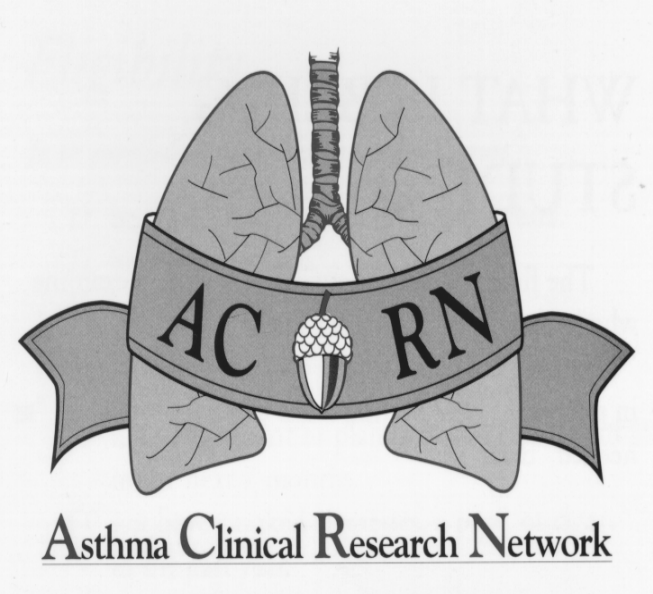

SLiMSIT

Salmeterol and **L**eukotriene **M**odifiers vs. **S**almeterol and **I**CS **T**reatment

A study of subjects with moderate, persistent asthma, comparing the effects of 14 weeks of treatment with the combination of an inhaled corticosteroid and a long-acting beta-agonist with the combination of a leukotriene modifier and a long-acting beta-agonist.

Protocol, v2.3

March 6, 2003

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I. HYPOTHESIS TO BE TESTED

In patients with moderate persistent asthma, the combination of a long-acting beta-agonist and an inhaled corticosteroid is superior to the combination of a long-acting beta-agonist and a leukotriene modifier as judged by treatment failure (a need for intensification of asthma therapy).

II. SECONDARY HYPOTHESES TO BE TESTED

The combination of a long-acting beta-agonist and an inhaled corticosteroid is superior to the combination of a long-acting beta-agonist and a leukotriene modifier in asthma control as measured by a scoring of symptoms, beta-agonist use, AM and PM peak expiratory flow, and FEV_1.

III. EXPLORATORY HYPOTHESES

1. The occurrence of treatment failure and changes in the indicators of airflow obstruction (FEV_1, AM PEF, PM PEF) occurring during the study are related to baseline values for the degree of bronchial reactivity, the percentage of eosinophils and concentration of tryptase in induced sputum samples, and the concentration of nitric oxide (NO) in exhaled air.

2. The occurrence of treatment failure and changes in the indicators of airflow obstruction are associated with changes from baseline in indices of airway inflammation during treatment.

3. In subjects with moderate, persistent asthma, changes in the values of indicators of airflow obstruction (FEV_1, AM PEF, PM PEF), of "asthma control," and of "asthma-related quality of life" in response to specific therapy are related to as-yet-to-be identified variations in genetic loci in candidate genes thought to be important in asthma severity.

IV. BACKGROUND AND RATIONALE

A. Introduction

Inhaled corticosteroids are extremely effective in asthma, and they have broad anti-inflammatory effects (1-4). Due to these properties, national guidelines recommend their use in persistent asthma (1). In the past decade, two new classes of pharmacotherapeutic agents, the long-acting beta-agonists and the leukotriene modifiers, have become available. The introduction of these new agents poses several new dilemmas for the practitioner, who must advise patients on the relative value of these new agents as compared with inhaled corticosteroids. This is particularly true since concerns regarding possible systemic adverse effects continue to limit the appeal of inhaled corticosteroids to many patients. Indeed, despite the current guidelines, and despite the broad anti-inflammatory effects of inhaled corticosteroids, a leukotriene receptor

antagonist, montelukast, is currently the most widely prescribed controller therapy for patients with asthma in the United States (data from Scott-Levin 2/5/01).

To date, studies that could provide the underpinning of evidence-based recommendations for the role of inhaled corticosteroid therapy relative to these new agents have been lacking. As reviewed below, we previously have investigated the ability of one these agents--the long-acting beta-agonist, salmeterol-- to substitute for inhaled corticosteroids. We now propose to define the role of combining these two new agents in the treatment of persistent asthma.

B. Long-acting beta-agonists vs. inhaled corticosteroids

In the SOCS (**S**almeterol **O**ff **C**ortico**S**teroids) and SLIC (**S**a**L**meterol +/- **I**nhaled **C**orticosteroids) trials (51,52), the ACRN addressed the question of whether a long-acting beta-agonist, salmeterol, could substitute for an inhaled corticosteroid. In the SOCS trial, we found that even in relatively mild patients, well controlled on a moderate dose of inhaled corticosteroids, a long-acting beta-agonist could not replace inhaled corticosteroids. We noted that despite apparent similar degrees of control (as assessed by pulmonary function, beta-agonist use, asthma symptom scores, and quality of life scores), when judged by asthma treatment failures (defined as a need for intensification of asthma therapy), long-acting beta-agonists could not be substituted as monotherapy in these patients. Further, the increase in treatment failures with salmeterol monotherapy was reflected by a corresponding rise in sputum inflammatory indices. We observed a rise in sputum eosinophils, sputum eosinophil cationic protein, and sputum tryptase. In SLIC we found that salmeterol permitted reduction of ICS dose, but it could not substitute entirely for inhaled corticosteroids. We concluded that long-acting beta-agonists could not be used in place of inhaled corticosteroids as monotherapy in patients with mild-to-moderate asthma.

C. Rationale for combining long-acting beta-agonists and leukotriene modifiers

The anti-asthmatic actions of the leukotriene modifiers suggest that they may complement the effect of the long-acting beta-agonists. In contrast to the long-acting beta-agonists, whose action is primarily on smooth muscle, the leukotriene modifiers act by interfering with the action or production of the fatty acid derived mediators of inflammation known as leukotrienes (5). In addition to causing airway narrowing through activation of airway smooth muscle, the leukotrienes are known to produce airway edema, inflammatory cell influx, and possibly increased mucus production (6-10). While apparently less effective than inhaled corticosteroids, leukotriene modifiers specifically have been shown to have effects similar to inhaled corticosteroids on inflammatory mediators that we associated with discontinuation of inhaled corticosteroids in the SOCS trial. These therapeutic agents have been shown to reduce exhaled nitric oxide levels (11-13), circulating and sputum eosinophils (11,14,15), and other mediators of airway inflammation (16,17) which increased in the salmeterol arm of the SOCS study. Additionally, they may also reduce

levels of sputum tryptase (18), a marker that increased when inhaled corticosteroids were withdrawn in the SOCS/SLIC trial.

In addition to potentially complementing the activity of long-acting beta-agonists through their activity on inflammatory indices, the leukotriene modifiers appear to complement the physiologic and clinical effects of long-acting beta-agonists. In the SOCS/SLIC study we observed that the long-acting beta-agonists improved indices of airway function but did not provide significant protection from asthma treatment failures. Use of the leukotriene modifiers has been shown to decrease asthma treatment failures and exacerbations requiring corticosteroids (19-24). Studies with the leukotriene modifiers show that they appear to be less effective than inhaled corticosteroids in increasing airway caliber, but they improve asthma control as reflected by frequency of exacerbations, days without symptoms and loss of work (15,25). In addition, while the bronchoprotective effects of long-acting beta-agonists decrease with regular use, leukotriene receptor antagonists do not result in such tolerance (26,27).

The apparent complementary mechanisms of action of the long-acting beta-agonists and the leukotriene modifiers (bronchodilatory and anti-inflammatory) suggest that when used together the combination could provide additive control of asthma. In support of this concept is a recent study that demonstrated an additive effect of salmeterol and montelukast in inhibiting AMP-induced bronchoconstriction (28). In fact, the degree of shift of reactivity associated with the combination of montelukast and salmeterol in this study was equivalent to that reported by others in association with inhaled corticosteroid monotherapy (29). Further, as “add-on therapy” to ICS, both agents have been shown to provide additional benefit in patients who are inadequately controlled on inhaled corticosteroids alone (25,30-34).

Taken together, these data suggest that combining a leukotriene modifier and a long-acting beta-agonist will provide effectiveness that exceeds that of either agent used alone. However, the precise role for such a combination remains unclear, especially in light of other combinations therapies for asthma which will soon be available.

D. Are inhaled corticosteroids, used alone, the standard to which new therapies should be compared?

Given that the combination of a long-acting beta-agonist and a leukotriene modifier represents a potentially effective bronchodilatory and anti-inflammatory combination, to which therapy should this combination be compared? The ACRN SOCS/SLIC trials compared a long-acting beta-agonist to an inhaled corticosteroid alone. We initially proposed that we compare the combination of a long-acting beta-agonist and a leukotriene modifier to an inhaled corticosteroid. However, over the past five years, it has become evident that in addition to the potential complementary effects of long-acting beta-agonists with leukotriene receptor antagonists, long-acting beta-agonists clearly complement the effects of inhaled corticosteroids. For example, in patients poorly controlled on inhaled corticosteroids, the combination of a long-acting beta-agonist and an inhaled corticosteroid has been shown to provide better clinical control than further

increasing the dose of inhaled corticosteroids(30,31,35-37). Multiple studies also have shown that the combination of a long-acting beta-agonist and an inhaled corticosteroid is superior to either therapy alone with respect to lung function, symptom-free days and rescue albuterol use (38-40). Furthermore, Pauwels and colleagues have demonstrated that the combination of the long-acting beta-agonist formoterol and a low dose inhaled corticosteroid is essentially as effective as a higher dose of inhaled corticosteroid alone (41). Subsequently, a recent study by Nielsen et al has even demonstrated that the addition of a long-acting beta-agonist to inhaled corticosteroid can result in a reduction in inhaled corticosteroid dose (42).

The increased effectiveness of the combination of a long-acting beta-agonist and an inhaled corticosteroid vs. the use of the same dose of an inhaled corticosteroid has spurred the development of delivery systems that deliver both agents together. These systems have been introduced in Europe and will soon be available in the United States. They have been well received by both patients and physicians. Since consensus-based guidelines recommend that when inhaled corticosteroids are used, that the minimal dose be used, we expect the combination of a long-acting beta-agonist and an inhaled corticosteroid combination to become the default standard in cases where inhaled corticosteroids are recommended. Considering these developments, we also believe the combination represents the standard against which other proposed agents should be compared.

E. Research Questions

In summary, as we attempt to re-formulate guidelines for the optimal treatment of asthma, inhaled corticosteroids clearly remain the most effective treatment for persistent asthma. Combining inhaled corticosteroids with long-acting beta-agonists permits lower doses of inhaled corticosteroids to be used for control of asthma. However, concerns regarding possible systemic adverse effects continue to limit the appeal of inhaled corticosteroids to many patients and physicians. Considering the potential synergy that might be produced by the combination of a long-acting beta-agonist and a leukotriene modifier, the question that remains unanswered is whether the combination of a long-acting beta-agonist and an inhaled corticosteroid is superior to the former combination. We will study subjects with a minimum of moderate persistent asthma to address the following questions:

1. Is the combination of a long-acting beta-agonist and an inhaled corticosteroid superior to the combination of a long-acting beta-agonist and a leukotriene modifier as reflected by treatment failures (a need for intensification of asthma therapy)?

2. Is the combination of a long-acting beta-agonist and an inhaled corticosteroid more effective than the combination of a long-acting beta-agonist and a leukotriene modifier as judged by physiological outcome measures such as AM and PM peak expiratory flow, FEV_1, and bronchial reactivity to methacholine?

3. Is the combination of a long-acting beta-agonist and an inhaled corticosteroid more effective than the combination of a long-acting beta-agonist and a leukotriene

modifier as judged by secondary clinical outcomes, such as “as-needed” beta-agonist use, quality of life score, and symptom free days?

4. If the combination of a long-acting beta-agonist and an inhaled corticosteroid is more effective, is the increased effectiveness predicted by baseline markers of inflammation that we studied in the SOCS trial – sputum eosinophils, exhaled nitric oxide, and sputum tryptase?

V. SPECIFIC AIMS, PROTOCOL AND TIMELINE

A. Specific Aims

1. Principal Aims

To determine, in subjects with moderate persistent asthma, whether the combination of the long-acting beta-agonist salmeterol administered by dry powder inhaler (DPI) and the inhaled corticosteroid beclomethasone HFA administered by MDI is superior to the combination of the long-acting beta-agonist salmeterol administered by DPI and the leukotriene modifier montelukast administered by mouth with respect to asthma control **as defined by time until treatment failure**.

2. Secondary Aims

1. To determine, in subjects with moderate persistent asthma, whether the combination of the long-acting beta-agonist salmeterol and the inhaled corticosteroid beclomethasone HFA is superior to the combination of the long-acting beta-agonist salmeterol and the leukotriene modifier montelukast **as assessed by effect on FEV_1, AM peak expiratory flow rate , PM peak expiratory flow rate, peak expiratory flow rate variability, and PC_{20}**.

2. To determine whether the combination of the long-acting beta-agonist salmeterol and the inhaled corticosteroid beclomethasone HFA is superior to the combination of the long-acting beta-agonist salmeterol and the leukotriene modifier montelukast with regard to reductions in **markers of asthma inflammation,** including the number and type of inflammatory cells in the sputum and the concentration of inflammatory mediators and markers in sputum and exhaled gas.

3. Exploratory Aims

To determine the relationship between:

1) hypothesized **physiological predictors** of outcomes;
2) various **markers of airway inflammation**;

—and;

a) the occurrence of **treatment failure**;

b) changes in the **indicators of airflow obstruction** during randomized treatment.

These physiological predictors and markers will include:

1. Methacholine PC_{20} at the end of the run-in
2. The degree of sputum eosinophilia (sputum eosinophil %) at the end of run-in
3. The concentration of NO in exhaled air at the end of run-in and the change in NO over the first three weeks of treatment
4. The concentration of tryptase in the induced sputum at the end of run-in

4. Other Aims

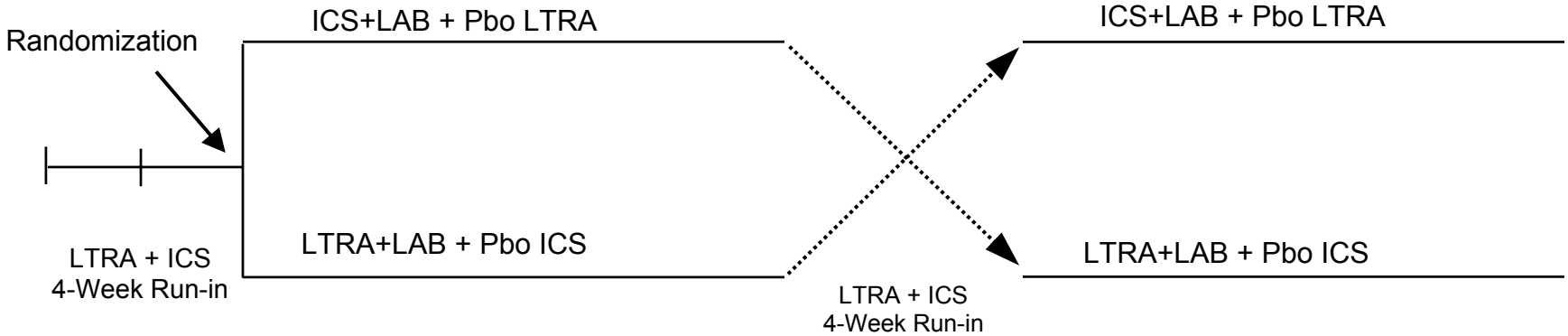
In an exploratory manner, we will also examine the relationship between the changes in the indicators of airflow obstruction and asthma control in the response to specific types of therapy and single nucleotide polymorphisms in genetic loci thought to be related to the occurrence of asthma or its severity. To this end, genomic DNA will be isolated from each subject to be examined for potentially important polymorphisms identified via gene discovery in the future.

B. Protocol Overview

A double-blind, placebo-controlled, cross-over study is proposed with time to treatment failure as the primary endpoint. Prior to each double-blind treatment phase, subjects will undergo a 4-week run-in period with combined inhaled beclomethasone HFA and the leukotriene receptor antagonist (LTRA) montelukast. This will be followed by 14 weeks of double-blind treatment. The study is presented schematically below.

C. Protocol Design

1. Study Schematic and Description



V	1	2	3		4			5			6		6a-c+	7		8			9			10
W	**0**	**2**	**4**	**6**	**7**	**9**	**11**	**13**	**15**	**17**	**18**	**20**		**22**	**24**	**25**	**27**	**29**	**31**	**33**	**35**	**36**
	PT LE IC Med Hx DD Blood S	S AR CD/DD DR Mch EPFMQ EPFMR EDEM-C	NO S/PSS PT Mch SI AR CD/DD DR	P	NO S/PSS DR ACQ AQLQ ASUI EPFMR EDEM-C	P	P	NO S/PSS AR CD/DD DR ACQ AQLQ ASUI EPFMR EPFMQ EDEM-C	P	P	NO PT Mch SI DR ACQ AQLQ ASUI EPFMR EPFMQ EDEM-C		S AR CD/DD DR LE EPFMR EPFMQ EDEM-C	NO S/PSS PT Mch SI AR CD/DD DR ACQ AQLQ ASUI EPFMR EPFMQ C		NO S/PSS AR CD/DD DR ACQ AQLQ ASUI EPFMR EPFMQ EDEM-C		P	NO S/PSS AR CD/DD DR ACQ AQLQ ASUI EPFMR EPFMQ EDEM-C	P	P	NO PT Mch SI LE ACQ AQLQ ASUI EPFMR EPFMQ EPFMC EDEM-C EDEM-X
B* Mch	ST EPFMD EPFMQ EDEM-I		ACQ AQLQ ASUI EPFMR EPFMQ EDEM-C		EDEM-C			EDEM-C														

ACQ = Asthma Control Questionnaire; **AQLQ** = Asthma Quality of Life Questionnaire; **AR** = Adherence Review; **ASUI** = Asthma Symptom Utility Index; **B**= Bronchodilator Response Assessment; **Blood** = Blood Collection for Genetic Analysis and IgE/Eos Determination; **CD** = Collect Symptom Diary; **DD** = Dispense Symptom Diary; **DR**=Symptom Diary Review; **EDEM-C**=eDEM Monitor Compliance Check; **EDEM-I**=eDEM Monitor Instruction; **EDEM-X**= Collect eDEM monitor; **EPFMC**= Collect Electronic Peak Flow Monitoring Device; **EPFMD**= Dispense Electronic Peak Flow Monitoring Device; **EPFMQ**=QC Electronic Peak Flow Monitoring Device; **EPFMR**= Review Electronic Peak Flow Monitoring Device; **IC**= Informed Consent; **ICS**=Inhaled CorticoSteroid (beclomethasone HFA); **LAB**=Long-Acting Beta-agonist (salmeterol); **LE**=Long Exam; **LTRA**=LeukoTriene Receptor Antagonist (montelukast); **Mch**=Methacholine Challenge; **Med Hx**=Medical History; **NO** = Exhaled Nitric Oxide Measurement; **P**=Phone Contact; **Pbo**=Placebo; **PSS**=Post-Salmeterol Spirometry; **PT**=Pregnancy Test; **S**=Spirometry; **SI**=Sputum Induction; **ST** =Allergen Prick Skin Testing; **V**=Visit; **W**=Week; *****= As indicated (see eligibility criteria); **+** = Visits scheduled as-needed during post-treatment failure washout period

During the initial 4-week run-in period, subjects who meet NAEP criteria for moderate persistent asthma (see section VI.A.) will receive single-blind treatment with combined inhaled beclomethasone HFA by metered dose inhaler (MDI) and the LTRA montelukast. The purpose of the run-in is to stabilize subjects on a standardized controller regimen that is likely to be effective in most subjects and to obtain baseline information on symptoms, beta-agonist use, and PEF for use in defining treatment failure (primary outcome, see section VII for definition) for each subject over the duration of the study. The final visit of this run-in period will serve as the baseline for comparison for the study variables measured during the initial treatment phase. The use of both beclomethasone HFA and montelukast during the run-in period will allow us to study a broader population of asthmatics rather than biasing the study with responders to one class of therapy (i.e. either inhaled steroids or LTRAs) alone.

At the end of the initial run-in, subjects with stable asthma symptoms will be randomized to one of two treatment regimens to be used for 14 weeks: 1) daily oral placebo, twice daily inhalation of salmeterol 50 μg by DPI, and twice daily inhalation of beclomethasone HFA 80 μg by MDI; or 2) once daily oral montelukast 10 mg, twice daily inhalation of salmeterol 50 μg by DPI, and twice daily inhalation of placebo. Active beclomethasone HFA and the beclomethasone HFA placebo will be given via a metered dose inhaler delivery device, while salmeterol will be given by a dry powder inhaler (DPI) Diskus®. A computer interface will randomize subjects into the two treatment groups, stratifying by clinical center and FEV_1 at the randomization visit (<80% versus ≥80% of predicted FEV_1).

During the first treatment period, subjects will make three visits to the clinic over 14 weeks. These visits will include re-assessment of the study variables and careful monitoring for increasing asthma symptoms and potential treatment failure. Subsequently, subjects will enter a second run-in period where they will receive single-blind treatment with inhaled beclomethasone HFA and oral montelukast as in the first run-in period. At the end of the second run-in period, subjects will cross over to the alternate treatment regimen for the second 14-week treatment phase which includes three visits to the clinic. These visits will again include re-assessment of the study variables and careful monitoring for increasing asthma symptoms and potential treatment failure. Frequent contact with the subject will be maintained throughout the study; no more than 2 weeks will pass between clinic visits and/or phone contacts with subjects. Subject adherence will be monitored, as will subject safety, potential treatment failure status and asthma deterioration. If these phone contacts indicate that a subject has experienced a treatment failure as evidenced by an increase in their asthma symptoms, increased albuterol use, decreased PEFRs, or other criteria, they will be asked to return for the clinic for additional assessment.

D. Timeline

Study details are described in the sections that follow. The timeline for completion of this study is:

Development	2/02-7/02
Recruitment (2.5 subjects/center/month)	8/02-7/03
Final subject visit	4/04
Analysis	4/04-8/04

VI. INCLUSION AND EXCLUSION CRITERIA

A. Inclusion Criteria (at Visit 1)

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

2. Clinical history consistent with asthma.

3. $FEV_1 \geq 40\%$ of predicted.

   For subjects not regularly using inhaled corticosteroids and/or leukotriene receptor antagonists at Visit 1, maximum FEV_1 is 80% of predicted.

4. Bronchial hyper-responsiveness or reversible airflow obstruction as defined by:

   a) For subjects with an $FEV_1 \geq 55\%$ of predicted, a 20% reduction in FEV_1 in response to a concentration of inhaled methacholine $\leq$ 8 mg/ml (PC_{20} $FEV_1 \leq$ 8mg/ml) at Visit 1.

   b) For subjects with an $FEV_1 \geq 40\%$ and <55% of predicted, a $\geq 12\%$ improvement in FEV_1 after aerosolized albuterol at Visit 1.

5. 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.

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

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

Table 1. Drugs to be withheld throughout the study	Washout prior to Visit 1
Leukotriene receptor antagonists, except as provided in study	None
Inhaled steroids, except as provided in study	None
Intranasal steroids, except nasal fluticasone	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	≥ 2 weeks
ACE inhibitors and Angiotensin II antagonists	> 2 weeks
Inhaled beta-adrenergic agonists (intermediate-acting, e.g., albuterol, terbutaline, metaproterenol, pirbuterol, bitolterol), except as 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 (1st generation)	≥ 72 hours
Cetirizine	≥ 7 days*
Supplemental magnesium (> 100 mg/day)	≥ 5 days
Drugs withheld prior to pulmonary function and/or methacholine challenge, per MOP	**Specified time period**
Albuterol (study RESCUE drug)	≥ 6 hours
Salmeterol (study drug)	≥ 12 hours
Fexofenadine (Allegra)	≥ 48 hours
Chlorpheniramine (Chlor-Trimeton)	≥ 48 hours
Loratadine (Claritin)	≥ 48 hours
Pseudoephedrine (Sudafed) and Oxymetazoline (Afrin)	≥ 48 hours
Methylxanthine-containing foods or beverages (e.g., coffee, tea)	≥ 6 hours

*Because 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 subject is eligible for study participation. If not, the subject requires a longer washout period (possibly weeks) prior to re-testing and enrollment.

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.

2. Chronic use of any medication other than study beta-agonists, leukotriene modifiers, and inhaled corticosteroids, except oral contraceptives and other hormonal forms of contraceptives (i.e., DepoProvera-7, Norplant-7), estrogen / progesterone replacement therapy for post-menopausal women, vitamins, nasal fluticasone (2 sprays each nostril (50 μg each spray), QD) at a stable dose throughout the entire study (see MOP), acetaminophen, non-steroidal anti-inflammatory medications (e.g., aspirin, naproxen, ibuprofen, cox2 inhibitors), thyroid replacement medications, lipid-lowering medication, or medium and low potency topical cutaneous steroids.

Additional allowable medications include:

- calcium supplements
- nasal saline spray
- topical eye preparations for allergic eye symptoms (e.g. antihistamines, NSAIDs, or antiallergic compounds)
- diuretics
- specific antihypertensives (e.g. calcium channel blockers, clonidine, etc.)
- acyclovir
- chlorpheniramine, fexofenadine, and loratadine (48 hour washout prior to visits)
- pseudoephedrine and oxymetazoline (48 hour washout prior to visits)
- antibiotics for acne
- stool softeners and bulk laxatives

3. Use of Pulmicort Respules for treatment of asthma.

4. Lung disease other than asthma.

5. Established or suspected diagnosis of vocal cord dysfunction.

6. Significant medical illness other than asthma.

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

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

9. History of life-threatening asthma requiring treatment with intubation and

mechanical ventilation within the past 10 years.

10. Hyposensitization therapy other than an established maintenance regimen.

11. Inability, in the opinion of the investigator or clinical coordinator, to coordinate use of the delivery devices used in the study (dry powder inhaler [DPI] or metered dose inhaler [MDI]).

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

13. Inability to use an electronic peak flow monitoring (EPFM) device correctly for recording peak flow measurements.

C. Inclusion Criteria For Randomization (Visit 3)

1. Ability of the subject to measure his/her AM and PM PEF on schedule using the EPFM device and to accurately transcribe the PEF measurements onto his/her diary cards at least 85% of the time during the last two weeks of the initial run-in (i.e., interval between visit 2 and visit 3).

D. Exclusion Criteria For Randomization (Visit 3)

1. FEV_1 < 85% of that obtained at Visit 1.

2. FEV_1 < 55% of predicted.

3. Treatment failure or significant exacerbation of asthma during the run-in period (see sections VII and IX for definition of treatment failure/exacerbation during run-in period).

4. Inability to comply with regular use of beclomethasone HFA (missed more than 15% of doses in last two weeks of run-in as reflected by Doser® device).

5. Inability to comply with LTRA tablet administration (missed more than 15% of doses in last two weeks of run-in as reflected by eDEM monitor).

6. Failure to record peak flow measurements and symptoms in a symptom diary more than 15% of required times during the last two weeks of the run-in period.

7. Presence at Visit 3 of any of the exclusion criteria stipulated for Visit 1 (see section VI.B. above). Note: Respiratory tract infections that do not

cause the subject to meet treatment failure criteria are not considered exclusionary.

8. Use of an average of ≥16 puffs of albuterol per 24 hours during last week of run-in (week 4).

VII. OUTCOME VARIABLES

A. Primary Outcome: Treatment Failure

To compare the effects of therapy with the different regimens, the primary outcome variable will be the time to treatment failure during each double-blind treatment period. Using survival analysis methods, we will compare treatment failures occurring during treatment with the beclomethasone HFA-salmeterol combination with those occurring during treatment with the montelukast-salmeterol combination. Specific criteria as outlined below (section VII.A.2.) will also be used to distinguish between treatment failure due to poor asthma control and dropout for any other reasons.

1. Criteria for Treatment Failure During the Initial Run-in Period

Treatment failure during the run-in periods ***will not*** establish endpoints for the SLiMSIT protocol, as treatment failure **endpoints** will only be attained during the double-blind treatment periods. However, treatment failure criteria will be applied during the run-in periods to ensure asthma stability prior to entry into the double-blind treatment periods of the trial. Criteria for treatment failure during the second run-in period are identical to criteria for the double-blind treatment periods (see section VII.A.2 below). Criteria for treatment failure during the initial run-in period follow. Subjects who meet treatment failure criteria during the initial run-in will be dropped from the study (see section VI.D).

*At-home measurements: **

1. Pre-bronchodilator PEF ≤ 65% of baseline on any 2 of 3 consecutive, scheduled measurements. Baseline is defined during the initial run-in (weeks 1-4) as:

Weeks 1-2: Best of three consecutive, technically acceptable blows on the EPFM device at Visit 1

Weeks 3-4: Mean value of AM pre-bronchodilator PEF recorded on symptom diary during the first two weeks of the run-in (weeks 1 and 2)

2. Post-bronchodilator PEF ≤ 80% of baseline despite 60 minutes of rescue beta-agonist treatment (≥6 puffs of albuterol in one hour). Post-bronchodilator PEF may be taken at any time of day. Baseline is defined during the initial run-in (weeks 1-4) as in criterion #1 above.

3. An increase in PRN albuterol use of 8 puffs per 24 hours over baseline use for a period of 48 hrs, or ≥16 puffs/24 hrs for 48 hrs. Baseline is defined during the initial run-in (weeks 1-4) as:

Weeks 1-2: The historical average daily albuterol use obtained at Visit 1

Weeks 3-4: Average daily use during the first two weeks of the run-in (weeks 1 and 2)

* Subjects will be instructed to contact the ACRN study site immediately should any of these events occur.

In-clinic measurements:

1. Pre-bronchodilator FEV_1 values on two consecutive sets of spirometric determinations (i.e., visits 2,3) that are ≤80% of the baseline pre-bronchodilator value obtained at Visit 1.

2. The subject will also meet treatment failure criteria if the following set of circumstances occurs:

If the pre-bronchodilator FEV_1 value at Visit 2 or Visit 3 is ≤80% of the pre-bronchodilator value obtained at Visit 1, the subject should be given albuterol (≥6 puffs in one hour) to assess the degree of reversibility in his/her airflow obstruction. These values must be reported to the physician responsible for the care of the ACRN subject on that day. If the physician determines that the subject's response to the bronchodilator is satisfactory, and the subject's clinical condition is stable, the subject may continue in the study, as usual, provided he/she returns to the ACRN study site in 24-96 hours for repeat spirometry. In addition, the clinic coordinator or designee shall telephone the subject every 24 hours to assess his/her condition. No additional provocative procedures (e.g., methacholine challenge, sputum induction) scheduled for that study day should be performed. At the additionally scheduled visit within the next four days, the repeat spirometric pre-bronchodilator FEV_1 value must be >80% of the pre-bronchodilator value obtained at visit 1; if not, the subject will be considered a treatment failure at that time. If spirometric values are within the acceptable range, all procedures for the previously scheduled visit shall be performed according to the MOP and the subject will continue on his/her study medications, as usual.

Additional Treatment Failure Criteria:

1. Any use of oral, parenteral, or non-study-related inhaled corticosteroids related to the treatment of the subject's asthma.

2. Occurrence of an asthma exacerbation during the run-in period (see section IX.C.1).

3. Need for emergency treatment or urgent care visit at a medical facility that is related to, or complicated by, the subject's asthma and which results in corticosteroid treatment or hospitalization for an acute asthma exacerbation.

4. Physician clinical judgment for safety reasons.

2. Criteria For Treatment Failure During Double-blind Treatment Periods and Second Run-In Period (Post-Randomization)

Treatment failure status will be defined as the occurrence of one or more of the following:

*At-home measurements: **

1. Pre-bronchodilator PEF ≤ 65% of baseline on any 2 of 3 consecutive scheduled measurements. Baseline is defined during the post-randomization period as:

Mean value of AM pre-bronchodilator PEF recorded on symptom diary during the last two weeks of the initial run-in period (weeks 3 and 4)

2. Post-bronchodilator PEF ≤ 80% of baseline despite 60 minutes of rescue beta-agonist treatment (≥6 puffs of albuterol in one hour). Post-bronchodilator PEF may be taken at any time of day. Baseline is defined during the post-randomization period as in criterion #1 above.

3. An increase in PRN albuterol use of ≥ 8 puffs per 24 hours over baseline use for a period of 48 hrs, or ≥16 puffs/24 hrs for 48 hrs. Baseline is defined during the post-randomization period as:

Average daily use during the last two weeks of the initial run-in (weeks 3 and 4)

* Subjects will be instructed to contact the ACRN study site immediately should any of these events occur.

In-clinic measurements:

1. Pre-bronchodilator FEV_1 values on two consecutive sets of spirometric determinations that are ≤80% of the baseline pre-bronchodilator value obtained at Visit 3. A subject will meet this criterion if he or she experiences pre-bronchodilator FEV_1 values at two consecutive post-randomization visits (e.g., Visits 4 and 5) that are ≤80% of the baseline value.

2. The subject will also meet treatment failure criteria if the following set of circumstances occurs:

If the pre-bronchodilator FEV_1 value at a visit is ≤80% of the pre-bronchodilator value obtained at Visit 3, the subject should be given albuterol (≥6 puffs in one hour) to assess the degree of reversibility in his/her airflow obstruction. These values must be reported to the physician responsible for the care of the ACRN subject on that day. If the physician determines that the subject's response to the bronchodilator is satisfactory, and the subject's clinical condition is stable, the subject may continue in the study, as usual, provided he or she returns to the ACRN study site in 24-96 hours for repeat spirometry. In addition, the clinic coordinator or designee shall telephone the subject every 24 hours to assess his or her condition. No additional provocative procedures (e.g., methacholine challenge, sputum induction) scheduled for that study day should be performed. At the additionally scheduled visit within the next four days, the repeat spirometric pre-bronchodilator FEV_1 value must be >80% of the pre-bronchodilator value obtained at visit 3; if not, the subject will be considered a treatment failure at that time. If spirometric values are within the acceptable range, all procedures for the previously scheduled visit shall be performed according to the MOP and the subject will continue on his/her study medications, as usual.

3. Post-bronchodilator FEV_1 value that is ≤80% of the baseline post-bronchodilator value obtained at Visit 3. This criterion does not apply to visit 6a, 6b, 6c, etc. as no post-bronchodilator value is obtained at these visits.

Additional Treatment Failure Criteria:

1. Any use of oral, parenteral, or non-study-related inhaled corticosteroids related to the treatment of the subject's worsened asthma.

2. Occurrence of an asthma exacerbation (see section IX.C.1) during the post-randomization period.

3. Need for emergency treatment or urgent care visit at a medical facility that is related to the subject's asthma and which results in corticosteroid treatment or hospitalization for an acute asthma exacerbation.

4. Physician clinical judgment for safety reasons.

3. Study Visit Management after Treatment Failure

Subjects who achieve treatment failure or exacerbation status will be medically treated according to protocol outlined below (Section IX.C.). This will include open-label inhaled corticosteroids (MDI beclomethasone HFA 160 μg/day) or oral corticosteroids (if the treating physician deems it necessary). As outlined in section IX.C, open-label inhaled corticosteroids will be given for two weeks. *For subjects already receiving inhaled corticosteroids as part of their double-blind study regimen, this will represent a doubling of the inhaled corticosteroid dose; for subjects not already using inhaled corticosteroids, this*

will represent initiation of these agents. If oral corticosteroids are prescribed, the recommended taper is outlined in section IX.C.

Study inhalers of salmeterol and inhaled corticosteroids (or placebo) and study tablets of montelukast (or placebo) will be continued unless the treating physician can document that treatment failure status was causally related to taking study medication. Subjects will continue to participate in the study for the remainder of the protocol. For safety reasons, all subjects will be seen at the clinical center 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 (first treatment phase) or Visit 7 (second treatment phase). However, subjects who achieve treatment failure status in the weeks prior to Visit 7 (beginning of second double-blind treatment period) may need to delay entering the second treatment period until a sufficient period of time has passed to allow for washout of treatment failure medications. In particular, Visit 7 will be delayed until at least six weeks have passed since the documentation of treatment failure status and, if prescribed, until at least six weeks have passed since the last dose of oral prednisone. To ensure comparability of treatment received during the two run-in periods, Visit 7 also will be delayed until at least four weeks have passed since the last dose of open-label inhaled corticosteroids. If a subject falls into the required washout period at the time that he or she was originally scheduled to complete Visit 7, then a Visit 6a will be performed in place of Visit 7. At Visit 6a spirometry will be performed, diary cards will be reviewed and dispensed, and the subject's asthma symptoms will be assessed. The subject will be given additional single-blind run-in medications to last for two weeks. If, after Visit 6a, more than two additional weeks are required to meet the minimum washout period, then a visit 6b will be scheduled for two weeks after Visit 6a. If necessary, additional interim visits will be scheduled at two-week intervals during the washout period. When less than two weeks are needed to meet all washout requirements, Visit 7 will be scheduled for two weeks following the current visit. All necessary washouts will be reviewed and confirmed prior to performing Visit 7 procedures.

B. Secondary Outcomes

Secondary outcomes to be studied will include changes in clinical and physiological outcomes, as well as quality of life scores and pharmacoeconomic outcomes during each of the two treatment periods. Physiologic/clinical parameters to be analyzed within the two treatments will include changes in FEV_1, AM and PM peak flow, mean peak flow variability (PM-AM peak flow difference normalized by the average of the AM and PM peak flow), and methacholine airway responsiveness. For each of these parameters, values obtained at visits during and at the end of each period will be compared with those obtained at visit 3 or 7 (at the end of four weeks of run-in with beclomethasone HFA-montelukast). These changes also will be compared across treatment regimens. Daily data obtained from diary cards will be summarized and analyzed as weekly averages. A longitudinal data analysis will be applied to each of these parameters (section XI.E) so that the end of each treatment period will be compared to those parameters measured at

the end, or the last two weeks (where appropriate, e.g. PEF and symptoms), of each run-in period.

Using asthma symptom diaries, quality of life measurements, including asthma symptom scores and rescue medication use, will also be assessed during the different treatment periods and compared across treatments.

The primary pharmacoeconomic outcomes for this trial will be "symptom-free" and "albuterol rescue-free" days, calculated from the symptom diaries. In addition, the Asthma Symptom Utility Index (ASUI) (43) will be administered at all regular post-randomization Visits, 3-10. The ASUI is a brief easy-to-administer symptom assessment scale used as a preference-based outcome measure in cost-effectiveness studies in asthma. This 11-item questionnaire (with two-week recall validity) reflects symptom gradations as well as medication side effects, weighted according to patient preferences.

To determine if differences in efficacy during therapy, or differences in the duration of therapeutic benefit after initiation of treatment reflect differences in anti-inflammatory activity, several inflammatory markers will be studied at Visits 3, 6, 7, and 10. Induced sputum samples will be obtained; they 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. Another inflammatory marker to be studied in an exploratory fashion will include measurement of levels of expired NO. The values for each of these parameters obtained at Visit 3 and 7 (beginning of each blinded treatment period) will be considered the baseline for each treatment and used as a basis for comparison with regard to visits 6 and 10 (end of each blinded treatment period), respectively, to assess the effects of the different therapies.

Further, in an exploratory fashion, we will examine the relationship between the acute changes in FEV_1, PEF, and expired NO over the first three weeks of randomized treatment period in relation to treatment failure. We also will examine the relationship between exhaled NO and the changes in the physiologic parameters followed during the study that occur in each of the treatment periods.

VIII. PROTOCOL

A. Number of Subjects Needed: Sample Size

The primary response variable is the time until treatment failure. Therefore, the primary statistical analysis will be based on an intent-to-treat proportional hazards regression analysis. The SLiMSIT protocol has eligibility criteria that are similar to those used for the SOCS/SLIC trials. In the SLIC trial, subjects experienced an 8.1% treatment failure rate after 14 weeks of combined treatment with an inhaled corticosteroid and a long-acting beta-agonist, while the subjects treated with inhaled corticosteroids **alone** in the SOCS trial had a 5.6% failure rate. The addition of salmeterol to ICS has been shown to

decrease the rates of exacerbations by 40% in one study (41) to by greater than 70% as demonstrated in another study (44). Adopting the more conservative estimate of reduction (40%), the anticipated treatment failure rate in the SOCS group would be 2.2% and thus the anticipated treatment failure rate for the subjects during treatment with ICS and salmeterol in the entire SLIMSIT study would be 5% (based on the observation in SOCS/SLIC that 50% of screened subjects were allotted to each trial). Based on its collective clinical experience, the Steering Committee has stated that the combination of a long-acting beta-agonist and an inhaled corticosteroid would be judged to be clinically superior to the combination of a long-acting beta-agonist and a leukotriene antagonist unless the latter combination was able to reduce the rate of treatment failure to 15% or less. In the Steering Committee's opinion, in the setting of the combination of a long-acting beta-agonist and an inhaled corticosteroid reducing the treatment failure rate to 5%, and the inability of the combination of a long-acting beta-agonist and a leukotriene antagonist to reduce treatment failure to a rate of 15% or less, the clinical significance of such a difference would be compelling enough to form the basis of a recommendation that the former therapy be used in place of the latter in the treatment of patients with moderate persistent asthma.

The primary response variable is the time until treatment failure. Because of the crossover design, each subject will have a time until treatment failure, or a censoring time, for each of the two treatment regimens. France, Lewis, and Kay describe the statistical analysis for such a situation (45). The basic statistical analysis involves the comparison of the two treatment failure/censoring times within each subject using a proportional hazards regression. The hazard function for subject i at time t, i=1,2,…,n, is

$$\lambda_i(t;\mathbf{x}) = \lambda_{0i}(t)\exp(\mathbf{x}'\boldsymbol{\beta}) \qquad [1]$$

where $\lambda_{0i}(t)$ represents the baseline hazard function for subject i, $\mathbf{x}$ represents a vector of regressors, and $\boldsymbol{\beta}$ represents a vector of parameter coefficients.

For each subject, a treatment preference is identified in terms of whether the subject performed better on one treatment regimen versus the other. If the subject has a censoring time on both treatment regimens, then that subject has no treatment preference. Therefore, the trial results can be summarized as a trinomial response:

n_A = # subjects who prefer treatment regimen A
n_B = # subjects who prefer treatment regimen B
$n - n_A - n_B$ = # subjects for whom a preference cannot be determined (treatment failures occurring within 2 days of each other in the two treatment arms will be considered "ties")

For convenience, define

θ_A = Pr[a subject prefers treatment regimen A]
θ_B = Pr[a subject prefers treatment regimen B]

In the absence of period effects, the hazard ratio reduces to

$$\Lambda = \theta_A/\theta_B \qquad [2]$$

and its maximum likelihood (ML) estimator reduces to

$$\Lambda_{ML} = n_A/n_B \qquad [3]$$

Thus, in the absence of period effects, the statistical analysis is comparable to McNemar's test for paired binary data. In the presence of period effects, the ML estimator is the geometric mean of the ratios of treatment regimen preferences within each of the two sequences, i.e.,

$$\Lambda_{ML} = \{(n_A/n_B)_{seq_AB}(n_A/n_B)_{seq_BA}\}^{1/2} \qquad [4]$$

The ML estimator in [4] can be generalized if there are other categorical covariates of interest. If there are continuous covariates of interest, then the proportional hazards regression model in [1] can be applied.

McNemar's test will provide the basis for the sample size calculation. Although there might be some important covariates that affect the hazard ratio, e.g., period, gender, etc., their impact on the sample size calculation is negligible. This is because the covariates tend to improve precision while sacrificing degrees of freedom.

Nam has derived a sample size formula for the matched-pairs design in which McNemar's test is applicable (46).

The ACRN Steering Committee anticipates a 15% or greater treatment failure rate for the salmeterol-LTRA combination and a 5% treatment failure rate for the beclomethasone HFA-LTRA combination. Therefore, this represents the effect size in the matched-pairs analysis. A total sample size of 180 randomized subjects, based on a two-sided, 0.05 significance level test and allowing for a 10% withdrawal rate, yields more than 90% statistical power for distinguishing between treatment failure rates of 5% and 15%. In fact, there is 90% statistical power under these circumstances to distinguish between treatment failure rates of 5% and 14%. The sample size of 180 randomized subjects provides 90% statistical power for other effect sizes as well:

Beclomethasone HFA-LTRA Failure Rate	Salmeterol-LTRA Failure Rate	Difference	Ratio
2%	10%	8%	5.00
3%	12%	9%	4.00
4%	13%	9%	3.25
5%	14%	9%	2.80
6%	16%	10%	2.67
7%	17%	10%	2.43

8%	18%	10%	2.25
9%	19%	10%	2.11
10%	20%	10%	2.00

The total sample size of 180 randomized subjects corresponds to 30 randomized subjects for each of the six ACRN clinical centers.

B. Recruitment

Harvard Clinical Center/Boston

The Asthma Clinical Research Center at the Brigham & Women's Hospital utilizes three primary resources for identifying and recruiting potential subjects as described below.

Research Patient Database: The Asthma Clinical Research Center at the Brigham and Women's Hospital has a database of over 1,500 asthmatics who have expressed interest in participating in research. All of these subjects have completed questionnaires regarding their asthma and medication use. In addition, many have undergone physiological screening. The database is screened based on entry criteria, and subjects are contacted in a manner approved by the IRB to ascertain their interest in participation.

Advertisements: We utilize IRB-approved radio and newspaper advertisements to inform potential subjects of our studies and solicit participation. In addition, we use posters in selected locations.

Asthma Patient Lists: Following IRB guidelines, the center has permission to contact patients with a diagnosis of asthma to ascertain these patients' possible interest in participating in asthma studies. Lists generated at the Brigham & Women's Hospital contain over 5,000 such patients. In the past, we have also used patient lists from the Harvard Pilgrim Health Care HMO. The latter list can be screened by medication use to preliminarily identify patients with specific patterns of medication use.

National Jewish Medical and Research Center/Denver

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

1. National Jewish 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 at least 15% are in the mild 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 10-15% are in the mild category. Ninety-seven additional children were seen in follow-up.

National Jewish Center 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 Asthma Research Pool

There are over 600 asthma patients (not followed in the NJC outpatient clinic) who 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.

3. Denver Health Medical Center

Dr. James Fisher, Head of Pulmonary Medicine, is supporting our efforts by helping us to recruit from the asthmatic patient population at Denver General. This is a large county hospital whose patient population comprises mainly Hispanic and African-American people.

4. Denver Veterans Administration Hospital

Dr. Clifford W. Zwillich, Head of Medicine, will support this grant. The V.A. hospital has a large outpatient clinic of patients with asthma.

5. Denver Kaiser Permanente HMO

Dr. Timothy Collins is the Director of Pulmonary Medicine and Dr. John Williams is the Director of Allergy at Kaiser. Drs. Collins and Williams have been actively involved in supporting research at NJ in the past by referring us patients. Their groups will continue to play an active role.

University of Wisconsin/Madison

The Asthma/Allergy Clinical Research Program of the University of Wisconsin maintains an ongoing computer database of potential subjects with asthma and allergic diseases who are interested in future research participation. These individuals have been screened and/or participated in previous clinical studies with our unit. Their names have been generated in response to extensive newspaper advertisements, physician referrals, radio advertisement campaigns, community health screening events, and by email communications to the entire student enrollment of the University of Wisconsin (approximately 40,000 students); all advertisement modalities have been approved by the Human Subjects Committee. Approximately 85% of the subjects in this database have “mild-to-moderate persistent asthma” and are, therefore, eligible for ACRN protocols. The following patient data is maintained: birth date, gender, ethnic background, duration of asthma, childbearing and contraceptive use status, smoking history, atopic status (including allergy skin testing results if previously performed), pulmonary function tests, concurrent medical history, asthma and non-asthma medication use, methacholine test results, and exercise challenge test results (if previously performed). When additional subjects are needed, referrals from physicians in the University of Wisconsin Clinics and Physicians Plus network are solicited.

Even though this database serves as the foundation of our recruitment efforts, the Madison ACRN site has utilized some additional approaches to target minority recruitment. We have utilized a marketing expert to coordinate and oversee our overall efforts in recruiting and retaining minorities. He is uniquely qualified for this task due to his combined professional and personal background (he is an ethnic minority, has a long history of asthma, and has participated in previous asthma studies with our unit). As a result of his efforts, we have advertised widely in newspapers and other publications that target ethnic minorities, established contacts with various ethnic community, university, church, and business groups, and conducted community-based asthma programs. We will continue our annual asthma screening services for all of the incoming University of Wisconsin freshmen athletic teams, which has been highly successful. Historically, retention of students in our asthma studies has been excellent, especially if contacted early upon arrival to the campus. These individuals discover that study participation serves as an ongoing source of quality medical management for their asthma. In addition, we will utilize published examples of successful retention strategies such as frequent payment of subject honoraria as study landmarks are achieved and study participant group social events. Study visits will be carefully planned and scheduled to avoid exam-time and university calendar breaks.

Harlem Hospital Center, Columbia University, New York City

The ACRN clinical center at Harlem Hospital Center draws study participants from four sources, including the Chest Clinic, the Emergency Department, the general community, and through advertising and outreach efforts. We advertise through local radio stations, newspapers, and newsletters of local churches and other community based organizations. In addition, we disseminate information about inclusion criteria for specific studies through ongoing outreach activities with volunteers in the AHA! (Asthmatics Helping Asthmatics) support and advocacy group, and through educational efforts in the community, including a series of asthma educational workshops.

The Chest Clinic, an outpatient pulmonary clinic in Harlem Hospital Center, sees a diverse group of patients with asthma. Patients learn about research at the Lung Center and about opportunities for participation in clinical trials, during their clinic visits.

The Harlem Hospital Center Emergency Department (ED) sees an average of eight adult patients per day for asthma. Through the REACH (Reducing Emergency Asthma Care in Harlem) project, we have been recruiting study participants at the ED. We have successfully recruited and interviewed 380 patients from the ED for that project, and most are currently being followed. One-third to one-half of REACH participants may be classified with mild-intermittent or mild-persistent asthma (self-reported symptoms, by NAEP guidelines criteria).

Responses to inquiries about participation in research studies are answered by a dedicated phone line that is manned during business hours and answered by voicemail at all other times. A research assistant responds to each inquiry immediately, using a screening instrument that inquires about potential respondents' contact information,

demographics, smoking history, and medical history. Our database includes 1,600 individuals with physician-diagnosed asthma.

Thomas Jefferson Medical College/Philadelphia

Patients are recruited for clinical trials at the Jefferson Center through two primary mechanisms: (1) local advertising and (2) identification in the asthma patient registry (database). Local advertising takes advantage of the printed, as well as the audio-visual, media. Printed media include posters placed in public information centers of local colleges and universities, as well as brochures sent to selected physicians in the Philadelphia area. Printed advertising is placed in local neighborhood newspapers and occasionally in the *Philadelphia Inquirer*. Audio-visual media advertisements are also placed in public service announcements on television and radio. All advertising in the printed and audio-visual media has prior approval of the Institutional Review Board.

The Jefferson patient registry (database) has been maintained since 1992 and currently contains 3,100 patients. The patient registry infrastructure includes a computer network linking those divisions of the institution that serve significant numbers of asthmatic patients (pulmonary medicine, family medicine, pediatric and adult allergy, and general internal medicine). Personal computers in each outpatient clinic site are linked to a dedicated file server located in the clinical research offices of the Pulmonary Division. The network operates on Novell Netware 3.22, and the database application is a customized version of Approach for Windows. The database provides a graphic interface for data entry. Fields for demographic information, smoking history, allergic history, medication used, pulmonary function tests, other laboratory tests, and other diagnoses are provided. Designated personnel are able to access the database and perform searches based on any field or combination of fields to define subsets of patients who qualify for particular research studies. The data coordinator is responsible for maintaining the database, assuring its accuracy, and keeping it current. It is estimated that 300-400 new asthmatic patients are seen each year, while a smaller number become inactive due to relocation, change of health care provider, etc. Once identified in the database, patients potentially eligible for a specific study are contacted by the nurse coordinator who explains the study and ascertains the patient's interest. If interested, the patient is seen in the clinical research laboratories where more detailed evaluations are made.

University of California/San Francisco

The approach to recruiting subjects with mild asthma for research studies at the San Francisco Center relies heavily on community advertising and on maintaining a database of subjects who have participated in previous studies, who have come for a "characterization" visit, or who have expressed interest in participating. Advertisements are placed in editions of the *San Francisco Chronicle*, the *San Francisco Examiner*, the *Bay Guardian*, and in small neighborhood and college campus newspapers. We post numerous fliers on bulletin boards on the UCSF campus, in community health centers, at campuses of local colleges and universities in the Bay Area, and we broadcast advertisements on local radio stations. We make frequent presentations to different

physician groups on and off campus describing our research studies and the enrollment criteria for future studies. Responses to these advertisements are made to a dedicated telephone number equipped with voice mail. A dedicated recruiter, Lila Glogowsky, either responds herself or directs other staff (technicians and clinical coordinators) to respond to each inquiry to obtain basic information about the subject's demographics and about the severity, duration, required treatment, and frequency of symptoms of asthma. Subjects who appear to meet entry criteria for a study are then referred to a study coordinator, who then contacts the subject to schedule a "characterization visit" in which details of the medical history and medication use are obtained, and spirometry (before and after albuterol administration), and skin testing is performed. To date, over 3,000 subjects have been screened for the database. We have met the goals for recruitment of women and of members of ethnic minorities in all studies so far.

C. Drug Supplies

Drug supplies for this study will include an inhaled corticosteroid (beclomethasone HFA MDI), a long-acting beta-agonist (salmeterol xinafoate DPI), a leukotriene modifier (montelukast), albuterol sulfate rescue, and placebos (tablets and MDI inhalers). LTRAs were chosen and not 5-lipoxygenase inhibitors because the currently-available 5-lipoxygenase inhibitor must be taken four times each day and is associated with liver function test elevations. Except albuterol, all drugs will be administered in a single-blind fashion during the run-in periods. During the treatment periods, beclomethasone HFA and the montelukast will be double-blind, while salmeterol will be open-label. The ACRN will work with contractors and coordinate with the DCC to ensure proper packaging, masking and coding of drugs.

D. Adherence and Monitoring

In order to determine subject adherence with beclomethasone HFA dosing during the single-blind run-in and double-blind treatment periods, a Doser® device will be attached to each inhaler. This device registers each actuation of the MDI and stores a daily history that will be reviewed at each clinic visit. To monitor subject adherence with salmeterol dosing during the treatment periods, the built-in counter on each DPI will be examined to determine the number of inhalations that were used. The Doser® and the salmeterol counter allow for objective measurement of the number of puffs used. A major limitation of these devices is their inability to discriminate between actual doses taken and "dose-dumping". They also do not yield much information regarding timing of doses and the ability of subjects to comply with the dosing schedule required by the study. As a secondary source of compliance information, subjects' diary cards will be examined for the number of puffs of each medication recorded for each day. This information will be compared to PEF measurements electronically recorded and date/time-stamped from the EPFM device. Because subjects are instructed to perform their morning and evening peak flow maneuvers right before taking their study medications, timing of PEF monitoring can be used as a surrogate for timing of dosing with study medications.

Limitations of this mechanism for monitoring adherence are accuracy of the subject's recall and honesty, because the timing or confirmation of dosing cannot be verified directly.

Adherence with regular use of montelukast will be assessed using the eDEM electronic Drug Exposure Monitor. The eDEM monitor records the date and time the subject opens the medication bottle in which the montelukast (or placebo) will be stored.

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, status of asthma control, use of asthma and non-asthma medications, and health status in the previous six weeks will be determined. An overview of the goals of the study, the visit structure and procedures involved will be presented. If the subject appears to fulfill entry criteria and is interested in study participation, Visit 1 may be scheduled. If the subject is taking an excluded asthma medication regularly, a pre-study visit will be scheduled, informed consent obtained, and the subject evaluated by the study investigator as to the appropriateness of drug withdrawal for the necessary washout interval prior to Visit 1. If warranted, the investigator may request additional pre-study visits for evaluation of asthma stability during this period.

Visit 1, Week 0

Subjects 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 that has been approved by the ACRN as well as by the local IRB (if this was not done at Visit 0). A medical history and vital signs will be obtained and a physical examination will be performed. Urine will be collected for a pregnancy test in females of child bearing potential (see Section VIII.F (Protocol Table) for timetable of visits and data collected).

If the individual qualifies for the study based on these data, allergy skin testing, spirometry, and methacholine challenge (if permissible by PFT criteria) or bronchodilator response assessment (see Eligibility Criteria, section VI.A) will be carried out according to protocols outlined in the ACRN MOP. Blood samples will be drawn for eosinophil and IgE determination. All data will be recorded electronically and on forms supplied by the ACRN.

If, based on the information gathered to this point, the subject meets the specific entry criteria, he or she will be entered into the first run-in phase of the trial, and blood will be drawn for DNA extraction and genetic analysis. If the subject meets the criteria for “triad analysis” (a diagnosis of asthma and either both identifiable biological parents or an

identifiable parent plus an identifiable biological sibling), consent will be obtained to contact family members for blood draws in the event that the subject becomes randomized in SLiMSIT.

Subjects will be given a single-blind inhaled corticosteroid MDI (beclomethasone HFA diproprionate, 80 μg/puff) with Doser® to be used one puff twice a day, single-blind LTRA tablets (montelukast) with eDEM monitor, an electronic peak flow monitoring (EPFM) device, and an albuterol "open-label" inhaler to be used for rescue treatment. Prior to distribution, the EPFM device readings will be checked using a Jones Flow-Volume calibrator. Only EPFM devices whose readings are within a specified range of the Jones will be distributed. Subjects will be taught how to use their EPFM devices, eDEM monitor, and MDI. They will be instructed to measure peak flow and then use their corticosteroid inhaler immediately upon arising (between 0500 and 1000 hrs) and at bedtime (between 2000 and 0100 hrs.), and to wash their mouth out after each use. They will be instructed to take their LTRA tablet following their bedtime dose of corticosteroid. Subjects will be instructed to record and circle peak flow values obtained less than two hours after use of inhaled albuterol on their diary cards. The use of diary cards will be explained and subjects will be given an appropriate supply. Subjects will be instructed to return to the clinical center in two weeks.

Visit 2, Week 2

Subjects 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 and methacholine challenge will be performed. The subject's EPFM device 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 noted using the protocol outlined by the ACRN. A thorough assessment of treatment failure criteria will be carried out and identified failures will be documented and treated according to protocol. (Subjects who achieve failure status at this point in the study will be terminated from further study participation; however, they will be followed until their treatment failure conditions have resolved.) Diary cards will be reviewed and new ones dispensed; EPFM device and eDEM data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. Subjects will be checked for signs of oral candidiasis. Single-blind montelukast tablets and inhaled beclomethasone HFA will be issued. Open-label albuterol rescue drug will be dispensed, if necessary. Subjects will be instructed to return to the clinical center in two weeks.

Visit 3, Week 4; Randomization and Beginning of Treatment Period 1

Subjects 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. The subject will complete the Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Asthma Symptom Utility Index. The subject's EPFM device 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 noted using the protocol outlined by the ACRN. A thorough assessment of treatment failure criteria will be carried out and identified failures will be documented and treated according to protocol. (Subjects who achieve failure status at this point in the study will be terminated from further study participation; however, they will be followed until their treatment failure conditions have resolved.) Diary cards will be reviewed; EPFM device and eDEM data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed. The subject will be assessed for evidence of oral candidiasis.

If adherence criteria and study entry criteria are met, urine will be collected for a pregnancy test in females of child-bearing potential. NO collection and baseline spirometry will be obtained and subjects then will inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated. Methacholine challenge and sputum induction then will be performed, in that order.

If the subject continues to meet the inclusion criteria for the study, and he or she fulfills the criteria for randomization, the subject will be randomized to one of the two double-blind treatment arms (inhaled salmeterol plus montelukast or inhaled salmeterol plus inhaled beclomethasone HFA) using a computer application provided by the DCC. Based upon this randomization, all subjects will receive a coded, double-blind "regular use" inhaler containing beclomethasone HFA or its placebo and an open-label regular use Diskus® inhaler containing salmeterol, both to be taken twice a day. Subjects will be instructed to take their "regular use" inhalers at the same time each evening and morning (on rising and retiring), and to measure their peak flow at the same time each morning and evening prior to dosing. Subjects will also receive a three weeks' supply of montelukast or its placebo (coded, double-blind), and they will be instructed to take one tablet each evening following dosing with their inhalers. All subjects will receive open-label albuterol inhalers to be used as-needed for rescue treatment and a supply of prednisone to be used for emergency rescue treatment. New study-specific diary cards will be issued. Subjects will be instructed to return to the clinical center in three weeks.

Phone Contact, Week 6

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Visit 4, Week 7

Subjects 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. Prior to Visits 4-6 and 8-10, subjects will withhold their rescue inhaler for ≥ 6 hours, as for all visits, and salmeterol for ≥ 12 hours; this withhold period will facilitate both AM and PM visits and will maximize the consistency of the time at which post-bronchodilator spirometry is performed relative to salmeterol use. The subject will complete the Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Asthma Symptom Utility Index. NO collection and baseline spirometry will be obtained and the subjects then will inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated.

The subject's EPFM device 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. A thorough assessment of treatment failure criteria will be carried out and identified failures will be documented and treated according to protocol. Diary cards will be reviewed and new ones dispensed; EPFM device and eDEM monitor data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. The subject will be assessed for evidence of oral candidiasis. Salmeterol and blinded study medicines will be dispensed; open-label rescue albuterol will be dispensed, if needed. Subjects will be instructed to return to the clinical center in six weeks.

Phone Contact, Week 9

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Phone Contact, Week 11

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Visit 5, Week 13

Subjects 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. Prior to Visits 4-6 and 8-10, subjects will withhold their rescue inhaler for ≥ 6 hours, as usual, and salmeterol for ≥ 12 hours; this withhold period will facilitate both AM and PM visits and will maximize the consistency of the time at which post-bronchodilator spirometry is performed relative to salmeterol use. The subject will complete the Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Asthma Symptom Utility Index. NO collection and baseline spirometry will be obtained and the subjects then will inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated.

The subject's EPFM device 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. A thorough assessment of treatment failure criteria will be carried out and identified failures will be documented and treated according to protocol. Diary cards will be reviewed and new ones dispensed; EPFM device and eDEM monitor data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. The subject will be assessed for evidence of oral candidiasis. Salmeterol and blinded study medicines will be dispensed; open-label rescue albuterol will be dispensed, if needed. Subjects will be instructed to return to the clinical center in five weeks.

Phone Contact, Week 15

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Phone Contact, Week 17

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Visit 6, Week 18; End of Treatment Period 1 and Beginning of Run-In 2

Subjects 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. The subject will complete the Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Asthma Symptom Utility Index. Urine will be collected for a pregnancy test in females of child-bearing potential. Prior to Visits 4-6 and 8-10, subjects will withhold their rescue inhaler for ≥ 6 hours, as usual, and salmeterol for ≥ 12 hours; this withhold period will facilitate both AM and PM visits and will maximize the consistency of the time at which post-bronchodilator spirometry and methacholine challenge are performed relative to salmeterol use. NO collection and baseline spirometry will be obtained and subjects then will inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated and a methacholine challenge and a sputum induction will be performed, in that order.

The subject's EPFM device 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 noted using the protocol outlined by the ACRN. A thorough assessment of treatment failure criteria will be carried out and identified failures will be documented and treated according to protocol. Diary cards will be reviewed and new ones dispensed; EPFM device and eDEM data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed. The subject will be assessed for evidence of oral candidiasis.

The double-blind study inhalers and tablets and open-label salmeterol inhalers will be collected. New single-blind inhalers containing beclomethasone HFA 80 μg/puff and new single-blind montelukast tablets will be dispensed as subjects enter the second run-in period. Open-label rescue albuterol will be dispensed. New study-specific diary cards will be issued. Subjects will be instructed to return to the clinical center in four weeks.

Phone Contact, Week 20

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Visits 6a, b, c (As needed to meet medication wash-out requirements following treatment failure or exacerbations during treatment period 1 or run-in 2)

Subjects who fulfill treatment failure criteria during the first treatment period or the second run-in period are required to wait at least six weeks from the documentation of treatment failure/exacerbation status, at least six weeks from the last dose of oral prednisone, if prescribed, and at least four weeks from the last dose of open-label inhaled corticosteroids before beginning the second double-blind treatment period (Visit 7). To accommodate these washout periods, subjects may require additional time between Visits 6 and 7. To ensure that subjects continue to comply with study run-in medications and procedures, and to monitor the subjects' symptoms and lung function, additional visits (6a, 6b, 6c, etc.) will be scheduled at two-week intervals during the washout period, prior to Visit 7. When less than two additional weeks of washout are required, the subject will be scheduled for Visit 7. The washout interval ensures that baseline symptoms and lung function have been re-established before the subject enters the second treatment period.

For interim visits 6a, 6b, 6c, etc., subjects 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 measured. The subject's EPFM device 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 noted using the protocol outlined by the ACRN. The subject will be monitored for exacerbation and treatment failure conditions. Diary cards will be reviewed and new ones dispensed; EPFM device and eDEM data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. A long physical exam will be performed, and the subject will be assessed for evidence of oral candidiasis. New single-blind inhalers containing beclomethasone HFA 80 μg/puff and new single-blind montelukast tablets will be dispensed. New study-specific diary cards will be issued. Subjects will be instructed to return to the clinical center in two weeks, either for another interim visit or for Visit 7, as appropriate.

Visit 7, Week 22; Cross-over; End of Run-In 2 and Beginning of Treatment Period 2

Subjects 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. The subject will complete the Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Asthma Symptom Utility Index. The subject's EPFM device 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 noted using the protocol outlined by the ACRN. The subject will be monitored for treatment failure and exacerbation conditions. Diary cards will be reviewed and new ones dispensed; EPFM device and eDEM data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed. The subject will be assessed for evidence of oral candidiasis. Urine will be collected for a pregnancy test in females of child-bearing potential.

NO collection and baseline spirometry will be obtained and subjects then will inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated. Methacholine challenge and sputum induction then will be performed, in that order as outlined in the ACRN MOP.

Based on the subject's initial randomization at Visit 3, he or she will cross over to receive the alternate treatment regimen in his or her randomized sequence. All subjects will receive one coded, double-blind "regular use" inhaler and one open-label Diskus® inhaler to be taken twice a day; inhaler one will contain beclomethasone HFA or its placebo and inhaler two will contain salmeterol. Subjects will be instructed to take their "regular use" inhalers at the same time each evening and morning (on rising and retiring), and to measure their peak flow at the same time each morning and evening, prior to dosing. Subjects also will receive a three weeks' supply of montelukast or its placebo (coded, double-blind), and they will be instructed to take one tablet each evening following dosing with their inhalers. Subjects will receive open-label albuterol inhalers for rescue treatment, and each subject's supply of prednisone will be monitored and replenished, if necessary. New study-specific diary cards will be issued. The subject's visit schedule for visits 8-10 will be generated, using the Visit 7 date as the reference point. This ensures that all subjects have equal follow-up during both treatment phases. Subjects will be instructed to return to the clinical center in three weeks.

Phone Contact, Week 24

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Visit 8, Week 25

Subjects 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. Prior to Visits 4-6 and 8-10 subjects will withhold their rescue inhaler for ≥ 6 hours, as usual, and salmeterol for ≥ 12 hours; this withhold period will facilitate both AM and PM visits and will maximize the consistency of the time at which post-bronchodilator spirometry is performed relative to salmeterol use. The subject will complete the Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Asthma Symptom Utility Index. NO collection and baseline spirometry will be obtained and the subjects then will inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated.

The subject's EPFM device 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. A thorough assessment of treatment failure criteria will be carried out and identified failures or exacerbations will be documented and treated according to protocol. Diary cards will be reviewed and new ones dispensed; EPFM device and eDEM monitor data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. The subject will be assessed for evidence of oral candidiasis. Salmeterol and blinded study medicines will be dispensed; open-label albuterol rescue drug will be dispensed, if needed. Subjects will be instructed to return to the clinical center in six weeks.

Phone Contact, Week 27

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Phone Contact, Week 29

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Visit 9, Week 31

Subjects will return to the clinical center at the same time of day as on week 0 $\pm$ 2 hours. If scheduling permits, all subsequent visits will occur within a $\pm$ 2-hour window on the study day. Prior to Visits 4-6 and 8-10, subjects will withhold their rescue inhaler for $\geq$ 6 hours, as usual, and salmeterol for $\geq$ 12 hours; this withhold period will facilitate both AM and PM visits and will maximize the consistency of the time at which post-bronchodilator spirometry is performed relative to salmeterol use. The subject will complete the Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Asthma Symptom Utility Index. NO collection and baseline spirometry will be obtained and the subjects then will

inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated.

The subject's EPFM device 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. A thorough assessment of treatment failure criteria will be carried out and identified failures or exacerbations will be documented and treated according to protocol. Diary cards will be reviewed and new ones dispensed; EPFM device and eDEM monitor data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. Guidelines will be reviewed as needed. The subject will be assessed for evidence of oral candidiasis. Salmeterol and blinded study medicines will be dispensed; open-label albuterol rescue drug will be dispensed, if needed. Subjects will be instructed to return to the clinical center in five weeks.

Phone Contact, Week 33

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Phone Contact, Week 35

Subjects will be contacted by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the patient. The coordinator will attempt to determine whether the subject is showing signs of treatment failure. Specifically, the coordinator will inquire about rescue beta-agonist use, peak flow measurements, and symptoms. If it is determined that the subject fulfills criteria for treatment failure, then they will be scheduled for a visit at the clinical center within 24 hours for evaluation and initiation of treatment.

Visit 10, Week 36; End of Treatment Period 2

Subjects will return to the clinical center at the same time of day as on week 0 ± 2 hours. Prior to Visits 4-6 and 8-10, subjects will withhold their rescue inhaler for ≥ 6 hours, as usual, and salmeterol for ≥ 12 hours; this withhold period will facilitate both AM and PM visits and will maximize the consistency of the time at which post-bronchodilator spirometry and methacholine challenge are performed relative to salmeterol use. The subject will complete the Asthma Control Questionnaire, Asthma Quality of Life

Questionnaire, and Asthma Symptom Utility Index. Urine will be collected for a pregnancy test in females of child-bearing potential. NO collection and baseline spirometry will be obtained and the subjects then will inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated and a methacholine challenge and a sputum induction will be performed, in that order.

The subject's EPFM device will be tested against the Jones Flow-Volume calibrator and results documented. Adverse events will be noted using the protocol outlined by the ACRN. A thorough assessment of treatment failure criteria will be carried out and identified failures or exacerbations will be documented and treated according to protocol. Diary cards will be reviewed; EPFM device and eDEM data will be uploaded. Clinical center personnel will assess adherence by reviewing the appropriateness of the timing of medication use, peak flow recording and symptom recording. A long physical exam will be performed.

All subjects will return their "regular use" inhalers, study tablets, eDEMs, and EPFM devices.

Visit 91-99 (Additional study visits required during the post-randomization period for safety and assessment of treatment failure conditions due to worsening asthma symptoms)

When a subject experiences worsening asthma symptoms between regularly scheduled SLiMSIT visits, he or she will be instructed to contact the ACRN site immediately. The subject will be scheduled for an additional study visit for purposes of assessing his or her condition and evaluating treatment failure criteria. At this extra visit, the subject will complete the Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Asthma Symptom Utility Index. NO collection and baseline spirometry will be obtained and the subject then will inhale 50 μg of salmeterol from a Diskus® labeled for this purpose. One hour after salmeterol dosing, spirometry will be repeated.

Adverse events will be sought and noted using the protocol outlined by the ACRN. A thorough assessment of treatment failure criteria will be carried out and identified failures will be documented and treated according to protocol. Diary cards will be reviewed and new ones dispensed; EPFM device data will be uploaded. A long exam will be performed. Open-label rescue albuterol will be dispensed, if needed. Subjects will be instructed to return to the clinical center for their next regularly scheduled visit.

F. Protocol in Tabular Form

	Run –In #1			Treatment Period #1			Run –In #2	Treatment Period #2				Visit 91-9+
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**6a-c***	**7**	**8**	**9**	**10**	**91-99**
Week	**0**	**2**	**4**	**7**	**13**	**18**		**22**	**25**	**31**	**36**	**5-36**
Window		±4d	±4d	±4d	±4d	-4d		+4d	±4d	±4d	±4d	N/A
Informed Consent	X											
Randomization			X									
Dispense Prednisone			X									
Medical History	X											
Long Physical Exam	X						X				X	X
Blood Draw for Genetic Analysis	X											
Blood Draw for IgE/Eosinophils	X											
Allergy Skin Test	X											
Pregnancy Test	X		X			X		X			X	
NO Collection			X	X	X	X		X	X	X	X	X
Spirometry	X	X	X	X	X	X	X	X	X	X	X	X
Post-Salmeterol Spirometry			X	X	X	X		X	X	X	X	X
Methacholine Challenge	X*	X										
Post-Salmeterol Methacholine Challenge			X			X		X			X	
Sputum Induction			X			X		X			X	
Bronchodilator Assessment	X*											
Treatment Failure Assessment		X	X	X	X	X	X	X	X	X	X	X
Adverse Events Assessment		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
Peak Flow QC	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
Dispense / Collect Medicines	X	X	X	X	X	X	X	X	X	X	X	
Quality of Life Questionnaire			X	X	X	X		X	X	X	X	X
Pharmaco-economic Outcomes			X	X	X	X		X	X	X	X	X
Final Med Collect											X	

*As indicated, see eligibility criteria and protocol

+Additional study visits for treatment failure assessment due to worsening asthma symptoms

G. Risks/Benefits

This study compares two usage strategies for three currently approved pharmaceutical products. The risks associated with use of a long-acting beta-agonist without inhaled corticosteroid include worsened asthma control as we saw in the SOCS/SLIC trial. However, we will be treating subjects with an inhaled corticosteroid or a leukotriene receptor antagonist in addition to the long-acting beta-agonist. Thus, we anticipate fewer problems with severe deteriorations in asthma control. Further, subjects with worsened asthma symptoms may take albuterol as needed for these symptoms and will have prednisone at home in case of emergencies. To ensure the safety of individuals whose asthma worsens during this period, specific criteria have been developed for assigning "treatment failure" status, and for initiating appropriate asthma therapy.

Risks of short-term inhaled corticosteroid use include oral candidiasis and dysphonia. Risks associated with the use of an LTRA include rare reports of increased liver function tests and what appear to be idiosyncratic occurrences of liver failure. Since these occurrences have been so rare (three cases in several million years of patient use) and do not appear to have been predicted by monitoring of liver enzymes, but rather were preceded by clinical symptoms of illness, subjects will be cautioned to report any symptoms associated with liver dysfunction.

There may 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

1. Primary Outcomes

We anticipate that the results of this trial will serve as a foundation for evidence-based recommendations regarding the treatment of patients with moderate persistent asthma. We expect that when subjects use a combination of a long-acting beta-agonist and an inhaled corticosteroid (LAB/ICS), they will experience a very low rate of treatment failures. Based on our experience in SOCS/SLIC, at 14 weeks (a time when treatment failure rates had stabilized) we expect a 5% or smaller rate of treatment failures (a need for intensification of asthma care) in the LAB/ICS phase. We expect that the combination of a long-acting beta-agonist and a leukotriene antagonist (LAB/LTRA) will reduce the rate of treatment failure well below the 40+% rate we saw with salmeterol alone in SLIC patients, and that this combination will likely reduce the failure rate below the 24% rate we observed in SOCS patients. The exact degree of the reduction with this combination is unclear and is the major question to be answered by the trial.

The interpretation of possible outcomes in this trial depends on the thresholds for clinical significance. The Steering Committee considered these thresholds. In the SLIC study comparing inhaled corticosteroids alone to a long-acting beta-agonist, we had anticipated treatment failure rates for the inhaled corticosteroids alone along the lines predicted for

LAB/ICS in this study. In that situation (the SLIC study), after Steering Committee deliberation, we determined that the inhaled corticosteroid arm would be superior to the long-acting beta-agonist arm unless the long-acting beta-agonist arm was able to reduce the treatment failure rate to 20% or less (a halving of the expected rate of treatment failures expected in the placebo arm). In our present study we would predict that a salmeterol alone arm would produce a 30-35% failure rate based on the combined rates we saw in SOCS/SLIC. With these considerations in mind, and based on its collective clinical experience, the Steering Committee made the following determination:

Assuming that LAB/ICS produced a treatment failure rate of 5% or less, LAB/ICS would be judged to be clinically superior to LAB/LTRA unless the latter combination was able to reduce the rate of treatment failure (a need for intensification of asthma care) to 15% or less. Further, if the treatment failure rate in the inhaled corticosteroid containing arm were greater (>5%-10%), the Committee felt that the inhaled corticosteroid arm would be judged to be clinically superior unless the LAB/LTRA arm were able to produce a treatment failure rate no more than 10% greater than LAB/ICS (e.g. 6% vs. 16%).

We therefore adjusted our sample size so that we have 90% power to detect a statistical difference between LAB/ICS and LAB/LTRA in such scenarios.

Thus, in the Steering Committee's opinion, if a combination of a long-acting beta-agonist and an inhaled corticosteroid reduced the treatment failure rate to 5% or less, the clinical significance of the inability of LAB/LTRA to reduce the treatment failure to a rate of 15% or less, would be compelling enough to form the basis of a recommendation that the former therapy be used in place of the latter in the treatment of patients with moderate persistent asthma. Further, if the LAB/ICS treatment failure rate were 5% -10%, the clinical significance of the inability of LAB/LTRA to produce a treatment failure rate no more than 10% higher (e.g. 7% vs. 17%), would be grounds for a similar conclusion.

In summary, we hypothesize that LAB/ICS will reduce treatment failure rates to 5% or below and that LAB/LTRA will not be able to reduce the rate to below 15%. Further, if LAB/ICS reduces the rate to 5% to 10%, we hypothesize that we will see more than a 10% greater treatment failure rate in the LAB/LTRA group. In both of these cases, we would judge the LAB/ICS treatment efficacy to be such that it would be the clinically recommended treatment.

It is possible that LAB/LTRA will, in fact, reduce the rate of treatment failures to $\leq$15%. In this case, regardless of statistical significance, it is the clinical judgment of the members of the Steering Committee that the clinical significance of a $\leq$5% or less rate of treatment failures (for the combination of a long-acting beta-agonist and an inhaled corticosteroid) and a $\leq$15% rate of treatment failures (for LAB/LTRA) would be such that other considerations such as patient and physician preferences, and/or perceived concerns, could play an important role in a decision as to which therapy to use. Further, if the treatment failure rate in LAB/LTRA group were greater than 15%, but the treatment failure rate in the combination of LAB/ICS were greater than expected (e.g., 6-10%), such that a treatment difference of less than 10% were found (e.g., 8% vs. 17%), then, regardless of

statistical significance, it was our clinical judgment that, once again, other considerations such as patient and physician preferences and/or perceived concerns could play an important role in a decision as to which therapy to use. Similar judgments regarding thresholds for clinical superiority guided our determination in the SLIC study.

2. Secondary Outcomes

The primary outcome we are evaluating in this study is treatment failure (i.e., a need for intensification of asthma therapy). Our experience in the SOCS trial demonstrates the limitations of using indices of airway caliber alone to assess asthma control. Secondary outcome measures will include other markers of asthma control (e.g., symptoms, use of rescue albuterol, quality of life scores), as well as indices of airway function (FEV_1, AM and PM peak expiratory flow, PC_{20Mch}). In the SOCS trial, we found that despite a decrease in symptoms and medication use, and an improvement in indices of airway caliber with salmeterol use alone that was equal to the effects seen with inhaled corticosteroids, asthma treatment failures occurred at a much higher rate with salmeterol. As mentioned in the **Background,** almost the opposite pattern is seen with leukotriene receptor antagonists. Prior data suggest that there may be a disparity between the effects of leukotriene receptor antagonists on airway function (less effect) vs. the effect on secondary outcomes such as symptoms and control of treatment failures (greater salutary effect) ((15), Israel et al. Unpublished observations). Thus, it is possible that we will observe no clinically significant difference in treatment failures between the treatment regimens, while we may observe differences in effects of the two combinations on FEV_1 and peak flow, and possibly less of a difference in the regimens when it comes to symptoms and quality of life scores. These results would imply that the airway effects of the inhaled corticosteroids and the “anti-asthmatic” effects (that is, effects on treatment failures, quality of life, and symptoms) of the inhaled corticosteroids may occur through distinct mechanisms. This would be in concert with findings that demonstrate that inhaled corticosteroid airway effects peak early, but their effect on methacholine reactivity continues to increase with persistent use (47).

The effect of the different treatment regimens on our exploratory inflammatory outcomes will also be of interest. Our hypothesis is that LAB/ICS is superior to LAB/LTRA and that we will see a clinically important difference in treatment failures. In this case, the pattern of differences among the inflammatory outcomes would be informative. Markers of inflammation that are associated with treatment failure may be candidates for additional investigation as indicators of pathways that play a critical role in asthma pathobiology. Further, if we find dissociations between treatment failures and other indices of asthma control, such as airway function, symptoms, or methacholine reactivity, then their association with particular markers may give us insight into the pathophysiological mechanisms that govern these particular manifestations of the asthmatic diathesis.

Since data examining the distribution of responses to inhaled corticosteroids and to leukotriene receptor antagonists ((20,19), ACRN MICE study, unpublished data) suggest that a significant percentage (perhaps one quarter to one third) of subjects may not respond to either inhaled corticosteroids, or alternatively to a leukotriene receptor

antagonist, we also propose to examine whether baseline physiologic parameters such as FEV_1 or methacholine reactivity and/or early changes in these parameters during treatment allow us to predict those individuals who may respond. The outcomes of this analysis may permit us to provide practice guidance as to which patients may be most likely to respond to a particular regimen, or how to more quickly assess whether a patient is responding to a particular regimen. In this regard, we plan to explore the importance of polymorphisms of genes, or regulatory regions in the genome, with putative relevance to the control and development of components of asthma as they relate to the ability to predict responses to the different treatment regimens.

In summary, regardless of outcome, the study will provide important information to guide the choice of therapeutic agents for persistent asthma. Concerns regarding the long-term effects of inhaled corticosteroids persist. This study will provide the basis for definitive evidence-based recommendations regarding the use of these newer controller agents as compared to the most effective method of using inhaled corticosteroids (i.e., with a long-acting beta-agonist). The effect of the combination of a long-acting beta-agonist and a leukotriene antagonist on inflammatory markers, in the context of their clinical outcomes, will provide further information regarding the relevance of these markers to different aspects of asthma control.

IX. ADVERSE EVENTS

A. Definitions

An adverse event shall be defined as any detrimental change in the subject'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 IX.C.2.

A Serious Adverse Event is any adverse experience occurring at any dose that:

a) **Results in death**, or

b) **Is life-threatening** (places the subject/patient, in the view of the investigator, at immediate risk of death from the experience as it occurred. Note: this does not include an adverse experience that, had it occurred in a more severe form, might have caused death), or

c) **Results in persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions), or

d) **Results in or prolongs an existing inpatient hospitalization** (hospitalized is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. Note: hospitalization (including hospitalization for an elective procedure) for a preexisting

condition which has not worsened does not constitute a serious adverse experience.), or

e) **Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis)

Other important medical events that may not result in death, not be life-threatening, or not require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject/patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

While **cancer and overdose** are not included in the ICH E2A definition of "serious" which has been adopted by worldwide regulators, the ACRN will record these events and report them to the DSMB.

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 subject is no longer able to participate in the study effectively. Subjects experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness 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 a Clinical Adverse Event form and will include the following information:

- Description of the illness (ICD-9 code)
- Dates of illness
- Treatment of illness (medications, doses, dates)
- Whether hospitalization or emergency treatment was required
- Treatment outcome

C. Adverse Events Related to Asthma

1. Definition

During the course of the study subjects 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 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 increase in symptoms may be brief and self-limited, or it may be of sufficient severity as to warrant documentation as an asthma exacerbation.

Although any increase in symptoms or changes in PEF should be carefully monitored by the subject, the clinic coordinator, and the physician, alterations in asthma stability will be considered as constituting an 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. The reference point for PEF comparisons will be as follows:

During the initial run-in period (weeks 1-4):

Weeks 1-2: Best of three consecutive, technically acceptable blows on the EPFM device at Visit 1

Weeks 3-4: Mean value of AM pre-bronchodilator PEF recorded on symptom diary during the first two weeks of the run-in (weeks 1 and 2)

During double-blind treatment periods and run-in 2 (weeks 5-36):

Mean value of AM pre-bronchodilator PEF recorded on symptom diary during the last two weeks of the initial run-in period (weeks 3 and 4)

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

An increase in "as-needed" beta-agonist use of ≥ 8 puffs per 24 hours over baseline use for a period of 48 hours.

Baseline use during the initial run-in period (weeks 1-4):

Weeks 1-2: The historical average daily albuterol use obtained at Visit 1

Weeks 3-4: Average daily use during the first two weeks of the run-in (weeks 1 and 2)

Baseline use during double-blind treatment periods and run-in 2 (weeks 5-36):

Average daily use during the last two weeks of the initial run-in (weeks 3 and 4)

or

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

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

Because less significant changes in symptoms and/or PEF may precede more severe alterations in asthma stability, a series of rescue algorithms has been developed to address the various clinical presentations that may occur. Once any of these rescue interventions leads to the administration of corticosteroids, the subject also will be considered to have developed an asthma exacerbation. In addition, if in the opinion of the treating physician, corticosteroid therapy is warranted regardless of any antecedent measurements of pulmonary function (PEF, FEV_1, etc.), value for symptom score, or frequency of rescue beta-agonist use, the subject will be considered to have developed an asthma exacerbation.

Any subjects who have had an asthma exacerbation will be considered to have attained treatment failure status.

The time in which an asthma exacerbation develops in relationship to the schedule of the SLiMSIT protocol will affect the manner in which future clinic visits, medication adjustments, and diagnostic studies are scheduled or performed. The scheduling of these events is outlined in section VII.A.3.

Subjects developing an asthma exacerbation during the initial run-in period will be terminated from study participation and may re-enroll after the exacerbation has fully resolved. Subjects developing an exacerbation during the second run-in period may return to the trial with the schedule of visits outlined in section VII.A.3.

Asthma exacerbations that occur following randomization will be managed according to the following rescue algorithms. Subjects will be placed on open-label ICS at the dose used during the run-in periods. During medical management of the exacerbation, other trial medication will be continued, unless the treating physician considers it appropriate to suspend such therapy until the exacerbation resolves. Reinstitution of trial medications will occur when the exacerbation has resolved at the discretion of the investigator. A record of all medications, dosages, and frequency of occurrence will be kept during exacerbations. Additional visits and procedures will be scheduled as outlined in section VII.A.3.

2. Rescue Algorithms

Rescue algorithms will be applied in cases where an exacerbation 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 (48). Albuterol, inhaled steroids, and oral prednisone are the principal medications for rescue management. Subjects will be instructed in their use for home management, and supplies of albuterol

and prednisone will be provided throughout the study. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician.

a) Home Care

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

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

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

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

b) Physician's Office or Emergency Room Treatment

- Subjects will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the subject's PEF and/or FEV_1 is less than 25% predicted or if the subject shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles, sampling of arterial blood for respiratory gas analysis is indicated, with appropriate action taken depending on the results obtained.

- When treated in the physician's office or the hospital emergency room, subjects should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 minutes over the first 60 minutes.

- If the PEF increases to >65% of reference level after the first 60 minutes, the subject can be discharged to continue treatment at home. Prednisone or open-label inhaled corticosteroids (beclomethasone HFA 160 μg/day) may be administered at the discretion of the physician to augment therapy.

- If symptoms persist and PEF remains <65% of reference level, nebulized albuterol should be continued as often as every hour and further treatment with oral or parenteral corticosteroids should be considered (prednisone, 60 mg orally; methylprednisolone, 60 mg iv bolus). Monitoring of PEF or spirometry should

continue every hour. Within four hours of treatment, a decision should be made regarding subject disposition.

- If PEF increases to >65% reference level within four hours, the subject can be discharged to continue treatment at home. Home treatment should include an 8-day course of prednisone followed by open-label inhaled corticosteroid treatment (see below).

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

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

c) Prednisone Treatment

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

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

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

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

d) Inhaled Corticosteroid Treatment

Inhaled corticosteroid dosing for worsened asthma symptoms during the SLiMSIT trial will be open-label beclomethasone HFA MDI 80 μg, 1 puff BID for two weeks. For subjects already receiving inhaled corticosteroids as part of their double-blind study regimen, this will represent a doubling of the inhaled corticosteroid dose; for subjects not already using inhaled corticosteroids, this will represent initiation of these agents.

D. Adjustments of Trial Medications During Asthma Exacerbations

Subjects will be placed on open-label ICS at the dose used during the run-in period (as indicated above). Trial drugs will be continued during exacerbations unless the treating physician considers it appropriate to suspend such therapy until the exacerbation resolves. Reinstitution of trial medications may occur when the exacerbation has resolved at the discretion of the investigator. A record of all medications, dosages, and frequency of occurrence will be kept during exacerbations.

E. Study Center Visits Following Exacerbations

If the subject receives open-label inhaled or systemic steroids for an exacerbation, regular follow-up evaluations will continue as outlined in section VII.A.3.

F. Criteria for Discontinuing Subjects Due to Treatment Failure Status

Criteria for assigning treatment failure status during the double-blind treatment period are described in section VII.A.2. Subjects who are assigned "treatment failure" status will continue to participate in the protocol as outlined in Section VII.A.3.

G. Adverse Events as Outcome Variables

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

- Hospitalization for asthma
- Emergency Department visits for asthma
- Unscheduled physician/clinic visits for asthma
- Use of corticosteroids
- Treatment failure

H. Cost and Payment

Each subject will be paid an amount determined by their local center. For subjects 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 subject not withdrawn.

X. DATA RECORDING

All data including the informed consent, history, physical examination, results of allergy skin testing, vital signs, results of pregnancy tests, adverse events, confirmation of medication dispensation, methacholine challenge testing, sputum induction, and quality of life testing will be recorded on forms prepared by the ACRN DCC. Initial data entry will be performed at each clinical center and forms will be forwarded to the DCC for

confirmatory entry. Results from pulmonary function tests and the EPFM device will be transmitted electronically to the DCC. All data will be stored and analyzed at the DCC.

XI. STATISTICAL DESIGN AND ANALYSIS

A. Data Collection and Data Management

Each center will have a computer configuration that includes a PC terminal, a printer, and a modem. This setup will give each center the capability of logging directly into the ACRN secure web site with the modem as a backup if the connection is not possible. Though this setup is installed primarily to allow for distributed data entry into a centralized and secure database at the ACRN web site, menu options also will 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 will 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 ACRN secure web site 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.

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 subjects are taking inhaled corticosteroid, LTRA, or placebo. Treatment medication for each subject will be packaged together and labeled with a unique number. The contents of the packages will be known only by limited personnel at the DCC who are not involved in data processing or analysis. These packages, and single-blind corticosteroid inhalers and LTRA for the run-in 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 treatment sequences as 1 and 2, and only limited personnel within the DCC will know the identity of 1 and 2.

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 visit packet of medications for the study participant, he or 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 or she checked to make sure the appropriate inhalers and LTRA were distributed to the participant.

C. Randomization

When a subject 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 subject requires randomization. Subjects will be randomized to sequences in this crossover design. 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 subject is eligible. If so, the clinic coordinator will be given a packet number from which all medication for that subject will be dispensed. In order to maintain security of the randomization schedules, the data manager at the DCC will receive automatically a notice from the ACRN network server that a subject has been randomized. If no follow-up information is forthcoming on such a subject, the data manager will contact the clinic coordinator concerning the status of the subject.

D. Stratification

The randomization scheme will be stratified according to clinical center because differences among clinical centers typically yield a large amount of variability. To ensure that sequence groups are balanced with respect to baseline lung function, the randomization scheme also will be stratified by FEV_1 percent of predicted (<80% versus $80%) at the randomization visit. In addition, each clinical center will be restricted to enroll no more than 25% of its target sample size of subjects to be between the ages of 12 and 18.

E. Statistical Analysis

The primary response variable in the SLiMSIT trial is the time until treatment failure. Because of the crossover design, each subject will have a time until treatment failure, or a censoring time, for each of the two treatment regimens. France, Lewis, and Kay describe the statistical analysis for such a situation (45). The basic statistical analysis involves the comparison of the two treatment failure/censoring times within each subject using a proportional hazards regression. The hazard function for subject i at time t, i=1,2,…,n, is

$$\lambda_i(t;\mathbf{x}) = \lambda_{0i}(t)\exp(\mathbf{x}'\beta) \qquad [1]$$

where $\lambda_{0i}(t)$ represents the baseline hazard function for subject i, $\mathbf{x}$ represents a vector of regressors, and β represents a vector of parameter coefficients.

For each subject, a treatment preference is identified in terms of whether the subject performed better on one treatment regimen versus the other. If the subject has a censoring time on both treatment regimens, then that subject has no treatment preference. Therefore, the trial results can be summarized as a trinomial response:

n_A = # subjects who prefer treatment regimen A
n_B = # subjects who prefer treatment regimen B
$n - n_A - n_B$ = # subjects for whom a preference cannot be determined (treatment failures occurring within 2 days of each other in the two treatment arms will be considered "ties")

For convenience, define

θ_A = Pr[a subject prefers treatment regimen A]
θ_B = Pr[a subject prefers treatment regimen B]

In the absence of period effects, the hazard ratio reduces to

$$\Lambda = \theta_A/\theta_B \qquad [2]$$

and its maximum likelihood (ML) estimator reduces to

$$\Lambda_{ML} = n_A/n_B \qquad [3]$$

Thus, in the absence of period effects, the statistical analysis is comparable to McNemar's test for paired binary data. In the presence of period effects, the ML estimator is the geometric mean of the ratios of treatment regimen preferences within each of the two sequences, i.e.,

$$\Lambda_{ML} = \{(n_A/n_B)_{seq_AB}(n_A/n_B)_{seq_BA}\}^{1/2} \qquad [4]$$

The ML estimator in [4] can be generalized if there are other categorical covariates of interest. If there are continuous covariates of interest, then the proportional hazards regression model in [1] can be applied.

With respect to secondary outcome variables, mixed-effects linear models will be applied to conduct a longitudinal data analysis (48,49). The mixed-effects linear model is appropriate even though the design is a crossover and not parallel (50). The objective of the longitudinal data analysis is to assess the difference in the changes between the end of the treatment period and the end of the run-in period for the two treatment regimens. Covariates can be included in the model, such as clinical center and gender. It will be important to include such effects and determine their impact on the treatment regimen comparisons.

One of the underlying assumptions for the model is that the responses will behave linearly during each active treatment period. This assumption will be investigated graphically and if it is determined that it is not viable, then alternative models will be considered, such as a piecewise-linear model or a cell means model, over each of the active treatment periods. PROC MIXED of SAS Version 8.1 will be invoked for performing the longitudinal data analysis.

The intent-to-treat (ITT) paradigm will be invoked as the major form of statistical analysis for the secondary outcome variables. The ITT analysis requires that all available data be included in the data analysis, even data from subjects who have experienced treatment failure. It is possible that the ITT analysis will yield a small bias because subjects who experience treatment failure are provided emergency medications. Supplemental statistical analyses will be conducted in which data are truncated after the occurrence of treatment failures.

One issue with the inflammatory markers (expired NO, sputum eosinophils, etc.) is that they tend to display nonnormal distributions, especially with a relatively modest amount of clumping at zero. In the SOCS trial, such variables were analyzed via nonparametric tests, namely, Wilcoxon signed-rank tests for changes within a treatment group and Wilcoxon rank sum tests for comparing the changes across treatment regimens. A similar nonparametric approach will be applied for the inflammatory outcomes in this trial.

No interim analysis of efficacy is planned for this trial because most of the recruitment goal will have been attained by the time one-half of the randomized subjects have completed the study (or withdrawn consent) and provided data for both treatment periods of the crossover trial. Informal analysis of safety will be presented to the ACRN Data and Safety Monitoring Board (DSMB).

The DSMB also will receive any reports of serious adverse events as they occur throughout the course of the trial.

F. Effect Size Calculations

The target sample size is 180 randomized subjects for this crossover trial, as described in section VIII.A for the primary outcome variable of time to treatment failure. The following table illustrates the effect sizes that can be detected for the secondary outcome variables (crossover trial design, 90% statistical power, 0.05 significance level, 10% withdrawal rate), based on variability estimates from the ACRN SOCS trial. The differences in the table refer to the differences in the changes between the end of the treatment period and the end of the run-in period for the two treatment regimens.

Outcome	Between-Group
FEV_1 (L)	0.23
AM PEF (L/min)	35
PC_{20} (doubling dilutions)	0.51
PM PEF (L/min)	35
PEF variability (%)	2.6%
Sputum eosinophils (%)	2.3%
Supernatant tryptase (ng/ml)	2.1
Exhaled nitric oxide (ppb)	3.1

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