

ASTHMA CLINICAL RESEARCH NETWORK

Tiotropium Bromide as an Alternative to Increased Inhaled Corticosteroid in Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroid (TALC)

**Study Protocol
Version 28.0
June 9, 2009**

A study to determine if tiotropium bromide/1x inhaled corticosteroid is superior to twice the dose (2x) of an inhaled corticosteroid in patients inadequately controlled on a lower dose (1x) of inhaled corticosteroid.

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I. PROPOSED TRIAL

We propose an exploratory 52 week, randomized, double-blind, three-period cross-over trial to determine if the addition of tiotropium bromide is superior to doubling the dose of an inhaled corticosteroid in patients with moderately severe asthma who are not adequately controlled on a lower dose of inhaled corticosteroid alone (primary exploratory research hypothesis). We further propose to determine if the addition of tiotropium bromide is not inferior to the addition of a long-acting beta-agonist in patients with moderately severe asthma who are not adequately controlled on a lower dose of inhaled corticosteroid alone (secondary exploratory research hypothesis).

II. HYPOTHESES TO BE TESTED IN THIS TRIAL

A. Primary Research Hypothesis (Exploratory)

In patients with moderately severe asthma not well controlled on an inhaled corticosteroid alone, addition of tiotropium bromide (tiotropium bromide/1xICS) is superior to doubling the dose of an inhaled corticosteroid (2xICS) in terms of improvement in pulmonary function, specifically AM peak expiratory flow (AM PEF).

B. Additional Endpoints for the Primary Research Hypothesis

In patients with moderately severe asthma not well controlled on an inhaled corticosteroid alone, addition of tiotropium bromide is superior to doubling the dose of inhaled corticosteroid in terms of the following outcomes:

1. Additional Physiological Variables

- a. FEV₁

2. Indices of Asthma Control and Quality of Life

- a. Asthma symptoms
- b. Asthma-control days (Days without any asthma symptoms or rescue albuterol use (except for preventative treatment for exercise))
- c. Rescue inhaler use
- d. Asthma control assessed by the Asthma Control Questionnaire (ACQ)
- e. Asthma symptoms as assessed using the Asthma Symptom Utility Index (ASUI)
- f. Asthma-specific quality of life (AQLQ) and coordinator and patient preference for study medications (CPQ)
- g. Asthma exacerbations (Increased asthma symptoms which result in use of oral corticosteroids, increased inhaled corticosteroids, or additional medications for asthma. We will also enumerate hospitalizations, ER visits, and unscheduled visits to a medical provider.)

- h. Good Asthma Control defined by a composite index that takes into account measures of pulmonary function, asthma symptoms, rescue beta-agonist use, need for additional asthma medications, and asthma exacerbations, similar to that used in the GOAL (Gaining Optimal Asthma Control) study (Bateman 2004)

3. Biomarkers of Inflammation

- a. Exhaled nitric oxide (eNO)
- b. Markers of oxidative stress (e.g. de-aerated pH) in exhaled breath condensates (eBC)
- c. Markers of inflammation (e.g. cysteinyl leukotrienes) and other biological markers in exhaled breath condensates (eBC)
- d. Sputum eosinophilia/eosinophil cationic protein (ECP)

C. Secondary Research Hypothesis (Exploratory)

In patients with moderately severe asthma not well controlled on an inhaled corticosteroid alone, addition of tiotropium bromide (tiotropium bromide/1xICS) is not inferior to the addition of a long-acting beta agonist (long-acting beta agonist/1xICS) in terms of improvement in pulmonary function, specifically AM peak expiratory flow (AM PEF).

D. Additional Endpoints for the Secondary Research Hypotheses

In patients with moderately severe asthma not well controlled on an inhaled corticosteroid alone, addition of tiotropium bromide is not inferior to the addition of a long-acting beta agonist in terms of the following outcomes:

1. Additional Physiological Variables

- a. FEV₁

2. Indices of Asthma Control and Quality of Life

- a. Asthma symptoms
- b. Asthma-control days (Days without any asthma symptoms or rescue albuterol use (except for preventative treatment for exercise))
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- g. Asthma exacerbations (Increased asthma symptoms which results in use of oral corticosteroids, increased inhaled corticosteroids, or additional medications for asthma. We will also enumerate

hospitalizations, ER visits, and unscheduled visits to a medical provider.)

- h. Good Asthma Control defined by a composite index that takes into account measures of pulmonary function, asthma symptoms, rescue beta-agonist use, need for additional asthma medications, and asthma exacerbations, similar to that used in the GOAL (Gaining Optimal Asthma Control) study (Bateman 2004)

3. Biomarkers of Inflammation

- a. Exhaled nitric oxide (eNO)
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- d. Sputum eosinophilia/eosinophil cationic protein (ECP)

E. Comparison of Addition of a Long-Acting Beta Agonist to Doubling the Dose of an Inhaled Corticosteroid

The rationale for comparing, in patients inadequately controlled on an inhaled corticosteroid alone, the addition of tiotropium to doubling the dose of the inhaled corticosteroid, stems from observations that the addition of a long-acting beta agonist is superior to doubling the dose of the inhaled corticosteroid (Greening 1994; Woolcock 1996; Shrewsbury 2000; Greenstone 2005). This protocol will also permit comparing the addition of a long-acting beta agonist to doubling the dose of the inhaled corticosteroid. This comparison is not a study endpoint but will be evaluated as a measure of internal study validity in comparison with previously reported comparisons of the addition of a long-acting beta agonist to doubling the dose of the inhaled corticosteroid.

F. Additional Exploratory Research Hypotheses

1. Bronchodilator reversibility with 4 puffs of albuterol will predict that subset of patients who will be able to gain and maintain asthma control with a long-acting beta-agonist. We have reasoned that the major benefit from both a long-acting beta-agonist and an anti-cholinergic agent would be due to their bronchodilator activity. Therefore, we predict that a positive response to a short-acting beta-agonist will be predictive of those patients who respond to the addition of a long-acting beta-agonist controller in this protocol.

2. Bronchodilator reversibility with 4 puffs of ipratropium bromide will predict that subset of patients who will be able to gain and maintain asthma control with tiotropium bromide. We have reasoned that the major benefit from both a long-acting beta-agonist and an anticholinergic agent would be due to their bronchodilator activity. Therefore, we predict that a positive response to a short-acting anticholinergic will be predictive of

those patients who respond to the addition of tiotropium bromide when used as a controller in this protocol.

A lower resting heart rate (lowest quintile versus highest quintile), suggesting greater cholinergic tone, will also predict that subset of patients who will be able to gain and maintain asthma control with tiotropium bromide. This hypothesis stems from previous observations and hypotheses which suggest that greater cholinergic tone, as reflected in a lower resting heart rate, is important in asthma pathogenesis, at least in some asthmatic patients (Nadel 1984; Lemanske 1990).

3. A significant number of asthmatics will gain and maintain asthma control with either a long-acting beta-agonist or tiotropium, but not with both agents. This hypothesis stems from the observations that many patients do not respond to asthma medications, including our most efficacious class of agent, inhaled corticosteroids (Szeffler 2002). In addition, it has been reported that when comparing the response of asthmatics to two different classes of asthma medications (inhaled corticosteroid and a leukotriene receptor antagonist), some patients respond to one medication, some to the other, some to both, and some to neither (Szeffler 2005; Zeiger 2006). Therefore, performing "responder analyses" to determine which patients respond to which class of medication, is an important first step in attempting to predict which asthma medications an individual asthmatic patient will respond to.

4. Asthmatics with specific alterations (single nucleotide polymorphisms and/or haplotypes) in the beta-2 adrenergic receptor, the M₃ muscarinic receptor, and genes in the glucocorticoid pathway will respond positively and negatively to a long-acting beta-agonist, tiotropium bromide and an inhaled corticosteroid. The first exploratory pharmacogenetic hypothesis to be tested states that asthmatics homozygous for arginine at the 16th amino acid position of the beta-2 adrenergic receptor ("arg16arg" patients), will respond better to tiotropium bromide/1xICS than to long-acting beta agonist/1xICS.

III. BACKGROUND AND RATIONALE

A. Need for Controllers in Addition to Long-Acting Beta-Agonists for Use in Combination with Inhaled Corticosteroids

National and international asthma treatment guidelines recommend inhaled corticosteroids as the initial controller therapy for all severities of asthma that warrant daily treatment with a controller (NAEPP 1997; NAEPP 2002; GINA 2005). When treatment with low-moderate doses of inhaled corticosteroids is not sufficient to gain and maintain asthma control, they suggest adding a second controller, rather than escalating the inhaled corticosteroid dose to high levels.

Therapeutic options for an additional controller include a long-acting beta-agonist, a leukotriene modifier, and theophylline. Adding a long-acting beta-agonist to a low-moderate dose of inhaled corticosteroid has been shown to provide better asthma

control than increasing the inhaled steroid dose by a factor of 2 or more in both individual studies (Greening 1994; Woolcock 1996) and meta-analyses (Shrewsbury 2000; Greenstone 2005). Adding either a leukotriene modifier (Price 2003; Ducharme FM 2002) or theophylline (Evans 1997; Ukena 1997) has also been reported to provide benefits. For example, adding a leukotriene modifier has been shown to be approximately equivalent to doubling the dose of an inhaled corticosteroid (Price 2003). Adding theophylline has been shown to provide approximately a 4% additional improvement in percent predicted FEV₁ and FVC when compared with doubling the dose of the inhaled corticosteroid (Evans 1997). Comparing these three interventions, it is generally agreed that adding a long-acting beta-agonist provides the greatest benefit (Nelson 2000; Ringdal 2003; Kankaanranta 2004).

However, it is important to note that long-acting beta-agonists may not be an appropriate choice for all patients. Some patients may have adverse effects with regular beta-agonist use. These adverse effects may range from merely troublesome or annoying, to severe. Minor side effects can include tremor or tachycardia, effects which may occur more frequently at the extremes of age, and which may not require discontinuation of the medication.

However, more severe adverse effects may also occur. Retrospective analyses of the ACRN's SOCS (Lazarus 2001) and SLIC (Lemanske 2001) trials suggest that asthmatics homozygous for arginine at the 16th amino acid position of the beta-2 adrenergic receptor appear to be at increased risk for adverse asthma outcomes when taking salmeterol regularly, whether they are taking salmeterol monotherapy (SOCS trial) or taking salmeterol in combination with an inhaled corticosteroid (SLIC trial) (Wechsler 2006). In addition, analyses of the GlaxoSmithKline SMART trial suggest that some asthmatics might be at increased risk for severe adverse events (severe asthma episodes, death) when taking salmeterol (Knobil 2003; Nelson 2006; Martinez 2005). These concerns were the topic of both an FDA Advisory Board meeting (July 13, 2005) and an FDA Public Health Advisory (November 18, 2005). Therefore, it would appear prudent to explore possible alternatives to long-acting beta-agonists for use in combination with inhaled corticosteroids as additional information about the safety of long-acting beta-agonists in subgroups of asthmatics is being collected and analyzed.

B. Tiotropium Bromide

Whether anticholinergic agents are useful for asthma treatment, and conditions under which they might provide benefit, are not clear. A Cochrane Review of anticholinergic agents for chronic asthma in adults reported that there is "no justification for routinely introducing anticholinergics as part of an add-on treatment for patients whose asthma is not well controlled on standard therapies" (Westby 2004). This review focused almost exclusively on ipratropium bromide, and usually as an add-on agent to the bronchodilator albuterol. The Cochrane Review also concluded that "this does not exclude the possibility that there may be a sub-group of patients who derive some benefit and a trial of treatment in individual patients may still be justified" and that "the role of long term anticholinergics such as tiotropium bromide has yet to be established

in patients with asthma." Consistent with our evolving understanding of the role of anticholinergics in asthma treatment is the conclusion of a recent systematic review with meta-analysis which "strongly suggests that the addition of multiple doses of inhaled ipratropium bromide to β_2 agonists is indicated as the standard treatment in children, adolescents, and adults with moderate to severe exacerbations of asthma in the emergency setting" (Rodrigo 2005).

Mechanisms by which anti-cholinergics and beta-agonists could provide benefits in patients with asthma might differ. However, bronchodilation is likely to be an important aspect by which both agents provide benefit. It has been reported that both classes of agents provide equivalent bronchodilation when maximal doses are given in patients with COPD (Easton 1986; Higgins 1991) or asthma (Higgins 1991).

Tiotropium bromide is a new generation anticholinergic agent that is structurally related to ipratropium bromide (Barnes 2000 and 2001). It shows a slow dissociation from the muscarinic M_3 receptor found on bronchial smooth muscle, and therefore, has a long duration of action (> 24 hours) (Barnes 2000 and 2001). It is inhaled as a dry powder and was approved by the FDA for the treatment of bronchospasm associated with COPD in January 2004.

In general, anticholinergics are safe for most patients with COPD. A recent meta-analysis of 22 randomized trials containing more than 15,000 participants with COPD suggested that anticholinergic agents reduce respiratory deaths by 70% (relative risk 0.3, CI 0.1 to 0.8) while beta-agonists increased respiratory deaths more than 2-fold (relative risk 2.5, CI 1.1 to 5.5) when compared to placebo (Salpeter 2006a, quoted in Salpeter 2006b).

Tiotropium is contraindicated in patients with narrow angle glaucoma, prostatic hypertrophy, bladder-neck obstruction, and renal insufficiency. The Lung Health Study reported an increase in deaths and hospitalizations for cardiovascular disease and coronary artery disease in the smoking intervention plus ipratropium inhaler group when compared with the smoking intervention plus placebo inhaler group, and that "the differences approached statistical significance" (Anthonisen 2002). An abstract described a cohort of Manitoba residents ≥ 35 years of age with asthma, COPD or chronic bronchitis who were dispensed ipratropium and described increased risk for first hospitalization for cardiovascular events including supraventricular tachycardia (~1.33), myocardial infarction (~1.24), and heart failure (~1.79), but not stroke, with similar increased risks for patients taking beta-agonists (Macie 2004). This study has yet to be published. The demographics of patients at risk and whether on not they had pre-existing cardiovascular disease or risk factors for cardiovascular disease, are not clear.

No safety database is available to determine the safety of tiotropium in asthma. Therefore study participants will be closely monitored for evidence of adverse cardiovascular and other adverse events, and patients with either coronary artery disease or a history of myocardial infarction will be excluded from the trial.

Information concerning the use of tiotropium in asthma is limited. A PubMed search (on 21 November 2005) for "Tiotropium AND Asthma" yielded 25 references. Only a few of these involved asthma, mainly concerning the effect of tiotropium on airway responsiveness (O'Connor 1996; Terzano 2004). While one company report to the FDA suggested that tiotropium bromide has shown no efficacy in asthma, recent conversations with employees of Boehringer-Ingelheim suggest a renewed interest in considering tiotropium bromide for use in asthmatics (R Wise, on behalf of the American Lung Association's, Asthma Clinical Research Centers, personnel communication). Because of its long duration of action, potent bronchodilatory activity, excellent safety profile, and a mechanism of action distinct from beta-adrenergic agonists, tiotropium would appear to be an excellent candidate to investigate as a possible alternative to either increasing the inhaled corticosteroid dose, or adding a long-acting beta-agonist, in patients inadequately controlled on an inhaled corticosteroid alone. Important concerning the questions posed in this protocol, we are not aware of any trials studying anticholinergic agents as add-on agents in patients taking an inhaled corticosteroid with inadequate asthma control.

IV. PROTOCOL OVERVIEW

A. Protocol Design

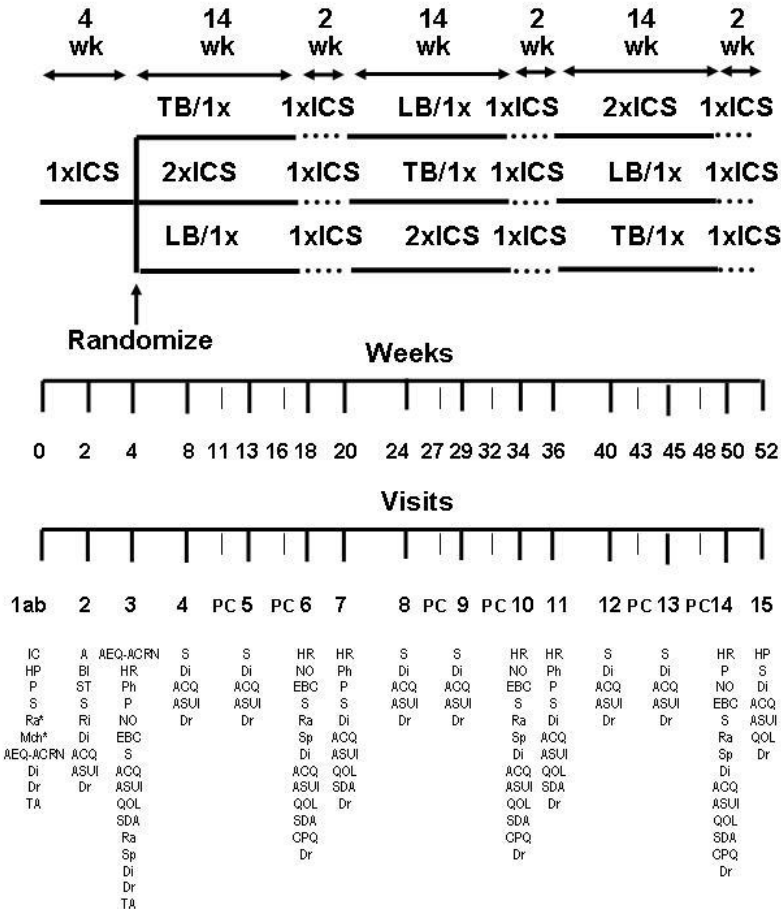
This protocol uses a common 4 week run-in period with the BASALT protocol to maximize patient recruitment for these complementary studies. BASALT will study asthmatics controlled on low-dose inhaled corticosteroids, while TALC will enroll patients who are sub-optimally controlled and, therefore, require additional treatment to gain asthma control.

During the first visit(s) (Visit 1a,b) patients will have the trials explained to them, then sign an informed consent. If they meet enrollment criteria, asthma will be confirmed either by beta-agonist reversibility testing or by airway challenge with methacholine. They will then enter a common run-in period where they will receive open-label low dose inhaled corticosteroid (1xICS) for 4 weeks. Visit 2 will take place 2 weeks later, mainly to assess subject adherence to study procedures and subject safety. Visit 3 will take place after an additional 2 week period to reinforce study procedures and to perform selected study procedures. Data collected between Visits 2 and 3 (from subject diaries: PEF, symptoms, rescue medication use (14 days prior to Visit 3, or entire interval between Visits 2 and 3 if less than 14 days)) and at Visit 3 (spirometry), will be used as baseline data. Data obtained at Visit 3 will determine if the subject meets allocation criteria and, if he/she does, the protocol into which he/she will be allocated (BASALT or TALC). During Visit 3 subjects will complete baseline study procedures, including reversibility testing with albuterol (measured with subjects on open-label inhaled corticosteroid (1xICS)). After subjects have been allocated into the TALC protocol, have met all eligibility criteria, and have completed all procedures at Visit 3, they will be randomized.

In TALC, subjects will be randomized to one of three treatment arms: 1) Inhaled corticosteroid alone at twice the dose (2xICS) used in the common run-in period; 2) Inhaled corticosteroid at the same dose (1xICS) used in the run-in period plus tiotropium bromide (tiotropium bromide/1xICS); 3) Inhaled corticosteroid at the same dose (1xICS) used in the run-in period plus a long-acting beta-agonist (long-acting beta-agonist/1xICS), for 14 weeks of double-blind, placebo-controlled treatment. This will be followed by a 2-week open-label wash-out period on 1xICS, followed by randomization to 14 weeks of double-blind, placebo-controlled treatment to a second treatment arm, which will be followed by a 2-week open-label wash-out period on 1xICS, followed by randomization to 14 weeks of double-blind, placebo-controlled treatment to the third treatment arm, followed by a final 2-week open-label wash-out period on 1xICS.

The Protocol Schema is shown below. It is a simplified version of the actual protocol because it shows only three different treatment groups. As described in the Statistical Analysis section of the protocol, six different treatment sequence groups will be used in the protocol to control for study period and sequence effects.

B. Protocol Schema (including study procedures) is shown below.



*Ra-albuterol reversibility testing and/or *Mch-methacholine bronchoprovocation will be done according to ACRN protocols. Subjects who are eligible for methacholine challenge will undergo this test at Visit 1a¹. If asthma diagnosis is not confirmed, these subjects may return for albuterol reversibility testing at Visit 1b at the investigator’s discretion. Visits 1a and 1b will occur on different days. Subjects who are ineligible to perform methacholine challenge at Visit 1a will undergo albuterol reversibility testing at that visit.

Abbreviations used in the protocol include the following: PC-phone contact; IC-informed consent; HP-medical history, physical examination; Ph – short physical exam; P-pregnancy test; NO-eNO measurement; EBC-exhaled breath condensate collection; Ra-reversibility testing with 4 puffs albuterol; Mch-methacholine bronchoprovocation; AEQ-ACRN-3 question ACRN Asthma Evaluation Questionnaire (Appendix 1); Di-diary dispensing, review; Dr-drug dispensing, adherence; A-specific adherence check; BI-blood for IgE level, eosinophils, DNA; ST-skin test; Ri-reversibility testing with 4 puffs ipratropium bromide; ACQ-asthma control questionnaire; ASUI-asthma symptom utility index; HR-resting heart rate as measured by ECG or oximeter (ECG required at Visit 3);

¹ Alternatively, a historic PC₂₀ from an ACRN methacholine challenge performed within 6 months of the Visit 1 date by an ACRN-certified technician may be used to qualify the subject.

QOL-asthma-specific quality of life questionnaire (AQLQ); S-spirometry; CPQ-coordinator and patient questionnaire: assessment of study drug and effectiveness of blind; SDA-sleep and daytime alertness questionnaire; SP-sputum induction; TA-technique assessment (drug delivery device).

C. Primary Outcome Variable

The primary outcome variable chosen for both the Primary Hypothesis (comparison of tiotropium bromide/1xICS to 2xICS in a superiority design) and the Secondary Hypothesis (comparison of tiotropium bromide/1xICS to long-acting beta-agonist/1xICS in a non-inferiority design) is AM PEF measured in the morning before administration of study drugs at the trough of the dosing period for each drug. Power analyses have also been performed for FEV₁ and asthma control days.

D. Secondary Outcome Variables

Additional secondary outcomes will be used to compare the efficacy of tiotropium bromide/1xICS to 2xICS and tiotropium bromide/1xICS to a long-acting beta-agonist/1xICS in terms of Physiological Variables (FEV₁), Indices of Asthma Control and Quality of Life (asthma symptoms, asthma-control days, rescue inhaler use, asthma control as assessed by the Asthma Control Questionnaire [ACQ], asthma symptoms as assessed using the Asthma Symptom Utility Index [ASUI], asthma-specific quality of life [AQLQ], asthma exacerbations [increased asthma symptoms which result in use of oral corticosteroids, increased inhaled corticosteroids, or additional medications for asthma, plus hospitalizations, ER visits, unscheduled medical care visits], and Good Asthma Control defined by a composite index), and Biomarkers of Inflammation (exhaled nitric oxide [eNO], markers of oxidative stress [e.g. de-aerated pH in exhaled breath condensates], markers of inflammation [e.g. cysteinyl leukotrienes] and other biological markers in exhaled breath condensates, and sputum eosinophilia and eosinophil cationic protein).

While measurement of AM PEF, FEV₁, asthma symptoms, asthma-control days, rescue inhaler use, ACQ, ASUI, AQLQ, and asthma exacerbations are well validated asthma endpoints, measurement of Good Asthma Control defined by a composite index and the biomarkers of inflammation are clearly exploratory endpoints.

E. Good Asthma Control as an Outcome

A new secondary outcome used in this trial is the ability to gain and maintain Good Asthma Control. This is a composite outcome which takes into account asthma exacerbations/unscheduled healthcare visits for asthma, symptoms, rescue beta-agonist use, a measure of pulmonary function, and adverse effects related to study medication use. Similar criteria have been used infrequently in the past, an exception being the GOAL study, in which escalating doses of fluticasone propionate, either alone or in combination with salmeterol, were used to try to reach the guideline ideal "Complete Asthma Control" (Bateman 2004). This outcome has the potential to define asthma control across several domains, all of which have been considered important goals for asthma treatment (NAEPP 1997; NAEPP 2002; GINA 2005). We will explore how robust this outcome is, in comparison with more traditional asthma outcomes, in this protocol.

Good Asthma Control is defined as:

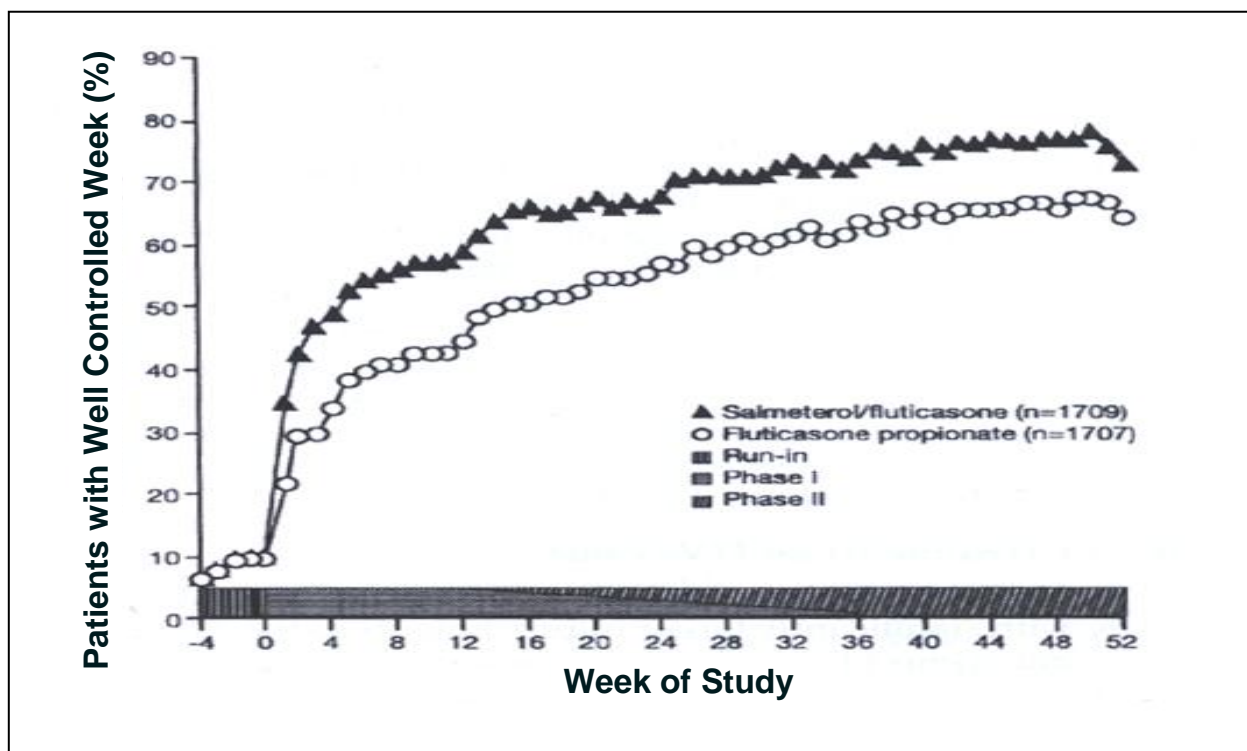
The presence of all of the following:

1. No asthma exacerbation. An exacerbation is defined as an increase in asthma symptoms which results in use of oral corticosteroids, increased inhaled corticosteroids, or additional medications for asthma.
2. No nocturnal awakening due to asthma.
3. No study medication-related adverse effect rated > mild by the patient.
4. Physician judgment that the patient's level of asthma control is adequate for the patient to remain in the trial.
5. Patient agrees to remain in the trial and does not withdraw from the study because of lack of satisfaction with asthma treatment.

The presence of 2 of 3 of the following:

1. Pre-bronchodilator AM PEF which does not fall to <80% of baseline on any 2 consecutive, scheduled measurements. Baseline is defined as the mean value of AM pre-bronchodilator PEF recorded on the symptom diary for the 2 weeks (14 days) prior to randomization (Visit 3) or entire interval between Visits 2 and 3 if less than 14 days.
2. No excessive rescue inhaler use, defined as use on 3 or more days/week or on 5 or more occasions per week (not counting premedication for exercise). An occasion is equivalent to a dosing session, regardless of the number of albuterol puffs taken.
3. No average symptom score > 1 on 3 or more days/week. (Symptom score 0-3 scale: none, mild, moderate, severe)

All of these factors will be assessed each week during the trial to determine whether or not patients meet criteria for "Good Asthma Control" for each week of the 14 week treatment periods. The figure below shows the percent of patients with "Well Controlled Asthma Weeks" in the GOAL study in patients treated with fluticasone alone (open circles) and fluticasone/salmeterol combination (closed triangles). As noted above, these criteria were very similar to the "Good Asthma Control" criteria listed above. Well Controlled Weeks increased rapidly during the first 4 weeks of treatment, then continued upward at a lesser rate during the last 48 weeks of therapy. These data suggest that 14 weeks of treatment should be sufficient to define asthma control in this manner, and that limited additional benefit would be obtained by increasing treatment duration to, for example, 16 or 20 weeks.



F. Biomarkers in Exhaled Breath (eNO and Biomarkers in Exhaled Breath Condensate) and Sputum

1. Research Question. We hypothesize that elevated levels of inflammatory markers in airway samples obtained non-invasively (increased levels of eNO, lower EBC pH, increased EBC leukotriene levels, increased sputum eosinophils, increased sputum eosinophil cationic protein [ECP]) will predict, or correlate with, clinically important measures indicating a loss of asthma control (deterioration in lung function, decrease in asthma control days, increased symptoms or rescue beta-agonist use, increased asthma exacerbations).

2. Primary Outcomes. Increased levels of eNO, lower EBC pH, increased EBC leukotriene levels, increased sputum eosinophils, and increased sputum eosinophil cationic protein [ECP] and their relationship to, or correlation with, clinically important measures indicating a loss of asthma control (deterioration in lung function, decrease in asthma control days, increased symptoms or rescue beta-agonist use, increased asthma exacerbations).

3. Clinical Importance. Airway inflammation is thought to be a primary factor in asthma pathogenesis. Clinical parameters (symptoms, rescue beta-agonist use) and physiological measures (measures of airflow obstruction) often do not correlate with measures of airway inflammation. Having a simple, non-invasive method to measure

and follow airway inflammation to use as an adjunct in guiding asthma therapy would represent a major advance in the treatment of asthma.

4. Discussion. The use of measurements of exhaled nitric oxide (eNO) to either assess ongoing airway inflammation (Lazarus 2001; Wechsler 2006), or to guide therapy (Smith 2005), is an area of continuing investigation and interest. While instruments are now commercially available to perform these measurements and billing codes are available to request reimbursement, evidence to support its routine use in clinical practice is lacking. This protocol will add to our understanding of the potential usefulness of measurements of eNO in asthma management.

Measurements in exhaled breath condensate (EBC) have been included because this is an easily obtainable, non-invasive test which might be useful to monitor airway inflammation non-invasively (Mutlu 2001; Montuschi 2002; Hunt 2002; Vaughan 2003). We hypothesize that a decrease in EBC pH and increase in EBC inflammatory markers will be associated with worse asthma control. The use of EBC for this purpose is the focus of one of the ACRN young investigator training supplements.

Sputum eosinophilia has been a useful predictor of asthma exacerbations in a number of clinical trials, including those performed by the ACRN (Deykin 2005). We will perform limited examination of induced sputum to see if the presence of sputum eosinophilia, and increased sputum eosinophil cationic protein, continues to predict treatment failure.

G. Exploratory Pharmacogenetic Studies

1. Research Question. We hypothesize that asthmatic patients with specific alterations (single nucleotide polymorphisms [SNPs] and/or haplotypes) in the beta-2 adrenergic receptor, the M₃ muscarinic receptor, and genes in the glucocorticoid pathway will demonstrate either positive or negative effects on measures of asthma control (lung function, asthma control days, symptoms or rescue beta-agonist use, asthma exacerbations) when treated with 2xICS, tiotropium/1xICS, and/or long-acting beta-agonist/1xICS. Proof of principle for this hypothesis is already available, as alterations in the beta-2 adrenergic receptor have been associated with worse asthma outcomes in a variety of end-points when patients were taking a beta-agonist regularly (Israel 2000; Israel 2004; Wechsler 2006), and patients with alterations in the corticotropin-releasing hormone receptor 1 (*CRHR1*) have been shown to have increased responses to inhaled corticosteroids (Tantisira 2004.).

2. Primary Outcomes. Alterations (single nucleotide polymorphisms [SNPs] and/or haplotypes) in the beta-2 adrenergic receptor, the M₃ muscarinic receptor, and genes in the glucocorticoid pathway and their association with either a positive or negative effect on measures of asthma control (lung function, asthma control days, symptoms or rescue beta-agonist use, asthma exacerbations), as either discrete or continuous variables, when asthmatics are treated with 2xICS, tiotropium/1xICS, and/or long-acting beta agonist/1xICS.

3. Clinical Importance. The eventual goal for asthma therapy is to devise an optimal, and customized, therapeutic strategy for each individual asthmatic patient, taking into account phenotypic characteristics of their disease, their response to different classes of asthma medications, risks for adverse versus beneficial effects from any specific asthma medication, and their personal, lifestyle preferences. Pharmacogenetic factors will undoubtedly be important in customizing asthma treatment for individual asthmatic patients. These exploratory pharmacogenetic association studies are a necessary first step for reaching this goal.

4. Discussion. We propose exploratory pharmacogenetic studies to determine if asthmatics with specific alterations (single nucleotide polymorphisms and/or haplotypes) in the beta-2 adrenergic receptor, the M₃ muscarinic receptor, and genes in the glucocorticoid pathway will respond positively and negatively to a long-acting beta-agonist, tiotropium bromide, and an inhaled corticosteroid. These studies will be performed at Wake Forest University and Harvard University, in collaboration with the Data Coordinating Center, and will be done with resources provided by investigators at Wake Forest and Harvard, including a collaborative pharmacogenetics grant. DNA sequencing reactions are performed using the ABI dye terminator chemistry as previously described, with sequencing of products using an ABI 3730 XL DNA Analyzer (Applied Biosystems, Inc., Foster City, CA) (Hawkins 2004). Genotyping is performed using the MassARRAY genotyping system (Sequenom Inc., San Diego, CA, USA) (Hawkins 2004).

a. Beta-2 Adrenergic Receptor

Recent reports of adverse effects associated with the use of beta-agonists (Israel 2000; Israel 2004; Knobil 2003; Nelson 2006) prompted us to re-sequence the beta-2 adrenergic receptor in an attempt to define SNPs and haplotypes in this gene that could be associated with adverse asthma outcomes. Forty-nine SNPs, 21 of which were novel, were identified after sequencing the gene in 669 individuals from two ethnic groups (429 Caucasians and 240 African Americans) (Hawkins...Peters...Bleecker, manuscript submitted). Based on these results we plan to examine five regions which occur with a high enough frequency to have a reasonable chance to find significant associations: 1) polymorphism +46 resulting in Gly¹⁶Arg at amino acid position 16 of the β_2 -adrenergic receptor; 2) 5' distal open reading frame (LC1) polymorphisms -654 [Glu¹¹²Lys], -468 [His¹⁷⁴Asp], -367 Pro²⁰⁷Pro], that affect amino acid changes in the potential LC1 polypeptide; 3) leader cistron (BUP region or LC2) polymorphism -47 [Cys>Arg]; 4) poly C repeat number at ± 1269 (small repeats:10-12 repeats vs large repeats: 13-15 repeats); and 5) 3' untranslated region polymorphisms ± 523 , ± 1053 , ± 1239 .

b. M₃ Muscarinic Receptor

We have recently re-sequenced the M₂ and M₃ muscarinic receptors in a panel of 72 individuals consisting of asthmatics and non-asthmatics of Caucasian, African American, and Hispanic origin (Hawkins 2006 [abstract]). We identified sixteen

polymorphisms in *CHRM2* (M_2 receptor), with eight polymorphisms with a minor allele frequency > 0.10 . The most frequent polymorphisms in *CHRM2* were located in the 3' untranslated region of the gene. Minor allele frequencies for *CHRM2* individual polymorphisms were similar between all ethnic groups and between asthmatics and controls. Six polymorphisms were identified in *CHRM3* (M_3 receptor); however only two polymorphisms with a minor allele frequency > 0.10 were identified in Caucasians and US Hispanics, and only one polymorphism with a minor allele frequency >0.10 was identified in African Americans. No coding changes were found in either gene. We will examine those alterations with a minor allele frequency >0.10 .

c. Genes of the Glucocorticoid Pathway

Gregory Hawkins, PhD, an Associate Professor of Medicine at Wake Forest University School of Medicine has recently been awarded a R21 award to examine genes in the glucocorticoid pathway in asthmatics and controls. Led by his efforts, we have completed preliminary sequencing of steroid complex genes *Hsp90 1 α* , *Hsp90 1 β* , *Hsc70*, *Hsp70* (A1A and A1B), *STIP1* (Hop), *Hsp40* and *p23* in 46 severe asthmatics. In addition, we have also been able to sequence two additional genes encoding the immunophilins *FKBP51* and *FKBP52* in 46 severe asthmatics. These two additional components of the steroid receptor complex are involved in transport of the activated receptor across the nuclear membrane. We have also sequenced the genes for *Hsp70* (A1A and A1B) in a screening panel of consisting of asthmatics and non-asthmatics from Caucasian, African American, and US Hispanic origin. To our knowledge, this is the first comprehensive sequencing of the *Hsp70* genes which defines the uniqueness of these genes.

In our collaboration with Dr. Scott Weiss at Channing Laboratory (Brigham and Women's Hospital and Harvard Medical School), we have used the polymorphisms in the steroid complex genes identified by re-sequencing and additional polymorphisms identified in dbSNP and the HapMap project and tested for association for changes in lung function in adult asthmatics and response to steroid therapy. Fifty-nine polymorphisms in *Hsp90 1 α* , *Hsp90 1 β* , *Hsc70*, *Hsp70*, *STIP1* (Hop), *Hsp40*, *p23*, *FKBP51*, and *FKBP52* were genotyped in a study of 470 asthmatic adults randomized to once daily Flunisolide or conventional inhaled corticosteroid therapy. The outcome measures included both baseline FEV1 and percent change in FEV1 after 8 weeks of treatment. Significant associations in *STIP1* were found for baseline FEV1 [SNPs rs4980524, $p=0.006$; rs2236647, $p=0.008$; rs6591838, $p=0.009$; and rs2236648, $p=0.03$]; % change in FEV1 [SNP rs1011219, $p<0.001$] and change in FEV1 % predicted [SNPs rs6591838, $p=0.03$; and rs1011219, $p=0.008$]. Haplotype analysis of *STIP1* indicates a single haplotype associated with % change FEV1 (8 weeks) ($p=0.007$). An additional 3 window sliding haplotype association tests indicates that polymorphisms rs4980524 and rs6591838, both intronic SNPs, are driving the primary haplotype association results. Additional association was measured for % change in FEV1 for *HSC70* [SNPs rs2276074, $p=0.04$; and rs2236658, $p=0.04$]. These data suggests that *STIP1* and *HSC70* may have important roles in predicting and/or regulating lung function.

V. INCLUSION AND EXCLUSION CRITERIA

A. Inclusion and Exclusion Criteria for Entry (Visits 1a and 1b)

1. Inclusion Criteria (Visit 1)

- a. Male and female subjects, ages 18 and older
- b. Clinical history consistent with asthma
- c. FEV₁ > 40% predicted
- d. Asthma confirmed either by²:
 - (1) Beta-agonist reversibility to 4 puffs albuterol ≥ 12% OR
 - (2) PC₂₀ FEV₁ methacholine ≤ 8 mg/ml NOT on an inhaled corticosteroid, or ≤ 16 mg/ml ON an inhaled corticosteroid
- e. Need for daily controller therapy (i.e., inhaled corticosteroids, leukotriene modifiers, and/or long-acting beta-agonists) as shown by either
 - (1) Used or received prescription for asthma controller during past yearOR
 - (2) Symptoms more than twice a week if not on asthma controller
- f. If on inhaled steroids (any drug at any dose not exceeding the equivalent of 1000 mcg fluticasone daily), subject must have been on a stable dose for at least 2 weeks
- g. Ability to provide informed consent, as evidenced by signing a copy of the consent form approved by the Committee on Human Research of the study institution
- h. Non-smoker (total lifetime smoking history < 10 pack-years; no smoking for at least 1 year)

2. Exclusion Criteria (Visit 1)

- a. Use of any drug prohibited in the trial (any non-study drug which could be used for the treatment of asthma, beta-blockers, MAO inhibitors, and macrolide antibiotics). Use of any drugs listed in Table 1 below during the designated washout period prior to screening visits and/or Visits 1a/1b, or intention to take any of these drugs during the study.

² Alternatively, a historic PC₂₀ from an ACRN methacholine challenge performed within 6 months of the Visit 1 date by an ACRN-certified technician may be used to satisfy this entry criterion.

Table 1. Drugs to be withheld throughout the study and washout periods prior to Visits 1a/1b

Exclusionary Drugs	Washout prior to Visits 1a and 1b
Leukotriene receptor antagonists	≥ 4 weeks
Inhaled steroids, except as provided in study	None
Oral steroids	≥ 6 weeks
Cromolyn/Nedocromil	≥1 weeks
Oral beta-adrenergic agonists	> 1 week
Monoamine oxidase inhibitors	> 4 weeks
Macrolide antibiotics	≥ 4 weeks
Oral beta-adrenergic blockers	≥ 2 weeks
Inhaled beta-adrenergic agonists (intermediate-acting, e.g., albuterol, terbutaline, metaproterenol, pirbuterol, bitolterol), except as provided in study	≥ 6 hours
Salmeterol/formoterol, except as provided in study	≥ 48 hours
Anticholinergics, except as provided in study	≥ 48 hours for ipratropium; ≥ 72 hours for tiotropium
Short-acting theophylline (e.g., Slophyllin tablets)	≥ 12 hours
Long-acting theophylline (e.g., Theo-Dur, Slo-bid)	≥ 24 hours
Ultra long-acting theophylline (e.g., Theo-24, Uniphyll)	≥ 48 hours
Anti-IgE therapy	≥ 6 months

Table 2. Drugs allowed during the study with washouts required prior to study visits

Drugs withheld prior to pulmonary function and/or methacholine challenge, per MOP	Specified withhold period
Albuterol (study RESCUE drug)	≥ 6 hours
Long-acting beta-agonist (study drug)	≥ 12 hours ⁺
Tiotropium (study drug)	≥ 24 hours ⁺
Antihistamines	≥ 48 hours; see ACRN skin testing MOP for required holds prior to Visit 2 skin test
Oral decongestants (pseudoephedrine and others)	≥ 48 hours
Nasal decongestants (oxymetazoline (Afrin) and others)	≥ 6 hours
Methylxanthine-containing foods or beverages (e.g., coffee, tea)	≥ 6 hours
Alcohol-containing foods or beverages	≥ 6 hours

⁺ For post-randomization TALC visits, subjects will hold their AM dose of tiotropium study drug and long-acting beta-agonist study drug until after completion of the applicable study visits.

- b. Patients taking contraindicated drugs listed in package insert of study drugs
- c. Chronic use of any medications other than beta-agonists or inhaled corticosteroids, except:
 - oral contraceptives and other hormonal forms of contraceptives (i.e., DepoProvera-7, Norplant-7)
 - estrogen / progesterone replacement therapy for post-menopausal women
 - vitamins and calcium supplements/osteoporosis medications (e.g. alendronate (Fosamax), etc.)
 - any intranasal corticosteroid at a stable dose throughout the entire study (see MOP)
 - acetaminophen
 - non-steroidal anti-inflammatory medications (e.g., aspirin, naproxen, ibuprofen, cox2 inhibitors)
 - thyroid replacement medications
 - lipid-lowering medication
 - stable dose medical therapy for well-controlled hypertension and well-controlled diabetes, except those medications specifically excluded in the MOP
 - medium and low potency topical cutaneous steroids
 - nasal saline spray/nasal Cromolyn/Atrovent spray

- topical eye preparations for allergic eye symptoms (e.g. antihistamines, NSAIDs, or antiallergic compounds)
 - diuretics and specific antihypertensives (e.g. calcium channel blockers, angiotensin receptor blockers, clonidine, etc. See MOP)
 - acyclovir and all anti-herpetic medications
 - antihistamines (48 hour washout prior to visits; see ACRN skin testing MOP for required washouts prior to Visit 2 skin test)
 - oral decongestants (pseudoephedrine and others) (48 hour washout prior to visits)
 - antibiotics for acne (except macrolides)
 - stool softeners and bulk laxatives/hemorrhoid treatment
 - anxiolytics/antianxiety medications -chronic stable dose
 - nasal decongestants (e.g., oxymetazoline (Afrin)) (6 hour washout prior to visits)
 - antidepressants (except monoamine oxidase (MAO) inhibitors)-at stable chronic dose; must be reviewed for anticholinergic side effects and approved by center PI
 - migraine medications (e.g. Imitrex, etc.)
 - non-macrolide antibiotics
 - antacids
 - select CNS stimulants/appetite suppressants (See MOP)
 - H₂ blockers(e.g. ranitidine, cimetidine, famotidine, nizatidine) for GERD
 - Proton pump inhibitors (e.g. omeprazole, lansoprazole, esomeprazole) for GERD
 - hair growth preparations
 - analgesics for acute/chronic pain management with MD discretion
- d. Lung disease other than asthma, including COPD and chronic bronchitis
 - e. Established or suspected diagnosis of vocal cord dysfunction
 - f. Significant medical illness other than asthma, including unstable/severe coronary artery disease or a history of myocardial infarction within 6 months of enrollment
 - g. History of respiratory tract infection within the previous 4 weeks.
 - h. History of a significant exacerbation of asthma in the previous 4 weeks
 - i. History of life-threatening asthma requiring treatment with intubation and mechanical ventilation within the past 5 years
 - j. Hyposensitization therapy other than an established maintenance regimen
 - k. Inability, in the opinion of the investigator or clinical coordinator, to coordinate use of the delivery devices used in the study
 - l. Pregnancy. If potentially able to bear children, not using an acceptable form of birth control (see study MOP).

B. Inclusion and Exclusion Criteria for Allocation to TALC or BASALT (Visit 3)

1. Criteria Required for Allocation (Visit 3)

- a. Ability to measure AM PEF on schedule using electronic peak flow meter (EPFM) and to complete the study diary correctly $\geq 75\%$ of the time during the interval between visits 2 and 3
- b. Adherence with study medication dosing $\geq 75\%$ of the time during the interval between visits 2 and 3
- c. No asthma exacerbation requiring use of oral corticosteroids or additional asthma medications (including increased inhaled corticosteroids) during the run-in period
- d. $FEV_1 > 40\%$ predicted

2. Criteria for Allocation to BASALT (Visit 3)

- a. Pre-bronchodilator $FEV_1 > 70\%$ predicted **AND**
- b. Score on the ACRN Asthma Evaluation Questionnaire (Appendix 1) of 0 or 1 on ALL 3 questions. (In reviewing your asthma control during the last 2 weeks, you have had: symptoms 5 or less days per week, AND rescue inhaler use 5 or less days per week, AND nocturnal awakenings for asthma once per week or less.)

3. Criteria for Allocation to TALC (Visit 3)

- a. No medical contraindications for tiotropium use (narrow angle glaucoma, prostatic hypertrophy, bladder-neck obstruction, renal insufficiency)
- b. Failure to meet both allocation criteria for BASALT. That is:
 - (1) Pre-bronchodilator $40 < FEV_1 \leq 70\%$ predicted **OR**
 - (2) Score on the ACRN Asthma Evaluation Questionnaire (Appendix 1) of 2 or 3 on any 1 (or more) of the 3 questions. (In reviewing your asthma control during the last 2 weeks, you have had: symptoms 6 or more days per week, OR rescue inhaler use 6 or more days per week, OR nocturnal awakenings for asthma two nights per week or more.)

C. Exclusion Criteria for Randomization in TALC (Visit 3)

1. Inability, in the opinion of the investigator or clinical coordinator, to coordinate use of the delivery devices (i.e., HandiHaler, Diskus, MDI) used in the study.

2. Presence at Visit 3 of any of the exclusion criteria stipulated for Visit 1 (Note: Respiratory tract infections that do not cause the subject to meet exacerbation criteria are not considered exclusionary.)

VI. PROTOCOL DETAIL AND VISIT STRUCTURES

Visit 1a,b, Week 0

The goal of this (these) visit(s) is to explain the study to potential subjects, obtain informed consent from the subject, and to determine if the subject meets general entry criteria.

The diagnosis of asthma is confirmed either by bronchodilator reversibility testing ($\geq 12\%$ with 4 puffs albuterol) or airway hyperresponsiveness to methacholine (PC_{20} $FEV_1 \leq 8$ mg/ml for patients NOT on an inhaled corticosteroid, ≤ 16 mg/ml for patients ON an inhaled corticosteroid at a stable dose for at least 2 weeks).

- Subjects who qualify for methacholine challenge ($FEV_1 \geq 55\%$ predicted) will complete a methacholine challenge at Visit 1a³. If the subject's PC_{20} does not confirm the asthma diagnosis (Visit 1a), then the subject will undergo albuterol reversibility testing on a separate day/visit (Visit 1b) at the investigator's discretion.
- Subjects who do not qualify for methacholine challenge ($FEV_1 < 55\%$ predicted) will complete albuterol reversibility testing at Visit 1a. If the subject does not meet the eligibility criteria, no further visits will be scheduled.

Study procedures are explained for eligible subjects and they are given open-label inhaled corticosteroid (1xICS) to begin taking. At this point the subject officially enters the common run-in period.

Procedures Performed

Informed Consent

Medical History and Physical Examination (performed by physician)

Pregnancy Test (for all women of child-bearing potential)

Spirometry

Reversibility Testing with Albuterol and/or Methacholine Bronchoprovocation (if qualifying historic PC_{20} is unavailable)

Asthma Evaluation Questionnaire - ACRN

Diary Dispensation and Explanation of Study Procedures

Drug Dispensation (open-label low dose inhaled corticosteroids (1xICS))

ICS Drug Delivery Device Technique Assessment

Electronic Peak Flow Meter (EPFM) Dispensation

³ Alternatively, a historic PC_{20} from an ACRN methacholine challenge performed within 6 months of the Visit 1 date by an ACRN-certified technician may be used to qualify the subject.

Visit 2, Week 2

The purpose of this visit is to assess subject adherence with study procedures and to perform additional study procedures.

Procedures Performed

Review of Subject Adherence
Blood Draw for IgE Level, Blood Eosinophils, and DNA Isolation
Allergy Skin Testing
Spirometry
Reversibility Testing with Ipratropium Bromide (4 puffs)⁴
Diary Collection/Review and Review of Study Procedures
Asthma Control Questionnaire
Asthma Symptom Utility Index
Diary Dispensation
Drug Collection/Dispensation (open-label low dose inhaled corticosteroids (1xICS))

Visit 3, Week 4

The purpose of Visit 3 is to determine which protocol subjects will be allocated to (either BASALT or TALC) based on the results of the pre-bronchodilator FEV₁ % predicted, and the Asthma Evaluation Questionnaire developed by the ACRN, and to perform additional study procedures. At Visit 3 subjects will perform reversibility testing to albuterol (4 puffs) and sputum induction while on the open label inhaled corticosteroid. Coordinators will then perform final review of study procedures and drug dispensing. Subjects will be randomized in TALC at the completion of Visit 3.

Procedures Performed

Asthma Evaluation Questionnaire – ACRN
Resting Heart Rate (ECG required for measurement at Visit 3)
Brief Physical Exam
Pregnancy Test (for all females of child-bearing potential)
Collection of Exhaled Nitric Oxide
Collection of Exhaled Breath Condensate
Spirometry
Reversibility Testing with Albuterol (4 puffs)
Sputum Induction
Asthma Control Questionnaire
Asthma Symptom Utility Index
Asthma-Specific Quality of Life Questionnaire
Sleep and Daytime Alertness Questionnaire (SDAQX)

⁴ Subjects who have a history of allergy to soy or peanuts will not be administered ipratropium for reversal testing at Visit 2.

Diary Review/Collection and Review of Study Procedures and Adherence
Run-in Drug Collection
Drug Delivery Device (HandiHaler, Diskus, etc.) Technique Assessment
Drug Dispensation (double-blind treatment period 1 medications) and Proper Use
Explanation
Diary Dispensation
Randomization

Visit 4, Week 8

Continuing treatment with study drugs for the first treatment period.

Procedures Performed

Spirometry
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Diary Dispensation
Drug Collection/Dispensation (double-blind treatment period 1 medications)

Phone Contact, Week 11

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If the subject is exhibiting signs of an asthma exacerbation, then he/she will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment, if necessary. The Asthma Control Questionnaire (ACQ) will be administered at each phone contact.

Visit 5, Week 13

Continuing treatment with study drugs for the first treatment period.

Procedures Performed

Spirometry
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Diary Dispensation
Drug Collection/Dispensation (double-blind treatment period 1 medications)

Phone Contact, Week 16

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If the subject is exhibiting signs of an asthma exacerbation, then he/she will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment, if necessary. The Asthma Control Questionnaire (ACQ) will be administered at each phone contact.

Visit 6, Week 18

This visit ends the first treatment period and will begin the washout from the first study treatment regimen.

Procedures Performed

- Resting Heart Rate (ECG or oximeter)
- Collection of Exhaled Nitric Oxide
- Collection of Exhaled Breath Condensate
- Spirometry
- Reversibility Testing with Albuterol (4 puffs)
- Sputum Induction
- Diary Collection/Review and Review of Study Procedures and Adherence
- Asthma Control Questionnaire
- Asthma Symptom Utility Index
- Asthma-Specific Quality of Life Questionnaire
- Sleep and Daytime Alertness Questionnaire (SDAQX)
- Coordinator Patient Questionnaire (Study Drug Preference and Assessment of Study Blind)
- Diary Dispensation
- Drug Collection (double-blind treatment period 1 medications)
- Drug Dispensation (open-label washout 1xICS)

Visit 7, Week 20

This visit marks the end of the washout and beginning of the second treatment period.

Procedures Performed

- Resting Heart Rate (ECG or oximeter)
- Brief Physical Exam
- Pregnancy Test (for all females of child-bearing potential)

Spirometry
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Asthma Specific Quality of Life Questionnaire
Sleep and Daytime Alertness Questionnaire (SDAQX)
Diary Dispensation
Drug Collection (open-label washout 1xICS)
Drug Dispensation (double-blind treatment period 2 medications)

Visit 8, Week 24

Continuing treatment with study drugs for the second treatment period

Procedures Performed

Spirometry
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Diary Dispensation
Drug Collection/Dispensation (double-blind treatment period 2 medications)

Phone Contact, Week 27

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If the subject is exhibiting signs of an asthma exacerbation, then he/she will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment, if necessary. The Asthma Control Questionnaire (ACQ) will be administered at each phone contact.

Visit 9, Week 29

Continuing treatment with study drugs for the second treatment period.

Procedures Performed

Spirometry
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire

Asthma Symptom Utility Index
Diary Dispensation
Drug Collection/Dispensation (double-blind treatment period 2 medications)

Phone Contact, Week 32

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If the subject is exhibiting signs of an asthma exacerbation, then he/she will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment, if necessary. The Asthma Control Questionnaire (ACQ) will be administered at each phone contact.

Visit 10, Week 34

This visit ends the second treatment period and will begin the wash-out from the second study treatment regimen.

Procedures Performed

Resting Heart Rate (ECG or oximeter)
Collection of Exhaled Nitric Oxide
Collection of Exhaled Breath Condensate
Spirometry
Reversibility Testing with Albuterol (4 puffs)
Sputum Induction
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Asthma-Specific Quality of Life Questionnaire
Sleep and Daytime Alertness Questionnaire (SDAQX)
Coordinator Patient Questionnaire (Study Drug Preference and Assessment of Study Blind)
Diary Dispensation
Drug Collection (double-blind treatment period 2 medications)
Drug Dispensation (open-label washout 1xICS)

Visit 11, Week 36

This visit marks the end of the wash-out and beginning of treatment for the third treatment period.

Procedures Performed

Resting Heart Rate (ECG or oximeter)
Brief Physical Exam
Pregnancy Test (for all females of child-bearing potential)
Spirometry
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Asthma Specific Quality of Life Questionnaire
Sleep and Daytime Alertness Questionnaire (SDAQX)
Diary Dispensation
Drug Collection (open-label washout 1xICS)
Drug Dispensation (double-blind treatment period 3 medications)

Visit 12, Week 40

Continuing treatment with study drugs for the third treatment period.

Procedures Performed

Spirometry
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Diary Dispensation
Drug Dispensation (double-blind treatment period 3 medications)

Phone Contact, Week 43

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If the subject is exhibiting signs of an asthma exacerbation, then he/she will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment, if necessary. The Asthma Control Questionnaire (ACQ) will be administered at each phone contact.

Visit 13, Week 45

Continuing treatment with study drugs for the third treatment period.

Procedures Performed

Spirometry
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Diary Dispensation
Drug Dispensation (double-blind treatment period 3 medications)

Phone Contact, Week 48

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If the subject is exhibiting signs of an asthma exacerbation, then he/she will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment, if necessary. The Asthma Control Questionnaire (ACQ) will be administered at each phone contact.

Visit 14, Week 50

This visit ends the third treatment period and will begin the wash-out from the study drugs.

Procedures Performed

Resting Heart Rate (ECG or oximeter)
Pregnancy Test (for all females of child-bearing potential)
Collection of Exhaled Nitric Oxide
Collection of Exhaled Breath Condensate
Spirometry
Reversibility Testing with Albuterol (4 puffs)
Sputum Induction
Diary Collection/Review and Review of Study Procedures and Adherence
Asthma Control Questionnaire
Asthma Symptom Utility Index
Asthma-Specific Quality of Life Questionnaire
Sleep and Daytime Alertness Questionnaire (SDAQX)
Coordinator Patient Questionnaire (Study Drug Preference and Assessment of Study Blind)
Diary Dispensation
Drug Collection (double-blind treatment period 3 medications)
Drug Dispensation (open-label washout 1xICS)

Visit 15, Week 52

This visit will conclude the study.

Procedures Performed

Physical Examination (performed by a physician)

Spirometry

Diary Collection/Review and Review of Study Procedures and Adherence

Asthma Control Questionnaire

Asthma Symptom Utility Index

Asthma-Specific Quality of Life Questionnaire

Drug Collection (open-label washout 1xICS)

Exit Interview/ACRN Satisfaction Questionnaire

VII. STATISTICAL DESIGN AND ANALYSIS

A. Statistical Design

The TALC trial is designed as a three-way crossover comparing Treatment Regimens A, B, and C, where

- Treatment Regimen A: 1 × ICS + tiotropium
- Treatment Regimen B: 2 × ICS
- Treatment Regimen C: 1 × ICS + long-acting beta-agonist (LABA)
- Treatment Regimen R: 1 × ICS

Treatment Regimen R is listed because it is administered during the run-in and run-out periods of the TALC crossover design.

The primary research hypothesis of the TALC trial is that Treatment Regimen A is superior to Treatment Regimen B. The secondary research hypothesis is that Treatment Regimen A is not inferior to Treatment Regimen C. The comparison of Treatment Regimen B to Treatment Regimen C is not of major interest because the superiority of Treatment Regimen C over Treatment Regimen B with respect to pulmonary function already has been demonstrated in previous studies. Nevertheless, a comparison of Treatment Regimen B to Treatment Regimen C will be performed as a check of internal validity.

Each study participant will be randomized to one of the six treatment regimen sequences. Randomization will be stratified by clinical center. Because the TALC trial invokes a crossover design, further stratification based on prognostic variables is not necessary. Each treatment period will consist of 14 weeks of therapy. There will be a four-week run-in period prior to randomization and a two-week run-out period following each of the three treatment periods. Treatment Regimen R, of a lower dose of inhaled corticosteroid (1 × ICS) will be administered to the participants during the run-in and run-out periods. Thus, the length of the TALC trial is 52 weeks for each participant. The following table illustrates the study design:

<u>Sequence</u>	<u>4 weeks Run-in</u>	<u>14 weeks Period #1</u>	<u>2 weeks Run-out</u>	<u>14 weeks Period #2</u>	<u>2 weeks Run-out</u>	<u>14 weeks Period #3</u>	<u>2 weeks Run-out</u>
<u>ABC</u>	R	A	R	B	R	C	R
<u>BCA</u>	R	B	R	C	R	A	R
<u>CAB</u>	R	C	R	A	R	B	R
<u>ACB</u>	R	A	R	C	R	B	R
<u>BAC</u>	R	B	R	A	R	C	R
<u>CBA</u>	R	C	R	B	R	A	R

The statistical design for this three-way crossover trial is uniform with respect to sequence and period effects, and it is balanced with respect to first-order carryover effects. Thus, the design can account for period and sequence effects, as well as homogeneous carryover effects. The two-week run-out periods should provide

adequate protection against heterogeneous carryover effects for tiotropium and LABA. Using all of the data from the repeated measurements within each treatment period, however, will yield an analysis that can account for heterogeneous carryover effects.

B. Statistical Analyses

The primary outcome variable for the TALC trial is the change over the 14-week treatment periods with respect to AM PEF. Important secondary outcomes are changes over the 14-week treatment periods with respect to FEV₁ and the proportion of asthma-control days. Other secondary outcomes in the TALC trial include changes over the 14-week treatment periods with respect to indices of Asthma Control and Quality of Life (asthma symptoms, asthma symptom free days, rescue inhaler use, asthma control as assessed by the Asthma Control Questionnaire [ACQ], asthma symptoms as assessed using the Asthma Symptom Utility Index [ASUI], asthma-specific quality of life [AQLQ], asthma exacerbations [increased asthma symptoms which results in use of oral corticosteroids, increased inhaled corticosteroids, or additional medications for asthma, plus hospitalizations, ER visits, unscheduled medical care visits]), Good Asthma Control defined by a composite index, Biomarkers of Inflammation (exhaled nitric oxide [eNO], markers of oxidative stress [e.g. de-aerated pH] in exhaled breath condensates, and markers of inflammation [e.g. cysteinyl leukotrienes in exhaled breath condensate and sputum eosinophilia and eosinophil cationic protein] and other biological markers in exhaled breath condensates).

Variables that are measured daily from the subject diary cards, e.g., PEF, PEF variability, symptoms, and rescue medication use, will be averaged between visits to create the outcome variables used in longitudinal data analyses described below. Because some subjects required an extended period between visits 2 and 3 in the run-in due to delays in receiving TALC drug supplies, the visit 3 (baseline) average will use data from the 14 days prior to the visit (if there are more than 14 days between visits 2 and 3).

All primary and secondary statistical analyses will follow the intention-to-treat principle, although there may be some exploratory analyses that do not.

The primary research hypothesis that Treatment Regimen A is superior to Treatment Regimen B is expressed as the following hypothesis testing problem:

$$H_0: \mu_A = \mu_B \text{ versus } H_1: \mu_A > \mu_B$$

where μ_A and μ_B represent the population means for Treatment Regimens A and B, respectively. This test will be performed at the 0.025 significance level because it is one-sided.

The secondary research hypothesis that Treatment Regimen A is not inferior to Treatment Regimen C is expressed as the following hypothesis testing problem:

$$H_0: \mu_A \leq \mu_C - \Delta \text{ versus } H_1: \mu_A > \mu_C - \Delta$$

where μ_A and μ_C represent the population means for Treatment Regimens A and C, respectively, and where Δ is a positive number and represents the boundary for non-inferiority (discussed further in the next section). This test also will be performed at the 0.025 significance level because it is one-sided.

A mixed-effects linear model adapted for the TALC crossover design will provide the basis of statistical analysis for each outcome variable (Laird 1982; Jennrich and Schluchter 1986; Vonesh and Chinchilli 1977). Fixed-effects terms in the model will include clinical center (the stratifying variable), treatment regimen, sequence, and period. The trivariate outcome from each participant (a set of three correlated treatment responses) will be accommodated via an unstructured 3×3 variance matrix in the longitudinal model. Restricted maximum likelihood estimates will be determined for the treatment effects represented by μ_A , μ_B , and μ_C (adjusted for clinical center, sequence, period, and homogeneous carryover effects) via SAS PROC MIXED statistical software.

The primary statistical analysis, however, will be performed via the mixed-effects linear model by fitting an intercept-slope model to the repeated measurements within each treatment period. This model is preferred because it will (1) use all of the available data and (2) be resistant to the presence of heterogeneous carryover effects, if they exist. The presence of heterogeneous carryover effects for the three treatment regimens will be assessed by comparing the measurements at the onset of each treatment period (after the 4-week run-in period or 2-week run-out periods).

Because TALC invokes a crossover design with each participant receiving all three treatment regimens, an interesting exploratory analysis will consist of determining the optimal treatment regimen, with respect to AM PEF, FEV₁, and asthma-control days, for each participant who completes the trial. The effect sizes listed in the next section will be used to determine whether an optimal treatment regimen exists for a particular participant. For example, if a participant displays an AM PEF for one treatment regimen that is more than 10.6 L/min for the other two treatment regimes, after adjusting for center, period, sequence, and carryover effects in the model, then that particular participant will have displayed an optimal treatment regimen. Thus, there will be a categorical response with respect to each of the three outcome variables as to the frequency of optimality for each of the three treatment regimens. These frequencies will be compared qualitatively and quantitatively via frequency tables and categorical data analysis, and additional exploratory analyses will examine the frequencies within selected subgroups.

C. Sample Size Calculations

The original target sample size for the TALC trial was 224 randomized participants, but in May 2009 the Steering Committee requested, and the Data and Safety Monitoring Board approved of, a reduced sample size of 210 randomized participants. The justification for the target sample size of 210 randomized participants appears below.

Sample size calculations based on continuous outcomes require estimates of variability. The ACRN SLIC trial recruited a similar type of participant as is planned for the TALC trial, so it provides standard deviations of response changes for AM PEF, FEV₁, and proportion of asthma-control days (Lemanske 2001). The SLIC trial invoked a parallel design, however, unlike the crossover design in the TALC trial.

The total number of randomized participants for testing the primary hypothesis of superiority in a crossover trial, assuming a one-sided, α -significance level test with $1 - \beta$ statistical power, is approximated by

$$N = (z_{1-\alpha} + z_{1-\beta})^2 \{2\sigma^2(1 - \rho)\} / \delta^2$$

where $z_{1-\alpha}$ and $z_{1-\beta}$ are percentiles from the standard normal distribution, σ is the standard deviation for the change in response, ρ is the correlation for the change in response between two treatment periods in the crossover design, and δ is the desired effect size for the change in response. Because of the one-sided nature of the test of superiority, α is chosen as 0.025. Although ρ cannot be estimated from the SLIC trial because it invoked a parallel design, other ACRN trials that invoked crossover designs (BARGE, SMOG) displayed estimated correlations for changes in AM PEF and FEV₁ between treatment periods that exceeded 0.65. As a conservative approach, ρ is chosen as 0.60 in the sample size calculation. Finally, the sample size formula is inflated by a factor of 1.11 to account for a 10% withdrawal rate.

The effect size for AM PEF of 10.6 L/min in the superiority comparison of 1xICS/tiotropium bromide versus 2xICS is smaller than those we have used in other ACRN trials, such as our beta-agonist trial (BAGS, Drazen 1996). In that and similar trials we have reasoned that an AM PEF difference in the range of 20 to 25 L/min would be robust and clinically meaningful. However, that and similar trials compared active drug treatment (regularly scheduled albuterol) with regularly scheduled placebo. Because we will compare 1xICS/tiotropium bromide to an active comparator (2xICS) we reasoned that a smaller effect size for superiority would be appropriate. After evaluating all primary and secondary endpoints, clinicians will be able to determine whether the clinical benefits of 1xICS/tiotropium bromide, including a possible AM PEF difference of 10.6 L/min, makes 1xICS/tiotropium bromide an attractive alternative to doubling the dose of an inhaled ICS in individual asthmatic patients.

The sample size formula for testing non-inferiority within the crossover design is similar to that of the superiority sample size formula given above, except that the non-inferiority boundary, Δ , replaces the effect size, δ . For the TALC protocol, the ACRN decided to set the effect size δ , equal to the non-inferiority boundary Δ . Ordinarily, the effect size is at least twice as large as the non-inferiority boundary. However, due to the increased concern about the use of long-acting β -agonists, and because of the smaller effect size chosen in the superiority comparison of 1xICS/tiotropium bromide with the active comparator, 2xICS, we thought this effect size was appropriate. From a clinical point of view, the Steering Committee felt that a larger bound of non-inferiority should be utilized

since clinicians would be willing to accept a wider boundary due to the safety concerns associated with the use of long-acting beta-agonists.

The following table summarizes the levels of statistical power for testing (1) superiority of Treatment Regimen A to Treatment Regimen B, and (2) non-inferiority of Treatment Regimen A to Treatment Regimen C, with respect to changes in AM PEF, FEV₁, and the proportion of asthma-control days when 210 participants are randomized. The sample size of 210 randomized participants was chosen because it yields at least 90% statistical power for testing the primary hypothesis of superiority and the secondary hypothesis of non-inferiority. Again, the treatment regimens are as follows:

Treatment Regimen A: 1 × ICS + tiotropium
 Treatment Regimen B: 2 × ICS
 Treatment Regimen C: 1 × ICS + long-acting beta agonist (LABA)

Statistical Power for a Sample Size of 210 Randomized Subjects

<u>Change in Response</u>	<u>St Dev (SLIC)</u>	<u>Effect Size</u>	<u>Superiority Stat Power (A vs. B)</u>	<u>Non-Inferiority Bound</u>	<u>Non-Inferiority Stat Power (A vs. C)</u>
AM PEF (L/min)	50.0	10.6	90%	10.6	90%
FEV ₁ (L)	0.40	0.09	93%	0.09	93%
Asthma-control days (proportion)	0.32	0.07	90%	0.07	90%

This sample size also provides the opportunity to evaluate and analyze subgroup responses to add-on anticholinergic therapy. This is especially true for individuals who are Arg/Arg homozygotes at the 16th amino acid locus in the ADRβ2 gene. In particular, there will be 80% statistical power within the Arg/Arg subgroup for detecting clinically meaningful effects in AM PEF (27 L/min), FEV₁ (0.22 L), and Asthma-Control Days (17.0%).

D. Missing Data

Because of the possibility of drop-outs and other missed visits, there will be some missing data. The statistical models and analyses that are planned for the primary and secondary outcomes assume that the data are missing-at-random (MAR). Because likelihood-based methods will be applied, MAR data still yield valid estimates. Although not expected, if it appears that the MAR assumption is not reasonable, then non-ignorable statistical analyses, such as pattern-mixture modeling, will be applied.

E. Interim Analyses and Data Monitoring

A formal interim analysis of efficacy data is not planned. The ACRN (II) Data and Safety Monitoring Board (DSMB), however, will be monitoring all of the safety data throughout the course of the trial and will be notified within 72 hours of any serious adverse events (SAEs) that occur.

VIII. DRUG SUPPLIES

A. Post-Randomization Drugs

All subjects will take three study drugs in a triple-dummy design during the 14-week treatment periods of the trial: an ICS bid at 1X or 2X (blinded for dose); tiotropium or tiotropium placebo q am; and LABA or LABA placebo bid. All subjects and study personnel will be blinded to study drug treatment during the three 14-week treatment periods of the crossover design. Subjects will receive an inhaler containing 1XICS to be taken 2 puffs bid during the 2-week washout periods (open-label).

This trial will require three post-randomization drug inhalers (inhaled corticosteroid, tiotropium/tiotropium placebo, long-acting beta-agonist/long-acting beta-agonist placebo).

Boehringer-Ingelheim will provide tiotropium bromide (18 µg of tiotropium bromide dry powder delivered with a Handihaler™) and matching placebo. Tiotropium bromide will be administered once daily in the morning.

We have chosen to use salmeterol (50 µg delivered via the Diskus™ device), for which we have previously identified a manufacturer of matching placebo, as the long-acting beta-agonist for this trial. Salmeterol will be administered twice daily in the morning and at night (1 puff BID).

Teva Pharmaceutical Industries, Ltd. will supply inhaled corticosteroid and matching placebo (beclomethasone dipropionate-HFA, QVAR). Beclomethasone dipropionate-HFA will be administered twice daily, in the morning and at night, at a dose of 80 µg (2 puffs of 40 µg each) twice daily for the 1xICS dose and 160 µg (2 puffs of 80 µg each) twice daily for the 2xICS dose.

B. Rescue Inhaler

Albuterol will be used as the rescue drug in this protocol.

IX. ADHERENCE AND MONITORING

Efforts will be made to determine subject adherence with medication dosing during the study. A Doser[®] device will be attached to each QVAR MDI inhaler. The Doser[®] device registers each actuation of the MDI and stores a daily history that will be reviewed at each clinic visit. Diskus inhalation counters also will be employed. The built in counters on these DPI devices will be examined to determine the number of inhalations that were used.

For the tiotropium/placebo which is supplied in blister packs, subjects will be asked to return both used and unused blister packs which will be tabulated by the coordinator to assess dosing compliance. Used capsules will be discarded to avoid exposing subjects and clinical coordinators to excess drug powder.

The Doser[®], the DPI counters, and assessment of used blister packs allow for objective measurement of the number of puffs used. A major limitation of these devices is their inability to discriminate between actual doses taken, and “dose-dumping.” They also do not yield much information regarding the timing of doses and the ability of subjects to comply with the dosing schedule required by the study.

As a secondary source of compliance information, subjects’ diary cards will be examined for the number of puffs of each medication recorded for each day. This information will be compared to PEF measurements electronically recorded and date/time stamped from the EPFM device. Because subjects are instructed to perform their peak flow maneuvers right before taking their study medications in the morning and evening, timing of PEF monitoring can be used as a surrogate for timing of dosing with study medications. Limitations of this mechanism for monitoring adherence are accuracy of the subjects’ recall and honesty, because the timing or confirmation of dosing cannot be verified directly.

X. RISKS AND BENEFITS

A. Risks

In many ways, the design of this protocol mirrors current clinical practice. Asthmatic patients who are judged to require daily controller therapy are first placed on an inhaled corticosteroid, as recommended by current guidelines (NAEPP 2002; GINA 2005). If this initial treatment does not result in good asthma control, treatment is escalated by increasing the inhaled corticosteroid dose or by adding a second asthma controller.

1. Risks During Run-In, Wash-Out/Run-In, and Run-Out with Patients taking Inhaled Corticosteroids Alone. The TALC and BASALT trials, which include patients with mild and moderate asthma, use a common run-in period to identify patients who are either reasonably well controlled with an inhaled corticosteroid (for BASALT) and suboptimally controlled (for TALC). Therefore, the run-in period using 1xICS reflects accepted clinical practice. Because TALC patients will have been identified to have

suboptimal asthma control on 1xICS they would appear to be at greatest risk during the Run-In, Wash-Out/Run-In, and Run-Out periods. Risks during this time are minimized by setting the initial Run-In period at 4 weeks during which each patient will be seen every 2 weeks, and by setting the Wash-Out/Run-In, and Run-Out periods at 2 weeks each. In addition, we have set the criteria for suboptimal asthma control as either an $FEV_1 \leq 70\%$ predicted, or clinical criteria (symptoms, rescue inhaler use, nocturnal awakenings due to asthma) slightly less than the threshold defined for "mild persistent asthma" (NAEPP 1997, 2002) (symptoms or rescue inhaler use 6 days per week would qualify a patient for TALC).

2. Risks Associated with Tiotropium Bromide Use. Any patient with a contraindication for tiotropium bromide use (narrow angle glaucoma, prostatic hypertrophy, bladder-neck obstruction, renal insufficiency) will be excluded from the trial. In addition, we will exclude any patients with coronary artery disease or a history of a myocardial infarction. We anticipate that tiotropium will provide substantial benefit in many patients in this trial because of its relatively potent bronchodilator activity (Barnes 2000, 2001). This reasoning is based on our view that the benefit provided by long-acting beta-agonists is also likely rendered primarily because of their bronchodilator activity, because we doubt that they display important anti-inflammatory effects *in vivo*. While the ACRN has observed that measures of pulmonary function do not always track with other important asthma outcomes (asthma exacerbations, treatment failure, for example), this occurred in a trial which studied salmeterol monotherapy (Lazarus 2001). When a second controller (a long-acting beta-agonist, a leukotriene modifier, or theophylline) has been added to an inhaled corticosteroid, the additional benefit derived appears to be proportional to the observed improvement in pulmonary function (Ringdal 2003; Kankaanranta 2004). Therefore, we do not anticipate significant increased risk because of the use of tiotropium in this trial, having excluded patients with contraindications for tiotropium bromide use.

B. Benefits and Benefit/Risk Analysis

Because not all patients respond to beta-adrenergic agents, and some appear to have adverse effects associated with their use, there is a need for additional controller medications which can be used in conjunction with an inhaled corticosteroid, when inhaled corticosteroid monotherapy does not provide adequate asthma control. This protocol will determine in these patients: 1) whether tiotropium bromide/1xICS is superior to 2xICS; and 2) whether tiotropium bromide/1xICS is not inferior to long-acting beta agonist/1xICS. If tiotropium bromide were found to be effective when used in this manner, important benefits for asthma patients would be anticipated. Because we estimate the risks associated with this protocol to be low, we judge the potential benefit/risk ratio associated with this work to be highly favorable.

XI. ANTICIPATED RESULTS

While the primary outcome measure selected for this trial is AM PEF, the trial has also been powered for, and has excellent power for, outcomes of FEV₁ and Asthma Control Days. These physiologic outcomes (AM PEF and FEV₁) will be interpreted in terms of the consistency of outcomes across secondary outcomes, particularly patient-centric outcomes which include Asthma Control Days and Good Asthma Control (similar to the GOAL study, Bateman 2004).

There are several possible outcomes for this study. 1) Tiotropium bromide/1xICS could be found to be superior to 2xICS and non-inferior to long-acting beta agonist/1xICS; 2) Tiotropium bromide/1xICS could be found to be not superior to 2xICS and non-inferior to long-acting beta agonist/1xICS; 3) Tiotropium bromide/1xICS could be found to be not superior to 2xICS and inferior to long-acting beta agonist/1xICS.

If tiotropium bromide/1xICS were found to be superior to 2xICS and non-inferior to a long-acting beta agonist/1xICS, we would have identified a safe and effective alternative to a long-acting beta agonist to be used in combination with an inhaled corticosteroid for patients with asthma.

If tiotropium bromide/1xICS were found to be not superior to 2xICS but non-inferior to a long-acting beta agonist/1xICS, we would have identified a possible alternative to a long-acting beta agonist to be used in combination with an inhaled corticosteroid for patients with asthma. This question would require further investigation. Examination of a comparison of 2xICS with long-acting beta agonist/1xICS, which has been included as a measure of internal validity, might prove insightful. We would predict long-acting beta agonist/1xICS to be superior to 2xICS, based on published studies (Greening 1994; Woolcock 1996; Shrewsbury 2000; Greenstone 2005). If this were not true, we would not expect tiotropium bromide/1xICS to be found superior to 2xICS. Reasons for this finding would need further investigation. An examination of secondary study outcomes might provide insight into the effectiveness of the various treatment regimes examined in this protocol.

If tiotropium bromide/1xICS were found to be not superior to 2xICS and significantly worse than a long-acting beta-agonist/1xICS, the trial could have been considered to have "failed." However, we will have obtained important new information concerning the effectiveness, or lack thereof, of tiotropium bromide for asthma treatment. However, it is possible, and perhaps even likely, that small subgroup(s) of patients will be identified that respond to tiotropium bromide/1xICS. For example, if 224 patients are randomized in this trial, 1/6 of them or approximately 38 patients should be homozygous for arginine (arg16arg) at the 16th amino acid position of the beta-2 adrenergic receptor, and approximately 40% (approximately 90 patients) homozygous gly16gly. Based on our retrospective analyses of the SOCS and SLIC data by beta-2 adrenergic receptor genotype (arg16arg versus gly16gly) (Wechsler 2006), this should provide a sufficient number of patients to determine whether a treatment strategy which avoids a long-acting beta-agonist is effective for arg16arg patients. We have avoided designing this

trial only for this subgroup of patients (arg16arg), because it is likely that there are other classes of asthmatics who either have adverse asthma outcomes, or would prefer an alternative to a long-acting beta-agonist, for asthma treatment. Post-hoc association studies with some of our exploratory endpoints (e.g., substances in exhaled breath condensate) may help to identify tiotropium bromide "responders." Our exploratory pharmacogenetic analyses may provide additional insight into patients who respond to one class of asthma controller but not another. Finally, because of the cross-over design employed in this protocol, we will identify patients who respond to one of the treatment strategies, two of the treatment strategies, all 3 strategies (2xICS, tiotropium bromide/1xICS, long-acting beta-agonist/1xICS), and none of strategies.

XII. RECRUITMENT STRATEGIES

Each clinical center involved in the ACRN was chosen based on documentation for patient availability, among other things. It is, however, worthy to note the specific plans of each center.

A. Harvard - Brigham and Women's Hospital, Boston, MA

The Boston Center has used a variety of recruitment methods to meet and exceed recruitment goals of previous ACRN studies.

Over the past five years, we have compiled an internal database of approximately 1500 individuals with asthma who are interested in participating in asthma studies. All of these individuals contacted us and expressed interest about asthma studies within the past year, and have been evaluated by our staff for participation in ongoing and future asthma clinical research studies.

The Boston site actively recruits subjects using a variety of external media. All methods are IRB-approved and include postcard mailings to area zip codes, newspaper advertisements, and broadcast e-mails and internet postings

Brigham and Women's Hospital has introduced a new clinical research tool called the BWH Research Patient Database Registry (RPDR) that allows researchers with proper IRB approval to query the hospital's patient database for potential research subjects. We recently queried this system and identified approximately 30,000 patients with a diagnosis of asthma. With permission from their primary care physician, patients may be contacted about current asthma research. We are in the process of developing tools to reach these patients through their physicians. Access to the physician database will further expand our capability to recruit asthmatic patients of differing severities.

B. National Jewish Asthma Research Center, Denver, CO

There are over 400 asthma subjects (not followed in the National Jewish outpatient clinic) that have participated in research studies conducted at the Denver Center. Many

of these subjects have been through various medication studies and bronchoscopies with lavage/biopsies. Their FEV1s range from 30-110% of predicted.

1. Denver Health Medical Center – Dr. James Fisher, Head of Pulmonary Medicine, is supporting efforts of the Denver Center by helping to recruit from the asthmatic subject population at the Denver Health Medical Center. This is a large county hospital whose subject population comprises mainly Hispanic and African-American people.

2. Denver Veterans Administration Hospital – Dr. Carol Welsh, Pulmonary faculty member, will support this grant. The VA hospital has a large outpatient clinic of patients with asthma, but not chronic obstructive pulmonary disease.

3. Denver Kaiser Permanente HMO – Dr. Timothy Collins is the Director of Pulmonary Medicine and Dr. John Williams is the Director of Allergy at Kaiser. Drs. Collins and Williams have been actively involved in supporting research at National Jewish in the past by referring subjects. Their groups will continue to play an active role in clinical research support.

C. University of Texas Medical Branch, Galveston, TX

The University of Texas site has developed an infrastructure to support all clinical and translational trials (Translational Research Unit for Asthma, Immunology, and Respiratory Diseases [TRU/AIR]). It is directed by Dr. William Calhoun, and ably assisted by Drs. Andrew Grant and Bill Ameredes. Each of these investigators has more than 13 years of experience with clinical and translational trials. The TRU/AIR is headed by Lisa Nemeth, RN, CCTC, who has more than 12 years of experience with multicenter clinical trials. The TRU/AIR includes several technicians, nurses, and a respiratory therapist who serve as Clinical Study Coordinators.

We recruit from the local and regional population using print and electronic media; all advertising and posting materials are approved in advance by the IRB at UTMB. In addition, we recruit from APICS Divisional (Allergy, Pulmonary, Immunology, Critical Care, and Sleep) clinics, which number more than eight ½ day clinics per week. Volunteers who express interest in response to any of the recruiting channels are recorded in a local data base.

Further, Dr James Goodwin, who directs the Sealy Center on Aging, and an NIH funded population study, has agreed to make available his database information on demographically characterized populations of subjects in the Southeast Texas region in support of the ACRN.

The population in our catchment area is about 35% Caucasian, 35% Hispanic/Latino, and 30% African American. Existing population databases have demographic characteristics similar to the population statistics.

D. Washington University, St. Louis, MO

The St. Louis site actively recruits subjects using a variety of external media. All methods are IRB-approved. These include newspaper advertisements in the local and minority newspapers, the University newspaper, posting flyers throughout the medical school campus, and the university website called "Volunteer for Health." This is a service the University offers to match interested volunteers with current clinical trials at the medical school. This service has a website, and anyone can access this with the web address.

Over the past 10 years, Dr. Castro has compiled an internal database of more than 400 individuals with asthma who are interested in participating in asthma studies. All of these individuals have contacted us and have expressed an interest in participating in an asthma study. These individuals have been evaluated by our staff for participation in ongoing and future asthma clinical research studies.

E. University of California, San Diego, CA

Recruitment activities at UCSD Clinical Trials Center is multi-faceted and includes a computerized database with current and previously enrolled subjects, direct advertising, and community outreach programs such as educational lectures on asthma, attendance at health fairs with staff conducting pulmonary screening tests. All activities, flyers and advertisements are approved by the UCSD Human Research Protection Program prior to initiation.

The UCSD Clinical Trials Center database has over 500 asthmatics who have been previously enrolled or expressed an interest in participating in a clinical trial. Interested subjects are entered into the database with fields for demographic, medical, medication, and pulmonary function tests. Quarterly newsletters and flyers are mailed to the subjects with specific information on trials and to maintain accurate contact information of the individuals.

In addition, this application is supported by the Naval Medical Center and Kaiser Permanente Healthcare whose directors (Drs. Warren Lockette and Michael Schatz) are faculty members at UCSD. The Clinical Investigation Department (CID), at Naval Medical Center, San Diego (NMCS D) is directed by Warren Lockette, M.D. and is dedicated to fostering training and research in both basic and patient-oriented research at the Naval Medical Center, San Diego. Dr. Lockette collaborates with the CTC recruitment program to recruit subjects from the active and retired navy community in San Diego for CTC studies. The NMCS D has 700,000 outpatient visits each year and serves as a provider of primary care to 260,000 patients living within an easy commute, i.e. a 40-mile radius of the hospital.

Kaiser Permanente Healthcare: Dr. Schatz is the Director of the Allergy Division of the Kaiser Permanente Healthcare of Southern California, Permanente Medical Group and a faculty member at UCSD. In San Diego alone, they serve over 600,000 members with

over 11,000 identified asthmatic subjects. Kaiser-Permanente has a fully operational computerized pharmacy records system, which provides identification of patients using anti-asthma medications. This system will be used to access patients with asthma under the care of primary care physicians and nurses. In addition, because of freeway access to UCSD and traffic, the CTC has been successful in recruiting from southern Los Angeles, Orange and Riverside Counties. Kaiser members living in that region will also be recruited. Dr. Schatz has previously collaborated with Dr. Wasserman on NIH-sponsored research projects and will continue this active collaboration and contribute to the recruitment for the ACRN protocols.

F. University of California, San Francisco, CA

Study population: The UCSF center's recruitment of asthmatic subjects relies on community advertising and on maintaining a database of subjects who have participated in previous studies, come for a "characterization" visit, or expressed interest in participating. They advertise in the San Francisco Chronicle, the Bay Guardian, and in neighborhood and college newspapers. They also advertise on "Craigslist," a Web-based bulletin board on local radio and television stations. They post fliers on neighborhood and campus bulletin boards, and present our studies to physician groups. Responses to these advertisements are made to a dedicated telephone number. A dedicated recruiter, Lila Glogowsky, responds to each inquiry to obtain basic information about demographics and about asthma severity, duration, and treatment. She schedules apparently qualified subjects for a "characterization visit" in which a coordinator obtains a detailed history and performs spirometry and skin testing.

Subject Characterization: The UCSF center's methods for characterizing subjects conform to national guidelines (e.g. spirometry), to widely accepted custom (e.g. methacholine challenge), or to its own standards as the center developing the method (eg., sputum induction and analysis). They have adopted standardized questionnaires for assessing asthma symptom severity, asthma control, and asthma-related quality of life. They have developed questionnaires on asthma history, patterns of health care utilization, and domestic exposure to allergens.

The recruitment/characterization program is supported by a data-base program ("File-Maker Pro") on a dedicated server. Phenotypic information is now stored on >5,000 potential subjects of a variety of ethnic backgrounds (64% Caucasian, 13% African American, 7% Hispanic, 10% Asian and 6% other).

Subjects at the University of California San Francisco: In addition to community advertising, subjects are recruited, especially those with severe asthma, from clinical programs overseen by UCSF faculty at Moffitt, S.F. Veteran's Administration, S.F. General, and Mt. Zion Hospitals. The faculty is responsive to approaches from colleagues conducting clinical trials and there has been collaboration with the Division of General Internal Medicine to recruit for specific protocols. This Division follows approximately 18,000 patients, of whom 8% (2,683) have a primary or secondary diagnosis of asthma (ICD-9 493.00, 493.01, 493.10, 493.11, 493.20, 493.21). Of these

asthmatic patients, 48% are White, 20% Asian/Pacific Islander, 10% Latino, 16% African American, and 1% Native American. Sixty-four percent are female.

G. University of Wisconsin, Madison, WI

The Allergy Research Program of the University of Wisconsin maintains a file of potential subjects with mild to moderate asthma who are interested in future research participation. These individuals have been screened and/or participated in previous asthma studies. The following information is maintained: birth date, gender, ethnic background, age of asthma diagnosis, childbearing status, atopic status (including results of skin testing if performed previously), concurrent medical history, asthma and non-asthma medications. Approximately 85% of subjects in this database have "mild to moderate" asthma. This database of subjects will be used as the primary source of recruitment for this protocol. If additional subjects are needed, they will be recruited via U.W. Human Subjects committee-approved, newspaper advertising and from the U.W. Allergy Clinic subject population as well as the U.W. Sports Medicine Clinic, U.W. Student Health, V.A. Allergy Clinic, and the Northeast Family Practice Clinic.

H. Wake Forest University Health Sciences Center, Winston-Salem, NC

The Cloverdale Clinical Research Center at Wake Forest University Health Sciences and the Center for Human Genomics maintains a screening database of approximately 1075 subjects with asthma. These are subjects who have called our clinic expressing interest in participating in asthma research studies. Some have been screened for or have participated in past research studies at our site. The following information is maintained on these subjects as it is obtained: gender, age, ethnic background, medical history, asthma history, skin testing results, exhaled breath condensate results, exhaled NO results, methacholine challenge testing results, pulmonary function, sputum induction results, bronchoscopy results, chest x-ray results, and medication usage. Should additional subjects be needed beyond this database of potential subjects, we continuously advertise for potential subjects using television, radio, and newspaper and flyer advertising (all advertising is IRB approved), as well as recruitment from the Wake Forest University Health Sciences Pulmonary and Allergy Clinics through our Primary and Sub-Investigators.

I. Columbia University Medical Center, New York, NY

Columbia University Medical Center is the main hospital providing service to the 265,000 residents of Washington Heights/Inwood and to many of the 712,541 people living in Northern Manhattan.

The Asthma Center at Columbia maintains a comprehensive database of all individuals who have responded to our recruitment efforts for asthma studies since 1996. To date, this database consists of over 1,800 asthmatic individuals who have expressed an interest in study participation. Their names have been generated in response to newspaper and radio advertisements, physician referrals, posting and distribution of

flyers and community health screening events. All of these subjects have completed phone questionnaires regarding their asthma and medication use; additional information maintained includes age, gender, duration of asthma and demographic details. Approximately 20% of these individuals have been screened at the Columbia University Asthma Center and have had pulmonary function testing performed. Potential study subjects will be identified through screening of this actively updated database and potentially eligible subjects will be contacted in a manner approved by the IRB.

The John Edsall/John Wood Asthma Center at Columbia Presbyterian Medical Center sees approximately 1,200 patient visits per year. Approximately 90% of visits were from patients living or working in Northern Manhattan, approximately 80% are insured by Medicaid. The severity of asthma varies among these patients; approximately 21 % are in the mild category, 63 % are in the moderate category and 16 % are in the severe category, 57% of the patients are atopic as determined by history or skin testing; IgE levels have been measured in the majority. 76% of patients followed at this clinic are female. Approximate demographic makeup of patients is 83% Hispanic, 14 % African American and 3 % other, including Caucasian. Patients included on this database are actively followed in the Asthma Center at Columbia and their asthma is well characterized. These patients have a longstanding relationship with providers in the clinic and have participated in many asthma clinical studies.

Advertisements: We plan to utilize IRB approved newspaper and radio advertisements to inform potential subjects of our studies. We have had success with recruiting subjects through advertisements in newspapers that target ethnic minorities living in Northern Manhattan, the South Bronx and surrounding areas. We will also advertise in media that reaches individuals city-wide. Responses to advertisements will be answered by a dedicated phone line to be manned during business hours and answered by voicemail at other times. A research assistant will respond to each inquiry immediately, using a screening instrument. We plan to regularly post and advertise our studies at the four colleges located in Northern Manhattan. We will also distribute flyers throughout the community on a regular basis, display posters at gathering places such as stores, laundromats, eating establishments and at community centers. Flyers advertising clinical studies will continue to be distributed along with educational materials at all asthma workshops and seminars. We have found these relatively low budget strategies to be highly effective.

Community Awareness of Clinical Trials: Efforts of the Columbia University Asthma Coalition to empower residents of Northern Manhattan by educating them about asthma and the ability to control the disease through lifestyle changes and with controller medications is likely to set the stage for interest in participation in clinical trials. As a result of outreach efforts, we have established contacts with various ethnic community, university, church and business groups and have conducted many community based asthma programs. The close collaboration with community based organizations that we have developed through our Asthma intervention program has resulted in referrals into clinical trials. Our advertisement posters are regularly displayed within these organizations, and staff working at the community based organizations have referred

patients to us for research participation, often as a means of allowing uninsured individuals to receive asthma medications and monitoring.

J. Duke University Medical Center, Durham, NC

Duke University recently opened the Duke Asthma, Allergy and Airway Center, a 13,000 square foot facility designed for the evaluation of clinical and research patients with airway disease. We are in the process of creating a HIPAA and IRB-approved database to capture clinical data from patients receiving care at the asthma center. Recruitment efforts focus primarily on Durham but also include Chapel Hill, Research Triangle Park and Raleigh. Durham County has a diverse population that includes 39% African Americans, 11% Hispanics and 3% Asian Americans. Subjects are recruited using print media (advertisements in the local newspapers), radio advertisements and television. The recruitment of African Americans and Hispanics is accomplished through advertisements at community events.

XIII. ADVERSE EVENTS

A. Definition

An adverse event shall be defined as any detrimental change in the subject's condition, whether it is related to an exacerbation of asthma or to another unrelated illness. Adverse events related to asthma exacerbation will be managed according to rescue algorithms outlined below.

B. Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal from the trial if the illness is considered significant by the investigator or if the subject is no longer able to effectively participate in the study. Subjects experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness also are recorded. Examples of minor intercurrent illnesses include acute rhinitis, sinusitis, upper respiratory infections, urinary tract infections, and gastroenteritis. Medications are allowed for treatment of these conditions in accordance with the judgment of the investigator.

Documentation of an adverse event unrelated to asthma will be recorded on a Clinical Adverse Event Report Form and will include the following information:

- Description of the illness
- Dates of illness
- Treatment of illness (medications, doses, dates)
- Whether hospitalization or emergency treatment was required
- Treatment outcome

C. Adverse Events Related to Asthma: Asthma Exacerbation

During the course of the study, subjects may experience an increase in asthma symptoms. While an increase in asthma symptoms may be brief and self-limited, any increase in symptoms or changes in PEF should be carefully monitored by the subject, the clinic coordinator, and the physician. During the course of the study, symptoms may be of sufficient severity so as to warrant documentation as an asthma exacerbation.

1. Asthma Exacerbation: Definition

For this protocol, an asthma exacerbation is defined as the development of an increase in asthma symptoms (e.g. cough, chest tightness, or wheezing) which results in an increase in asthma controller medications, typically inhaled corticosteroids and/or oral or parenteral corticosteroids.

Subjects developing an asthma exacerbation during the initial run-in period will be terminated from study participation and may re-enroll after the exacerbation has fully resolved. Subjects developing an exacerbation during the wash-out/run-in period prior to the second and third double-blind treatment periods may return to the trial with the schedule of visits outlined in section XIII.E below.

Once an asthma exacerbation has occurred, the subject should contact the clinic coordinator and/or be evaluated at the study site or the nearest medical emergency facility as quickly as possible.

2. Management of Exacerbations

Asthma exacerbations that occur following randomization will be managed according to the rescue algorithms described below. During medical management of the exacerbation, other trial medication will be continued, unless the treating physician considers it appropriate to suspend such therapy until the exacerbation resolves. Reinstitution of trial medications will occur when the exacerbation has resolved at the discretion of the investigator. A record of all medications, dosages, and frequency of occurrence will be kept during exacerbations.

Rescue algorithms will employ open label medication, including albuterol.

a. Rescue Algorithms

Once an asthma exacerbation has occurred, the subject should contact the clinic coordinator and/or be evaluated at the study site or the nearest medical emergency facility as quickly as possible.

Because less significant changes in symptoms and/or PEF may precede more severe alterations in asthma stability, a series of rescue algorithms has been developed to address the various clinical presentations that may occur. Once any of these rescue interventions leads to the administration of oral or increased inhaled corticosteroids, the subject also will be considered to have developed an asthma exacerbation. In addition, if in the opinion of the treating physician, corticosteroid therapy is warranted regardless of any antecedent measurements of pulmonary function (PEF, FEV₁, etc.), value for symptom score, or frequency of rescue beta-agonist use, the subject will be considered to have developed an asthma exacerbation.

Asthma exacerbations that occur following randomization will be managed according to the following rescue algorithms. Treatment for worsened asthma symptoms will be the addition of open-label inhaled corticosteroid at the dose used during the run-in periods, or a higher dose. During medical management of the exacerbation, other trial medication will be continued, unless the treating physician considers it appropriate to suspend such therapy until the exacerbation resolves. Reinstitution of trial medications will occur when the exacerbation has resolved at the discretion of the investigator. A

record of all medications, dosages, and frequency of occurrence will be kept during exacerbations.

Rescue algorithms will be applied in cases where an exacerbation fails to resolve or PEF is not improved to $\geq 70\%$ of reference level within 48 hours after increasing as-needed rescue albuterol use. Rescue algorithms are based on recommendations from the NAEPP Guidelines for the Diagnosis and Management of Asthma. Albuterol, inhaled steroids, and oral prednisone are the principal medications for rescue management. Subjects will be instructed in their use for home management, and supplies of albuterol and prednisone will be provided throughout the study. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician.

1) Home Care

Asthma exacerbations will be recognized by an increase in symptoms and by a corresponding drop in PEF below baseline level. Subjects will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity.

Baseline PEF levels are defined as follows:

- During the first 2 weeks of the run-in period (interval between Visits 1 and 2), baseline is defined as the spirometer PEF value (converted to liters/min) associated with the best FEV1 obtained during baseline spirometry at Visit 1.
 - During the last 2 weeks of the run-in, baseline is the average AM PEF during the interval between Visits 1 and 2 established from diary cards returned at Visit 2.
 - Following randomization at Visit 3, baseline is the average AM PEF during the interval between Visits 2 and 3.
- Subjects who recognize increased symptoms and/or a fall in PEF to $< 70\%$ of baseline level will use albuterol by MDI, 2-4 puffs every 20 minutes up to 60 minutes if needed, and then every 4 hours, or less, if needed. Subjects will be instructed to use the as-needed “rescue” albuterol inhaler for treatment.
 - If the PEF does not increase to $\geq 70\%$ baseline level or if symptoms are not improved after the first 60 minutes of albuterol therapy, the subject should contact the investigator or their primary care physician or seek care in the emergency department.
 - Failure of albuterol to control or maintain PEF $\geq 70\%$ of baseline level may necessitate the use of corticosteroids (see below).

- Subjects who use ≥ 10 puffs of rescue albuterol per day (not including preventative puffs) for a period of 48 hours should contact the investigator. Use of corticosteroids may be necessary (see below).

2) Physician's Office or Emergency Room Treatment

- Subjects will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the subject's PEF and/or FEV₁ is less than 25% predicted or if the subject shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles, sampling of arterial blood for respiratory gas analysis is indicated, with appropriate action taken depending on the results obtained.
- When treated in the physician's office or the hospital emergency room, subjects should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 minutes over the first 60 minutes.
- If the PEF increases to $\geq 70\%$ of reference level after the first 60 minutes, the subject can be discharged to continue treatment at home. Prednisone or open-label inhaled corticosteroids may be administered at the discretion of the physician to augment therapy.
- If symptoms persist and PEF remains $<70\%$ of reference level, nebulized albuterol should be continued as often as every hour and further treatment with oral or parenteral corticosteroids should be considered (prednisone, 60 mg orally; methylprednisolone, 60 mg iv bolus; or dose deemed appropriate by the treating physician). Monitoring of PEF or spirometry should continue every hour. Within four hours of treatment, a decision should be made regarding subject disposition.
- If PEF increases to $\geq 70\%$ reference level within four hours, the subject can be discharged to continue treatment at home. Home treatment should include an 8-day course of prednisone followed by open-label inhaled corticosteroid treatment (see below).
- If PEF remains $>40\%$ but $<70\%$ of reference level, an individualized decision should be made to hospitalize the subject for more aggressive therapy or to continue therapy at home with a course of prednisone followed by inhaled corticosteroids.
- If PEF is $<40\%$ reference level after repeated albuterol treatments, the subject should be admitted to the hospital unless, in the physician's best judgment, alternative treatment could suffice.

3) Prednisone Treatment

In this protocol, prednisone will be used when, in the judgment of the investigator, acute exacerbations cannot be controlled by albuterol and inhaled corticosteroid therapy. Indications for prednisone therapy include the following:

- To achieve stable control of symptoms and optimize pulmonary function once asthma exacerbation status is achieved.
- For follow-up management after discharge from the physician's office, emergency room, or hospital for an acute exacerbation.

The dose of prednisone used during an acute exacerbation shall consist of 60 mg as a single dose every day for three days (or that which is deemed appropriate by the treating physician), followed by a 10 mg/day taper over the next five days. The decision to initiate or to continue a course of prednisone beyond eight days is left to the discretion of the physician.

4) Inhaled Corticosteroid Treatment

Inhaled corticosteroid dosing for worsened asthma symptoms during the TALC trial will be the addition of open-label inhaled corticosteroid at the dose used during the run-in periods, or a higher dose at the physician's discretion.

D. Adjustments of Trial Medications during Asthma Exacerbations

Trial drugs will be continued during exacerbations unless the treating physician considers it appropriate to suspend such therapy until the exacerbation resolves. Reinstitution of trial medications may occur when the exacerbation has resolved at the discretion of the investigator. A record of all medications, dosages, and frequency of occurrence will be kept during exacerbations.

E. Study Center Visits Following Exacerbations

If the subject receives open-label inhaled or systemic steroids for an exacerbation, regular follow-up evaluations will continue as outlined in the original protocol schema (intent-to-treat). All medications used to treat exacerbations will be recorded and entered into the study database. For safety reasons, all subjects will be seen at the clinical center within one week (+3d) from the day they have been categorized as experiencing an asthma exacerbation. Following this "safety" visit, subsequent protocol visits generally will continue in accordance with the visit schedule established at Visit 3 (first treatment period), Visit 7 (second treatment period) or Visit 11 (third treatment period), with some exceptions as discussed below.

The time in which an asthma exacerbation develops in relationship to the schedule of the TALC protocol will affect the manner in which future clinic visits, medication

adjustments, and diagnostic studies are scheduled or performed. The scheduling of these events is outlined below.

Subjects developing an asthma exacerbation during the initial run-in period (weeks 1-4) will be terminated from study participation and may re-enroll after the exacerbation has fully resolved.

Subjects who experience an exacerbation in the weeks prior to Visit 7 (beginning of second double-blind treatment period) or Visit 11 (beginning of third double-blind treatment period) may need to delay entering the double-blind treatment period until a sufficient period of time has passed to allow for washout of rescue medications. In particular, Visit 7 (or 11) will be delayed until at least 4 weeks have passed since the documentation of exacerbation status and, if prescribed, until at least 4 weeks have passed since the last dose of oral prednisone, and, if prescribed, until at least 2 weeks have passed since the last dose of rescue open-label inhaled corticosteroid. If a subject falls into the required washout period at the time that he or she was originally scheduled to complete Visit 7 (or 11), then an additional safety visit will be performed prior to Visit 7 (or 11). At that safety visit, spirometry will be performed, diary cards will be reviewed and dispensed, and the subject's asthma symptoms will be assessed. The subject will be given additional wash-out/run-in medications to last for 2 weeks. If, after this safety visit, more than 2 additional weeks are required to meet the minimum washout period, then a second safety visit will be scheduled. If necessary, additional interim visits will be scheduled at 2-week intervals (+3 day window) during the wash-out period. Interim visits will be designated Visits 6A, 6B, 6C, etc. (or 10A, 10B, 10C, etc.), as needed. When less than 2 weeks are needed to meet all washout requirements, Visit 7 (or 11) will be scheduled for 2 weeks following the current visit. All necessary washouts will be reviewed and confirmed prior to performing Visit 7 (or 11) procedures.

F. Criteria for Achieving Dropout Status

- Subject becomes pregnant
- Subject withdraws consent

G. Criteria for Withdrawal from Study Due to Asthma Exacerbation

For safety reasons, subjects will end the study treatment regimen during any of the three double-blind treatment periods if they have two asthma exacerbations requiring treatment with oral or parenteral corticosteroids. Blinded study drugs will be discontinued during treatment of the second exacerbation. At the conclusion of oral or parenteral corticosteroids as treatment for the second exacerbation subjects will be placed on open-label 2xICS for 1 week followed by open-label 1xICS for 1 week. If subjects cannot tolerate this two week treatment period (1 week with 2xICS, 1 week with 1xICS) because of an asthma exacerbation, they will be withdrawn from the study. Subjects who are stable after the two week treatment period will continue on open-label 1xICS alone for the remainder of the treatment period. Subjects who experience a third

exacerbation (by any criteria) during a given treatment period will be withdrawn from the study for safety reasons.

H. Adverse Events as Outcome Variables

During exacerbations, the following variables will be recorded and used as additional outcome measures:

- Hospitalization
- Emergency room visits
- Unscheduled physician/clinic visits
- Number of subjects having an asthma exacerbation

XIV. COSTS, LIABILITY AND PAYMENT

All tests will be performed without cost to the participating subjects. Since this is a trial using a well-established asthma treatment, liability for subject care costs incurred by subjects during the course of the trial will in most cases be borne by the subject or the insurer. Details of the National Institutes of Health policies concerning this issue can be found in NIH Documents # 5305 and 6352-2, *Research Patient Care Costs Supported by NIH Sponsored Agreements*, which are in the *ACRN Manual of Operations*.

Each subject will receive financial compensation within FDA guidelines for participation in an amount determined by the local center. For subjects who drop out, payments will be pro-rated for the length of time they stayed in the study, but payment will not be made until the study would have been completed had the subject not dropped out.

XV. DATA RECORDING

Recording of all data including the informed consent, history, physical examination, results of allergy skin testing, vital signs, electrocardiogram, results of pregnancy tests, adverse events, confirmation of medication dispensation, methacholine challenge testing, and questionnaires will be recorded on forms prepared by the ACRN Data Coordinating Center. Initial data entry will be done at each Clinical Center and forms will be forwarded to the DCC for confirmatory entry. Results from pulmonary function tests will be transmitted electronically to the DCC. All data will be stored and analyzed at the DCC.

XVI. EQUIPMENT

Manuals of Procedures (MOPs) have been developed and have been in use for performance of all ACRN procedures (spirometry, methacholine challenge, NO collection, etc) including ACRN equipment calibration.

Equipment to be used in the TALC trial include the following:

1. Skin Testing

The Multi-Test II provided by Lincoln Diagnostics, Inc. will be used for skin testing. The Multi-Test II device is a sterile, disposable, multiple test applicator used to administer skin-test substances. This device meets OSHA guidelines for technician protection, and it provides a lower coefficient of variation than similar devices and than a bifurcated small pox needle.

2. Exhaled Breath Condensate

Equipment (R tube and condensers) are supplied by Respiratory Research, Inc.

3. Exhaled nitric oxide

NIOX machine provided by Aerocrine, Inc.

4. Spirometry

Spirometry equipment has been provided by QUANTUM Research, Inc. The spirometry equipment has been customized for ACRN.

5. Peak-Flow Meter

The AM1 device by Viasys will be used. The AM1 device will provide daily measurements of peak flow and also will provide compliance checks.

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XVIII. PROTOCOL IN TABULAR FORM

Visit	1a ⁵	1b ⁶	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Week	0	0	2	4	8	13	18	20	24	29	34	36	40	45	50	52
Window (reg/ext) (Days)			±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5
Study Phase	Open-Label ICS Run-In		14-Week Double-Blind Treatment Period 1				14-Week Double-Blind Treatment Period 2				14-Week Double-Blind Treatment Period 3			Wash out		
Allocation to TALC				X												
Randomization				X												
Informed Consent	X															
Medical History	X															
Long Physical Exam	X															X
Short Physical Exam				X				X				X				
Venipuncture for IgE /Eosinophils			X													
Venipuncture for genetic analysis (optional)			X													
Skin Testing ⁷			X													
Heart Rate Assessment (ECG or oximeter; ECG required at Visit 3)				X			X	X			X	X			X	
Urine Pregnancy Test	X			X				X				X			X	
Exhaled NO (ENO) Collection				X			X				X				X	

⁵ Subjects who have prebronchodilator FEV₁ ≥ 55% of predicted and are eligible to perform a methacholine challenge will undergo the challenge at Visit 1a to determine eligibility; subjects who are ineligible to perform the methacholine challenge will undergo albuterol reversibility testing with 4 puffs of albuterol at Visit 1a.

⁶ Subjects who do not meet methacholine PC₂₀ criteria at Visit 1a may return for albuterol reversibility testing (4 puffs) for eligibility assessment at Visit 1b at the study investigator's discretion.

⁷ If a subject is unable to perform the allergy skin test at Visit 2 due to necessary drug washouts per the ACRN Skin Testing MOP, he/she may perform this procedure at a later TALC visit.

Visit	1a ⁵	1b ⁶	2	3	4	5	6	7	8	9	10	11	12	13	14	
Week	0	0	2	4	8	13	18	20	24	29	34	36	40	45	50	52
Window (reg/ext) (Days)			±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5
Exhaled Breath Condensates (EBC) Collection				X			X				X				X	
Spirometry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Albuterol Reversal (4 puffs)	(X)	X		X			X				X				X	
Ipratropium Reversal (4 puffs) ⁸			X													
Methacholine Challenge ⁹	(X)															
Sputum Induction				X			X				X				X	
Asthma Evaluation Questionnaire (AEQ)	X			X												
ACQ/ASUI Questionnaire			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Asthma QOL Questionnaire (AQLQ)				X			X	X			X	X			X	X
Sleep and Daytime Alertness Questionnaire (SDAQX)				X			X	X			X	X			X	
Coordinator/Patient Questionnaire (CPQ)							X				X				X	

⁸ Subjects who have a history of allergy to soy or peanuts will not be administered ipratropium for reversal testing at Visit 2.

⁹ Alternatively, a historic PC₂₀ from an ACRN methacholine challenge performed within 6 months of the Visit 1 date by an ACRN-certified technician may be used to qualify the subject.

Visit	1a ⁵	1b ⁶	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Week	0	0	2	4	8	13	18	20	24	29	34	36	40	45	50	52
Window (reg/ext) (Days)			±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5
ACRN Satisfaction Questionnaire (final study visit; optional)																X
MDI/HandiHaler/ Diskus Technique Assessment	X	X		X												
Medication Dispensing	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Medication Review			X	X	X	X	X	X	X	X	X	X	X	X	X	X
EPFM Dispensing	X	X														
Diary Card Dispensing	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Diary Card Review			X	X	X	X	X	X	X	X	X	X	X	X	X	X

XIX. APPENDIX 1 - ACRN Asthma Evaluation Questionnaire

ASTHMA CLINICAL RESEARCH NETWORK

ASTHMA EVALUATION QUESTIONNAIRE

Rev 3.0 28 August 2006

Please consider your last two weeks of asthma control in answering these questions. Check the box next to the response that best describes your asthma symptoms.

1. In the past two weeks, how often have you experienced asthma symptoms?

- 0 Less than or equal to 2 days a week
- 1 3 to 5 days per week
- 2 6 or more days per week, but not continual
- 3 Continual (multiple times every day)

2. In the past two weeks, how often have you used your rescue beta-agonist medicine (e.g., albuterol (Proventil, Ventolin)), aside from preventive use prior to exercise?

- 0 Less than or equal to 2 days per week
- 1 3 to 5 days per week
- 2 6 days per week
- 3 At least once per day (daily)

3. In the past two weeks, how often have you awakened at night due to asthma symptoms?

- 0 No awakenings or awakened 1 night during the 2 weeks
- 1 1 night per week
- 2 2 or 3 nights per week
- 3 4 or more nights per week