol®. Descriptive statistics of the type and frequency of all AEs will be generated, including 95 confidence intervals.

13.8 Evaluation of Response

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

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

13.9 Interim Analysis

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

13.10 Ancillary Studies

None.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

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

14.2 Other Required Documents

14.2.1 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. Documentation of "Good Clinical Practice" training is required for all study personnel listed on the FDA Form 1572.

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

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form

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

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

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will

not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

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

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

14.5 Submission of Regulatory Documents

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

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

E-mail Submissions:
regulatory@ccsainc.com

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

14.6 Other

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

15. FINANCING, EXPENSES, AND/OR INSURANCE

Study procedures performed during study visits will be covered by the study budget. Research tests, including biomarker evaluations, will not be billed to the subject. Subjects may incur minimal out-of-pocket expenses for transportation but will not be charged for study agent or any study-related activities. If subjects complete the study, they will receive a total of $525, to be used at their discretion for out of pocket cost such as transportation. If they do not complete the study, the amount will be prorated for completed visits as follows: $75 for Visit 2, $50 for Visit 3, $100 for Visit 4, $50 for Visits 5 and 6, and $200 for Visit 7. If injury occurs, medical care will be provided and charged to the subject's insurer.

REFERENCES

1. Leon X, Quer M, Diez S, Orus C, Lopez-Pousa A, and Burgues J. Second neoplasm in patients with head and neck cancer. *Head & neck.* 1999;21(3):204-10.
2. Lee DH, Roh JL, Baek S, Jung JH, Choi SH, Nam SY, et al. Second cancer incidence, risk factor, and specific mortality in head and neck squamous cell carcinoma. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery.* 2013;149(4):579-86.
3. Day GL, Blot WJ, Shore RE, McLaughlin JK, Austin DF, Greenberg RS, et al. Second cancers following oral and pharyngeal cancers: role of tobacco and alcohol. *Journal of the National Cancer Institute.* 1994;86(2):131-7.
4. Lippman SM, and Hong WK. Second malignant tumors in head and neck squamous cell carcinoma: the overshadowing threat for patients with early-stage disease. *International journal of radiation oncology, biology, physics.* 1989;17(3):691-4.
5. Khuri FR, Kim ES, Lee JJ, Winn RJ, Benner SE, Lippman SM, et al. The impact of smoking status, disease stage, and index tumor site on second primary tumor incidence and tumor recurrence in the head and neck retinoid chemoprevention trial. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2001;10(8):823-9.
6. Gilpin EA, and Pierce JP. Demographic differences in patterns in the incidence of smoking cessation: United States 1950-1990. *Ann Epidemiol.* 2002;12(3):141-50.
7. Slaughter DP, Southwick HW, and Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. *Cancer.* 1953;6(5):963-8.
8. Chainani-Wu N. Diet and oral, pharyngeal, and esophageal cancer. *Nutrition and cancer.* 2002;44(2):104-26.
9. Pavia M, Pileggi C, Nobile CG, and Angelillo IF. Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. *The American journal of clinical nutrition.* 2006;83(5):1126-34.
10. Day GL, Shore RE, Blot WJ, McLaughlin JK, Austin DF, Greenberg RS, et al. Dietary factors and second primary cancers: a follow-up of oral and pharyngeal cancer patients. *Nutrition and cancer.* 1994;21(3):223-32.
11. Bravi F, Bosetti C, Filomeno M, Levi F, Garavello W, Galimberti S, et al. Foods, nutrients and the risk of oral and pharyngeal cancer. *British journal of cancer.* 2013;109(11):2904-10.
12. Fowke JH. Head and neck cancer: a case for inhibition by isothiocyanates and indoles from cruciferous vegetables. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation.* 2007;16(4):348-56.
13. Burge-Bottenbley A, and Shklar G. Retardation of experimental oral cancer development by retinyl acetate. *Nutrition and cancer.* 1983;5(3-4):121-9.
14. Lippman SM, Garewal HS, and Meyskens FL, Jr. Retinoids as potential chemopreventive agents in squamous cell carcinoma of the head and neck. *Preventive medicine.* 1989;18(5):740-8.
15. Hong WK, Endicott J, Itri LM, Doos W, Batsakis JG, Bell R, et al. 13-cis-retinoic acid in the treatment of oral leukoplakia. *The New England journal of medicine.* 1986;315(24):1501-5.
16. Hong WK, Lippman SM, Itri LM, Karp DD, Lee JS, Byers RM, et al. Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. *The New England journal of medicine.* 1990;323(12):795-801.
17. Khuri FR, Lee JJ, Lippman SM, Kim ES, Cooper JS, Benner SE, et al. Randomized phase III trial of low-dose isotretinoin for prevention of second primary tumors in stage I and II head and neck cancer patients. *Journal of the National Cancer Institute.* 2006;98(7):441-50.
18. Leeman-Neill RJ, Seethala RR, Singh SV, Freilino ML, Bednash JS, Thomas SM, et al. Inhibition of EGFR-STAT3 signaling with erlotinib prevents carcinogenesis in a chemically-induced mouse model of oral squamous cell carcinoma. *Cancer prevention research.* 2011;4(2):230-7.
19. Perkins TM, and Shklar G. Delay in hamster buccal pouch carcinogenesis by aspirin and indomethacin. *Oral surgery, oral medicine, and oral pathology.* 1982;53(2):170-8.
20. Cornwall H, Odukoya O, and Shklar G. Oral mucosal tumor inhibition by ibuprofen. *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons.* 1983;41(12):795-800.

21. Tanaka T, Nishikawa A, Mori Y, Morishita Y, and Mori H. Inhibitory effects of non-steroidal anti-inflammatory drugs, piroxicam and indomethacin on 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in male ACI/N rats. *Cancer letters.* 1989;48(3):177-82.
22. Shiotani H, Denda A, Yamamoto K, Kitayama W, Endoh T, Sasaki Y, et al. Increased expression of cyclooxygenase-2 protein in 4-nitroquinoline-1-oxide-induced rat tongue carcinomas and chemopreventive efficacy of a specific inhibitor, nimesulide. *Cancer research.* 2001;61(4):1451-6.
23. Laine L, Bombardier C, Hawkey CJ, Davis B, Shapiro D, Brett C, et al. Stratifying the risk of NSAID-related upper gastrointestinal clinical events: results of a double-blind outcomes study in patients with rheumatoid arthritis. *Gastroenterology.* 2002;123(4):1006-12.
24. Mulshine JL, Atkinson JC, Greer RO, Papadimitrakopoulou VA, Van Waes C, Rudy S, et al. Randomized, double-blind, placebo-controlled phase IIb trial of the cyclooxygenase inhibitor ketorolac as an oral rinse in oropharyngeal leukoplakia. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2004;10(5):1565-73.
25. Bertagnolli MM. Chemoprevention of colorectal cancer with cyclooxygenase-2 inhibitors: two steps forward, one step back. *The Lancet Oncology.* 2007;8(5):439-43.
26. Papadimitrakopoulou VA, William WN, Jr., Dannenberg AJ, Lippman SM, Lee JJ, Ondrey FG, et al. Pilot randomized phase II study of celecoxib in oral premalignant lesions. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2008;14(7):2095-101.
27. Saba NF, Hurwitz SJ, Kono SA, Yang CS, Zhao Y, Chen Z, et al. Chemoprevention of head and neck cancer with celecoxib and erlotinib: results of a phase ib and pharmacokinetic study. *Cancer prevention research.* 2014;7(3):283-91.
28. Prochaska HJ, Santamaria AB, and Talalay P. Rapid detection of inducers of enzymes that protect against carcinogens. *Proceedings of the National Academy of Sciences of the United States of America.* 1992;89(6):2394-8.
29. Kensler TW. Chemoprevention by inducers of carcinogen detoxication enzymes. *Environmental health perspectives.* 1997;105 Suppl 4:965-70.
30. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochemical and biophysical research communications.* 1997;236(2):313-22.
31. Zhang Y, and Tang L. Discovery and development of sulforaphane as a cancer chemopreventive phytochemical. *Acta pharmacologica Sinica.* 2007;28(9):1343-54.
32. Hong F, Freeman ML, and Liebler DC. Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. *Chemical research in toxicology.* 2005;18(12):1917-26.
33. Kensler TW, Egner PA, Agyeman AS, Visvanathan K, Groopman JD, Chen JG, et al. Keap1-nrf2 signaling: a target for cancer prevention by sulforaphane. *Topics in current chemistry.* 2013;329:163-77.
34. Fahey JW, Zhang Y, and Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proceedings of the National Academy of Sciences of the United States of America.* 1997;94(19):10367-72.
35. Kensler TW, Chen JG, Egner PA, Fahey JW, Jacobson LP, Stephenson KK, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2005;14(11 Pt 1):2605-13.
36. Egner PA, Chen JG, Wang JB, Wu Y, Sun Y, Lu JH, et al. Bioavailability of Sulforaphane from two broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, China. *Cancer prevention research.* 2011;4(3):384-95.
37. Kensler TW, Ng D, Carmella SG, Chen M, Jacobson LP, Munoz A, et al. Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China. *Carcinogenesis.* 2012;33(1):101-7.
38. Egner PA, Chen JG, Zarth AT, Ng DK, Wang JB, Kensler KH, et al. Rapid and sustainable detoxication of airborne pollutants by broccoli sprout beverage: results of a randomized clinical trial in China. *Cancer prevention*

research. 2014;7(8):813-23.
39. Bauman JE, Zang Y, Sen M, Li C, Wang L, Egner PA, et al. Prevention of Carcinogen-Induced Oral Cancer by Sulforaphane. *Cancer prevention research.* 2016;9(7):547-57.
40. Shapiro TA, Fahey JW, Wade KL, Stephenson KK, and Talalay P. Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2001;10(5):501-8.
41. Shapiro TA, Fahey JW, Dinkova-Kostova AT, Holtzclaw WD, Stephenson KK, Wade KL, et al. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. *Nutrition and cancer.* 2006;55(1):53-62.
42. Fahey JW, Talalay P, and Kensler TW. Notes from the field: "green" chemoprevention as frugal medicine. *Cancer prevention research.* 2012;5(2):179-88.
43. Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, et al. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. *Proceedings of the National Academy of Sciences of the United States of America.* 2002;99(11):7610-5.
44. Xu C, Huang MT, Shen G, Yuan X, Lin W, Khor TO, et al. Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. *Cancer research.* 2006;66(16):8293-6.
45. Cornblatt BS, Ye L, Dinkova-Kostova AT, Erb M, Fahey JW, Singh NK, et al. Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. *Carcinogenesis.* 2007;28(7):1485-90.
46. Zhang Y, Kensler TW, Cho CG, Posner GH, and Talalay P. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proceedings of the National Academy of Sciences of the United States of America.* 1994;91(8):3147-50.
47. Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, et al. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America.* 2001;98(6):3410-5.
48. Ohkoshi A, Suzuki T, Ono M, Kobayashi T, and Yamamoto M. Roles of Keap1-Nrf2 system in upper aerodigestive tract carcinogenesis. *Cancer prevention research.* 2013;6(2):149-59.
49. Yuan JM, Stepanov I, Murphy SE, Wang R, Allen S, Jensen J, et al. Clinical Trial of 2-Phenethyl Isothiocyanate as an Inhibitor of Metabolic Activation of a Tobacco-Specific Lung Carcinogen in Cigarette Smokers. *Cancer prevention research.* 2016;9(5):396-405.
50. Shapiro TA, Fahey JW, Wade KL, Stephenson KK, and Talalay P. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 1998;7(12):1091-100.
51. Fahey JW, Zalcmann AT, and Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry.* 2001;56(1):5-51.
52. Brooks JD, Paton VG, and Vidanes G. Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. *Cancer Epidemiol Biomarkers Prev.* 2001;10(9):949-54.
53. Myzak MC, Tong P, Dashwood WM, Dashwood RH, and Ho E. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Exp Biol Med (Maywood).* 2007;232(2):227-34.
54. Jones SB, and Brooks JD. Modest induction of phase 2 enzyme activity in the F-344 rat prostate. *BMC Cancer.* 2006;6:62.
55. Zhang Y, Munday R, Jobson HE, Munday CM, Lister C, Wilson P, et al. Induction of GST and NQO1 in cultured bladder cells and in the urinary bladders of rats by an extract of broccoli (Brassica oleracea italica) sprouts. *J Agric Food Chem.* 2006;54(25):9370-6.
56. Yoxall V, Kentish P, Coldham N, Kuhnert N, Sauer MJ, and Ioannides C. Modulation of hepatic cytochromes P450 and phase II enzymes by dietary doses of sulforaphane in rats: Implications for its chemopreventive activity. *Int J Cancer.* 2005;117(3):356-62.
57. Seow A, Shi CY, Chung FL, Jiao D, Hankin JH, Lee HP, et al. Urinary total isothiocyanate (ITC) in a population-

based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione S-transferase M1/T1/P1 genotypes. *Cancer Epidemiol Biomarkers Prev.* 1998;7(9):775-81.
58. Nugon-Baudon L, Rabot, S. Glucosinolates and glucosinolate derivates: implications for protection against chemical carcinogenesis. *Nutr Res Rev.* 1994;7(1):205-31.
59. Milford GFJ, Evans, E.J. Factors causing variation in glucosinolates in oilseed rape. *Outlook Agric.* 1991;20:31-7.
60. Mullin WJ, Sahasrabudhe, H.R. An estimate of the average daily intake of glucosinolate. *Nutr Rep Int.* 1978;18:273-9.
61. Wattenberg LW, Hanley, A.B., Barany, G., Sparnins, V.L., Lam, L.K.T., Fenwick, G.R. . In: Hayashi Y ed. *Diet, Nutrition and Cancer*. Tokyo: Japan Science Society Press; 1986:193-203.
62. Murashima M, Watanabe S, Zhuo XG, Uehara M, and Kurashige A. Phase 1 study of multiple biomarkers for metabolism and oxidative stress after one-week intake of broccoli sprouts. *Biofactors.* 2004;22(1-4):271-5.
63. Riedl MA, Saxon A, and Diaz-Sanchez D. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. *Clin Immunol.* 2009;130(3):244-51.
64. Christiansen B, Bellostas Muguerza N, Petersen AM, Kveiborg B, Madsen CR, Thomas H, et al. Ingestion of broccoli sprouts does not improve endothelial function in humans with hypertension. *PloS one.* 2010;5(8):e12461.
65. Bahadoran Z, Mirmiran P, Hosseinpanah F, Rajab A, Asghari G, and Azizi F. Broccoli sprouts powder could improve serum triglyceride and oxidized LDL/LDL-cholesterol ratio in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *Diabetes Res Clin Pract.* 2012;96(3):348-54.
66. Alumkal JJ, Slottke R, Schwartzman J, Cherala G, Munar M, Graff JN, et al. A phase II study of sulforaphane-rich broccoli sprout extracts in men with recurrent prostate cancer. *Invest New Drugs.* 2015;33(2):480-9.
67. Carmella SG, Chen M, Zarth A, and Hecht SS. High throughput liquid chromatography-tandem mass spectrometry assay for mercapturic acids of acrolein and crotonaldehyde in cigarette smokers' urine. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences.* 2013;935:36-40.
68. Haiman CA, Patel YM, Stram DO, Carmella SG, Chen M, Wilkens LR, et al. Benzene Uptake and Glutathione S-transferase T1 Status as Determinants of S-Phenylmercapturic Acid in Cigarette Smokers in the Multiethnic Cohort. *PloS one.* 2016;11(3):e0150641.
69. Egner PA, Kensler TW, Chen JG, Gange SJ, Groopman JD, and Friesen MD. Quantification of sulforaphane mercapturic acid pathway conjugates in human urine by high-performance liquid chromatography and isotope-dilution tandem mass spectrometry. *Chemical research in toxicology.* 2008;21(10):1991-6.
70. Piller M, Gilch G, Scherer G, and Scherer M. Simple, fast and sensitive LC-MS/MS analysis for the simultaneous quantification of nicotine and 10 of its major metabolites. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences.* 2014;951-952:7-15.
71. Kelsey KT, Nelson HH, Wiencke JK, Smith CM, and Levin S. The glutathione S-transferase theta and mu deletion polymorphisms in asbestosis. *Am J Ind Med.* 1997;31(3):274-9.
72. Alberts D, Ranger-Moore J, Einspahr J, Saboda K, Bozzo P, Liu Y, et al. Safety and efficacy of dose-intensive oral vitamin A in subjects with sun-damaged skin. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2004;10(6):1875-80.
73. Bartels PH, Krouse RS, Prasad AR, Yozwiak M, Liu Y, Bartels HG, et al. Actinic damage in histopathologically normal skin. *Anal Quant Cytol Histol.* 2008;30(6):316-22.

CONSENT FORM

Study Title for Study Participants:
Testing the effect of Avmacol® on the cancer causing substances of tobacco in heavy smokers

Official Study Title for Internet Search on http://www.ClinicalTrials.gov:
Clinical Study of Avmacol® for Detoxification of Tobacco Carcinogens in Heavy Smokers

Introduction

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 the research. Please take your time to make your decision about volunteering. You may discuss your decision with your friends and family. You can also discuss this study with your health care team. If you have any questions, you can ask your study doctor for more of an explanation. You should only agree to participate in this study when you are comfortable enough with the information so that you can make an informed decision about joining.

What is the usual approach to my status as a current smoker?

You are being asked to take part in this study because you are a current smoker. Smokers are at increased risk for developing lung, head and neck, and other cancers. Current smokers who do not take part in this study are usually followed closely by their doctor to watch for the development of cancer and may be offered help in stopping smoking.

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,
- you may choose to take part in a different study, if one is available,
- or you may choose to do nothing.

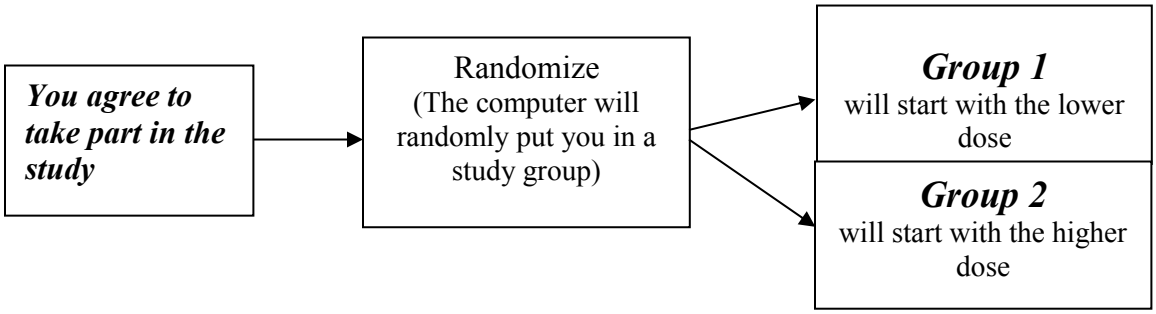
Why is this study being done?

The purpose of this study is to see if Avmacol®, a dietary supplement made from broccoli sprout and seed extract powder, can break down some of the cancer causing substances in tobacco smoke and produce substances that may protect cells from tobacco smoke-induced damage in current smokers. The study will also study inner nose and cheek cells for damage from tobacco smoke, and see if the genetic type of a certain detoxification enzyme affects the amount of cancer causing substances in tobacco that are broken down. As a dietary supplement, Avmacol® is not approved by the Food and Drug Administration for use in the treatment or prevention of disease. Its use in this study is investigational. The study is sponsored by the National Cancer Institute.

What are the study groups?

This study has two study groups. Group 1 will receive the study drug, Avmacol®, at a lower dose and then increase to a higher dose after a washout period (a period of not taking the study drug). Group 2 will first receive Avmacol® at a higher dose and then will decrease to a lower dose after a washout period. Approximately 61 subjects will participate in this study.

A computer will randomly put you in a study group—like a coin toss—to decide what group you get placed into. Once you are put in a group, you cannot switch to the other group.



How long will I be in this study?

You will be in the study for around 8 - 12 weeks. You will take Avmacol® for a period of 10-14 days, followed by a 10-14 day washout period (a period of not taking the study drug), and then take Avmacol® for another period of 10-14 days. Each period could be extended up to 21 days if there was any scheduling difficulties. Even if you do not finish the study, your doctor will continue to watch you for side effects and follow your condition for 10-14 days.

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

Before you begin the study:

You will need to have the following procedures to find out if you can be in the study at a **screening visit (Visit 1):**

- The study staff will go over this consent form and answer any questions you may have. Once you have signed it, the following procedures will be done.
- Brief physical exam including vital signs (height, weight, blood pressure, pulse and temperature). A clinician will listen to your heart and lungs and check your abdomen.
- Collection of approximately 2½ teaspoons of blood for routine laboratory tests.
- A review of your medical history and current medications.
- You will be given a questionnaire to complete about your past and current tobacco and alcohol use. It will take about 10 minutes to complete.
- Urine pregnancy test if you are a woman who could become pregnant.
- You will be given a symptom diary to record any illness or injury during the study.
- You will be given a urine collection kit consisting of the supplies and instructions to collect an overnight urine sample the night before your next visit to bring with you to the visit. This is done to measure the amount of substances from tobacco exposure and substances from Avmacol® in your urine.

If the exams, tests, and procedures show that you can take part in the study, and you choose to, then you will need the following extra procedures.

Intervention period 1 Baseline Visit (Start of study, Visit 2): You will be assigned to Study Group 1 or 2. Tests and procedures include:

- You will turn in your overnight urine collection.
- Vital signs (weight, blood pressure, pulse and temperature).
- Urine pregnancy test if you are a woman who could become pregnant.
- A review of your symptom diary and current medications.
- You will be asked about your tobacco use since the previous visit.
- Collection of approximately 2½ teaspoons of blood for research tests (to determine the genetic type of a certain detoxification enzyme).
- Collection of cells from your nose (to determine cellular damage from tobacco exposure). We will inspect the inside of your nose for any signs of bruising or issues that may make collection of the cells from your nose difficult. We will ask you to sit upright and to tilt your head back. We will brush the inside of your nose with a

soft brush to collect the cells. We will repeat this procedure for a second brushing.

- Cheek cell collection (to determine the change of a cellular substance related to the activity of Avmacol® in previous research, and to determine changes in the shape of the cell's nucleus related to tobacco exposure). This is done by rinsing your mouth with tap water for 10 seconds before collection. Each inner cheek will be brushed twice. A collection brush will be scraped 10 times across the inner cheek, then this will be repeated with a second brush. Then the opposite cheek will be collected in the same manner. Gentle counter-pressure will be made on the outside of your cheeks during these collections.
- You will be given a supply of the study medication and instructions on how to take them. You will be instructed to take the Avmacol® tablets with your evening meal with at least 8 oz of water and with food. Swallow the tablets whole, do not crush or chew them. If you miss a dose, do not take a double dose the next day, just take your normal dose the following day. Avmacol® tablets should be stored in a climate controlled area, away from light and moisture and out of reach of children.
- You will be given an Intake Calendar to mark each day you take study medication.
- If you miss the overnight urine collection, you will be asked to collect the overnight urine the night of Visit 2 and turn in the urine the next day. A supply of the study medication will not be provided to you until you have turned in your overnight urine.

You will return to the clinic on Day 4, 5, 6, 7 or 8 for **Visit 3** to undergo the following procedures:

- A review of your symptom diary and current medications.
- You will be asked about your tobacco use since the previous visit.
- Cheek cell collection.
- You will be given a urine collection kit to collect an overnight urine sample the night before your next visit to bring with you to the visit.

You will return to the clinic at the end of Intervention Period 1for **Visit 4** to undergo the following procedures:

- You will turn in your overnight urine collection.
- Return unused study medication and Intake Calendar.
- Cheek cell collection.
- Collection of cells from your nose.
- Collection of approximately 2½ teaspoons of blood for research tests.
- A review of your symptom diary and current medications.
- You will be asked about your tobacco use since the previous visit.
- If you miss the overnight urine collection prior to the visit, you will be provided with additional study medication and be asked to continue to take the study medication the evening of Visit 4 and collect the overnight urine and turn in the urine the next day.
- You will be given a urine collection kit to collect an overnight urine sample the night before your next visit to bring with you to the visit.

After completing Intervention Period 1 of study medication, you will undergo a **Washout Period** for10-14 days.

After the Washout Period, you will return to the clinic for **Visit 5** to undergo the following procedures for Intervention Period 2 of study medication:

- You will turn in your overnight urine collection.
- Cheek cell collection.
- Collection of cells from your nose.
- Collection of approximately 2½ teaspoons of blood for research tests.
- A review of your symptom diary and current medications.
- You will be asked about your tobacco use since the previous visit.
- Urine pregnancy test if you are a woman who could become pregnant.
- You will be given a new supply of the study medication and an Intake Calendar.

- If you miss the overnight urine collection prior to Visit 5, you will be asked to collect the overnight urine the night of Visit 5 and turn in the urine the next day. A new supply of the study medication will not be provided to you until you have turned in your overnight urine.

You will return to the clinic on Day 4, 5, 6, 7 or 8 of Intervention Period 2 of medication for **Visit 6** to undergo the following procedures:

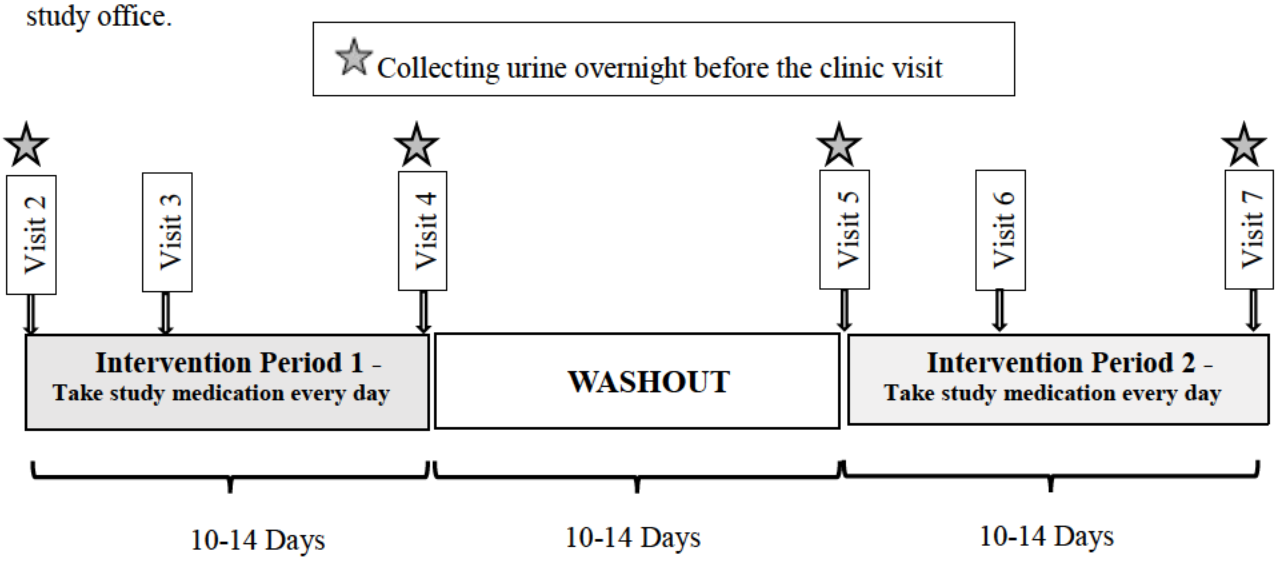
- A review of your symptom diary and current medications.
- You will be asked about your tobacco use since the previous visit.
- Cheek cell collection.
- You will be given a urine collection kit to collect an overnight urine sample the night before your next visit to bring with you to the visit.

You will return to the clinic at the end of Intervention Period 2 of medication for **Visit 7** to undergo the following procedures:

- You will turn in your overnight urine collection.
- Return unused study medication and Intake Calendar.
- Cheek cell collection.
- Collection of cells from your nose.
- Collection of approximately 2½ teaspoons of blood for routine laboratory tests.
- Collection of approximately 2½ teaspoons of blood for research tests.
- A review of your symptom diary and current medications.
- You will be given a questionnaire to complete about your current tobacco and alcohol use. It will take about 5-10 minutes to complete.
- If you miss the overnight urine collection prior to the visit, you will be provided with additional study medication and be asked to continue to take the study medication the evening of Visit 7 and collect the overnight urine and turn in the urine the next day.
- You will be offered information about a smoking cessation program called the Arizona Smokers Helpline.

You will be contacted prior to each study visit to remind you about the study visit and overnight urine collection.

You will be contacted 10-14 days after completing Intervention Period 2 of medication for a final review your symptom diary. You will be asked to mail your symptom diary to the study office in a stamped, addressed envelope provided by the study office.



As part of this study you will also be asked to answer questions about your tobacco and alcohol use, both before you begin the study and again at the End of Intervention visit. Researchers want to see if tobacco and alcohol use affects the side effects people might get while on this study, or if tobacco and alcohol use modifies the effects of the study agents.

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, for example about your tobacco and alcohol use. 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. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

The Avmacol® used in this study may affect how different parts of your body work, such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

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.

There is no recognized risk from consuming dietary supplements containing broccoli seed, including Avmacol®. Possible mild side-effects might include:

- flatulence (gas)
- an unpleasant taste
- nausea

These symptoms are not common and have occurred in less than 10% of healthy volunteers taking broccoli sprout extracts and Avmacol®.

There may be side effects of Avmacol® that are currently unknown. Everyone taking part in the study will be watched carefully for any side effects, including food intolerances. Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect. If important new side effects are found, the study doctor will discuss these with you.

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 ask you to stop taking the study medication for several days to try to reduce side effects.

Reproductive risks: The effects of this dietary supplement (composed of broccoli seed, broccoli sprout extract, and Vitamin C) on the developing human fetus are unknown, however are very unlikely to be harmful. Nonetheless, women and men who are able to conceive must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her study doctor immediately.

Risks of drawing blood: Possible risks associated with drawing blood are bleeding, bruising, infection, pain, and possibly fainting.

Risks of cell collection: Possible risks associated with the nose and cheek cell collection: The brushing of the inside of your nose and cheek using a soft brush can cause mild discomfort. We use a soft brush to obtain the cells from the inside of your nose and cheek. There may be a small amount of bleeding. If bleeding does occur, gentle pressure can be used to stop the bleeding.

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 current smokers. This study may help us learn things that could help people in the future.

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. 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.

For the tobacco and alcohol use questions, you can decide to not answer some or all of the questions. Your decision will not affect whether you can participate in the study, and it will not affect your relationship with your doctor or the study staff.

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 (National Cancer Institute), CIRB (Central Institutional Review Board, a group of people who review the research with the goal of protecting the people who take part in the study) or Food and Drug Administration.

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*).

What are the costs of taking part in this study?

The Avmacol® will be supplied at no charge while you take part in this study. The cost of study-specific exams, tests, and any other procedures will be paid for by the study.

You will receive monetary compensation for study participation. You will receive $25 once you have completed the Baseline visit (Visit 2) and turned in your overnight urine collection. You will receive an additional $500 at the end of the study upon completion of all required visits and procedures. If you are unable to complete the entire study, the amount of compensation will be based on how long you are in the study. You will receive $50 each for Visits 2 and 3, $100 for Visit 4, $50 each for Visits 5 and 6, and $200 for Visit 7. This money is to reimburse you for your time and help cover any cost you may have in being on the study.

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. You and/or your health plan will be charged for this treatment.

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. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. 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 University of Arizona study team including the study doctor and study personnel.
- The Central Institutional Review Board.
- The U.S. Food and Drug Administration and the National Cancer Institute.
- 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?

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 STUDIES SECTION:

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

There may be some samples (blood, urine, cheek cells and nose cells) remaining once the study is complete. The researchers would like to store and use your remaining samples for future medical research. The research that may be done is unknown at this time but your samples could help researchers 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.

You can take part in the main research study described above without giving your consent for your samples to be stored for future research.

WHAT IS INVOLVED?

If you agree to take part, here is what will happen next:

1) Your remaining samples will be stored at the University of Arizona until the end of the study.
2) After study completion, your remaining samples and some related information may be transferred to a central facility (Biobank) supported by the National Institutes of Health and stored in the Biobank along with samples and information from other people who take part.
3) Qualified researchers can submit a request to use the materials stored. A research committee 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.
4) Neither you nor your study doctor will be notified if/when research is conducted using your samples. Neither you nor your study doctor will be notified of the results of the research conducted.
5) 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?

1) 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.

   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.

   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.

2) 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.

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:

1) 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.
2) The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and the University of Arizona staff with access to the list must sign an agreement to keep your identity confidential.
3) Researchers to whom the University of Arizona study team 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.
4) Information that identifies you will not be given to anyone, unless required by law.
5) 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 in the optional studies. 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. 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 AND INFORMATION FOR FUTURE RESEARCH STUDIES:

Indicate your choice of "yes" or "no" for each of the following studies.

1. My remaining samples and related information may be kept in a Biobank for use in future health research.

Yes No

2. The information from my tobacco and alcohol use questionnaires may be used in future health research.

Yes No

3. 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

4. My genetic data and health information can be released, with no direct identifiers, into scientific databases.

Yes No

This is the end of the section about optional studies.

My Signature Agreeing to Take Part in the Main Study

I have read this consent form or had it read to me. 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 where I circled 'yes'.

_____

Participant's signature

_____

Date of signature

_____

Signature of person(s) conducting the informed consent discussion

_____

Date of signature

APPENDIX A
Karnofsky Performance Status Criteria

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
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.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B
Tobacco and Alcohol Assessment Questionnaires

TOBACCO ASSESSMENT – BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __/__ __/__ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

☐ Yes
☐ No → **Skip to Section B**
☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?

_____ Years old

3. How old were you when you first began smoking cigarettes regularly?

_____ Years old
☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

_____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

☐ Within 30 minutes
☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini
☐ Other, Please specify:_____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

- ☐ Yes
- ☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

- ☐ Yes In total, for about how many years?_____ If less than 1, write "1."
- ☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

- ☐ Yes → In total, for about how many years?_____ If less than 1, write "1."
- ☐ No

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Investigator Name (please print) _____

TOBACCO ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __/__ __/__ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to Question 3.**
☐ Never smoked → **Skip to Question 4**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a whole number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No (**End)**

5. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

6. Since your last visit, which of the following products have you used? ***Check all that apply***

- ☐ Cigarettes
- ☐ E-cigarettes or other electronic nicotine delivery system
- ☐ Traditional cigars, cigarillos or filtered cigars
- ☐ Pipes
- ☐ Waterpipe
- ☐ Hookah
- ☐ Clove cigarettes or kreteks
- ☐ Bidis
- ☐ Smokeless tobacco, like dip, chew, or snuff
- ☐ Snus
- ☐ Paan with tobacco, gutka, zarda, khaini
- ☐ Other, Specify_____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable

9. After the end of study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Investigator Name (please print) _____

ALCOHOL ASSESSMENT – BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?

   ☐ Yes
   ☐ No **(End)**
   ☐ Refused **(End)**
   ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?

   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)
   ☐ Week
   ☐ Month
   ☐ Year
   ☐ Refused
   ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?

   _____ (Enter the average number of drinks per day)

   ☐ Refused
   ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?

   _____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

   ☐ Refused
   ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?

   ☐ Yes
   ☐ No
   ☐ Refused
   ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?

   ☐ Within the past month (0 to 1 month ago)
   ☐ Between 1 and 3 months (1 to 3 months ago)
   ☐ Between 3 and 6 months (3 to 6 months ago)
   ☐ Between 6 and 12 months (6 to 12 months ago)
   ☐ Between 1 and 5 years (1 to 5 years ago)
   ☐ Between 5 and 15 years (5 to 15 years ago)
   ☐ More than 15 years ago
   ☐ Don't know/Not sure
   ☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?

   _____ (Enter the number of drinks a day)

   ☐ Refused
   ☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?

   _____ years

   ☐ Refused
   ☐ Don't know/Not sure

9. At what age did you begin drinking regularly?

   _____ years of age

   ☐ Refused
   ☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)

    ☐ Wine
    ☐ Liquor
    ☐ Beer
    ☐ Wine cooler

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Investigator Name (please print) _____

ALCOHOL ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. During the past 30 days, did you drink any alcoholic beverages?

☐ Yes
☐ No **(End)**
☐ Refused **(End)**
☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?

_____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)

☐ Week
☐ Month
☐ Refused
☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?

_____ (Enter the average number of drinks you had per day.)

☐ Refused
☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?

_____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

☐ Refused
☐ Do not know/Not sure

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Investigator Name (please print) _____