American Lung Association

Asthma Clinical Research Centers

The Study of Soy Isoflavones in Asthma

(SOYA)

R01 HL087987-01A2 (Smith) and R01 HL0088367-01A2 (Wise)
IND 104204

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Abstract

Asthma is a common disease that has a major impact on morbidity and health care costs. Although the prevalence and severity of asthma have increased over the last several decades, the specific causes remain unknown. One possible mechanism is a change in diet. Yet, epidemiological and interventional studies designed to identify a key nutrient or antioxidant vitamin that may be responsible for the increase in disease severity have produced inconsistent results. We recently reported and have since confirmed an association between low soy genistein intake and more severe asthma. We subsequently found that (1) *in vitro* the soy isoflavone genistein inhibits two key pathways that may contribute to asthma severity, human peripheral blood eosinophil leukotriene (LT) C_4 synthesis and myofibroblast differentiation, and (2) in a pilot study a soy isoflavone supplement reduces exhaled nitric oxide (eNO) and *ex vivo* LTC_4 synthesis in patients with inadequately controlled asthma. We now propose a clinical trial to test the novel hypothesis that dietary supplementation with soy isoflavones is an effective treatment in patients with poorly controlled asthma. The study will include 380 patients with low dietary soy intake, 12 years of age or older, who are taking either inhaled corticosteroids or leukotriene modifiers and have inadequately controlled asthma. Participants will be randomly assigned to treatment with either a soy isoflavone supplement (containing genistein, daidzein and glycitein; 100 mg daily of the glycoside forms) or placebo for six months. The primary outcome measure is forced expiratory volume in one second (FEV_1), an objective measure of lung function. Secondary outcomes include biomarkers of airway inflammation (eNO, exhaled breath condensate 8-isoprostane), measures of asthma symptoms (e.g., asthma control scores, unscheduled health care contacts, quality of life), and episodes of poor asthma control (EPAC). Tertiary outcomes include biomarkers of systemic inflammation (peripheral blood eosinophil counts and C-reactive protein and interleukin-6 levels). Treatment response will be correlated with plasma genistein concentrations. The results of this trial will increase understanding of the role of diet in asthma; could identify a novel, safe and relatively inexpensive treatment for patients with asthma; and potentially will have a substantial impact on public health in the United States.

1. Introduction

1.1 Title

The Study of Soy Isoflavones in Asthma (SOYA)

1.2 Sponsors

The American Lung Association; National Institutes of Health/National Heart Lung Blood Institute R01 HL087987-01A2 (Smith) and R01 HL0088367-01A2 (Wise), and Archer Daniels Midland Company will provide soy isoflavone tablets and matching placebo for the trial

1.3 Investigators and Study Centers

Network Chair

Bailey, William	University of Alabama at Birmingham

Participating Centers

Smith, Lewis	Illinois Consortium
Castro, Mario	Washington University
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Irvin, Charles	University of Vermont
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Hanania, Nicola	Baylor College of Medicine
Sundy, John	Duke University Medical Center
Katial, Rohit	National Jewish Health
Salzman, Gary	University of Missouri, KC
Lockey, Richard	University of South Florida
Busk, Michael	Indiana University
Karpel, Jill	Long Island Jewish Medical Center
Summer, Warren	Louisiana State University
Reibman, Joan	New York University
Mastronarde, John	Ohio State University
Wasserman, Stephen	University of California, San Diego
Wanner, Adam	University of Miami
Gerald, Lynn	University of Arizona
Teague, Gerald	University of Virginia

(additional clinics may be added)

Data Coordinating Center

Wise, Robert	Johns Hopkins University

1.4 Background and Significance

Current estimates are that between 5% and 12% of the U.S. population has asthma. Data from the multi-center Coronary Artery Risk Development in Young Adults study (1) revealed that asthma prevalence in young adults in the mid-1980s was approximately 8% and now may be closer to 12% (Smith and Jacobs, unpublished data, 2006). Several mechanisms have been proposed to explain this increase in asthma prevalence and severity, including changes in diet.

Decreased consumption of fresh fruits, green vegetables, potatoes and fresh fish, important sources of antioxidants and other essential nutrients, has occurred over the same time period and has been associated with a decrease in lung function in the general population (2-6).

Data from both epidemiological and mechanistic studies support a possible role for diet as a risk factor for asthma (7-11). Yet the contribution of specific nutrients and antioxidant vitamins remains controversial. Most epidemiological studies have focused on the effect of diet on asthma prevalence and incidence. In addition, there have been several intervention studies in which patients with asthma receive vitamin C or vitamin E supplements (12-15). Overall, the data do not support a beneficial effect of supplemental vitamin C (16), vitamin E, magnesium or selenium. Unfortunately, these studies were either underpowered or used patients with asthma whose disease was well-controlled and unlikely to show substantial improvement regardless of the intervention.

An alternative explanation for the inconsistent or negative findings with specific foods and antioxidant vitamins is the limited assessment of other potentially beneficial nutrients such as soy isoflavones. Soy isoflavones, which consist primarily of genistein and daidzein, are linked to a lower risk of several conditions including cardiovascular disease, osteoporosis, bone fracture and cancer (17-20). Genistein is the most abundant of the soy isoflavones and thus is likely to play a role in health and disease. Both soy isoflavones and genistein have a number of biological effects that could be beneficial to patients with asthma. It was shown in a broadly representative group of patients with asthma that those who consume soy isoflavones have higher lung function than those who do not (21). A recent report from Japan identified an association between high intake of soy isoflavones and a reduced prevalence of allergic rhinitis (22), a disease linked to asthma. One other study has explored the association between dietary intake of flavonoids and asthma in adults (23). That study failed to find an association between flavonoid intake and asthma prevalence or severity, but it did not specifically examine the effect of soy isoflavones. Recent in vitro data indicates that genistein, at levels achievable in plasma, inhibits human peripheral blood eosinophil cysteinyl leukotriene (LT) synthesis, an important mediator of asthma. Preliminary studies also reveal a potential effect on airway remodeling as well (24). In addition, relevant to its use as a possible treatment to asthma is the accumulating data that genistein has an excellent safety profile.

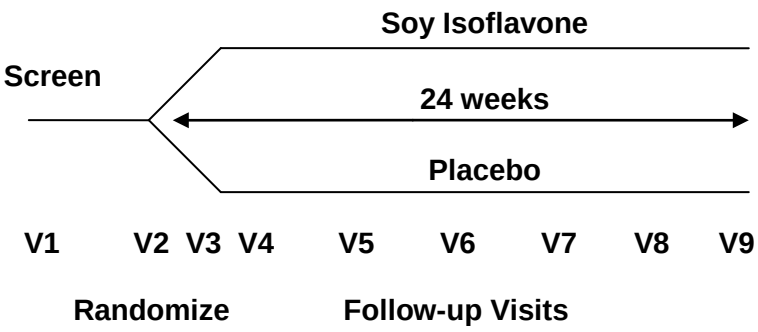
In contrast to the background dietary intake of antioxidant vitamins in the United States, soy isoflavone intake is very low. Although dietary intervention with soy foods is possible, the composition of specific soy foods varies substantially (25) and food supplements typically produce alterations in caloric intake that are difficult to control. An alternative approach is to provide soy isoflavone supplements. This makes it possible to systematically test the effect of soy isoflavone consumption on asthma severity.

Although multiple therapies are available to treat patients with asthma, there are problems with each one. As a result, new therapies are needed that are safe, effective, relatively inexpensive, likely to be used by patients with asthma and may have beneficial effects beyond asthma.

Given the magnitude of the problem, the available data, the association between soy genistein intake and asthma severity, recent in vitro mechanistic data, the remarkably low intake of soy isoflavones in our asthma population and the excellent safety profile and low cost of soy isoflavone supplements, we believe there is compelling rationale to investigate the effect of soy isoflavone supplements in patients with symptomatic asthma.

2. Study Design

The study is designed as a multi-site, randomized, double-masked, placebo-controlled, parallel group clinical trial. Three hundred and eighty (380) adults and children 12 years of age or older with symptomatic asthma will be randomly assigned to one of two treatment groups: oral isoflavone supplement (100 mg/day) or oral placebo for six months.



The study will include 9 visits. Visit 1 is the screening visit, visit 2 is the randomization visit, V3 is an interim phone visit after 2 weeks and visits 4-9 are data collection visits at 4 week intervals. The time from randomization to completion is 24 weeks.

2.1 Study Treatment

There will be two parallel treatment groups:

- Soy supplements (Novasoy®) that contain 50 mg of isoflavones (genistein, daidzein and glycitein), twice daily
- Matching placebo, twice daily

2.2 Hypothesis

The primary hypothesis is that patients with symptomatic asthma have improved lung function when treated with a soy isoflavone dietary supplement. Change in pre-bronchodilator FEV_1 allows us to evaluate a physiologic effect on lung function. The trial is designed with a conservative type 1 error rate of 2.5% to allow more robust analyses of secondary outcomes such as change in eNO and Asthma Control Score.

2.3 Eligibility Criteria

The general goal of patient selection is to enroll adults and children with poor (e.g., inadequate) asthma control whom physicians would consider need a change in or additional therapy to improve asthma control. Although a beneficial effect is most likely to be seen in those with little or no intake of soy foods, the published literature and our data indicate that intake is very low in the population we will be studying. Therefore, we will only exclude individuals who are currently taking soy isoflavone supplements or are consuming soy or soy-enriched foods (e.g., soy milk, tofu) at least once each week.

2.3.1 Inclusion Criteria

Age:

- Age 12 or older

Asthma

- Physician diagnosed asthma
- FEV_1 equal or greater than 50% predicted pre-bronchodilator

- At least 12% increase in FEV_1 15-30 minutes after inhaling 2-4 puffs of albuterol or positive methacholine challenge (20% fall in FEV_1 at less than 16 mg/mL) – either of these can be available from the previous 2 years
- Currently prescribed daily controller asthma medication(s) (e.g., inhaled corticosteroids and/or leukotriene modifier)

Poor asthma control (at least one of the following)

- A score of 19 or less on the Asthma Control Test (ACT)
- Use of beta-agonist for asthma symptoms two or more times per week
- Nocturnal awakening with asthma symptoms more than once per week
- Two or more episodes of asthma symptoms in the past 12 months with each requiring at least one of the following: emergency department visit, unscheduled physician visit, prednisone course, hospitalization

Smoking status

- Non-smoker for 6 months or longer
- Less than 10 pack-year smoking history

2.3.2 Exclusion Criteria

Pulmonary function

- FEV_1 less than 50% predicted pre-bronchodilator

Other major chronic illnesses

- Conditions which in the judgment of the study physician would interfere with participation in the study, e.g., non-skin cancer, endocrine disease including insulin-dependent diabetes mellitus, coronary artery disease, congestive heart failure, stroke, severe hypertension, renal failure, liver disorders, malabsorption disorders, immunodeficiency states, major neuropsychiatric disorder
- Active thyroid disease
- History of breast cancer, ovarian, or endometrial cancer
- History of physician diagnosis of chronic bronchitis, emphysema or COPD

Medication use

- Current consumption of soy isoflavone supplements
- Oral corticosteroid use within the past 6 weeks
- Use of tamoxifen
- Use of an investigational treatment in the previous 30 days

“Drug” allergy

- Known adverse reaction to genistein, other phytoestrogens, or soy products

Females of childbearing potential

- Pregnant or lactating – participants of appropriate age who might be pregnant at the time of enrollment will be screened and cannot participate if pregnant. Participants must agree to use effective contraception during the trial.

Non-adherence

- Inability or unwillingness to provide consent or, in the case of children, inability or unwillingness of the child to provide assent
- Inability to swallow study medication
- Inability to perform baseline measurements
- Completion of less than 10 of the last 14 days diary entries during screening period
- Inability to be contacted by telephone
- Intention to move out of the area within 6 months

Other

- Recent asthma exacerbation (within 6 weeks)
- Recent upper respiratory infection (within 2 weeks)
- Body weight less than 77 pounds (35 kg)
- Intake of soy or soy-enriched foods 1 or more times a week
- Change in diet over the past month or expected change in diet (e.g., will initiate weight loss diet) during the study

2.4 Outcomes

2.4.1 Primary Outcome

FEV_1 will be assessed by forced expiratory spirometry, using the Koko Spirometer (Ferris Respiratory, Louisville, CO), before and after two inhalations of albuterol. Pre- and post-BD FEV_1 will be measured at baseline and at every follow-up visit. Spirometry will be done according to ATS standards (26). The largest value at each session will be the study value.

2.4.2 Secondary and Tertiary Outcomes

- Exhaled Nitric Oxide (eNO), a measure of airway inflammation, will be measured at baseline and every follow-up visit using the Insight eNO System (Apieron, Menlo Park, California) according to the recommendations of the manufacturer, American Thoracic Society and European Respiratory Society (27)*. A small number of clinics (about 6-7) will perform a substudy and compare results using the Insight with results using the NIOX MINO monitor (Aerocrine, New Providence, NJ)*

- Asthma control will be measured with the Asthma Control Test (ACT) administered at each clinic visit. This instrument, which has been validated for ages 12-84 years, is a 4-week recall questionnaire that addresses issues of asthma control, symptoms, and nocturnal awakenings (28).

- Exhaled Breath Condensate (EBC) samples will be collected at baseline, 4 weeks and 24 weeks using the Rtube® (Respiratory Research, Charlottesville, VA) following published guidelines (29). Participants will wear nose clips and breathe comfortably at tidal volume for ten minutes into the Rtube chilled at -20°C with an aluminum sleeve. The typical condensate yield ranges from 700 to 1200 μl in children with asthma to up to 2000 μl in adults. The condensate sample will be placed on ice immediately after collection, 100 and 200 μl aliquots removed, labeled in cryotubes, and stored at -70°C until 8-isoprostane measurements are made. 8-isoprostane will be measured by EIA (Cayman Chemicals, Ann Arbor, MI) using a plate reader.

- Asthma diaries will be completed by participants throughout the study to record daily morning peak expiratory flow (PEF), daily asthma symptom scores, beta-agonist use, nocturnal asthma awakenings, asthma treatments and health care use.

- Episodes of poor asthma control (EPAC), which are determined from the daily asthma diaries as well as the interval asthma history obtained at each follow-up visit. They are defined by one or more of the following:
  - Decrease of > 30% in morning Peak Expiratory Flow (PEF) (from personal best) for 2 consecutive days (definite yellow zone event), OR
  - Addition of oral corticosteroid (e.g., prednisone) to treat asthma symptoms, OR

- Unscheduled contact with a health care provider (ED, physician office, hospital) for asthma symptoms, OR
- Increased use of bronchodilator rescue medication over baseline by 4 or more puffs of metered dose inhaler or 2 or more nebulizer treatments on one day.

- The Asthma Symptom Utility Index (ASUI) is a validated 2-week recall questionnaire that will be completed at all clinic visits (30).

- Asthma specific quality of life will be measured using two instruments – the Marks Asthma Quality of Life Questionnaire (Marks AQLQ), validated for 15 years and older, will be administered for those 17 years and older (31) and the Children's Health Survey for Asthma (CHSA-Child), validated for ages 7-16 years, will be administered to children 12 – 16 years old (32). These questionnaires will be completed at all clinic visits.

- Generic health-related quality of life (QOL) will be measured using two instruments – the Medical Outcomes Study SF36 (33) for those 18 years of age and older and the Child Health Questionnaire (CHQ-PF50) for those less than 18 years old will be completed at the screening visit and the final clinic visits. Both are widely used, have been correlated with asthma outcomes (34).

- Airway reactivity, if needed to establish eligibility, will be measured with methacholine challenge testing, following ATS guidelines using the dosimeter technique (35) before randomization. Five breaths each of doubling concentrations of methacholine (Provocholine™) are inhaled, starting at .03 mg/ml until a 20% or greater fall in FEV_1 occurs or a maximum of 16 mg/ml is inhaled. Results are computed as the logarithmic interpolated concentration that causes a 20% fall (PC_{20}) as well as the slope of the dose-response relationship. This test is contraindicated in participants with an $FEV_1 < 70\%$ of predicted.

  The dosimeter technique was selected over the tidal breathing method because of wider availability of equipment, less environmental contamination and comparability of the results (36). We will use the ATS 10-dose protocol.

- Treatment adherence will be assessed by pill counts at every follow-up clinic visit. Pill counts will not be done in the presence of participants to mitigate "dumping" behavior and therefore allow more accurate assessment of treatment use.

- Side-effects and toxicity are assessed by questionnaire and open-ended questions at each visit. Questions regarding potential estrogenic effect of isoflavone supplements will be asked at each visit. Side-effects that are thought possibly or probably related to study treatment and are considered more than mild (i.e., interfering with daily functioning) will result in temporary discontinuation of study treatment. Serious adverse events (SAE) are reported at any time that an investigator is aware of them.

- Interval history/Health care is recorded at each clinic visit. With participant permission, records of all hospitalizations and, if necessary, deaths are obtained for verification of diagnoses and assessment of safety issues.

- Baseline questionnaires are administered to ascertain demographics, general health, asthma symptoms and menstruation (Asthma in Females Questionnaire), medication use,

diet including soy intake (Block 2005 Food Frequency and Block Soy questionnaires), nasal symptoms, smoke exposure, asthma severity and asthma triggers. These data are collected at the screening and/or baseline visits and are used to determine eligibility, characterize the population, and classify asthma subtypes.

- Closeout questionnaires are administered at the last visit to determine global assessments of treatment, change in dietary intake of soy isoflavones during the study, adequacy of informed consent procedures, satisfaction with study procedures and personnel, effectiveness of the masking procedures and information about participation in future studies.

- Genistein Levels. Blood will be collected at visit 2 (randomization) before receiving any treatment. It will also be collected at visits 4 (after 4 weeks of treatment) and 9 (after 24 weeks of treatment), three hours after the morning dose of placebo or soy isoflavones. The plasma will be sent to Dr. John Lima (Nemours Children's Clinic, Jacksonville, FL) for analysis. Genistein levels will be measured using high performance liquid chromatography (37).

- Peripheral Blood Eosinophil Counts will be performed on whole blood at each site's clinical hematology laboratory at randomization, the 4 week and the 24 week clinic visits.

- Interleukin-6 (IL-6) and C-Reactive Protein (CRP) will be measured in serum from blood collected at randomization, 4 week and 24 weeks visits (V2, V4 and V9, respectively). IL-6 will be measured by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI) using a plate-reader. CRP will be measured by rate turbidometry using anti-CRP antibody coated particles and the IMMAGE Immunochemistry System and Calibratory 5 Plus (Beckman Coulter, Fullerton, CA). These assays will be performed at Northwestern University under the direction of Peter Sporn, MD and Lewis Smith, MD.

- Urinary LTE_4 Levels will be measured in urine collected at randomization, the 4 week and the 24 week clinic visits. This measure of cysteinyl leukotriene synthesis in the lungs of patients with asthma (38, 39) will be assayed using a commercially available enzyme immunoassay kit (Cayman Chemicals, Ann Arbor, MI). The assay has a sensitivity of less than 20 pg/ml. Data quality will be insured by doing duplicate measurements on each sample and re-analyzing 5% of the samples. These assays will be performed at Northwestern University under the direction of Peter Sporn, MD and Lewis Smith, MD,

- Genotyping will be performed on venous blood samples collected at visit 2 (randomization). Dr. John Lima (Nemours Children's Clinic, Jacksonville, FL) laboratory will perform the genotyping assessments. Dr. Lima will extract DNA from 1 mL of packed red cells using the PureGene isolation kit from Gentra (Minneapolis, MN), which should yield 5 to 15 μg of genomic DNA. An ancillary study will be led by Dr. Lima to explore the genetic determinants of the soy isoflavone effects.

2.5 Data Collection Schedule

Study procedures and approximate time commitment from the participants are shown below:

Screening Visit(s) (weeks -4 to -2) (4 hours)

- Explanation of study
- Obtain informed consent (and assent when appropriate)
- Baseline eligibility forms
- Brief physical examination
- Pregnancy test for persons of child-bearing potential
- Spirometry pre- and post-bronchodilator
- Asthma control, asthma and generic QOL, and diet questionnaires (Soy and generic)
- Asthma action plan
- Distribute baseline diary and portable peak flow meter
- Schedule methacholine challenge study, if needed for eligibility

Randomization Visit (week 0) (3 hours)

- Review consent, study expectations
- Review diaries
- Asthma action plan
- Interval medical history
- Pregnancy test for persons of child-bearing potential
- Review eligibility criteria
- Randomization
- Asthma control, QOL, diet, Asthma in Females, Sinus Nasal and Smoke Exposure in Children questionnaires
- Exhaled NO
- Exhaled breath condensate collection
- Spirometry pre- and post-bronchodilator
- Venous blood sample for genistein, systemic inflammatory markers and DNA
- Urine sample for LTE_4
- Adherence and diet counseling
- Treatment distribution
- Establish study visit schedule

Phone Visit (week 2) (15 minutes)

- Reinforce daily diary completion and PEF monitoring
- Adverse event screen
- Confirm first follow-up visit date

Interval visits (weeks 4, 8, 12, 16, 20) (2 hours)

- Return, review asthma diaries
- Asthma control, QOL questionnaires
- Adverse event screen
- Interval asthma/health history
- Exhaled NO
- Exhaled breath condensate collection (week 4)
- Spirometry pre- and post-bronchodilator
- Venous blood sample for genistein and systemic inflammatory markers (week 4)
- Urine sample for LTE_4 (week 4)
- Pill counts, adherence and diet counseling, and treatment distribution

Final visit (week 24) (3 hours)

- Return, review asthma diaries
- Asthma control, QOL and diet questionnaires
- Adverse event screen
- Interval asthma/health history
- Brief physical examination
- Exhaled NO
- Exhaled breath condensate collection
- Spirometry pre- and post-bronchodilator
- Venous blood sample for genistein and systemic inflammatory markers
- Urine sample for LTE_4
- Pill counts
- Close-out questionnaire including global assessments

Table 1: Data Collection Schedule

Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9
Time (wks)	-4 to -2	0	2	4	8	12	16	20	24
Consent, eligibility evaluation	●								
Block Food Frequency Questionnaire		●							●
Block Soy questionnaire	●	●							●
Asthma in Females Questionnaire (FQ)		●							
Sinus Nasal Questionnaire (SI)		●							
Smoke Exposure in Children Questionnaire		●							
Asthma Control (ACT, ASUI)	●	●		●	●	●	●	●	●
Asthma Quality of Life Measures (Marks AQLQ or CHSA-Child)	●	●		●	●	●	●	●	●
SF-36 or CHQ-PF50 screen	●								●
Methacholine challenge*	●								
Brief physical exam	●								●
Pregnancy Testing (if indicated)	●	●							
Randomization		●							
Treatment distribution		●		●	●	●	●	●	
Pill counts / Adherence Counseling				●	●	●	●	●	●
Blood for genotype (save)		●							
CRP, IL-6 levels (blood)		●		●					●
Eosinophil count (blood)		●		●					●
Genistein level (blood)		●		●					●
Instruction in diary, PEF, action plan	●	●	●	●	●	●	●	●	●
Return asthma diary		●		●	●	●	●	●	●
Interval Asthma / Health history		●	●	●	●	●	●	●	●
Adverse event screen			●	●	●	●	●	●	●
Exhaled nitric oxide (eNO)		●		●	●	●	●	●	●
Exhaled breath condensate (EBC)		●		●					●
Spirometry pre- and post-BD	●	●		●	●	●	●	●	●
Urinary LTE_4		●		●					●

*Only if needed to meet eligibility criteria

ACT – Asthma Control Test, ASUI – Asthma Symptom Utility Index, Marks AQLQ – Marks Asthma Quality of Life Questionnaire, CHSA-Child – Children's Health Survey for Asthma-Child version,
PEF – Peak Expiratory Flow, NO – nitric oxide. V# – visit number; V3 – phone visit

3 Study treatment

3.1 Description of Supplement

Soy isoflavone supplements (Novasoy®) and matching placebo will be provided by Archer Daniels Midland (ADM). Each isoflavone tablet contains 50 mg of soy isoflavones (genistein, daidzein and glycitin – approximately 32 mg as the aglycone form). Because the dose of isoflavones that will be used (100 mg) exceeds the level "Generally Recognized as Safe", which is 50 mg per day, the trial will be conducted under an IND (#104204) held by Robert Wise, MD, director of the ACRC Data Coordinating Center.

3.2 Delivery of Supplements

Isoflavone supplements and matching placebo will be packaged in one month supplies with a one-week surplus of tablets so that treatment can be continued if a participant misses or delays a visit. Bottles will be labeled with two-part, tear-off labels. The tear-off label will be affixed to a data collection form and treatment ID data from the label will be keyed so that accuracy of treatment dispensing is verifiable and auditable. Distribution, auditing and maintenance of treatment inventories at the clinical centers including monitoring of expiration dates will be managed as a feature of the data system.

Participants will be provided with a one month supply of treatment at baseline and each interval visit. One soy supplement tablet or matching placebo tablet will be swallowed twice daily. Participants will be instructed to bring the study treatment bottle(s) to each follow-up visit.

3.3 Side Effects of Supplements

Soy isoflavone supplements are available over the counter and are well tolerated. Data from previously published studies using various soy isoflavone preparations indicate no increase in adverse events over those experienced by the control or placebo group. In two studies in which soy isoflavones (100 mg/day) were given for a year to post-menopausal women (40, 41), adverse events were the same as those in the control group. Several single dose pharmacokinetic studies have been performed using higher doses of soy isoflavones and genistein (42, 43). Even at the high doses used in these studies (up to 16 mg/kg), there is only one report of a treatment-related skin rash in a patient with cancer (43).

Soy isoflavones are phytoestrogens (plant estrogens). They are structurally similar to 17β-estradiol, but with relatively weak estrogen-like effects. They may exert agonist or antagonist effects on various estrogen target tissues, acting as selective estrogen receptor modulators. Because of their estrogen-like effects, there has been concern that they could increase the risk of breast and endometrial cancer. However, the available data support a protective effect of soy isoflavones on a number of malignancies including breast cancer (44, 45) and endometrial cancer (46, 47). As a result we do not believe there is a need to exclude women with a family history of either breast or endometrial cancer. Individuals with previous or current breast, ovarian or endometrial cancer will not be enrolled since individuals with any non-skin cancer are excluded from participating in the study. Those taking Tamoxifen will also be excluded from participation. Because of a theoretical effect of soy isoflavones on thyroid hormone transport, we will exclude individuals who have a history of thyroid disease.

In addition, studies in young girls (48), healthy young men (49, 50), healthy young females (51, 52), and post-menopausal women (41, 53-55) have failed to identify consistent effects of soy isoflavones, in doses comparable to those we propose, on sex hormone levels, endothelial thickness, vaginal epithelium, sperm counts, and post-menopausal symptoms. One study has

identified a small increase in menstrual cycle length (51). In the interval health history we will include specific questions to monitor symptoms possibly related to estrogenic effects of isoflavone supplements: breast swelling and tenderness, and menstrual symptoms.

Little information is available on drug or food interactions with soy isoflavone supplements. To minimize potential interactions, all participants will be instructed to take the study treatment (soy isoflavone or placebo tablet) at the same time of day and without food. In addition, as they will not have other unstable medical conditions, there should be few instances where other medications are changed.

Adverse reactions to soy isoflavones will be minimized by monitoring side-effects at clinic visits. If possible side-effects become more than mild, study treatment will be discontinued, and this is recorded as a study outcome event for secondary analyses. If it is considered probable that the side-effects were the result of the treatment, study treatment will not be re-started. If it is considered possible or unlikely that the side-effects are the result of the study treatment, then the treatment will be re-started after the side-effects subside. If the same side-effects recur, the participant will be taken off the study treatment. For severe adverse events thought to be possibly or probably related to study treatment, the treatment will be withdrawn for the duration of the study.

3.4 Randomization

Participants will be randomly assigned with an equal allocation to receive either oral isoflavone supplement (100 mg/day) or oral placebo for 6 months. The treatment assignment will be double-masked, neither the participant nor the clinical center investigators will be informed of the treatment group. Randomization will be accomplished with an auditable, documented generation scheme that produces a reproducible order of assignment. Randomization will be stratified by center and each clinical center randomization schedule will be based on randomly permuted blocks of varying size to ensure appropriate balance between the treatment groups within a center. The randomization scheme will be written and controlled by the Data Coordinating Center (DCC).

Randomization will be carried out at Visit 2, after all eligibility criteria have been checked, the participant has signed consent for the study and baseline assessments are completed. An eligibility form will be entered into the web-based online data system. After the data system verifies eligibility, the participant will be assigned a multi-digit medication ID number. The participant will only receive study treatment labeled with that ID number throughout the trial. All interactions with the treatment assignment database will be logged. Only the drug distribution center and selected DCC personnel will know the actual treatment assignments.

3.5 Unmasking

Unmasking of the clinic or the participant to the treatment assignment may be required in some situations. If the participant becomes acutely ill and the treating physician requires knowledge of the container contents, the treatment assignment may be revealed. This may be accomplished in one of two ways, with the preference given to the first. 1) When the clinic learns of a request for unmasking from a participant or a participant's physician or a hospital emergency room, a phone call to request unmasking is made to the DCC. The DCC may then contact the participant or the physician directly to reveal the treatment assignment. Thus in some cases partial unmasking may occur, with the clinic staff remaining unaware of the treatment. In other cases the participant's physician may be told of the treatment assignment while both the clinic staff and participant remains masked. 2) If the DCC cannot be contacted

in an emergency, clinic personnel may unmask the treatment assignment by opening the tear-off label in the participant's folder. All instances of unmasking will be recorded and submitted to the Monitoring Board for review.

We anticipate that in most acute illnesses, knowledge of the treatment assignment would not be required for treatment. If necessary, the assigned treatment could be stopped until the emergency passes and re-instituted later.

3.6 Asthma Treatment

All participants are required to be taking an asthma controller medication daily in order to be eligible for the study (section 2.3.1). Participants will be asked to maintain the same controller medication regimen throughout the study. However, if a participant experiences an asthma exacerbation or other signs of significant worsening of asthma, the participant will be referred to their primary asthma care provider for re-evaluation of their asthma medication regimen. If the asthma care provider is not available in a timely fashion, the clinical center physician will evaluate the participant's treatment regimen and make recommendations for the future course. There are no restrictions on rescue medications and clinic personnel will ensure that all participants have a supply of rescue medications at the V2 visit.

4 Statistical Design

4.1 General Statistical Approach

Statistical analyses for all the primary evaluations of treatment effects will follow the intention-to-treat paradigm, which means that all randomized patients will be included in the treatment group to which they were assigned. Any randomized participant who does not have the requisite data on lung function will be accounted for and compared by assigned treatment group. Participants not able to be included in the intention-to-treat analyses will be compared to those who are included with respect to demographic and other characteristics.

4.2 Primary Analysis

The primary outcome for the trial is change from baseline to 24 weeks in pre-bronchodilator FEV_1, which was used in the sample size estimation as discussed below. The primary analysis will be performed using a linear mixed effects model of the change measure at 24 weeks unadjusted for additional covariates. The fixed effects will include indicator variables for treatment group (placebo = 0, treatment = 1) and visit time (baseline = 0, 24 weeks = 1). The random effects include random intercepts for clinics as well as an adjustment for the correlation between baseline and follow-up measures 56).

More powerful linear mixed effects modeling incorporating all available longitudinal patient data on FEV_1 will also be performed as a secondary analysis. Such models allow estimation of the rate of change in FEV_1 from baseline. These can be either constant or time dependant depending upon the inclusion of a single time covariate, multiple time point covariates or a function of time (e.g. quadratic). In addition, flexible covariance structures can be constructed via the random effects terms to allow for non-constant relationships between repeated measures (e.g. time dependent random effects – i.e. random slopes). Subgroup analyses, pre-specified below, will rely on the same approach by adding appropriate subgroup and treatment group interaction terms into the models.

4.3 Subgroup Analyses

Potential confounding and interaction will be evaluated by examining the effect of adjustments for baseline measures including demographic descriptor, intake of soy, use of non-soy

supplements, intake of Vitamin C, concurrent asthma treatment including use of leukotriene modifier medications, clinic, asthma severity, menstrual status, and body mass index on the estimated treatment effect. In addition, analysis by treatment adherence status will utilize interim outcomes to evaluate efficacy of the treatment in an unbiased manner. Of note, available data suggest that there are no substantial gender differences or differences between pre- and post-menopausal women when soy isoflavones are ingested (57, 58)

Treatment effects will be examined across subgroups by testing for interaction of the subgroup indicator variable with treatment group assignment. These tests usually have low power but are hypothesis generating and may inform interpretation of results. Additional power for subgroup effects will be obtained by using longitudinal data analysis with appropriate interaction terms, as described above.

4.4 Secondary Outcomes

Secondary outcomes for the SOYA trial includes change in eNO, Asthma Control Test score (ACT), EBC, EPAC rates, quality of life scores, PEF and physiological measures of pulmonary function as assessed by spirometry, and systemic markers of inflammation. In general, analyses for outcomes related to secondary hypotheses will follow the same analytic approach proposed for the primary outcome. Rates of EPACS will be evaluated using negative binomial models with random intercepts for clinics and individuals within clinics (56, 59). Binary outcomes such as occurrence of one or more EPAC and adverse events will be analyzed using logistic regression also with random intercepts for clinics and individuals within clinics.

A large number of comparisons are planned for the secondary outcomes and caution is needed in the reporting the interpretation of the results. We have selected eNO and ACT as most important of these outcomes because eNO serves as a biological marker of a treatment effect and ACT will allow us to estimate the clinical impact of treatment. For interpretation of the treatment effect on these two outcomes, we will use a Bonferroni correction for the 2-sided type I error rate of 0.025 (see section 4.7). Our primary focus for analysis of other outcomes such as C-reactive protein will be on the parameter estimates and confidence intervals as opposed to p-values as recommended by Wang et al (58). In addition, we will include a calculation of the expected number of false positives when reporting our results in order to guide interpretation. Several method of adjusting p-values for multiple comparisons exist, however no clear consensus as to the most appropriate method is available. Techniques such as those suggested by Wang et al and Lagakos will be considered (58, 59).

4.5 Adherence Analysis

Data from daily diaries, pill counts and blood levels of genistein, measured at 4 and 24 weeks, will be used to evaluate adherence to the treatment regimens. Evaluation of treatment effects in subgroups of adherent versus non-adherent participates will be performed. These analyses will also utilize time-dependent covariates to characterize adherence. Each participant's overall diet and dietary soy intake at baseline and at the end of the study will be analyzed using the Block Food Frequency and Soy Questionnaires to assess stability of diet and nutrient intake during the study. We will perform subgroup analyses to assess the effects of adherence or dietary change on the overall treatment effect.

4.6 Missing Data

Chi-squared and t-tests (or Kruskall-Wallis tests if appropriate) will be used to identify imbalances in the baseline covariates and treatment groups between individuals with and

without missing data. However, it is not possible to determine whether of not the data are MAR, MCAR or NMAR.

We assume ignorable non-response, which implies that the probability of missing data depends only upon the observed data, both covariates and previously observed response variable measurements. This falls under the definition of missing at random (MAR) provided by Rubin (60) and is less stringent than the requirement of missing completely at random (MCAR), which is the underlying assumption made when observations are omitted. One advantage of the linear mixed effects model is that, unlike GEEs, it can incorporate individuals with missing data and provide valid inference in the presence of ignorable non-response (61).

A variety of imputation techniques will be used to perform sensitivity analyses to compare with the primary analysis based upon linear mixed effects models. Simple techniques such as incorporating pessimistic and optimistic imputations will be included to assess the range of possible estimates. However, the primary focus will be multiple imputation which yields valid statistical inference in the presence of missing data. In essence multiple imputation acknowledges the uncertainty due to missing data, instead of simply ignoring it. Several complete data sets will be generated from modeled probability distributions over the missing observations given the observed data. The estimates from these generated data sets will be combined using the techniques presented in Rubin and Little (62). The uncertainty in the model parameter estimates incorporates the standard errors of the parameter estimates as well as the variability between the parameter estimates from the replicate data sets. A few statistical methods are available when there are non-ignorable missing data and these would be employed; however, all such methods involve strong assumptions that cannot be verified from the available data.

4.7 Justification of Sample Size

The study has 80% power to detect a difference in the change in pre-bronchodilator FEV_1 of 134 mL or greater (Table 2). Based on the excellent safety profile and low cost of the soy isoflavone supplements relative to current treatments, the consensus of the investigators is that an effect size of 5% or greater in the population mean FEV_1 would justify daily use of soy isoflavone supplements in patients with asthma. However, the effect on FEV_1 was not replicated in the small (n = 13) pilot study. In the pilot study, markers of inflammation, both airway and systemic, improved after consumption of genistein. Accordingly, we elected to use a smaller type 1 error rate, 2.5%, for sample size estimation. Furthermore, this strategy will allow us to evaluate treatment effects on the outcomes of eNO and ACT with more confidence. With a total sample size of 380 participants and a cumulative type 1 error rate of 2.5%, the study has 80% power to detect a 4 to 5% change in percent predicted FEV_1. In addition, the study is also reasonably powered to detect clinically meaningful differences for change in eNO and asthma control scores.

Table 2: Detectable treatment effects for total sample size *of 380* (Power=80%)

Outcome Measure	Standard Deviation	Detectable Treatment Effect
Primary Outcome		
FEV_1*	400 mL	134 mL
Secondary Outcomes		
eNO†	20 ppb	7 ppb
Asthma Control Score†	1.0	0.4

*Type 1, 2-sided error rate of 2.5%
†Type 1, 2-sided error rate of 1.25%.

Power to detect subgroup effects with formal test of interactions will be low. Hence, we will view these analyses as hypothesis generating. Subgroups of interest will include those defined by primary treatment for asthma, e.g., inhaled corticosteroids or leukotriene antagonists, isoflavone intake at baseline, intake of Vitamin C at baseline, asthma severity (as measured by percent predicted FEV_1) and demographic characteristics.

Calculations were performed based on t-tests using the PS Power package (63) for continuous and dichotomous measures. Detectable treatment effects were estimated for 80% power with a cumulative type 1 error rate of 2.5%; which is adjusted for 2 interim looks (see section 5.8). The sample size was increased by 10% to account for missing data and lost-to-follow-up.

4.8 Data and Safety Monitoring

The standing DSMB for ACRC network will be employed to review the SOYA trial. The Board includes nationally recognized experts in adult and pediatric asthma, clinical trials, and biostatistics.

The DSMB will meet twice a year, usually by teleconference, to review data from SOYA and other ongoing ACRC trials. The DSMB is also responsible for review of related issues, such as center performance standards or recruitment incentives. The ALA Scientific Review Group has established criteria for the DSMB to use to evaluate the performance of clinical centers. The DSMB may request more frequent meetings if necessary to fulfill its charge. It may also request additional safety reports on a more frequent basis. After each meeting, the DSMB will make formal recommendations regarding trial continuation and clinical performance. The DSMB recommendations will be submitted to participating center's IRB's.

The DSMB will review two planned interim analyses of the primary outcome measures. O'Brien-Fleming statistical stopping guidelines for efficacy will apply (64). These interim efficacy analyses will occur when approximately 25% and 50% of the data are complete or when approximately 100 and 190 patients have completed the follow-up, respectively. The absolute value of the boundary z-values are 4.86 ($P<0.0001$) and 3.34 ($P<0.0004$), respectively. The final analysis will have a critical z-value of +/-2.24, which corresponds to a two-sided, type 1 error rate of 2.5%.

A special responsibility of the DSMB is to review serious adverse events (SAEs), as defined by deaths, life threatening conditions, hospital admissions, adverse pregnancy outcomes or events requiring permanent discontinuation of the treatment. SAEs are to be reported to the DCC within 72 hours of notice, with follow up reporting until the event has terminated. SAEs will be reported to the Chairman of the DSMB or other designee in a timely fashion for review. This initial review will determine whether there is a recommendation for a change in the

protocol or halt to the study until such time as the matter can be considered by a quorum of the DSMB. In addition, the DCC will distribute reports of SAEs from one center to all of the centers for review by the local IRBs.

5 Protection of Human Subjects

5.1 Risks to the Participants

We expect the eighteen clinical centers will enroll 380 children and adults ages 12 years and up, of either sex who have poorly controlled asthma on at least one controller medication such as an inhaled corticosteroid and/or leukotriene modifier. The risks associated with the supplement were described in section 3.3 of this protocol

Pregnant women will be excluded from the study because of the uncertain effect soy isoflavones, which are phytoestrogens, may have on the fetus. In the event that a participant becomes pregnant during the study, she will be withdrawn from the study treatment and referred to her primary physician for further evaluation and management. The perinatal outcomes of any pregnancies will be reported. This will be considered an adverse event and reported to the DCC, DSMB, IRB and the manufacturer.

Overall, the study has few serious risks. The biggest risk is enrolling subjects with poorly (inadequately) controlled asthma. All study sites and investigators have experience enrolling and managing comparable subjects in asthma trials. Risks are minimized by 1) only enrolling individuals who at baseline are already receiving treatment with a controller medicine (e.g., inhaled corticosteroid, leukotriene modifier) and have stable disease as defined by the inclusion and exclusion criteria, 2) having a 2-week run-in period to confirm stability, 3) providing participants with some degree of baseline asthma education, 4) having frequent clinic and phone visits, and 5) giving them an asthma action plan that is individualized for the person's peak flow measurements and emergency treatment for study related issues. In addition, the investigators will not provide routine oversight of asthma medications. This is done to avoid conflict between the investigator and caretaker roles and to avoid bias in the outcome measures that rely on treatment decisions.

The asthma action plan is comprehensive and includes a temporary asthma action plan with instructions for its use at Visit 1. The "red", "yellow" and "green" zones will be based on peak flow readings at Visit 1. At Visit 2 the asthma action plan will be modified based on the participant's personal best peak flow readings over the last 14 days as recorded on the Diary Card. A computer system will calculate and print out new color zone cut-offs. The zones are the following: "green" (>80% of baseline personal best), "yellow" (50-79% of baseline personal best), or "red" (less than 50% of baseline personal best). The Asthma Action Card is the size of a credit card and has been used successfully in several previous ALA-ACRC asthma trials. The card instructs participants to seek medical attention immediately if they are experiencing a serious asthma attack. Participants who experience less serious asthma symptoms or a drop in peak flow into the yellow or red zone are instructed to take 2 puffs of their rescue inhaler and to wait 15 to 20 minutes. If symptoms persist or peak flow does not return to the green zone, they are instructed to take 2 more puffs of the inhaler and wait 15 to 20 minutes up to 2 more times. If after a total of 3 administrations of the rescue inhaler, symptoms persist or peak flow is not in the green zone, participants are instructed to seek medical care immediately.

Participants will undergo spirometry at each clinic visit. Occasionally individuals complain of light-headedness during the procedure. The risk of syncope in this study is minimized by having participants sit during the procedure.

Some participants will undergo methacholine challenge testing during screening to determine eligibility. This procedure involves the inhalation of progressively increased doses of methacholine to induce bronchoconstriction. This test has the potential of inducing a symptomatic asthma attack. The potential is minimized by starting with a diluent control followed by very low concentrations of the agent and gradually increasing the inhaled dose. The risk is minimized by monitoring spirometry after each concentration and stopping if the FEV_1 decreases by 20% or more. The risk of a serious reaction is minimized by treating the participants with inhaled albuterol, a bronchodilator, at the end of the test if they have more than a 10% decrease in FEV_1 and not permitting them to leave the testing area until their lung function improves to within the normal range or the pre-challenge value. Emergency medical equipment, including oxygen, bronchodilators, and atropine (a specific antidote), will be readily available.

Participants with a baseline FEV_1 less than 70% of predicted will not undergo methacholine challenge testing. Other absolute contraindications to methacholine challenge testing are any known sensitivity to methacholine chloride or other parasympathomimetics, or uncontrolled hypertension (systolic BP > 200 mm Hg, diastolic BP > 100 mm Hg). Relative contraindications include an inability to perform acceptable quality spirometry, pregnancy or nursing mothers. To minimize the risk of administering methacholine inappropriately, participants will undergo measurement of blood pressure and spirometry on the day of methacholine challenge testing. A physician must be available to treat any adverse response to methacholine challenge testing, which can only be done in a clinically secure area. Overall, the risk of methacholine challenge testing is low (65, 66). In the CAMP study, more than 1000 methacholine challenge studies were done in children with asthma without serious incident. In early studies, approximately 0.4% of participants had a drop in FEV_1 more than 50% from baseline, but in no case was it irreversible or led to hospitalization or emergency department treatment. When asked, about 25% of adult participants report cough or dyspnea and 10% report wheezing after methacholine challenge (66). The ALA-ACRC network has used this protocol to perform nearly 700 methacholine challenge studies in children or adults without serious adverse events.

Participants in this study will undergo serial measurements of exhaled nitric oxide (eNO) and exhaled breath condensate (EBC). The risks of these tests are minimal. Collection of exhaled NO requires that the participant, while seated, inhale slowly to total lung capacity and then exhale slowly against a resistance for 10 seconds. Both children and adults are able to perform this maneuver easily and without any complications. Collection of EBC requires that the participant breathe for 10 minutes into a low-resistance collection system. Individuals infrequently complain of fatigue while doing this test and often stop to take a break from the collection procedure. Such breaks do not have an important effect on the volume of breath condensate collected and thus are not discouraged. To minimize discomfort associated with these two procedures, a trained technician is present to both encourage and coach the participant.

Three blood samples will be drawn during the study (visits 2, 4 and 9). A maximum total volume of 100 ml will be obtained over 6 months – 40 ml at visit 2, 30 ml at visit 4 and 30 ml at visit 9. The risks of venipuncture include anxiety, brief pain during the needle insertion, local bruising at the collection site and infection. All participating clinics have personnel who are experienced performing venipuncture in adults and/or children. Because only a small volume of blood will be obtained, the risk of anemia is negligible.

A blood sample will be obtained for DNA. Although not proposed in this study, we anticipate submitting in the future an ancillary study exploring genetic determinants of genistein

absorption and effects. The risks of DNA analysis are primarily related to the possibility that an outside agency not approved by the participant might obtain and use the information in such a way that it would harm the participant. To minimize this risk, DNA samples will be identified only by a numeric identifier, and no genetic analysis will be performed other than that associated with genistein effect, asthma and airway diseases. These results will not be a part of a participant's general medical record. Genetic information or other information obtained from participants will not be supplied to any outside agency except as authorized by participants. Genetic information will be shared with other investigators in the network through study ID codes and not by name. Genetic results may be presented in publications and meetings but individual names will not be identified. We will advise participants that the U.S. Department of Health and Human Services has the right to inspect medical records relating to this research for the purposes of verifying data. Demographic information on the subjects is released only to characterize populations for the National Institutes of Health. Where data are shared with other research entities, it will comply with the HIPAA definition of a limited dataset, and appropriate IRB approvals and waivers will be obtained.

Three spot urine samples will be collected during the study (visits 2, 4 and 9). There are no risks associated with this procedure.

Each participant will complete questionnaires of health status and quality of life. There is always the risk that information from a study can be disseminated in ways that can risk the privacy of a person with attendant social and occupational harm. However, this risk is low for a common condition like asthma. Care, however, will be taken to ensure confidentiality. All transmission of data to the coordinating center for analysis is by study ID code only. Publication of results will involve aggregate data only so that individual participants cannot be identified. On occasion it may be necessary, for legal reasons, or for good clinical practice, for third parties such as the FDA, IRB or DSMB members to review medical records that are identified by name. This is not a common occurrence, and every effort will be made by the investigators to maintain confidentiality during such audits.

5.2 Recruitment and Consent Procedures

Participants will be recruited by each participating clinic by their own methods. These methods may include local American Lung Association campaigns, solicitation in physician offices, clinics, workplaces, schools and public media advertisements. All public advertisements are subject to approval by the local IRB and must indicate that it is a research study. The DCC will help coordinate recruitment among clinics and promote sharing of effective recruitment strategies within the network. The trial will be registered on www.ClinicalTrials.gov.

Potential enrollees and their legal guardian (for pediatric participants) will be approached either in person, by telephone or by mail to establish general eligibility criteria. A general description of the study, including frequently asked questions and a consent form will be provided prior to their initial screening visit. If the potential enrollees are interested in attending a screening visit, they will meet with the study coordinator and the local physician co-investigators to review the study and answer questions. They will be asked to sign consent, and if appropriate provide assent, and undergo the screening procedures at that visit. At the same visit, if they still qualify but need a methacholine test to meet eligibility criteria, that test will be scheduled prior to the randomization visit.

The consent form will be subject to approval by the clinical center IRB. A copy of the consent form will be given to each participant, and the signed original will be kept in the participant's research chart. Individual consent for the methacholine challenge test and genotyping may be required depending upon institutional requirements.

Subjects 12 to 17 years of age may also need to provide assent according to local IRB policies. The legal guardians of participants in this age range must also sign the consent form.

5.3 Inclusion of Children

Children twelve to twenty years of age will be included in this study. The ACRC network has sites that serve predominantly adults, predominantly children and a combination of the two. The net effect is the ability to enroll research participants from a wide age range, including large numbers of children. Those centers that enroll children are identified as pediatric specialty clinics within the ACRC network. Each of them maintains staff, facilities and laboratory support oriented towards children. The participating clinic directors and staff provide ongoing asthma care to pediatric patients, and have experience in the design, conduct and analysis of clinical trials in children. Most are affiliated with children's hospitals.

The lower age limit of children (twelve years) included in this study is based on the available data regarding the intake of soy isoflavones. Some human infants who consume soy based formulas ingest up to 4-8 mg/kg body weight (67). The total daily soy isoflavone dose of 100 mg (about 50% of which is genistein), permits us to enroll individuals weighing more than 25 kg. We decided to err on the conservative side by choosing a lower weight limit of 35 kg (77 pounds). Most children 12 years of age or older will exceed this weight limit.

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