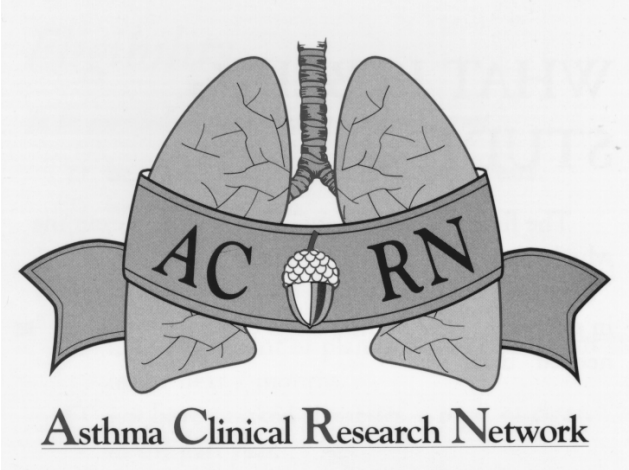


Salmeterol Off Corticosteroids (SOCS)

Study Protocol

Comparison of Clinical Benefit, Anti-Inflammatory Effect, and Duration of Benefit of an Inhaled Corticosteroid, a Long-Acting Beta-Adrenergic Agonist, and Placebo in Patients with Moderate Asthma.

Version 1.7
December 20, 1996

I. HYPOTHESIS TO BE TESTED

Proposed Null Hypothesis: In patients with moderate asthma whose symptoms are well-controlled by using an inhaled β-agonist on an "as needed" basis and an inhaled corticosteroid on a scheduled basis, continued treatment with the inhaled corticosteroid does not differ in efficacy from a change to therapy with a long-acting β-agonist.

This null hypothesis was proposed to examine the importance of anti-inflammatory therapy in altering symptoms, airway function, and bronchial reactivity in patients with moderate asthma.

II. BACKGROUND AND RATIONALE

A. Introduction

The approval of a long-acting, highly potent, selective β-agonist for use in the U.S. presents potential problems for the thoughtful practitioner, especially in the treatment of patients with moderately severe asthma requiring inhaled corticosteroids, as outlined in the NAEP's guidelines. The problems presented are those of knowing how to advise a patient on the relative values of an inhaled corticosteroid and a long acting β-agonist. If symptoms are well controlled on the combination of the two drugs, must therapy be continued with both of them? Does regular use of a long-acting β-agonist carry the risk of worsening asthma? Alternatively, can asthma be controlled with a long-acting β-agonist alone, eliminating the possible risks of local or systemic toxicity from long-term use of inhaled corticosteroids?

Little data are available to permit a reasoned approach to these issues, and it is the purpose of this proposed study to compare the efficacy of an inhaled corticosteroid alone with that of an inhaled long-acting β-agonist alone and with placebo, in subjects with moderate asthma both while they are taking the medications and during a period immediately after therapy is withdrawn.

It is not known how differences in the mechanisms of action of β-adrenergic agonists and inhaled corticosteroids translate into differences in efficacy in the long-term management of chronic asthma. ***Beta-agonists*** are extremely effective as bronchodilators and for three decades have been widely used as first-line therapy for patients with asthma. Recent studies have suggested that the long-term, regular use of inhaled β-agonists may contribute to a worsening of asthma control (1) or increase the risk of death or near-death from asthma (2). The mechanisms underlying the putative adverse effects of β-agonists are unknown, but speculation has focused on the possibility that β-agonist use might worsen underlying airway mucosal inflammation, whether because the bronchodilation produced by these agents permits patients to continue exposure to antigens and other materials that provoke inflammation or because the active agent or its enantiomer stimulates inflammation

(3, 4). Concern over the evidence of an adverse effect of β-agonists, coupled with a growing appreciation for the role of inflammation in the pathogenesis of asthma, has led to a change in the use of β-agonists. The NHLBI's Expert Panel on the Management of Asthma recommended that inhaled β-agonists continue to be used as required for the relief of symptoms, but that anti-inflammatory therapy be instituted in patients who require β-agonists on a daily basis (5). Sears has gone further in suggesting that reducing β-agonist use is an essential step in treating patients with chronic severe disease (6).

Inhaled corticosteroids are the anti-inflammatory agents most used for chronic therapy in adult asthmatics. The major action of corticosteroids in asthma remains a topic of debate, but mechanisms believed to be important include interference with arachidonic acid metabolism, inhibition of the directed migration and activation of inflammatory cells, inhibition of release of mediators, and increasing the responsiveness of β-adrenergic receptors on airway smooth muscle. Although increasing use of "high-dose" inhaled corticosteroid therapy has raised concerns about the potential for adverse effects with long-term use, standard doses of corticosteroids given by inhalation appear to be safe and effective (7, 8, 9, 10, 11).

These observations have shaped the view of the roles of β-agonists and corticosteroids in asthma therapy. Beta-agonists are viewed as symptomatic therapy, providing relief, but having no effect, or possibly worsening, the inflammatory mechanisms underlying the disease, whereas inhaled corticosteroids are viewed as "disease modifiers," acting on inflammatory mechanisms fundamental to asthma's pathogenesis. Support for the concept that these agents differ in their properties as "disease modifiers" is provided by a study showing the ability of an inhaled corticosteroid to modify asthma symptoms and airway function even after administration of the medication was stopped or reduced ("off effect"). In that study, the clinical benefit of an inhaled corticosteroid persisted 3 months after the completion of 12 months of therapy (12), whereas worsening of clinical control of asthma has been found even while β-agonists were being taken regularly (1). It has not been shown, however, that changes in airway mucosal inflammation produced by inhaled corticosteroids are responsible for the persistence of asthma control after treatment is discontinued.

Possible differences in the disease-modifying activities of inhaled corticosteroids and β-agonists have become of greater interest with the introduction of **long-acting beta-adrenergic agonists.** The molecular structure of these agents enables them to maintain β_2-adrenoceptor occupancy by anchoring to an adjacent exoreceptor site (13, 14). This linkage results in a prolongation of the duration of action. A single inhaled dose of **salmeterol** induces bronchodilation that lasts at least 12 hours in patients with asthma (15), and prevents bronchoconstriction induced by methacholine (16, 17, 18), histamine (19), allergens (20), hyperventilation with cold air (21), or exercise (22) for 12 hours or longer. In addition, salmeterol may have anti-inflammatory properties. Studies *in vitro* have shown that salmeterol inhibits release of inflammatory mediators from human lung

fragments for up to 20 hours, and that the duration and magnitude of this effect is greater with salmeterol at equipotent concentrations than with shorter-acting β_2-agonists (23, 24). Salmeterol attenuates microvascular leakage and inflammatory cell infiltration in guinea pig lung for 6 to 8 hours (25), and markedly inhibits mediator release from neutrophils (25) and alveolar macrophages (26) *in vitro*. Studies of human asthmatics suggest that salmeterol has anti-inflammatory effects *in vivo*, preventing antigen-induced early- and late-asthmatic responses and bronchial hyperresponsiveness (20), improving PEF for one week after salmeterol was discontinued (27), and decreasing circulating eosinophils (28). Some of these anti-inflammatory properties of salmeterol have been confirmed in asthmatics by bronchoscopy and bronchoalveolar lavage (29, 30). While early studies of the regular use of salmeterol suggest that it reduces the symptomatic severity of asthma (28, 31), the relative importance of its bronchodilating versus anti-inflammatory activities is not known.

Four recently reported, large clinical trials have examined the safety and efficacy of inhaled β-agonists in asthmatic subjects and have not found evidence of harm. D'Alonzo et al. reported that, in 322 asthmatic subjects, salmeterol inhaled twice daily for 3 months is more effective than albuterol inhaled four times daily. No deterioration of asthma control was observed with the use of salmeterol over a 3 month period (32). In addition, Greening et al. studied 429 asthmatic subjects who still had symptoms despite maintenance treatment with 200Φg twice daily of inhaled beclomethasone and found that the addition of salmeterol (50 Φg twice daily) for 6 months was more effective than higher dose beclomethasone therapy in improving morning and evening peak flow rates, use of rescue bronchodilator medications, and daytime and nighttime symptoms of asthma. There was no evidence that the addition of salmeterol resulted in deterioration of asthma control (33). In a similar study, Woolcock et al. reported that the addition of salmeterol as twice daily inhaled therapy was more effective than was doubling the dose of inhaled beclomethasone in improving peak expiratory flow and in reducing as needed use of albuterol in 739 asthmatic subjects with persistent symptoms despite regular use of beclomethasone (34). Woolcock also examined bronchial reactivity 3 and 14 days after completion of therapy and found that no treatment arm was associated with more than a doubling dose change in PC_{20}. Finally, Chapman et al. studied 341 asthmatics in a four-week randomized cross-over trial of salbutamol 200Φg four times daily for 2 weeks and as needed for two weeks (35). They found no significant difference in morning and evening peak flows between treatments but asthma symptoms and supplementary bronchodilator use were significantly less frequent when salbutamol was given regularly.

How the differences in the mechanisms of action of these different classes of pharmaceutical agents translate into differences in efficacy in the long-term management of chronic asthma is not yet known. The relative importance of anti-inflammatory versus bronchodilator activity might be inferred by analyzing the relationship between reductions in markers of inflammation in the airways at the completion of different therapies and the rate of recurrence of symptoms over the period immediately thereafter ("off effect"), for symptoms are more likely to recur quickly after chronic therapy with agents having only

bronchodilator action than after treatment with agents altering a fundamental pathogenetic mechanism. We thus propose a study of these different classes of therapeutic agents in the long-term treatment of asthma, comparing not only their efficacy in reducing symptoms, but also their effects on markers of inflammation in the airways, and on the rate of recurrence of symptoms after therapy is discontinued.

B. Specific Aims

This study is designed to examine the importance of inflammation and of anti-inflammatory therapy in moderate asthma.

The specific aims of this study are as follows:

To determine whether, in patients with moderate asthma, an inhaled corticosteroid and an inhaled long-acting β-adrenergic agonist with possible anti-inflammatory activity (salmeterol) differ from each other or from placebo in:

(1) efficacy in reducing symptoms, reducing bronchial reactivity, and improving peak flow
(2) efficacy in reducing the numbers of inflammatory cells and concentrations of inflammatory mediators recovered from the airways
(3) duration of therapeutic benefit after cessation of therapy
(4) systemic toxicity

This study also is designed to examine the safety and efficacy of methacholine challenge in the evaluation of subjects with moderate asthma.

C. Research Questions

The choice of pharmaceutical agents for the chronic treatment of a disease is based on an assessment of the relative efficacy regarding clinical endpoints, risks, and the potential for modifying the disease state. For patients with moderate asthma, physicians can choose among a small number of "anti-inflammatory agents", of which inhaled corticosteroids are the current "gold standard," but it is not known whether treatment with a long-acting β-agonist might not be equally or more effective. The evidence that asthma is an inflammatory disease suggests that anti-inflammatory therapy will be more effective at reducing symptoms and in modifying the course of disease than will alternate forms of treatment (without anti-inflammatory activity). The goal in this trial is to compare therapy with an inhaled corticosteroid alone with therapy with a long-acting β-agonist alone.

In testing the stated hypothesis, this study will examine patients with moderately severe asthma whose symptoms are well-controlled by treatment with an inhaled corticosteroid, to address the following research questions:

1) Over a 16 week period, does continuation of regular treatment with an inhaled corticosteroid have different effects on AM Peak Expiratory Flow (primary outcome variable) or on PM PEF, asthma symptom score, as needed β-agonist use, quality of life score, FEV_1, or bronchial reactivity to methacholine than does replacement of inhaled corticosteroid therapy by regular treatment with a long-acting β-agonist or with placebo?

2) Is there an association between changes in markers of inflammation and the "on effect" or "off effect" of therapy? (role of inflammation)

3) Do these three treatment regimens differ in the duration of protective effect over six weeks after therapy is discontinued, as reflected by the frequency of exacerbations of asthma and by the indices of asthma control used to compare effectiveness during the 16 weeks of continued treatment?

4) Is there a difference in the toxicity of the 2 treatment regimens compared with placebo, as reflected by a detectable difference in markers of systemic steroid effects?

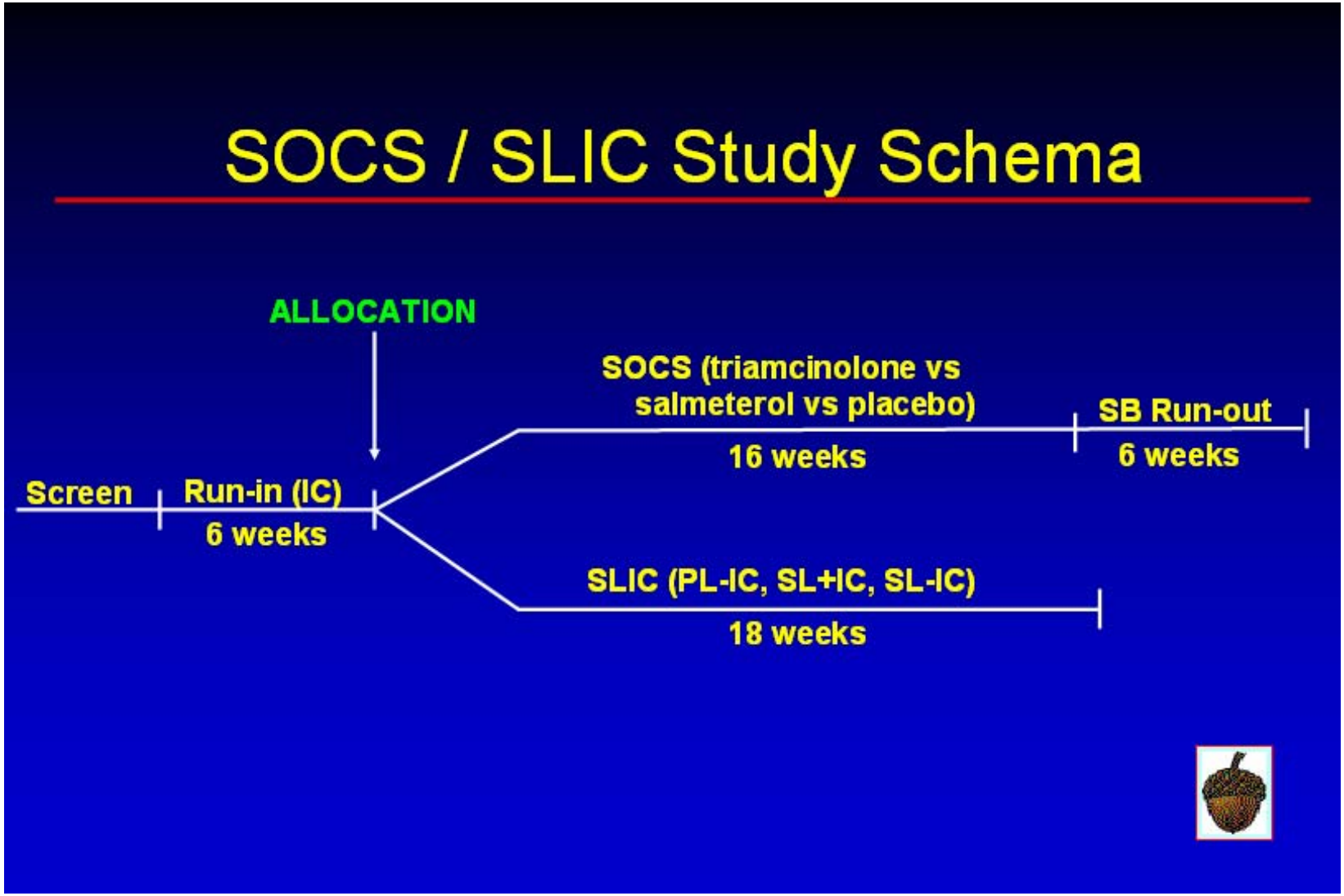
In addition to issues related to salmeterol therapy in moderate asthma, two additional questions regarding the safety and efficacy of methacholine challenge will be addressed:

5) Is there a difference in the frequency of asthma exacerbations following clinic visits at which methacholine challenge is performed, compared with visits at which it is not?

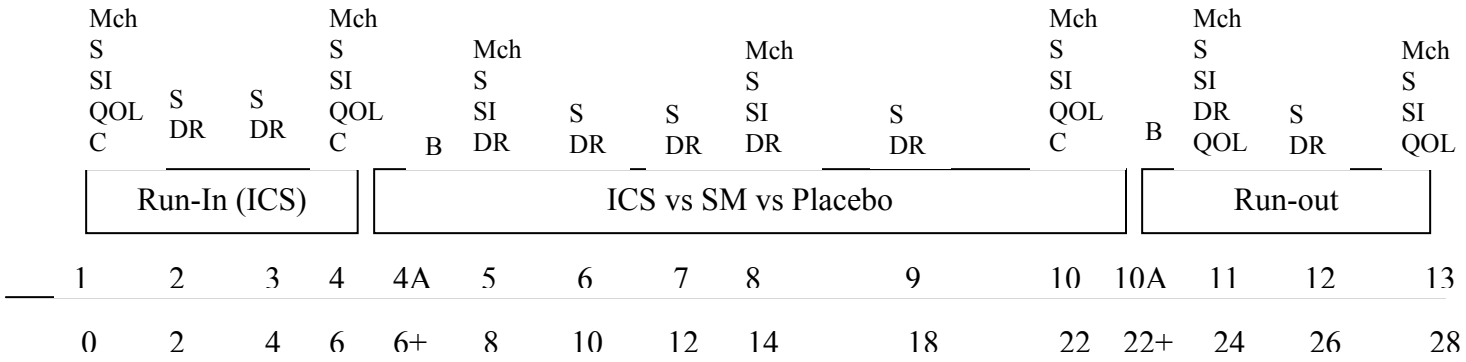
6) How does PC_{20} compare with other measures of asthma outcome, such as AM and PM PEF, FEV_1, symptom scores, rescue beta-agonist use, and quality of life scores?

D. Rationale for Choosing these Questions

Treatment guidelines suggest the use of "anti-inflammatory agents" for those patients who require β-agonists more than 2-3 times per day (5, 36). These recommendations are based upon observations suggesting that asthma is an inflammatory disease, and that the regular use of inhaled β-agonists may be deleterious. Despite these recommendations, many medical practitioners appear to be reluctant to recommend chronic therapy with an inhaled corticosteroid (37, 38, 39), perhaps because of concern over the possible risk of toxicity with long-term use (40, 41, 42, 43, 44, 45, 46). The availability in the US of long-acting inhaled β-agonists, which have enjoyed popularity in Europe because of their convenience and efficacy, may further encourage practitioners to prescribe β-agonists rather than anti-inflammatory agents. In this context, it is important to define the role of anti-inflammatory agents as "disease-modifying" therapy. The rationale for the chronic use of anti-inflammatory therapy would be supported by demonstrating in patients with moderate asthma that use of inhaled corticosteroids differed from treatment with long acting β-agonists either during the period of active treatment or for some period of time after cessation of treatment, and that this improvement was associated with a reduction in markers of inflammation.


SOCS / SLIC Study Schema
ALLOCATION
SOCS (triamcinolone vs salmeterol vs placebo)
16 weeks
SB Run-out
6 weeks
Screen
Run-in (IC)
6 weeks
SLIC (PL-IC, SL+IC, SL-IC)
18 weeks

Detailed Schematic:



Mch=Methacholine reactivity; SL=sputum Induction/analysis; DR=diary review; QOL = quality of life questionnaire; C=24 h urine cortisol; B=bronchial biopsy performed in subset; V=Visit number; W=Week number

General Study Design: This will be a 28-week, randomized, double-blind, prospective multi-center trial comparing in subjects with moderate asthma the efficacy of an inhaled corticosteroid (triamcinolone acetonide) with that of an inhaled long-acting β-adrenergic agonist (salmeterol xinafoate) and with placebo, during 16 weeks of therapy, and for 6 weeks following cessation of therapy. After a 6 week run-in period, during which they receive inhaled corticosteroid (triamcinolone, 4 puffs BID) and as-needed rescue albuterol, subjects will be randomized to receive either the inhaled corticosteroid (triamcinolone, 4 puffs BID) or salmeterol (2 puffs BID) or placebo (2 puffs BID) for the next 16 weeks. All subjects will be provided with albuterol inhalers to use as rescue medication throughout the study. Subjects will take the study medication for 16 weeks, then stop medication for an additional 6 week single-blind study period, to examine the "off effect" of the medications. Beginning with the run-in period, subjects will record in a daily diary their daytime and nighttime symptoms (dyspnea, wheeze, cough, each scored 0-3), AM and PM PEF, β-agonist use, and intercurrent illnesses and hospitalizations. They will visit the clinical center every 2-4 weeks for an interval history and physical examination, diary review, and spirometry. Bronchial challenge with methacholine and sputum induction for analysis of markers of inflammation will be performed upon entry into the study, at the end of the inhaled steroid run-in period (week 6), after 2 weeks (week 8) and 8 weeks (week 14) of double-blind therapy, at the end (week 22) of the 16 week double-blind period, and 2 and 6 weeks (week 28) after cessation of therapy. Those subjects unable to perform sputum induction adequately during Visit 4, will not be asked to attempt sputum induction for the remainder of the study. In addition, bronchial biopsies will be obtained in a subset of patients over the age of 18 at the end of the inhaled steroid run-in period (week 6) and at the end (week 22) of the 16 week double-blind period. Quality of life will be assessed upon entry into the study, at the end of the inhaled steroid run-in period (week 6), 2 (week 8) and 8 (week 14) weeks after the start and at the end (week 22) of the 16 week double-blind period, and 2 (week 24) and 6 (week 28) weeks after cessation of therapy, using a general survey instrument (Medical Outcomes Survey Short Form 36) and an asthma specific instrument (Juniper Asthma Quality of Life Questionnaire).

III. INCLUSION AND EXCLUSION CRITERIA

A. Inclusion Criteria (at Visit 1)

1. Male and female subjects, between the ages of 12 and 65 years.

2. For patients not already taking inhaled corticosteroids:

   a) FEV_1 # 80 % of predicted.

   b) Document from the preceding 6 months a ∃ 12% increase in FEV_1 after aerosolized albuterol.

3. For patients already taking inhaled corticosteroids:
    a) FEV_1 ∃ 40% of predicted.
    b). If FEV_1 is 40-80% of predicted, patient must:

    - document from the preceding 6 months ∃ 12% increase in FEV_1 after aerosolized albuterol

    OR

    - document from the preceding 6 months or demonstrate a 20% reduction in FEV_1 in response to a concentration of inhaled methacholine # 8mg/ml ($PC_{20}FEV_1$ # 8mg/ml).

    c) If FEV_1 is > 80% of predicted, patient must document from the preceding 6 months or demonstrate a 20 % reduction in FEV_1 in response to a concentration of inhaled methacholine # 8mg/ml ($PC_{20}FEV_1$ # 8mg/ml).

4. 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.

5. Non-smoker (total lifetime smoking history # 10 pack - years; no smoking for at least 1 year).

6. No smokeless tobacco use for at least one year.

B. Exclusion Criteria (at Visit 1)

1. Use of any drugs listed in Table 1 during the designated washout period prior to Visit 1, or intention to take the drug during the study.

Table 1. Drugs to be withheld throughout the study	**Washout prior to Visit 1**
Inhaled Steroids, except as provided in study	None
Oral Steroids	∃ 6 weeks
Cromolyn/Nedocromil	∃ 6 weeks
Oral beta-adrenergic agonists	∃ 1 week
Monoamine oxidase inhibitors	∃ 4 weeks
Tricyclic antidepressants	∃ 4 weeks
Beta-adrenergic blockers	∃ 4 weeks
ACE inhibitors	∃ 4 weeks
Inhaled beta-adrenergic agonists (intermediate-acting, e.g., albuterol, terbutaline, metaproterenol, pirbuterol, bitolterol), except provided in study	∃ 8 hours
Salmeterol, except as provided in study	∃ 48 hours
Anticholinergics	∃ 48 hours
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, Uniphyl)	∃ 48 hours
Antihistamines (for Astemizole and Loratidine, see below)	∃ 72 hours
Astemizole, Loratidine	∃ 72 hours *
Zafirlukast (Accolate)	> 6 weeks
Zileuton (Zyflo)	> 6 weeks
Drugs withheld prior to pulmonary function and/or methacholine challenge, per MOP	**Specified time period**
Albuterol	∃ 6 hours
Salmeterol Study Drug	∃ 48 hours
Terfenadine (Allegra)	∃ 48 hours
Chlorpheniramine (ChlorTrimeton)	> 48 hours
Methylxanthine-containing foods or beverages (e.g., coffee, tea)	∃ 8 hours
Alcohol-containing foods or beverages	∃ 8 hours

*Since these medications may remain in the body for weeks following administration, it is recommended that prick skin testing be performed in patients who are currently on

treatment. If a flare ∃ 5 mm develops following testing, the patient would be eligible forstudy participation. If not, the patient would need a longer washout period (possibly weeks) prior to retesting and enrollment.

2. Medication use: Chronic use of any medication other than β-agonists and inhaled corticosteroids, except oral contraceptives and other hormonal forms of contraceptives (i.e., DepoProvera[7], Norplant[7]), estrogens/progesterone replacement therapy for post-menopausal women, vitamins, nasal beclomethasone (2 puffs each nare, BID) at a stable dose throughout the entire study (see MOP), acetaminophen, non-steroidal anti-inflammatory medications (e.g., aspirin, naproxen, ibuprofen), thyroid replacement medications, terfenadine, anticholesterol medication, or medium and low potency topical cutaneous steroids.
   Allowable medications also include:
   a. calcium supplements
   b. nasal saline spray
   c. topical eye preparations for allergic eye symptoms (e.g. antihistamines, NSAIDs, or antiallergic compounds)
   d. diuretics
   e. specific antihypertensives (e.g. calcium channel blockers, clonidine, etc.)
   f. antibiotics for acne
   g. stool softeners, psyllium
   h. acyclovir
   i. chlorpheniramine (48 hour washout)
   j. pseudoephedrine (48 hour washout)

3. Lung disease other than asthma.

4. Established diagnosis of vocal cord dysfunction.

5. Significant medical illness other than asthma.

6. History of respiratory tract infection within the previous 6 weeks.

7. History of a significant exacerbation of asthma in the previous 6 weeks (see section VI for definition of significant exacerbation and guidelines for treatment).

8. History of life-threatening asthma requiring treatment with intubation and mechanical ventilation within the past 10 years.

9. Hyposensitization therapy other than an established maintenance regimen.

10. Inability, in the opinion of the investigator or clinical coordinator, to coordinate use of a metered dose inhaler (MDI).

11. Changes of ischemic heart disease or arrhythmia on screening ECG (not excluded for occasional, #3/min, atrial or ventricular premature contractions or clinically insignificant sinus bradycardia).

12. Pregnancy. If potentially able to bear children, not using an acceptable form of birth control (see ACRN MOP, Appendix One).

13. Inability, as evidenced through biological quality control testing, to correctly use an AirWatch™ device for recording peak flow measurements.

C. Inclusion Criteria For Randomization (at Visit 4)

1. FEV_1 > 80% of predicted.
2. Average variability $\frac{PM-AM}{1/2(PM+AM)}$ in PEF # 20% during the final two weeks of the run-in period (weeks 4-6).
3. Ability of the subject to measure his/her AM and PM PEFR on schedule using the AirWatch device, to appropriately mark the measurements using the post-medication marker, and to accurately transcribe the PEFR measurements onto his/her diary cards at least 85% of the time during the last two weeks of the run-in.

D. Exclusion Criteria For Randomization (at Visit 4)

1. Significant exacerbation of asthma during the run-in period (see section VI for definition of significant exacerbation and guidelines for treatment).

2. Inability to comply with regular use of Azmacort (use of scheduled MDI less than twice/day on more than 12 days in the run-in period, as reflected by the diary card.

3. Failure to record peak flow measurements and symptoms in a symptom diary on average more than two days/week during the run-in period.

4. Any changes with regard to any of the exclusion criteria identified for visit 1 (see section IIIB above).

5. Use of an average of ∃ 16 puffs of albuterol per 24 hours during last week of run-in (week 6).

E. Criteria For Assigning Treatment Failure Status During Double-blind Treatment Period

1. Requirement for 1 course of prednisone for treatment of asthma exacerbations (see section VI for definition of significant exacerbation and guidelines for treatment).

2. More than one Emergency Department or Urgent Care Visit for treatment of asthma exacerbation.
3. Hospitalization for treatment of asthma exacerbation.

4. Physician clinical judgement for safety reasons.

F. Criteria For Assigning Drop-out Status During Single-blind Run-out Period

1. FEV_1 # 50% of predicted and inability to reverse to within 5% of baseline FEV_1 (Visit 1 value).

2. Emergency Department or Urgent Care visit requiring treatment for asthma exacerbation.

3. PEF # 65% of Reference Level (average AM pre-bronchodilator PEF for two weeks prior to Visit 4) despite albuterol treatment.

4. An increase in symptoms associated with either:

   X an increase in "as-needed" β-agonist use of ∃ 8 puffs per 24 hours over baseline use for a period of 48 hours,

   Baseline defined during double-blind period: Average daily use during the last two weeks of the run-in period.

OR

   X use of ∃ 16 puffs of "as-needed" β-agonist per 24 hours for a period of 48 hours.

5. Use of oral or parenteral corticosteroids for an asthma exacerbation.

6. Physician clinical judgement for safety reasons.

G. Inhaled Corticosteroid Dose after Treatment Failure

1. If patients meet criteria for treatment failure status, their dose of inhaled corticosteroids will be increased to the dose they were receiving at Visit 4

(800 Φg/day) delivered by an open-label inhaler. Study inhalers of corticosteroids (or its placebo) will be returned to the clinical center. If they meet the criteria detailed in Section VI, they will be treated with appropriate rescue algorithms for an asthma exacerbation. Study inhalers of salmeterol (or placebo) will be continued unless the treating physician can document that treatment failure status was causally related to taking this medication. They will continue to participate in the study until its termination (intent-to-treat analysis), but no eliminations in inhaled corticosteroid dose will be attempted during this time period. For safety reasons, all subjects will be seen within one week (∀ 3d) from the day they have been categorized as achieving treatment failure status. Following this "safety" visit, subsequent protocol visits will continue in accordance with the visit schedule established at Visit 1 (see SOCS/SLIC MOP for details concerning possible replacement of scheduled protocol visits with treatment failure related visits).

IV. OUTCOME VARIABLES

For comparison of the effects of therapy, the primary outcome variable will be the change in AM peak expiratory flow from the final week of the run-in period (week 6) to the final week of the double-blind treatment period (week 22). A longitudinal data analysis will be applied in which a segmented linear model is fit (see section IX.E) and the estimated AM peak expiratory flow at the end of the treatment period will be compared to that at the end of the run-in period. With this type of analysis, all of the data between weeks 6 and 22 are used in evaluating AM peak expiratory flow (Daily data from post-randomization diary cards will be summarized and analyzed as weekly averages. Because subjects are instructed to withhold their study salmeterol or placebo for 48 hours prior to a visit, the affected peak flow values will be excluded). For comparison of the duration of benefit, the primary outcome variable will again be AM PEF, comparing the change from the final week of the run in period (week 6) to the second week and the final week in the off-treatment period (week 24 and week 28).

Peak flow from the run-out period will be estimated using a segmented linear model. Secondary endpoints will be other markers of asthma severity (FEV_1, symptom diaries, as needed use of albuterol, quality of life scores, PC20 to methacholine) and similar analyses will be applied. Other endpoints will be the number of exacerbations in the treatment and off-treatment periods and comparisons of markers of severity at different time points (to examine, for example, the rate of any deterioration in AM PEF after switching from ICS to long-acting β-agonist and the time course of any difference in the off-treatment periods). Using Kaplan-Meier survival analysis we will compare treatment failures between the triamcinolone, salmeterol and placebo groups during the double-blind treatment phase, with specific criteria (Section IIIE) to distinguish between treatment failure due to poor asthma control and drop-out for any other reasons.

To determine if differences in efficacy during therapy, or differences in the duration of therapeutic benefit after cessation of therapy, reflect differences in anti-inflammatory activity, induced sputum samples will be obtained at weeks 1, 6, 8, 14, 22, 24 and 28, and will be analyzed for total cell count and differential and concentrations of eosinophil cationic protein, and tryptase, as markers of airway inflammation, eosinophil activation, and mast cell activation, respectively. In addition, bronchial biopsies will be obtained in a subset of adult patients (n=45) at the end of the inhaled corticosteroid run-in period (week 6) and at the end of the 16 week double-blind period (week 22). These samples will be analyzed for eosinophils in epithelium and in submucosa and T-lymphocytes in submucosa. ACRN procedures for induced sputum and bronchoscopy and biopsy are in the Manual of Operations.

The primary analyses will be conducted as described, using longitudinal data analyses based on a segmented linear model. A secondary analysis will be performed by analyzing the rate of loss of subjects due to asthma exacerbations during the run-out period, analyzing deterioration by Kaplan-Meier survival curves.

V. PROTOCOL

A. Subjects

1. Sample Size. To have an 80% likelihood of detecting clinically significant changes in morning peak flow (See section IX.E), we estimate that a total of 150 subjects will be required. This sample size will detect a 15.7L/min difference in AM PEF in a 3-armed trial, assuming a withdrawal rate of 20%. This sample size also yields effect sizes for FEV_1 and PC_{15} of 0.17 L and 0.68 dose steps (less than 1 doubling dose) respectively.

The primary source for the estimate of variability used to calculate the sample size is the recently-published study by Haahtela et al. (47). This study defined a difference in AM PEF that occurred between week 52 of a 12 month double-blind treatment period (that followed a 2 year inhaled corticosteroid treatment period) and the average of a 4 week run-in period for 37 subjects randomized to treatment with an inhaled corticosteroid alone or placebo.

The sample size calculation for the subset of subjects in whom bronchoscopy and bronchial biopsies will be performed was based upon a recent study by Djukanovic (48) in which bronchial biopsies were obtained from 10 asthmatics before and after 6 weeks of therapy with an inhaled corticosteroid. We estimate that enrolling 9 subjects for bronchoscopy at each center (n=45 total) will allow us to detect significant changes in eosinophils and T-lymphocytes with 80% statistical power, allowing for a 20% withdrawal rate and a block randomization, to ensure that equal numbers of subjects in each treatment arm are randomized.

2. Subjects. This study will require a total of 150 subjects with moderate asthma. Patients will be recruited from the "standing" populations of the participating centers, by advertisement, and by referral from participating physicians. Patients will meet the inclusion criteria specified herein and not possess any of the exclusion criteria. Both heterogeneity of the study group and rapidity of recruitment are greatly facilitated by the involvement of several geographically dispersed study centers in a multi-center collaboration. At randomization there will be stratification according to ethnic group, gender, and age. Each center can recruit 25% of patients from the 12-18 years age group. Bronchoscopy will not be performed in subjects less than 18 years old. Every attempt will be made by each center to enroll approximately equal numbers of patients of either gender and to include in their enrolled patients at least 33% (up to 50 patients) from under-represented minorities (Native Americans, Asian-Pacific Islanders, Blacks, and Hispanics). Because bronchial reactivity is an important endpoint, subjects will also be stratified at randomization based upon PC_{20} at the end of the 6 week run-in period. This will insure an equal distribution of subjects with $PC_{20} < 2$mg/ml and $PC_{20} \exists 2$mg/ml. The Data Coordinating Center (DCC) will distribute monthly accrual reports for each clinical center, listing patients entered by age, gender, and ethnicity. This routine monitoring will allow early identification and resolution of problems in achieving demographic goals.

B. Recruitment

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.

Harvard Clinical Center/Boston

1. Need

Approximately 30 patients with moderate asthma are needed to fulfill the recruitment needs of this study at this center. We propose to use the population at Harvard Community Health Plan to achieve our enrollment goals.

2. Potential Participants Stratified by Severity

To assess the number of potential participants, computerized pharmacy records of all individuals who had been Plan members for at least 3 months, who were between 12 and 65 years of age, who had pharmacy benefits and who had received prescriptions for β-agonists plus inhaled steroids were selected. Such individuals were also retained in this category if they received concurrent prescriptions for one other asthma medication such as theophylline, cromolyn, or nedocromil. Severe asthma was operationally defined as being extant in those individuals who had received prescriptions for β-agonists plus 2 or more asthma drugs where one of these agents was oral or inhaled steroids. Prescription frequency was not considered when assigning severity categories between moderate and severe patients; failure to consider this may have resulted in an inappropriate assignment of severity category.

3. Results
9,885 asthmatic individuals were identified of whom 7,588 (76.7%) met the definition of mild asthma, 1,883 (19.0%) met the criteria for moderate asthma and 414 (4.3%) met the criteria for severe asthma.

4. Recruitment strategy
We will contact a fraction of the 1,883 individuals identified as having moderate asthma by the pharmacy search by letter. In this solicitation, attention will be paid to postal zip code to achieve the needed minority patients.

National Jewish Center/Denver

Research subject recruitment has been very successful for all types of asthma patients at the National Jewish Center for Immunology and Respiratory Medicine. The total of 30 subjects with one-half being female and one-third minority population will come from the following areas.

1. National Jewish Center Outpatient Clinic. The adult clinic saw 1,079 new asthmatic patients over the last year with 503 being from the Denver metropolitan area. Another 335 from the Denver area were seen in follow-up. The severity of asthma varies among these patients, but approximately 50% are in the moderate category. The pediatric clinic saw 490 new asthmatic children with 352 being from the Denver metropolitan area. Again, these patients were of varying severity, but about 40% are in the moderate category. Ninety-seven additional children were seen in follow-up. The National Jewish Center has changed markedly over the last decade. We have evolved from a primary inpatient facility with a small clinic to a very active outpatient service. Thus, we are seeing many more asthmatic patients of all degrees of severity.

2. National Jewish Center Asthma Research Pool. There are over 200 asthma patients (not followed in the NJC outpatient clinic) that have participated in our research studies. Many of these subjects have been through various medication studies and bronchoscopies with lavage/biopsies. Their FEV_1s range from 30-110% of predicted.
a. Denver General Hospital - Dr. Michael Hanley, Acting Head of Pulmonary Medicine, is supporting our efforts by helping us to recruit from the asthmatic patient population at Denver Health Medical Center. This is a large county hospital whose patient population comprises mainly Hispanic and African-American people.
b. Denver Veterans Administration Hospital. Dr. Carol Welch, acting Pulmonary Director, will support this grant. The VA hospital has a large outpatient clinic of patients with asthma, but not chronic obstructive pulmonary disease.
c. Denver Kaiser Permanente HMO. Dr. Timothy Collins is the Director of Pulmonary Medicine and Dr. William Marsh is the Director of Allergy at Kaiser. Drs. Collins and Marsh have been actively involved in supporting research at NJC in the past by referring us patients. Their groups will continue to play an active role.

d. Dr. Jay Markson is a pediatrician in a large inner city clinic and will support this grant by recruitment of patients.

University of Wisconsin/Madison

The Allergy Research Program of the University of Wisconsin maintains a file of potential subjects with moderate asthma (FEV_1 50-80%) 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 21% of subjects in this database have "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 patient population. Also, the U.W. Adolescent Clinic, U.W. Sports Medicine Clinic, U.W. Student Health, V.A. Allergy Clinic, Northteast Family Practice Clinic, Wingra Family Practice Clinic and Verona Family Practice Clinic will serve as other patient sources, in cooperation with the patient's primary physician. Referrals from the private allergy clinics in town will also be sought. Radio and television advertising may be utilized. Approved recruitment ads will be posted in churches, community centers, grocery stores, and U.W. Student Organization headquarters. Recruitment of women and minorities from the available pool will be emphasized. To improve the recruitment of people of color, especially African-Americans, a part-time community liaison has been hired to assist. This population is being targeted by placing advertisements and articles in community newspapers and journals. Community-based programs have been established in order to increase awareness about the University of Wisconsin Allergy Research Program's studies and to develop trust within the community. In addition, work is being done to try to eliminate any barriers that might prevent someone from participating such as lack of transportation.

Harlem Prevention Center/New York

Central Harlem has a residentially stable population of approximately 115,000, of whom 98% are African American or Hispanic, and 53% are women. The prevalence of asthma in Central Harlem is 3-4 times that in the U.S. population. Harlem Hospital and its network of community-based clinics, together comprise the Northern Manhattan Network. Through the Network, the Harlem Asthma Research Center (HARC) has identified more than 2,000 asthmatic patients who are in stable primary care relationships, and established collaborative arrangements with their primary care providers.

The Harlem Asthma Research Center will initially recruit participants in ACRN clinical trials through this network of collaborating providers. While the Center will specifically target people of color, it will never turn anyone away.
The investigators anticipate no difficulty in recruitment of women. Accrual of participants

will be monitored for all protocols. If targeted approaches are needed, the HARC will consider strategies which have been used successfully to recruit and sustain the participation of women in this community. These have included provision of transportation, meals, child care, home visits, utilizing peer educators, the formation of a woman's support group, culturally appropriate education efforts and linkages to support services.

Primary care physicians from the Northern Manhattan Network will approach their patients about their willingness to participate in the clinical trials. If they are interested, the screening and all follow-up visits will take place at the Harlem ACRN Clinical Center. Because asthma clinical trials will require procedures that are not performed routinely in primary care offices, appropriate procedures will be followed so that patients participate fully in ACRN protocols while staying in contact with their primary care providers as needed.

Thomas Jefferson Medical College/Philadelphia

All patients with a diagnosis of asthma currently cared for in the outpatient offices of the Division of Pulmonary Medicine and General Internal Medicine and the Departments of Family Medicine and Pediatrics are listed in a computerized data-base. Approximately 23% of asthmatics in this database have "moderate" asthma. Terminals located at each clinic site are linked to the ACRN file server located in the study coordinator's office. Patients fulfilling every criteria for a given study will be identified by the database, and personal contact will be made by the study coordinator for the purpose of explaining the study and enlisting their participation. If on initial contact, the patient agrees, they will return to the study center to verify entry qualifications and further discuss the study.

University of California/San Francisco

Our approach to recruiting subjects with asthma for research studies relies heavily on community advertising. We place advertisements in editions of the San Francisco Chronicle and Examiner, in small neighborhood newspapers, and on bulletin boards on the UCSF campus, in community health centers, and at campuses of colleges and universities in the Bay Area. We also place advertisements on two popular radio stations (one "soft rock" station; one "soul" station). Finally, we place fliers in the patient waiting areas of the Pulmonary Medicine and Allergy Clinics at the major teaching hospitals of UCSF (Moffitt-Long, San Francisco General Hospital, Ft. Miley V.A. Hospital, and Mt. Zion Hospital). Responses to these advertisements are made to a dedicated telephone number equipped with voice mail. We have hired a full-time recruiter to respond to each inquiry and to obtain basic information about the subject's demographics and about the severity, duration, required treatment, and frequency of symptoms of asthma. Subjects who pass this telephone screen and who are interested in proceeding are scheduled for a screening appointment in the laboratory. We have obtained permission from our institutional review board to perform basic, simple screening tests on potential research subjects to determine if they qualify for research studies. These tests include a focused medical history,

spirometry, prick skin testing with allergen mixes common to Northern California, and methacholine challenge.

To improve our recruitment of ethnic minorities, especially African Americans, we have opened a second, "satellite" research site in space leased by the UCSF General Clinical Research Center (GCRC) at Summit Hospital in Oakland, CA. This site was established by Dr. Curtis Morris, GCRC Director, for a study of the effects of potassium supplementation on hypertension in African Americans. Dr. Morris has generously allowed us to consult with his clinical research staff for identification of the publications, radio stations, physician practices, community health centers, and census tracts where they have been most successful in recruiting people of minority ethnicity for research studies. People who call to express interest are screened by telephone and by a characterization visit, as described above, except that all procedures are performed at the satellite research site.

To date, we have screened well over 500 subjects for our database. Of those screened at the Moffitt-Long site, less than 10% are members of ethnic minorities. Over 60% of those screened at the Oakland satellite are of this category.

C. Drug Supplies

Drug supplies for this study will consist of triamcinolone acetonide, salmeterol xinafoate, albuterol, and placebo MDIs. Rhône-Poulenc-Rorer has agreed to provide triamcinolone canisters with spacers, as well as matched placebo. Glaxo has agreed to provide salmeterol and a matched placebo, as well as albuterol. The ACRN will contract with ProClinical, Inc. to work with the DCC to ensure proper blinding and coding of drugs (see Section IXB). The participation of pharmaceutical companies in this protocol is limited to the provision of drugs and placebos. They have not participated in the design of the study.

D. Compliance and Monitoring

In order to determine subject compliance with Azmacort[7] usage during the run-in period, information recorded on subjects' diary cards regarding number of puffs taken from each inhaler each day will be reviewed at each visit. Limitations of this mechanism for monitoring compliance are accuracy of the subject's recall and honesty. For specifics on Azmacort usage adherence criterion, see Section III.D.

In order to determine subject compliance with study drug usage during the post-randomization period, information recorded on subjects' diary cards regarding number of puffs taken from each inhaler each day will be reviewed at each visit. Limitations of this mechanism for monitoring compliance are accuracy of the subject's recall and honesty.

E. Visit Structure

Visit 0, Prescreening

Subjects will be interviewed prior to protocol entry (either by phone or in person) regarding their asthma and medical history. Specifically, the current status of asthma control, use of asthma and non-asthma medications, and health status in the previous 6 weeks will be determined (see Section III.B). An overview of the goals of the study and the visit structure and procedures involved will be presented. If the patient appears to fulfill entry criteria, is interested in study participation, and is not taking oral corticosteroids or inhaled nedocromil or cromolyn, Visit 1 may be scheduled. If the patient is taking one of these asthma medications regularly, a pre-study visit must be scheduled, informed consent obtained, and the patient evaluated by the study investigator as to the appropriateness of drug withdrawal for the 6 weeks prior to Visit 1. If warranted, the investigator may request additional pre-study visits for evaluation of asthma stability during this 6 week period.

Visit 1, Week 0

Patients will visit their clinical center after having had verbal contact with one of the study investigators, or their representatives, concerning the general goal and outline of the trial. On this first visit, written Informed Consent will be obtained, using a document which has been approved by the ACRN as well as by the local IRB. A medical history, physical examination, allergy skin testing, vital signs, spirometry, and 12-lead electrocardiogram will be obtained. Urine will be obtained for a pregnancy test in females and blood will be drawn for determination of serum electrolytes and for DNA extraction and possible subsequent genetic analysis. (see Section V.F for timetable of visits and data collected).

If the individual qualifies for the study based on these data, methacholine challenge and sputum induction will be performed. Methacholine challenge will always be performed prior to sputum induction.

Allergy skin testing, spirometry, and methacholine challenge will be administered according to protocols outlined in the ACRN MOP. All data will be recorded electronically and on forms supplied by the ACRN.

If, based on the information gathered to this point, the patient meets the specific entry criteria, he/she will be entered into the (run-in phase of the) trial, and blood will be drawn for determination of serum electrolytes and for DNA extraction and genetic analysis. Patients will be given an "open label" regular use inhaler (triamcinolone acetonide, 100 Φg/puff) to be used 4 puffs twice a day, a peak flow measuring device (AirWatch^{TM}), and an albuterol "open-label" inhaler to be used for rescue treatment. Prior to distribution, the AirWatch readings will be checked using a Jones Flow-Volume calibrator. Only AirWatches whose readings are within a specified range of the Jones will be distributed. Patients will be taught how to use their AirWatches and MDI's. They will be instructed to measure peak flow and

then use their corticosteroid inhaler immediately upon arising and at bedtime (between 2000 and 0100 hrs.). Patients will be instructed to record and circle peak flow values obtained less than 2 hours after use of inhaled albuterol on their diary cards. The use of diary cards will be explained and patients will be given an appropriate supply. Patients will be instructed to return to the clinical center in 2 weeks.

Visit 2, Week 2

Patients will return to the clinical center at the same time of day as on week 0 ∀ 2 hours. If scheduling permits, all subsequent visits will occur within a ∀ 2-hour window on the study day. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones Flow-Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. Open label inhaled triamcinolone and albuterol will be issued as needed. Patients will be instructed to return to the clinical center in 2 weeks.

Visit 3, Week 4

Patients will return to the clinical center at the same time of day as on week 0 ∀ 2 hours. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones Flow-Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. Open label inhaled triamcinolone and albuterol will be issued as needed. Patients will be instructed to return to the clinical center in 2 weeks.

Visit 4, Week 6

Patients will return to the clinical center at the same time of day as on week 0 ∀ 2 hours. Quality of Life Questionnaires will be administered. Urine will be obtained for a pregnancy test in females. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed.

If at this time, in the opinion of the clinical center personnel, the patient understands and can follow the protocol adequately to participate in the study, spirometry will be performed

and the patient's peak flow data for the past two weeks will be reviewed. If the pre-bronchodilator FEV_1 is > 80% of predicted and the average variability in PEF during the final two weeks of the run-in is # 20%, then the patient is eligible for the SOCS protocol. All other patients are potentially eligible for the SLIC protocol. For patients who meet entry criteria for the SOCS protocol, methacholine challenge and sputum induction will be performed. Methacholine challenge will always be performed prior to sputum induction. If the patient continues to meet the inclusion criteria for the study, and fulfills the criteria for randomization, the ACRN DCC will be contacted and the patient will be randomized to one of the 3 double-blind treatment arms (inhaled triamcinolone BID, inhaled salmeterol BID, inhaled placebo BID). Based upon this randomization, all patients will receive two coded, double-blind "regular use" inhalers to be taken twice a day; inhaler one will contain triamcinolone or its placebo, inhaler two will contain salmeterol or its placebo. Patients will be instructed to take their "regular use" inhalers at the same time each evening and morning (approximately 1800 and 0600 hrs), and to measure their peak flow at the same time each morning. All patients will receive open label albuterol inhalers, to be used as needed for rescue treatment and predisone. New study specific diary cards will be issued to those subjects not undergoing bronchoscopy. Patients will be instructed to return to the clinical center in 2 weeks. Those patients who were selected and consented for bronchoscopy will have blood drawn for coagulation studies (PT, PTT, platelets) and will be instructed to return to the clinical center in 3 days. In these subjects, double-blind, regular use inhalers will be started at Visit 4A rather than at Visit 4. Run-in diary cards will be continued for bronchoscopy subjects until Visit 4A.

Visit 4A, Week 6 + 3 days

Those subjects who were selected and consented for bronchoscopy (n=9 in each center, except New York, n=3) will return to the Clinical Center approximately 3 days after visit 4. A brief interval history, physical examination, and spirometry will be performed to assess the patient's clinical status prior to bronchoscopy, and laboratory tests of coagulation will be reviewed. Bronchoscopy with bronchial biopsies will be performed, according to the protocol detailed in the ACRN MOP. Patients will be monitored after bronchoscopy until stable, as described in the MOP. Patients who undergo bronchoscopy will receive their coded, double-blind, "regular use" inhalers, to be taken twice a day, at this visit, rather than at Visit 4. New study specific diary cards will be issued. Patients will be instructed to return to the clinical center in 2 weeks.

Telephone Call, Week 7

Patients will be contacted by the clinical 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, as assessed by the patient. The coordinator will attempt to determine whether the patient is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue β-agonist use, peak flow measurements and symptoms. If it is determined that the patient fulfills criteria for

treatment failure, they will be advised to visit the clinical center within 24 hours for evaluation and initiation of treatment as specified by ACRN protocol (see Section VI.C). Otherwise, arrangements will be made for the patient to return to the clinic in 1 week.

Visit 5, Week 8

Patients will return to the clinical center at the same time of day as on week 0. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. Methacholine challenge will be performed. Sputum induction will then be performed, as long as the subject performed adequately at Visit 4. Methacholine challenge will always be performed prior to sputum induction. New "regular use" inhalers will be dispensed. Open label albuterol will be issued as needed. Patients will be instructed to return to the clinical center in 2 weeks.

Visit 6, Week 10

Patients will return to the clinical center at the same time of day as on week 0. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. New "regular use" inhalers will be dispensed. Open label albuterol will be issued as needed. Patients will be instructed to return to the clinical center in 2 weeks.

Visit 7, Week 12

Patients will return to the clinical center at the same time of day as on week 0. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. New "regular use" inhalers will be dispensed. Open label albuterol will be issued as needed. Patients will be instructed to return to the clinical center in 2 weeks.

Visit 8, Week 14

Patients will return to the clinical center at the same time of day as on week 0. Spirometry will be obtained. The patient's AirWatch will be tested against theJones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed;AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. Methacholine challenge will be performed. Sputum induction will then be performed, as long as the subject performed adequately at Visit 4. Methacholine challenge will always be performed prior to sputum induction. New "regular use" inhalers will be dispensed. Open label albuterol will be issued as needed. Patients will be instructed to return to the clinical center in 2 weeks.

Drug Swap Visit, Week 16

Patients will return to the clinical center 2 weeks after Visit 8. Both "regular use" inhalers will be exchanged during this visit. Diary cards will be reviewed to determine whether the patient is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue β-agonist use, peak flow measurements and symptoms. If it is determined that the patient fulfills criteria for treatment failure, the initiation of treatment as specified by ACRN protocol (see Section VI.C) will occur. Study medications will be adjusted accordingly.

Visit 9, Week 18

Patients will return to the clinical center at the same time of day as on week 0. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. New "regular use" inhalers will be dispensed. Open label albuterol will be issued as needed. Patients will be instructed to return to the clinical center in 2 weeks.

Drug Swap Visit, Week 20

Patients will return to the clinical center 2 weeks after Visit 9. Both "regular use" inhalers will be exchanged during this visit. Diary cards will be reviewed to determine whether the patient is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue β-agonist use, peak flow measurements and symptoms. If it is determined that the patient fulfills criteria for treatment failure, the initiation of treatment as specified by ACRN protocol (see Section VI.C) will occur. Study medications will be adjusted accordingly.

Visit 10, Week 22

Patients will return to the clinical center at the same time of day as on week 0. The Quality of Life Questionnaires will always be administered. Urine will be obtained for a pregnancy test in females. Spirometry and a 12-lead ECG will be obtained. Blood will be drawn for determination of serum electrolytes. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. Methacholine challenge will be performed. Sputum induction will then be performed, as long as the subject performed adequately at Visit 4. Methacholine challenge will always be performed prior to sputum induction. At this time, new "regular use" inhalers will be dispensed which will be a coded placebo. Open label albuterol inhalers will be issued as needed. New diary cards will be issued. Patients will be instructed to return to the clinical center in 2 weeks. Those patients who were selected and consented for bronchoscopy will have blood drawn for tests of coagulation (PT, PTT, platelets), and will be instructed to return to the Clinical Center in 3 days. In these subjects, new, "regular use" inhalers (a coded placebo) will be dispensed at visit 10A rather than at visit 10.

Visit 10A, Week 22 + 3 days

Those subjects who were selected and consented for bronchoscopy (n=9 in each center, except New York, n=3) will return to the Clinical Center approximately 3 days after visit 10. A brief interval history, physical examination, and spirometry will be performed to assess the patient's clinical status prior to bronchoscopy, and laboratory tests of coagulation will be reviewed. Bronchoscopy with bronchial biopsies will be performed, according to the protocol detailed in the ACRN MOP. Patients will be monitored after bronchoscopy until stable, as described in the MOP. At this time, new "regular use" inhalers (which will be a coded placebo) will be dispensed. Patients will be instructed to return to the clinical center in 2 weeks.

Telephone Call, Week 23

Patients will be contacted by the clinical 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, as assessed by the patient. The coordinator will attempt to determine whether the patient is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue β-agonist use, peak flow measurements and symptoms. If it is determined that the patient fulfills criteria for treatment failure, they will be advised to visit the clinical center within 24 hours for evaluation and initiation of treatment as specified by ACRN protocol (see Section VI.C). Otherwise, arrangements will be made for the patient to return to the clinic in 1 week.

Visit 11, Week 24

Patients will return to the clinical center at the same time of day as on week 0. Quality of Life Questionnaires will be administered. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. Methacholine challenge will be performed. Sputum induction will then be performed, as long as the subject performed adequately at Visit 4. Methacholine challenge will always be performed prior to sputum induction. New "regular use" inhalers will be dispensed. Open label albuterol will be issued as needed. Patients will be instructed to return to the clinical center in 2 weeks.

Telephone Call, Week 25

Patients will be contacted by the clinical 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, as assessed by the patient. The coordinator will attempt to determine whether the patient is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue β-agonist use, peak flow measurements and symptoms. If it is determined that the patient fulfills criteria for treatment failure, they will be advised to visit the clinical center within 24 hours for evaluation and initiation of treatment as specified by ACRN protocol (see Section VI.C). Otherwise, arrangements will be made for the patient to return to the clinic in 1 week.

Visit 12, Week 26

Patients will return to the clinical center at the same time of day as on week 0. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator and will be replaced if it does not meet defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed and new ones dispensed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. New "regular use" inhalers will be dispensed. Open label albuterol inhalers will be dispensed as needed. New diary cards will be issued. Patients will be instructed to return to the clinical study center in 2 weeks.

Telephone Call, Week 27

Patients will be contacted by the clinical 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, as assessed by the patient. The coordinator will attempt to determine whether the patient is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue β-agonist use, peak flow measurements and symptoms. If it is determined that the patient fulfills criteria for treatment failure, they will be advised to visit the clinical center within 24 hours for evaluation and initiation of treatment as specified by ACRN protocol (see Section VI.C). Otherwise, arrangements will be made for the patient to return to the clinic in 1 week.

Visit 13, Week 28

Patients will return to the clinical center at the same time of day as on week 0. Quality of Life Questionnaires will be administered. A complete physical examination will be performed. Urine will be obtained for a pregnancy test in females. Spirometry will be obtained. The patient's AirWatch will be tested against the Jones-Flow Volume calibrator to determine if it meets defined quality control standards. Adverse events will be sought and noted using the protocol outlined by the ACRN. Diary cards will be reviewed; AirWatch data will be uploaded. Clinical center personnel will review the appropriateness of the timing of medication use, peak flow recording and symptom recording. Methacholine challenge will be performed. Sputum induction will then be performed, as long as the subject performed adequately at Visit 4. Methacholine challenge will always be performed prior to sputum induction. All patients will return their "regular use" and "as needed" inhalers.

Treatment Failure Visit

In the event of a "treatment failure" (see Section III.F), patients will be instructed to come into the clinical center as soon as possible, preferably within 72 hours and ideally within 24 hours, in order to have their condition assessed. At this time, the patient will complete Quality of Life Questionnaires, undergo spirometry and a physical exam with adverse events assessment. Peak flow measures and rescue inhaler use will be reviewed on the diary cards. Any non-study drugs taken prior to this visit will be documented. If patients meet criteria for treatment failure status, their dose of inhaled corticosteroids will be increased to the dose they were receiving at Visit 4 (800 Φg/day) delivered by an open-label inhaler. Study inhalers of corticosteroids (or its placebo) will be returned to the clinical center. Study inhalers of salmeterol (or placebo) will be continued unless the physician can document that treatment failure status was causally related to taking this medication. They will continue to participate in the study until its termination (intent-to-treat analysis), but no elimination in inhaled corticosteroid dose will be attempted during this time period. For safety reasons, all subjects will be seen within one week (∀ 3d) from the day they have been categorized as achieving treatment failure status. Following this "safety" visit,

subsequent protocol visits will continue in accordance with the visit schedule established at Visit 1 (see SOCS/SLIC MOP for details concerning possible replacement of scheduled protocol visits with treatment failure related visits).

F. Protocol in Tabular Form

CS = Inhaled corticosteroid MDI; S = salmeterol MDI; β = albuterol MDI; P = placebo MDI

Variable	Run-in (ICS)					Double-Blind Treatment									Run-out			Treatment Failure
Visit	1	2	3	4	4A	5	6	7	8	Drug Swap	9	Drug Swap	10	10A	11	12	13	
Week	0	2	4	6	6+3 days	8	10	12	14	16	18	20	22	22+3 days	24	26	28	
Window		∀3 days	∀3 days	∀3 days	∀1 day	-2 +3 days	∀3 days	∀3 days	∀3 days	∀3 days	∀3 days	∀3 days	∀3 days	∀1 day	-2 +3 days	∀3 days	∀3 days	
"Regular" (BID) treatment	CS	CS	CS	CS or SM or P	CS or SM or P	CS or SM or P	CS or SM or P	CS or SM or P	CS or SM or P	CS or SM or P	CS or SM or P	CS or SM or P	P	P	P	P		CS and SM, (or its P)
"As needed" treatment	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β
Randomization				X														
Informed Consent	X			* X														
Medical History	X																	
Long Physical Exam	X																X	X
Allergy Skin Test	X																	
Short Physical Exam		X	X	X	X	X	X	X	X		X		X	X	X	X		
Spirometry	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X
ECG	X												X					
Pregnancy Test	X			X									X				X	
Adverse Events Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review Peak Flow Data		X	X	X		X	X	X	X	X	X	X	X		X	X	X	X

Variable	Run-in (ICS)					Double-Blind Treatment									Run-out			Treatment Failure
Visit	1	2	3	4	4A	5	6	7	8	Drug Swap	9	Drup Swap	10	10A	11	12	13	
Week	0	2	4	6	6+3 days	8	10	12	14	16	18	20	22	22+3 days	24	26	28	
Window		∀3 days	∀3 days	∀3 days	∀1 day	-2 +3 days	∀3 days	∀3 days	∀3 days	∀3 days	∀3 days	∀3 days	∀3 days	∀1 day	-2 +3 days	∀3 days	∀3 days	
BIOQC and Peak Flow Performance Check	X																	
Peak Flow QC	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X
Dispense/Review Diary Cards	X	X	X	** X	X	X	X	X	X		X		X	X	X	X	X	X
Dispense/Collect Medications	X	X	X	*** X	X	X	X	X	X	X	X	X	* X	X	X	X	X	X
Methacholine Challenge	X			X		X			X				X		X		X	
Sputum Induction	X			X		X			X				X		X		X	
Bronchoscopy					X									X				
Quality of Life Questionnaire	X			X									X		X		X	X
Electrolytes	X												X					
Perform Coag Studies ****				X									X					
Genetic Analysis /Blood Draw	X																	
Dispense Prednisone				X														

* Bronchoscopy subjects must sign informed consent for procedure.
** Bronchoscopy subjects do not receive SOCS specific diary cards; stay with run-in diary cards until Visit 4A.
*** Bronchoscopy subjects do not receive medication at this visit.
**** Bronchoscopy and bronchial biopsies and an associated blood draw performed only in a subset of 9 adults/center, except New York, n=3.

G. Risks/Benefits

This study compares two approved usage strategies for two currently approved pharmaceutical products. The risks associated with regular versus "as needed" use of β-agonists are minimal and include the possibility of a small increase in airway responsiveness and a minor loss of asthma control. A major multi-center study to compare regular versus as-needed β-agonist use is currently being conducted by the NHLBI ACRN. Two recently published studies suggest that the regular use of a long-acting β-agonist or of an inhaled corticosteroid, over at least the time period proposed for this study, is efficacious and is not associated with an increase in adverse reactions (11, 31). During the double-blind treatment period, subjects randomized to receive placebo may experience an increase in their asthma symptoms. Similarly, during the run-out period, subjects may experience an increase in their asthma symptoms. They may take albuterol as needed for these symptoms. To ensure the safety of individuals whose asthma worsens during this period, specific criteria have been developed for assigning "treatment failure" or "drop-out" status, and for initiating appropriate asthma therapy. There will be no direct benefit to individual subjects participating in this study. The results of this study may be of potential benefit to the entire group of patients with asthma, as it may lead to a better definition of guidelines for asthma therapy.

H. Anticipated Results

Based on differences in their mechanisms of action, we anticipate that the differences demonstrated in the responses to the 2 different active pharmacologic regimens will help to establish an algorithm for asthma therapy. If given to patients with moderate asthma who are not already taking inhaled corticosteroids, both regimens might be expected to decrease symptoms during the period of administration. However, in patients already stabilized with inhaled corticosteroids, the clinical response to the two regimens may differ.

The most likely scenario is that subjects randomized to inhaled corticosteroids will either maintain their clinical status (having been run-in on inhaled corticosteroids for 6 weeks) or may improve slightly with 16 additional weeks of continuous inhaled corticosteroid therapy; subjects switched from inhaled corticosteroids to salmeterol may be worse. We anticipate that those patients switched from inhaled corticosteroids to placebo will also be worse. However, this group provides an important control, as some patients who meet accepted criteria for inhaled corticosteroids may not require long-term treatment. We anticipate that inhaled corticosteroids will be associated with a decrease in markers of inflammation or with continued suppression of inflammatory markers in patients already taking inhaled corticosteroids chronically, but that salmeterol alone will have no anti-inflammatory effect (and markers suppressed by ICS during run-in will rise). We thus would predict that, upon cessation of therapy, symptoms in the 6 post-treatment weeks will be less severe after the inhaled corticosteroid than after the inhaled salmeterol and that these differences will correlate with differences in the reductions in inflammatory cells and the levels of ECP and

tryptase in induced sputum samples. We expect that the bronchial mucosal biopsies will validate the changes in induced sputum, showing reductions in eosinophils in the epithelium and submucosa, and T-lymphocytes in the submucosa. If the major effect of salmeterol is due simply to reversal of induced airway tone, then we would anticipate that symptoms and airflow obstruction will return sooner after stopping salmeterol than after inhaled corticosteroids.

The next most likely outcome is that both the inhaled corticosteroid and the salmeterol treatment groups will maintain their clinical baseline or will improve during the double-blind treatment period. Even if this occurs, we anticipate a difference in markers of inflammation. We expect that the inhaled corticosteroid regimen will be associated with suppression of inflammation but that salmeterol will not. This would suggest that reduction of inflammation is important in decreasing asthma symptoms (because inhaled corticosteroids do not have a bronchodilator effect), but that an anti-inflammatory effect is not critical (because salmeterol produces clinical improvement without a decrease in markers of inflammation). If the major effect of salmeterol is due simply to reversal of induced airway tone, then we would anticipate that symptoms and airflow obstruction will return sooner after stopping these drugs than after inhaled corticosteroids Alternatively, a similar "off effect" time course for both groups for the return of asthma symptoms and worsening of airway physiology, would suggest that an anti-inflammatory effect is important only while medication is being taken. Another possibility is that both active drug regimens
might be associated with suppression of inflammation, suggesting that both inhaled corticosteroids and salmeterol act, at least in part, via their anti-inflammatory effect. If salmeterol decreases airway inflammation, the effect on symptoms, airflow obstruction, and bronchial reactivity may be more sustained, resembling the response to inhaled corticosteroids. If both agents decrease markers of inflammation, with similar off-transients, salmeterol may be a reasonable alternative to inhaled corticosteroids.

Finally, it is possible that we will observe no difference in markers of inflammation, despite difference in clinical efficacy between the two active drug treatment regimens. This would suggest either that anti-inflammatory activity is not important in determining clinical efficacy, or that the markers we have chosen to measure are not responsible for the observed differences.

The primary outcome variable to be evaluated in this study is AM peak flow. This indicator, used commonly in asthma trials, was chosen because it is a recurrently gathered objective measure that provides a day-to-day index of asthma control. However, AM peak flow may be affected differently by the two pharmacologic agents used in this study. Salmeterol, because of its long duration of action, improves PEF and FEV_1 and inhibits methacholine-induced bronchoconstriction for up to 24 hours. Thus, improvement of AM peak flow in the salmeterol-treated group may reflect medication carryover; improved AM peak flow in the inhaled corticosteroid-treated group likely represents improved asthma control and decreased bronchial reactivity. Despite this potential for affecting AM peak flow by different mechanisms, we believe that AM peak flow is the appropriate primary outcome indicator,

providing a daily objective assessment of asthma status while subjects are taking medication. Secondary outcome variables will include other markers of asthma severity (symptom diaries, use of rescue albuterol, quality of life scores, asthma exacerbations) and objective measures of airway function (FEV_1, PC_{20Mch}). The latter will be performed following an appropriate medication hold, at 2-4 week interval visits at the clinical study centers. Recognizing the possibility that AM peak flow in the salmeterol-treated group may improve despite worsening asthma control, we have calculated the sample size to ensure adequate power not only to detect a clinically significant difference for AM peak flow, but also for FEV_1 and methacholine responsiveness (see Section IX).

This study will provide potentially important information for choosing therapeutic agents for therapy of chronic asthma. Recent data suggest that long-term regular use of β-agonists may be detrimental. Some patients are intolerant of or reluctant to use inhaled corticosteroids. Salmeterol has shown promise as an anti-asthma drug, and may act by its bronchodilator effect or by an anti-inflammatory mechanism. Understanding the significance of reduction in markers of inflammation in the airways with therapy, the duration of the "off effect," and the relative activity of salmeterol in altering these endpoints may be helpful in devising and timing therapy to prevent inflammation, and may provide insights into the appropriate endpoint of therapy.

VI. ADVERSE EVENTS

A. Definition

An adverse event shall be defined as any detrimental change in the patient's condition, whether or not it is related to an exacerbation of asthma or to another unrelated illness. Adverse events related to asthma exacerbations will be managed according to rescue algorithms outlined below in Section C.

B. Adverse Events Unrelated to Asthma

Adverse events due to intercurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the investigator or if the patient is no longer able to effectively participate in the study. Patients 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 are also 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 responsible physician.

Documentation of an adverse event unrelated to asthma will be recorded on an 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 Exacerbations

1. Definition

During the course of the study, patients may experience an exacerbation of asthma. An exacerbation of asthma is characterized by an increase in symptoms of cough, chest tightness, and wheezing and it is generally associated with a fall in PEF. It is recognized, however, that the PEF may be improved by use of a bronchodilator and that increased bronchodilator use may, in this case, be more reflective of the exacerbation than PEF. An exacerbation may be brief and self-limited, or it may be of sufficient severity as to warrant documentation as a significant asthma exacerbation. Although <u>any</u> increase in symptoms or changes in PEF should be carefully monitored by the patient, the clinic coordinator, and the physician, alterations in asthma stability will be considered as constituting a significant asthma exacerbation when PEF does not increase to > 65% of reference levels, or symptoms are not satisfactorily relieved, after the first 60 minutes of rescue beta agonist (albuterol) use. Albuterol may be used at a dose of 2-4 puffs every 20 minutes during this one hour time period. For the SOCS protocol, the reference point for PEF comparisons will be as follows:

During run-in period (weeks 1 $\leq$ 6): Weeks 1-2: Predicted PEF obtained from spirometry software at Visit 1

Weeks 3-6: Mean value of AM prebronchodilator PEF recorded during the first 2 weeks of the run-in (weeks 1 and 2)

During double-blind treatment (weeks 6 $\leq$ 22): Mean value of AM prebronchodilator PEF recorded during the last 2 weeks of the run-in period (weeks 5 and 6)

In addition, a significant asthma exacerbation will be identified if patients have a significant increase in symptoms associated with either:

An increase in "as needed" β-agonist use of $\geq$ 8 puffs per 24 hours over baseline use for a period of 48 hours,

Baseline defined during run-in period: Average daily use during the first two weeks of the run-in period.

Baseline defined during double-blind period: Average daily use during the last two weeks of the run-in period.

or

Use of $\exists$ 16 puffs of "as needed" β-agonist per 24 hours for a period of 48 hours.

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

Since 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 (Section VI 2). Once any of these rescue interventions leads to the administration of oral or parenteral corticosteriods, the patient will also be considered to have developed a significant 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, FEV, etc.), value for symptom score, or frequency of rescue beta agonist use, the patient will be considered to have developed a significant asthma exacerbation.

The time in which a significant asthma exacerbation develops in relationship to the schedule of the SOCS protocol will affect the manner in which future clinic visits, medication adjustments, and diagnostic studies are scheduled or performed.

Patients developing significant asthma exacerbations during the run-in period will be removed from the study. Once the exacerbation has resolved, the patient may be considered for re-enrollment, starting again at Visit 1 and undergoing randomization into either SOCS or SLIC.

X Significant asthma exacerbations which occur following randomization (double-blind treatment phase) and during the run-out period will be managed according to the following rescue algorithms. During medical management of the exacerbation, 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. Additional visits and procedures will be scheduled as needed.

2. Rescue Algorithms

Rescue algorithms will be applied in cases where an exacerbation, as defined in Section C.1., fails to resolve or PEF is not improved to > 65% of reference level within 48 hours after increasing as needed albuterol use. Rescue algorithms are based on recommendations from the NAEP Guidelines for the Diagnosis and Management of Asthma (NHLBI Publication No. 91-3042, 1991). Albuterol and oral prednisone are the principal medications for rescue management. Patients will be instructed in their use for home management and supplies of both 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.

a) Home Care:

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

- Patients who recognize increased symptoms and/or a fall in PEF # 65% reference level will use albuterol by MDI, 2-4 puffs every 20 min up to 60 min if needed, and then every 4 hours, or less, if needed. Patients will be instructed to use the as needed inhaler for treatment.

- If the PEF does not increase to > 65% reference level or if symptoms are not improved after the first 60 min of albuterol therapy, the patient should contact the investigator, their primary physician or seek care in the emergency department.

- Failure of albuterol to control or maintain PEF > 65% reference level may necessitate the use of corticosteroids (see below).

b) Physician's Office or Emergency Room Treatment:

- Patients will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the patient's PEF or FEV_1 are less than 25% predicted or if the patient 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, patients should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 min over the first 60 min.

$ If the PEF increases to > 65% reference level after the first 60 min, the patient can be discharged to continue treatment at home. Prednisone may be administered at the discretion of the physician to augment therapy (see C.2.c).

$ If symptoms persist and PEF remains < 65% 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). Monitoring of PEF or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding patient disposition.

$ If PEF increases to > 65% reference level within 4 hours, the patient can be discharged to continue treatment at home. Home treatment should include an 8-day course of prednisone (see C.2.c).

$ If PEF remains > 40% but < 65% reference level, an individualized decision should be made to hospitalize the patient for more aggressive therapy or to continue therapy at home with a course of prednisone.

$ If PEF is < 40% reference level after repeated albuterol treatments, the patient should be admitted to the hospital unless, in the physician's best judgment, alternative treatment could suffice.

c) Prednisone Treatment:

In this protocol, prednisone will be used when acute exacerbations cannot be controlled by albuterol therapy. Indications for prednisone therapy include the following:

X To achieve stable control of symptoms and optimize pulmonary function once treatment failure status is achieved.

X For follow-up management after discharge from the physician's office, emergency room, or hospital for an acute exacerbation.

- X An increase in "as needed" β-agonist use of ≥ 8 puffs per 24 hours over baseline use (baseline defined as average daily use over first 2 weeks of run-in period) for a period of 48 hours, or

- X Use of ≥ 16 puffs of "as needed" β-agonist per 24 hours for a period of 48 hours.

- X When PEF falls to < 50% reference level despite albuterol treatment.

The dose of prednisone used during an acute exacerbation shall consist of 60 mg as a single dose every day for 3 days, followed by a 10 mg/day taper over the next 5 days. The decision to initiate or to continue a course of prednisone beyond 8 days is left to the discretion of the physician.

D. Adjustment 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 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.

E. Study Center Visits Following Exacerbations

If the patient receives steroids for an exacerbation, regular follow-up evaluations will continue according to the original protocol.

F. Criteria for Discontinuing Patients Due to Asthma Exacerbations

1. Treatment Failure Status

Criteria for assigning treatment failure status during the double-blind treatment period are described in Section III.E. Patients who are assigned "treatment failure" status will continue to participate in the data gathering aspects of the protocol until the time they would have completed the trial.

2. Drop-out Status

Criteria for assigning drop-out status during the single-blind run-out period are described in Section III.F. In addition, patients may be assigned "drop-out" status during the double-blind treatment period if they become pregnant or if the patient withdraws consent.

G. Adverse Events as Outcome Variables

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

$Hospitalization
$Emergency Department Visits
$Unscheduled physician/clinic visits
$Number of subjects having an exacerbation as defined by prednisone use
$Treatment failure

VII. COST AND PAYMENT

All tests will be performed without cost to the participating patients.

Each patient will be paid an amount determined by their local center. For patients who withdraw, payments will be pro-rated for the length of time they stayed in the study, but payments will not be made until the study would have been completed had the patient not withdrawn.

VIII. DATA RECORDING

Recording of all data including the informed consent, history, physical examination, results of allergy skin testing, vital signs, electrocardiograms, results of pregnancy tests, adverse events, confirmation of medication dispensation, methacholine challenge testing, and quality of life testing will be recorded on forms prepared by the ACRN DCC. 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 and the metered dose inhaler (Chronolog) will be transmitted electronically to the DCC. All data will be stored and analyzed at the DCC.

IX. STATISTICAL DESIGN AND ANALYSIS

A. Data Collection and Data Management

Each center will have a computer configuration that includes an X-terminal, a postscript printer, and a modem. This will give each center the capability of logging directly into the DCC computing system over the Internet with the modem as a backup if the connection is not possible. Though this set-up is installed primarily to allow for distributed data entry into a centralized database on the ACRN project server at the DCC, menu options will also include sending electronic mail, downloading study documents such as forms and reports, and viewing a calendar of ACRN events. A sophisticated security system will limit access to qualified personnel and prevent corruption of the study database.

The DCC will be responsible for generating the data collection forms based on input from the clinical centers. Once the data collection forms have been filled out and reviewed, the Clinic Coordinator will log into the DCC computer system and enter the data. The advantage of this distributed data entry system is that the Clinic Coordinators will review the data a second time as they are entering it, which serves as another level of quality control. Forms will then be forwarded to the DCC for the second data entry and filing. The DCC will be responsible for identifying problem data and resolving inconsistencies.
Results from lung function tests will be sent directly to the DCC via a modem in the computer attached to the spirometer.

B. Masking

Careful procedures are required in order to maintain the triple-masking of the study participants, clinical center personnel, and DCC personnel as to whether individual patients are taking inhaled corticosteroid, salmeterol, or placebo. Treatment medication for each patient will be packaged together and labelled with a unique number. The contents of the packages will be known only to limited personnel at the DCC. These packages, and canisters for the run-in and withdrawal periods will be delivered to the Clinic Coordinators. Triple-masking, i.e., masking of the DCC personnel in addition to the study participants and clinical center personnel, will be employed so that the statistical analyses are not biased by preconceived notions. Until the time of manuscript preparation, DCC personnel will identify the randomized groups as A and B and C, and only the limited personnel within the DCC will know the identity of A, B, and C.

In order to decrease the likelihood of incorrect drug distribution, each coded package designated for a study participant will have a sheet of removable labels attached to it. When the Clinic Coordinator retrieves a canister for the study participant, he/she will remove one of the labels and attach it to the data collection form prior to mailing the form to the DCC. The Clinic Coordinator will initial across the label to indicate that he/she checked to make sure the appropriate canister was distributed to the participant.

C. Randomization

When a patient at a particular center is deemed eligible for the study, the Clinic Coordinator will log into the ACRN network server and indicate to the system that a patient requires randomization. After entering the pertinent information with respect to clinical center and eligibility criteria, the Clinic Coordinator will be asked to verify that all of the information has been reviewed carefully and the patient is eligible. If so, the Clinic Coordinator will be given a packet number, from which all medication for that patient will be dispensed. In order to maintain security of the randomization schedules, the data manager of the DCC will receive automatically a notice from the ACRN network server that a patient has been randomized. If no follow-up information is forthcoming on such a patient, the data manager will contact the Clinic Coordinators concerning the status of the patient.

At the time of randomization, patients will be questioned regarding their willingness to undergo bronchoscopy (Nine at each clinical center except three at New York). This procedure will have been described as a possibility in the original consent form. Those patients interested in bronchoscopy will sign an additional informed consent form specifically for bronchoscopy with bronchial biopsy. Clinical coordinators will then contact the DCC and patients will be randomized in such a way as to ensure that equal numbers of subjects are recruited from each treatment group. Because some subjects may choose not to undergo bronchoscopy, and because only a small subset of the total subjects in this study are needed for bronchoscopy, willingness to undergo bronchoscopy will not be a requirement for entry into the study. However, bronchoscopy will be offered to each consecutive patient randomized until a sufficient number have agreed so that the target goal of nine per center can be achieved.

D. Stratification

The randomization scheme will be stratified according to center because differences among clinical centers typically yields a large amount of variability. In addition, each clinical center will be restricted to enroll no more than 25% of its target sample size of patients to be between the ages of 12 and 18.

Within each PC_{20} subgroup (< 2 mg/ml, ≥ 2 mg/ml) of each clinical center an adaptive randomization scheme will be invoked in order to balance the treatment arms with respect to the following strata:

ethnic group (white, black, Hispanic, native American, Asian, Other)
gender (male, female)
age (adolescent, adult)

The strata of a particular patient are noted and the marginal frequencies of these strata are summed for each treatment arm. Then the patient is assigned to that treatment arm which has the smallest sum of marginal frequencies (49).

E. Statistical Analysis

In this protocol there is a 6-week run-in period with inhaled corticosteroids, a 16-week randomized double-blind period with 3 treatment arms, and a 6-week run-out period. The table in Section V.F lists the measurements taken at the 13 patient visits over this 28-week protocol.

The major research questions are posed in Section II.C. A discussion of the statistical analysis focuses on the primary research question, i.e., how do the 3 treatment arms compare with respect to change from the end of the run-in period to the end of the double-blind treatment period. The statistical analyses for the other research questions are similar. The response variables of interest for the primary research question include AM PEF (primary response variable), PM PEF, asthma symptom scores as needed β-agonist

use, quality-of-life scores, FEV_1, and bronchial reactivity to methacholine challenge (Daily data from post-randomization diary cards will be summarized and analyzed as weekly averages. Because subjects are instructed to withhold their study salmeterol or placebo for 48 hours prior to a visit, the affected peak flow values will be excluded).

A longitudinal data analysis will provide the most statistical power because it uses all the data from each patient visit. In particular, the random coefficient (also called the mixed-effects) linear model is suited for this situation (50, 51). The statistical model appropriate for the treatment period is

$$Y_{ijk} = \alpha_{ij} + x_{ijk}\beta_{ij} + \varepsilon_{ijk}$$

where
i = 1,2,3 denotes treatment arm
$j = 1,...,n_i$ denotes patient within treatment arm i
$k = 1,...,p_{ij}$ denotes Visit number for patient j within treatment arm i
Y_{ijk} = response at Visit k for patient j within treatment arm i
α_{ij} = intercept for patient j within treatment arm i
β_{ij} = time slope for patient j within treatment arm i
x_{ijk} = elapsed number of weeks between Visit k and Visit 4 (end of the run-in period) for patient j within treatment arm i
ε_{ijk} = random error at Visit k for patient j within treatment arm i

Other effects can be included in the model, such as center, center H slope, etc. It will be important to include such effects and determine their impact on the treatment arm comparisons. However, for the sake of illustrating the statistical approach for this trial, these are not discussed any further.

One of the underlying assumptions for the model is that the responses will behave linearly during the treatment period. This assumption will be investigated graphically and if it is determined that it is not viable, then the model will be modified to be piecewise-linear over the 16-week treatment period.

It is assumed that the $[\alpha_{ij}\ \beta_{ij}]$'s are independent and distributed according to a bivariate normal with mean vector $[\alpha_i\ \beta_i]$ and variance matrix with elements $\omega_{\alpha\alpha}$, $\omega_{\beta\beta}$, and $\omega_{\alpha\beta}$. The ε_{ijk}'s are independent and identically distributed according to a normal distribution with null mean and variance σ^2. The $[\alpha_{ij}\ \beta_{ij}]$'s and the ε_{ijk}'s are mutually independent. The variance components $\omega_{\alpha\alpha}$, $\omega_{\alpha\beta}$, and $\omega_{\beta\beta}$ are inter-patient variances and covariances for the random intercepts and slopes. The variance component σ^2 is the intra-patient variance for the intercept-slope model.

The primary research question is how the mean changes in response over the 16-week treatment period (Visit 10 vs. Visit 4) compare among the 3 treatment arms. This is equivalent to estimating β_1, β_2, and β_3, and testing the hypotheses

$$H_0 : 16\beta_1 = 16\beta_2, \quad H_0 : 16\beta_1 = 16\beta_3, \quad \text{and} \quad H_0 : 16\beta_2 = 16\beta_3$$

Details for performing restricted maximum likelihood (REML) estimation and empirical generalized least squares (EGLS) estimation of the intercepts, slopes, and variance components are provided elsewhere (50, 51). Both REML and EGLS estimation are available in PROC MIXED of SAS (52). A similar type of longitudinal data analysis, employing the models and assumptions described above, will be applied in order to determine how the treatment groups compare during the run-out period.

Treatment failure status is defined in Section III.E. Categorical data analysis will be applied to compare the treatment groups with respect to the number and types of treatment failures. A patient who is deemed a treatment failure will continue in the study, and data will be collected at visits beyond the treatment failure time. All data will be included in the longitudinal data analyses described above, regardless of treatment-failure status (intent-to-treat analysis). One consequence of the intent-to-treat analysis is the interpretation of comparisons to the placebo arm. A patient in the placebo arm is at higher risk for treatment failure than patients in the active treatment arms. If the placebo group does experience a higher treatment failure rate, then it will have proportionately more patients receiving prednisone and inhaled corticosteroids. This could result in improved measures of lung function for the placebo group, so that comparisons of the treatment arms to the placebo arm could be misleading. Therefore, the intent-to-treat analysis will be supplemented with secondary statistical analyses that exclude data collected after treatment failure occurrences.

Safety of methacholine challenge will be examined by analyzing the frequency of adverse events following clinic visits at which methacholine challenge is performed, compared with the frequency following visits at which it is not. McNemar's test will be applied for this purpose because of the correlated responses within each subject. The efficacy of methacholine challenge will be analyzed by correlating PC_{20} with other measures of asthma outcome, such as AM and PM PEF, FEV_1, symptom scores, rescue beta-agonist use, and quality of life scores. Correlation coefficient estimates and confidence intervals, specifically designed for longitudinal data, will be calculated for this purpose.

F. Effect Size Calculations

The Clinical Centers will recruit a total sample size of 150 patients (50 per treatment arm). However, it is expected that there will be a 20% withdrawal rate during the treatment period so that 120 patients are expected to complete the treatment period (40 patients per treatment arm). Effect sizes for various response variables are calculated that correspond to 120 patients completing the treatment period (although the longitudinal data analysis described above will incorporate the data from every randomized patient).

For the sake of simplicity, it is assumed that every patient has visits scheduled at the same set of times. In terms of the linear model described in the previous section, this means that the X_{ij1}'s are all the same (= X_1), the X_{ij2}'s are all the same (= X_2), etc. The slope estimate for each patient has variance

$$Var(\text{estimated slope for patient } j \text{ in group } i) = \omega_{\beta\beta} + \sigma^2/S_{XX}$$

where

$$S_{XX} = \sum_{k=1}(X_k - X^*)^2 \text{ and } X^* = (\sum_{k=1}X_k)/p$$

The effect size formula for a 2-sided test is as follows:

$$\text{Effect Size} = 16(z_{1-\alpha/2} + z_{1-\beta})\{(2/n)(\omega_{\beta\beta} + \sigma^2/S_{XX})\}^{1/2}$$

where
z_γ represents the 100γ percentile from the standard normal distribution
α represents the Type I error rate (100α% = significance level)
β represents the Type II error rate ($100(1-\beta)$% = statistical power)
n represents the number of patients per treatment arm who complete the trial
$\omega_{\beta\beta}$represents the inter-patient variance for the slopes
σ^2 represents the intra-patient variance for the intercept-slope model

One interim analysis is planned for this trial. Therefore, the interim and the final analyses should each impose a significance level of 0.029 (53). However, as evidenced by the 3 tests of hypotheses of interest, a Bonferroni correction yields that each of 3 tests at each of the interim and final analyses should impose a significance level of 0.0097. This yields $z_{1-\alpha/2} = 2.5875$ and requiring 80% statistical power yields $z_{1-\beta} = 0.84$.

The sample size n = 40 represents the number of patients per treatment arm that are expected to complete the trial when allowing for a 20% withdrawal rate for 50 randomized patients per treatment arm. Finally, the estimates of the inter- and intra-patient variability are taken from the placebo group in a 48-week trial (47) and listed in Table 2. The effect sizes are calculated for 6 response variables in Table 2, using the above formula.

Table 2: Effect sizes for 6 response variables under the assumption that 150 patients (50 per treatment arm) are randomized.

Response Variable	Elapsed Time (weeks)	S_{xx}	$(\omega_{\beta\beta})^{1/2}$	σ	Effect Size
A.M. PEF (L/min)	0, 1, 2, 3, 4, ... , 16	408.0	0.53	23.50	15.7
P.M. PEF (L/min)	0, 1, 2, 3, 4, ... , 16	408.0	0.44	23.80	15.4
Symptom Score	0, 1, 2, 3, 4, ... , 16	408.0	0.018	0.11	0.23
FEV_1 (L)	0, 2, 4, 6, 8, 12, 16	190.9	0.008	0.15	0.17
FVC (L)	0, 2, 4, 6, 8, 12, 16	190.9	0.008	0.19	0.20
PC_{15} (dose steps)	0, 2, 8, 16	155.0	0.053	0.20	0.68

The effect sizes listed in Table 2 are based on the assumption that the intercept-slope model is appropriate. If it is observed that a more complicated model is necessary, then the simplest approach will be to construct the differences between week 22 and 6. Such differences yield that $S_{xx} = 128$ for each response variable in Table 2. The effect sizes in this instance are more conservative, although still reasonable, and are listed in Table 3.

Table 3: Revised effect sizes for 6 response variables using differences between week 22 and week 6.

Response Variable	Effect Size
A.M. PEF (L/min)	26.3
P.M. PEF (L/min)	26.3
Symptom Scoire	0.25
FEV_1 (L)	0.19
FVC (L)	0.23
PC_{15} (dose steps)	0.69

G. Statistical Analysis and Sample Size for Biopsy Responses

The 3 response variables of interest from the biopsies are eosinophils in epithelium, eosinophils in submucosa, and T-lymphocytes in submucosa. The biopsies will be performed at Visit 4A (start of the double-masked treatment period) and at Visit 10A (end of the double-masked treatment period). The response variables represent counts of cells and typically display skewed distributions, so that a square-root transformation will be applied in order to normalize the measured responses. The change in square-root count between Visit 10A and Visit 4A will be calculated and subjected to two different analyses:

(1) a paired t test within each treatment arm in order to determine if the change in square-root counts are significantly different from zero;

(2) a one-way analysis of variance with multiple comparisons in order to determine if the treatment arms differ with respect to the change in the square-root counts.

Djukanovic, et al. (48) reported on a trial with 10 symptomatic atopic asthmatic patients who underwent a 6-week therapy with inhaled beclomethasone diproprionate. Biopsy was performed on these patients before and after the inhaled corticosteroid therapy. Sample means (and standard deviations) for the change in square-root counts from this report are 1.4 (1.5), 3.5 (2.6), and 5.1 (5.5), respectively, for eosinophils in epithelium, eosinophils in submucosa, and T-lymphocytes in submucosa.

In order to detect similar changes in the proposed trial using a 2-sided, 5% significance level, paired t test with 80% statistical power, 10, 5, and 10 patients per treatment arm are needed, respectively, for eosinophils in epithelium, eosinophils in submucosa, and T-lymphocytes in submucosa. Therefore, 10 patients per treatment arm are required for biopsy. To allow for block randomization and a 20% withdrawal rate, 9 subjects per clinical center, (except New York, 3 subjects) will be recruited for bronchoscopy and bronchial biopsy for a total of 48 patients.

X. REFERENCES CITED

1. Sears MR, Taylor DR, Print CG, Lake DC, Li Q, Flannery EM, Yates DM, Lucas MK, Herbison GP. Regular inhaled β-agonist treatment in bronchial asthma. Lancet 1990; 336:1391-1396.

2. Spitzer WO, Suissa S, Ernst P, Horwitz RI, Habbick B, Cockcroft D, Boivin J-F, McNutt M, Buist AS, Rebuck AS. The use of β-agonists and the risk of death and near death from asthma. N Engl J Med 1992; 326:501-6.

3. Page CP. One explanation of the asthma paradox: inhibition of natural antiinflammatory mechanism by β_2-agonists. Lancet 1991; 337:717-720.

4. Morley J, Sanjar S, Newth C. Viewpoint: untoward effects of β-adrenoceptor agonists in asthma. Eur Respir J. 1990; 3:228-233.

5. Sheffer AL. National Asthma Education Program. Expert Panel Report. National Institutes of Health, 1991:

6. Sears MR. Dose reduction of β-agonists in asthma. Lancet 1991; 338:1331-1332.

7. Kraan J, Koeter GH, Mark TWVD. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma. A comparison between budesonide and terbutaline. J Allergy Clin Immunol 1985; 76:628-636.

8. Kerrebijn KF, van Ess-Zandvliet EM, Neijens HJ. Effect of long-term treatment with inhaled corticosteroids and β-agonists on the bronchial responsiveness in children with asthma. J Allergy Clin Immunol 1987; 79:653-659.

9. Juniper EF, Kline PA, Vanzielehgem MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. Am Rev Respir Dis 1990; 142:832-836.

10. Bel EH, Timmers MC, Hermans J, Dijkman JH, Sterk PJ. The long-term effects of nedocromil sodium and beclomethasone dipropionate on bronchial responsiveness to methacholine in nonatopic asthmatic subjects. Am Rev Respir Dis 1990; 141:21-28.

11. Haahtela T, Jarvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander

K, Persson T, Reinikainen K, Selroos O, Sovijarvi A, Stenius-Aarniala B, Svahn T, Tammivaara R, Laitinen LA. Comparison of a β2-agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. N Engl J Med 1991; 325:388-392.

12. Juniper EF, Kline PA, VanzieleghEm MA, Hargreave FE. Reduction of budesonide after a year of increased use: a randomized controlled trial to evaluate whether improvements in airway responsiveness and clinical asthma are maintained. J Allergy Clin Immunol 1991; 87:483-489.

13. Bradshaw J, Brittain RT, Coleman RA. The design of salmeterol, a long-acting selective β2-adrenaceptor agonist. Br J Pharm 1987; 92:590P.

14. Brittain RT. Approaches to a long-acting, selective β2-adrenoceptor stimulant. Lung 1990; 168:111-114.

15. Ullman A, Svedmyr N. Salmeterol, a new long acting inhaled β2-adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. Thorax 1988; 43:674-678.

16. Derom E, Pauwels R, Van Der Straeten M. Duration of the protective effect of salmeterol on methacholine challenge of asthmatics. Am Rev Respir Dis 1990; 141:A469.

17. Derom EY, Pauwels RA, Van Der Straeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. J Allergy Clin Immunol 1992; 89:811-815.

18. Rabe KF, Jorres R, Nowak D, Behr N, Magnussen H. Comparison of the Effects of Salmeterol and Formoterol on Airway tone and Responsiveness over 24 hours in Bronchial Asthma. Am Rev Respir Dis 1993; 147:1436-1441.

19. Maconochie JG, Forster JK, Fowler P, Thomas M. An initial comparison of slameterol and salbutamol against histamine-induced bronchoconstriction in healthy subjects. Br j Clin Pharm 1988; 25:115P.

20. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. Lancet 1990; 336:1338-1342.

21. Malo J-L, Ghezzo H, Trudeau C, L'Archeveque J, Cartier A. Salmeterol, a new inhaled β_2-adrenergic agonist, has a longer blocking effect than albuterol on hyperventilation-induced bronchoconstriction. J Allergy Clin Immunol 1992; 89:567-574.

22. Newnham D, Ingram C, Earnshaw J, Palmer JBD, Dhillon DP. Duration of action

of inhaled salmeterol against exercise induced asthma. Am Rev Respir Dis 1991; 143:A29.

23. Butchers PR, Cousins SA, Vardey CJ. Salmeterol: a potent and long-acting inhibitor of the release of inflammatory and spasmogenic mediators from human lung. Br J Pharmacol 1987; 92:745P.

24. Butchers PR, Vardey CJ, Johnson M. Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. Br J Pharmacol 1991; 104:672-676.

25. Johnson A. The pharmacology of saalmeterol. Lung 1990; 168:115-119.

26. Baker AJ, Fuller RW. Anti-inflammatory effect of salmeterol on human alveolar macrophages. Am Rev Respir Dis 1990; 141:A394.

27. Ullman A, Hedner J, Svedmyr N. Inhaled salmeterol and salbutamol in asthmatic patients, an evaluation of asthma symptoms and the possible development of tachyphylaxis. Am Rev Respir Dis 1990; 142:571-575.

28. Lotvall J, Lunde H, Ullman A, Tšrnqvist H, Svedmyr N. Twelve months, treatment with inhaled salmeterol in asthmatic patients. Effects on β_2-receptor function and inflammatory cells. Allergy 1992; 47:477-483.

29. Dahl R, Pedersen B, Venge P. The influence of inhaled salmeterol on bronchial inflammation. A bronchoalveolar lavage study in patients with bronchial asthma. Am Rev Respir Dis 1991; 143:A649.

30. Pedersen B, Dahl R, Venge P. The effect of salmeterol on the early and late phase reaction to bronchial allergen challenge and postchallenge variation in bronchial hyperreactivity, blood eosinophils, and SE-eosinophil cationic protein. Am Rev Respir Dis 1991; 143:A649.

31. Pearlman DS, Chervinsky P, LaForce C, Seltzer JM, Southern DL, Kemp JP, Dockhorn RJ, Grossman J, Liddle RF, Yancey SW, Cocchetto DM, Alexander WJ, Van As A. A comparison of salmeterol with albuterol in the treatment of mild-to-moderate asthma. N Engl J Med 1992; 327:1420-1425.

32. D'Alonzo GE, Nathan RA, Henochowicz S, Morris RJ, Ratner P, Rennard SI. Salmeterol Xinafoate as Maintenance Therapy compared with Albuterol in Patients with Asthma. JAMA 1994; 271:1412-1416.

33. Greening AP, Ind PW, Northfield M, Shaw G. Added Salmeterol versus higher-

dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. Lancet 1994; 344:219-224.

34. Woolcock A, Lundback B, Ringdal OLN, Jacques LA. Comparison of the effect of Addition of Salmeterol with Doubling the Inhaled Steroid Dose in Asthmatic Patients. Am Rev Respir Dis 1994; :A280.

35. Chapman KR. Lancet 1994; 343(1379-1382)

36. Guidelines for the management of asthma in adults: II--Acute severe asthma. Statement by the British Thoracic Society, Researach Unit of the Royal College of Physicians of London, King's Fund Centre, National Asthma Campaign. Br Med J 1990; 301:797-800.

37. DaCruz DJ. Guidelines for management of asthma. Br Med J 1990; 301

38. Cotton P. Asthma consensus is unconvincing to many. JAMA 1993; 270:297.

39. Lask B. International consensus report on diagnosis and management of asthma. Clin Exp Allergy 1993; 23:155.

40. Toogood JH, Crilly RG, Jones G, Nadeau J, Wells GA. Effect of high-dose inhaled budesonide on calcium and phosphate metabolism and the risk of osteoporosis. Am Rev Respir Dis 1988; 138:57-61.

41. Toogood JH. Complications of topical steroid therapy for asthma. Am Rev Respir Dis 1990; 141:S89-S96.

42. Toogood JH, Jennings B, Hodsman AB, Baskerville J, Fraher LJ. Effects of dose and dosing schedule of inhaled budesonide on bone turnover. J Allergy Clin Immunol 1991; 88:572-580.

43. Hodsman AB, Toogood JH, Jennings B, Fraher LJ, Baskerville JC. Differential effects of inhaled budesonide and oral prednisolone on serum osteocalcin. J Clin Endocrinol Metab 1991; 72:530-540.

44. Geddes DM. Inhaled corticosteroids - benefits and risks. Thorax 1992; 47:404-407.

45. Monson JP. Systemic effects of inhaled steroids. Thorax 1993; 48:955-956.

46. Kiviranta K, Turpeinen M. Effect of eight months of inhaled beclomethasone diproprionate and budesonide on carbohydrate metabolism in adults with asthma. Thorax 1993; 48:974-978.

47. Haahtela T, Jarvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander K, Svahn T, Tammivaara R, Laitinen LA. Effects of Reducing or Discontinuing Inhaled Budesonide in Patients with Mild Asthma. N Eng J Med 1994; 331:700-705.

48. Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls A, Roche WR, Howarth P, Holgate ST. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. Am Rev Respir Dis 1992; 145:669-674.

49. Pocock SJ. Clinical Trials: A Practical Approach. New York: John Wiley & Sons, 1983.

50. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982; 38:963-974.

51. Vonesh EF, Carter RL. Efficient inference for a random coefficient growth curve model with unbalanced data. Biometrics 1987; 43:617-628.

52. SAS Institute. SAS Technical Report P-229 SAS/STAT Software: Changes and Enhancements. Cary, North Carolina: SAS Institute. 1992; 287-366.

53. Pocock SJ. Interim analysis for randomized clinical trials: the group sequential approach. Biometrics 1982; 38:153-162.